



**Babylon University
College of Medicine**

Study of Some Bacterial Isolates Associated with Leukocytospermia in Asthenospermic Patients in Hilla City

A Thesis

**Submitted to the Council of the College of Medicine,
University of Babylon, in Partial Fulfillment of the
Requirements for the Degree of Master in
Medical Microbiology**

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جامعة بابل
كلية الطب

دراسة بعض العزلات البكتيرية المصاحبة لتقيح السائل المنوي في مرضى وهن النطف في مدينة الحلة

رسالة

مقدمة الى مجلس كلية الطب /جامعة بابل
كجزء من متطلبات نيل درجة الماجستير في
علم الأحياء المجهرية الطبية

من قبل

حسين عليوي مطلب الدهموشي
بكالوريوس علوم حياة-أحياء مجهرية/جامعة بابل(2005)

إشراف

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Abstract

Abstract:

One hundred asthenospermic seminal fluid specimens were collected from 100 infertile males who referred to Babylon maternity and children hospital-infertility center during a period of six months (from November 2007 through April 2008). The age of patients ranged from (25-44) years with mean age of (32.11) years. The specimens were diagnosed as asthenospermic by the seminal fluid analyses (SFA) and the number of the pus cells/ml of the seminal fluid were also recorded.

It was found that 70 seminal fluid specimens had asthenospermia with leukocytospermia (First group). The rest 30 seminal fluid specimens had asthenospermia without leukocytospermia (Second group).

Regarding semen cultures the results showed that 61(87.1%) of specimens of First group revealed positive bacterial culture, whereas 9(12.9%) specimens of First group showed no bacterial growth even after incubation of 48 hours. All semen specimens of Second group revealed negative bacterial culture. Gram positive bacteria constituted 44(62.9%) while gram negative bacteria constituted 26(37.1%) of isolates. Coagulase negative staphylococci (CoNS) represented by *Staphylococcus epidermidis* & *Staphylococcus saprophyticus* were the common type of bacterial isolates 25(35.7%) followed by *Staphylococcus aureus* 19(27.2%), *Escherichia coli* 12(17.1%), *Enterobacter aerogenes* 8(11.4%), *Acinetobacter* spp 4(5.7%) and *Moraxella* spp 2(2.9%).

The virulence factors of bacterial isolates were investigated. Only 5(26.3%) isolates of *S. aureus* and 2(16.7%) isolates of *E. coli* were found to be able to form capsule. The results showed that all *S. aureus* isolates, 18(72%) isolates of CoNS and 5(41.7%) of *E. coli* isolates and 4(50%) of *E. aerogenes* isolates produce hemolysin. All isolates of *E. coli* and *Moraxella* spp were to be

Abstract

siderophore producers. 3(15.8%) of *S. aureus*, 17(68%) of CoNS, 6(75%) isolates of *E. aerogenes* and 3(75%) isolates of *Acinetobacter* spp produce siderophore.

Colonization factor antigens (CFA/III) were detected in all isolates of *S. aureus*, CoNS, *E. coli*, *E. aerogenes*, *Acinetobacter* spp. and *Moraxella* spp. (CFA/I) were expressed in 10(52.6%) isolates of *S. aureus*, 8(32%) isolates of CoNS, 8(66.7%) isolates of *E. coli*, 6(75%) isolates of *E. aerogenes*, 2(50%) isolates of *Acinetobacter* spp. and 1(50%) isolate of *Moraxella* spp.

The ability of bacteriocin production was found in 6(31.6%) isolates of *S. aureus*, 10(83.3%) isolates of *E. coli*, 2(25%) isolates of *E. aerogenes* and 1(50%) isolate of *Moraxella* spp. Lipase produced by 15(78.9%) and 7(28%) isolates of *S. aureus* and CoNS isolates respectively, while 9(75%) isolates of *E. coli*, 7(87.5%) isolates of *E. aerogenes* and 1(50%) isolate of *Moraxella* spp. produce lipase. Only 7(36.8%) isolates of *S. aureus* and 5(41.7%) isolates of *E. coli* were found to be protease producers.

The effects of some antibiotics on bacterial isolates were investigated. The results showed that, the bacterial isolates were highly susceptible to imipenem, meropenem and ciprofloxacin whereas exhibited moderate resistance to amikacin (42.7%), gentamycin (49%) and norfloxacin (49.3%). On the other hand bacterial isolates revealed high rate of resistance to amoxicillin (95.8%), ceftizoxime (89.7%), ceftazidime (86.7%), cefamandole (82.7%), doxycycline (72.4%), cefepime (72%), trimethoprim-sulfamethoxazole (66.3%), amoxicillin–clavulanic acid (61.1%) and tobramycin (58.5%).

بِسْمِ اللَّهِ الرَّحْمَنِ الرَّحِيمِ

((قَالُوا سُبْحَانَكَ لَا عِلْمَ لَنَا إِلَّا مَا

عَلَّمْنَا إِنَّكَ أَنْتَ الْعَلِيمُ الْحَكِيمُ))

مِثْقَالَ ذَرَّةٍ ۚ وَاللَّهُ عَلِيمٌ غَلِيبٌ

سُورَةُ الْبَقَرَةِ

الآيَةُ (32)

Examination committee

We, the examiner committee, certify that we have read the thesis entitled (**Study of Some Bacterial Isolates Associated with Leukocytospermia in Asthenospermic Patients in Hilla City**) and have examined the student (**Hussein Oleiwi Muttaleb Al-Dahmoshi**) in its contents, and that in our opinion it is accepted as a thesis for degree in Master of Science in **Medical Microbiology** with excellent estimation.

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الخلاصة

تم في هذه الدراسة جمع (100) عينة سائل منوي تعاني من وهن النطف من مائة ذكر عقيم راجعوا مركز العقم في مستشفى بابل للولادة والأطفال خلال ستة أشهر للفترة من تشرين الثاني 2007 لغاية نيسان 2008. وكانت أعمارهم تتراوح (25-44) سنة وكان متوسط أعمارهم (32.11). أظهر تحليل السائل المنوي (Seminal fluid analysis) إن هؤلاء المرضى كانوا يعانون من وهن النطف. كذلك تم الكشف عن حالات (Leukocytospermia) من خلال تحديد عدد الخلايا القحيية (WBCs count) الموجودة في عينة السائل المنوي لأولئك المرضى.

أظهرت نتائج هذه الدراسة أن (70) عينة من عينات السائل المنوي التي تعاني من وهن النطف احتوت على الخلايا القحيية بإعداد غير اعتيادية بلغت أكثر من 1×10^6 خلية قحيية/مليتر من السائل المنوي (المجموعة الأولى)، أما البقية (30) عينة سائل منوي فقد احتوت على أعداد طبيعية من الخلايا القحيية أقل من 1×10^6 خلية قحيية/مليتر من السائل المنوي (المجموعة الثانية).

أظهرت نتائج زرع السائل المنوي أن (61) (87.1%) من العينات (المجموعة الأولى) أعطت نتائج موجبة، حيث شكلت البكتريا الموجبة لصبغة غرام (44) (62.9%) من العزلات معظمها كانت من *Staphylococcus epidermidis* Coagulase negative staphylococci (CoNS) ممثلة بـ *Staphylococcus saprophyticus* حيث مثلت (25) (35.7%)، تلتها *Enterobacter aerogenes*, 12 (17.1%) *Escherichia coli*, 19 (27.2%) *aureus* 8 (11.4%) *Acinetobacter* spp, 4 (5.7%) و *Moraxella* spp 2 (2.9%) في حين (9) (12.9%) من العينات أعطت نتائج سالبة لزرع عينات السائل المنوي. من جانب آخر فإن كل عينات السائل المنوي للمجموعة الثانية أعطت نتائج سالبة.

خلال الدراسة تم الكشف عن بعض عوامل الضراوة التي تمتلكها العزلات البكتيرية حيث أظهرت النتائج أن (5) (26.3%) عزلات من *S. aureus* و (2) (16.7%) عزلة من *E. coli* كانت حاوية على المحفظة. وفيما يخص إنتاج الهيمولايسين والسيروفورات كانت النتائج كالآتي جميع عزلات *S. aureus* و (5) (41.7%) عزلات من *E. coli* وأربع عزلات من *E. aerogenes* كانت منتجة للإنزيم الحال للدم (هيمولايسين) من نوع بيتا. في حين كانت (18) (72%) عزلة فقط من CoNS منتجة للإنزيم الحال للدم من نوع الفا. جميع عزلات *E. coli* و *Moraxella*، ست عزلات من *E. aerogenes*، ثلاث عزلات من *Acinetobacter*، 3 (15.8%) عزلات من *S. aureus* و (17) (68%) عزلة من CoNS كانت منتجة للسيروفورات.

تم الكشف عن قابلية العزلات البكتيرية على امتلاك عوامل الاستيطان، وجد أن جميع عزلات *S. aureus*، CoNS، *E. coli*، *E. aerogenes*، *Moraxella*، *Acinetobacter* كانت تمتلك عامل الاستيطان الثالث في حين كانت (10) (52.6%) عزلات *S. aureus*، (8) (32%) عزلات CoNS، (8) (66.7%) عزلات *E. coli*، ست عزلات *E. aerogenes*، عزلتين *Acinetobacter* و عزلة واحدة *Moraxella* فقط تمتلك عامل الاستيطان الأول. فيما يخص إنتاج البكتريوسين فقد كانت (6) (31.6%) عزلات *S. aureus*، (10) (83.3%) عزلات *E. coli*، وعزلتين من *E. aerogenes* وعزلة واحدة *Moraxella* كانت منتجة للبكتريوسين.

بالنسبة لإنتاج بعض الأنزيمات المهمة في ضراوة البكتريا فقد أظهرت النتائج أن (15) (78.9%) عزلة *S. aureus*، (9) (75%) عزلات *E. coli*، (7) (28%) عزلات CoNS وسبع عزلات *E. aerogenes* وعزلة واحدة *Moraxella* لها القابلية على إنتاج إنزيم اللايباز. في حين أظهرت (7) (36.8%) عزلات *S. aureus* و (5) (41.7%) عزلات *E. coli* القابلية على إنتاج إنزيم البروتياز الخارجي.

أظهرت نتائج اختبار الحساسية للمضادات الحيوية إن العزلات البكتيرية كانت حساسة جدا لمضادات الامبيسين، الميروبينيم والسبروفلوكساسين في حين أظهرت مقاومة متوسطة لمضادات

اميكاسين (42.7%) , جنتاميسين (49%) و للنورفلوكساسين (49.3%). من جانب آخر فان أعلى نسبة مقاومة أظهرتها العزلات البكتيرية كانت لمضادات الاموكسيلين (95.8%) , سيفتيزوكسيم (89.7%) , سيفتازديم (86.7%) , سيفماندول (82.7%) , دوكسيساكيلين (72.4%) , سيفيفيم (72%) , ترايميثوبريم-سلفاميثوكسازول (66.3%) أموكسلين-كلافيلولانيك أسيد (61.1%) , توبراميسين (58.5%) ,

List of Contents

No.	Subject	Page
	List of contents	I
	List of tables	VI
	List of figures	VI
	Abbreviations	VII
Chapter One: Introduction and Literature Review		
1.1.	Introduction	1
1.2.	Literatures review	3
1.2.1.	Bacteriospermia	3
1.2.1.1.	Definition and etiology	3
1.2.1.2.	Effect of bacteriospermia on spermatozoa	4
1.2.1.3.	Spermagglutination by bacteria	6
1.2.2.	Male infertility	7
1.2.2.1.	Overview	7
1.2.2.2.	Male infertility nomenclature	7
1.2.2.2.1.	Azoospermia	7
1.2.2.2.2.	Asthenospermia	8
1.2.2.2.3.	Teratospermia	9
1.2.2.2.4.	Oligospermia	9
1.2.2.2.5.	Leukocytospermia	9
1.2.2.2.6.	Immunological infertility	10
1.2.3.	Bacteriospermia and male infertility	11
1.2.4.	Bacterial agents	12
1.2.4.1.	Gram positive bacteria	12
1.2.4.1.1.	Coagulase positive staphylococci (<i>Staphylococcus aureus</i>)	12
1.2.4.1.2.	Coagulase negative staphylococci (CoNS)	15

1.2.4.2.	Gram negative bacteria	16
1.2.4.2.1.	<i>Escherichia coli</i>	16
1.2.4.2.2.	<i>Enterobacter</i> spp.	17
1.2.4.2.3.	<i>Acinetobacter</i> spp.	18
1.2.4.2.4.	<i>Moraxella</i> spp.	20
1.2.5.	Virulence factors of bacteria associated with bacteriospermia	21
1.2.5.1.	Capsule formation	21
1.2.5.2.	Hemolysin production	22
1.2.5.3.	Siderophore production	22
1.2.5.3.1.	Phenolate-type siderophores	24
1.2.5.3.2.	Hydroxamate-type siderophores	24
1.2.5.4.	Coagulase production	24
1.2.5.5.	Protease production	25
1.2.5.6.	Lipase production	25
1.2.5.7.	Colonization factors	25
1.2.5.8.	Bacteriocin production	26
1.2.6.	Resistance of bacteria to antibiotics	28
1.2.6.1.	Genetic mechanisms of antibiotic resistance	28
1.2.6.2.	Biological mechanisms of antibiotic resistance	29
1.2.6.2.1.	Antibiotic destruction or antibiotic transformation	29
1.2.6.2.2.	Impermeability	30
1.2.6.2.3.	Receptor modification	30
1.2.6.2.4.	Antibiotic active efflux	31
1.2.6.2.5.	Alteration of metabolic pathway	31

Chapter two : Materials and Methods		
2.1.	Materials	32
2.1.1.	Patients	32
2.1.2.	Laboratory equipments	33
2.1.3.	Chemical materials	33
2.1.4.	Biological materials	34
2.1.5.	Antibiotic disks	34
2.2.	Methods	35
2.2.1.	Specimen collection	35
2.2.1.1.	Seminal fluid analysis	36
2.2.2.	Preparation of the reagents and solutions	36
2.2.2.1.	Catalase reagent	36
2.2.2.2.	Ready made reagents	36
2.2.2.3.	Phosphate buffer solution(PBS) (pH=7.3)	37
2.2.2.4.	Coppric sulphate solution (20%)	37
2.2.2.5.	Tannic acid solution (1%)	37
2.2.2.6.	D-mannose solution preparation (0.1 M)	37
2.2.2.7.	Urea solution (20%)	37
2.2.2.8.	Trichloroacetic acid (TCA) solution (5%)	37
2.2.3.	Preparation of culture media	38
2.2.3.1.	Blood agar medium	38
2.2.3.2.	Chocolate agar medium	38
2.2.3.3.	MacConkey agar medium	38
2.2.3.4.	Nutrient agar medium	38
2.2.3.5.	Mannitol salt agar medium	39
2.2.3.6.	Muller Hinton agar	39
2.2.3.7.	M9 medium	39
2.2.3.8.	Brain heart infusion(BHI) broth -glycerol medium	39

2.2.3.9.	Egg-yolk agar medium	39
2.2.3.10.	Urea agar medium	39
2.2.4.	Laboratory Diagnosis	40
2.2.4.1.	Microscopic examination and colonial morphology	40
2.2.4.2.	Physiological and biochemical tests	40
2.2.4.2.1.	Oxidase test	40
2.2.4.2.2.	Catalase test	40
2.2.4.2.3.	Coagulase test	41
2.2.4.2.4.	Mannitol fermentation test	41
2.2.4.2.5.	Urease(Christensen's) test	41
2.2.4.2.6.	Motility test	41
2.2.4.3.	Rapid identification system	42
2.2.5.	Virulence factors tests	42
2.2.5.1.	Capsule stain test (Hiss's Method)	42
2.2.5.2.	Hemolysin production test	43
2.2.5.3.	Siderophores production test	43
2.2.5.4.	Extracellular protease production test	43
2.2.5.5.	Haemagglutination test (HA)	43
2.2.5.6.	Bacteriocin production test	44
2.2.5.7.	Lipase production test	45
2.2.6.	Antimicrobial susceptibility test	45
2.2.7.	Preservation of bacterial isolates	45
2.2.8.	Statistical analysis	45
Chapter three: Results and discussion		
3.1.	Laboratory investigation	46
3.1.1.	Asthenospermia and leukocytospermia	46
3.1.2.	Bacterial isolates from asthenospermic specimens	46
3.2.	Pathogenicity of bacteria in asthenospermic patients	49

3.3.	Identification of bacterial isolates	52
3.3.1.	Gram positive bacteria	52
3.3.2.	Gram negative bacteria	53
3.4.	Virulence factors of the bacterial isolates	54
3.4.1.	Coagulase production	55
3.4.2.	Capsule production	55
3.4.3.	Hemolysin production	56
3.4.4.	Siderophore production	58
3.4.5.	Bacteriocin production	59
3.4.6.	Lipase production	60
3.4.7.	Extracellular protease production	60
3.4.8.	Colonization Factor Antigen(CFA)	61
3.5.	Effect of some antibiotics on bacterial isolates	62
Conclusions and Recommendations		
4.1.	Conclusions	81
4.2.	Recommendations	82
References		
	References	83
	Appendices	124

List of Tables

Table No.	Title	Page No.
2-1	Laboratory equipments	33
2-2	Chemical materials	33
2-3	Biological materials	34
2-4	Antibiotic disks	34
3-1	Distribution of asthenospermia, leukocytospermia and bacteriospermia	46
3-2	Distribution of bacterial isolates from patients with asthenospermia according to the isolates.	48
3-3	Conventional and rapid identification system (HiStph identification kit (Himedia /India) for gram positive bacteria.	52
3-4	Conventional and rapid identification system (Hi 25 Enterobacteriaceae identification kit Himedia /India) for gram negative bacteria.	53
3-5	virulence factor of gram positive bacterial isolate	55
3-6	virulence factor of gram negative bacterial isolate	57

List of Figures

Figure No.	Title	Page No.
3-1	Antibiotics resistance of gram positive isolates to penicillin, methicillin, oxacillin and vancomycin	64
3-2	Resistance of bacterial isolates to amoxicillin and amoxicillin-clavulanic acid	67
3-3	Resistance of bacterial isolates to cephalosporins	70
3-4	Resistance of bacterial isolates to carbapenems	72
3-5	Resistance of bacterial isolates to aminoglycosides	73
3-6	Resistance of bacterial isolates to Fluoroquinolones	76
3-7	Resistance of bacterial isolates to doxycycline and trimethoprim-sulfamethoxazole	78

List of Abbreviations

Abbreviations	Key
Abs	Antibodies
AK	Amikacin
AM	Amoxicillin
AMC	Amoxicillin-clavulanic acid
AmpC	β -lactamase type enzyme
ASA	antisperm antibodies
BHI	Brain heart infusion
CA-MRSA	Community-acquired, Methicillin-resistant <i>Staphylococcus aureus</i>
CAZ	Ceftazidime
CDC	Center of disease control
CFA	Colonization Factor Antigen
CFA/I	Colonization Factor Antigen-I
CFA/II	Colonization Factor Antigen-II
CFA/III	Colonization Factor Antigen-III
CFs	Colonization Factors
CIP	Ciprofloxacin
CN	Gentamycin
CoNS	Coagulase negative staphylococci
CPPS	chronic pelvic pain syndrome
D.W.	Distilled water
DNA	Deoxyribonucleic Acid
DO	Doxycycline
EARSS	European Antimicrobial Resistance Surveillance System
EMB	Eosin methylin blue
EPS	Extracellular polysaccharide

ESBL	Extended spectrum beta-Lactamase
ETEC	Enteroxogenic <i>Escherichia coli</i>
FEP	Cefepime
G+ve	Gram positive bacteria
gal	Galactose
gm	gram
G-ve	Gram negative bacteria
HPF	high power field
hrs.	hours
ICU	intensive care unit
IgG	Immunoglobulin G
IPM	Imipenem
kDa	Kilo dalton
M.W.	Molecular weight
MA	Cefamandole
MAGI	Male accessory gland inflammation
MDR	Multi-drug resistant
ME	Methicillin
<i>mecA</i>	Methicillin resistance gene
MEM	Meropenem
MFS	Major facilitator superfamily
MRCoNS	Methicillin -resistant coagulase negative staphylococci
MRSA	Methicillin resistant <i>Staphylococcus aureus</i>
MRS	Methicillin-resistant staphylococci
MSSA	Methicillin-sensitive <i>Staphylococcus aureus</i>
NCCLS	National committee for clinical laboratory standards
NNIS	National nosocomial infections surveillance system
NOR	Norfloxacin

ONPG	O-nitrophenyl galactoside
OX	Oxacillin
P	Penicillin G
PABA	Para-aminobenzoic acid
PBPs	Penicillin-binding proteins
PCF	Putative colonization factors
PRNG	Penicillin-resistant <i>Neisseria gonorrhoeae</i>
RBCs	Red blood cells
rpm.	Round per minute
rRNA	Ribosomal ribonucleic acid
SFA	Seminal fluid analysis
SPA	Sperm penetration assay
TBP	Transferrin binding protein
TCA	Trichloroacetic acid
TMP-SMX	Trimethoprim-sulfamethoxazole
TOB	Tobramycin
U	unit
UTIs	urinary tract infections
VA	Vancomycin
VISA	vancomycin-intermediate <i>Staphylococcus aureus</i>
VRCoNS	vancomycin resistant- coagulase negative staphylococci
VRSA	Vancomycin-resistant <i>Staphylococcus aureus</i>
WBCs	white blood cells
WHO	World health organization
ZOX	Ceftizoxime
β -lactam	Beta-lactam
μ g	Microgram

Dedication

To..

Soul of my brother ... (Muntadher).

Great heart ... My Father.

Great lady ... My mother.

Helper & supporter ... My brothers & friends.

Pure soul ... My wife.

Hussein

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Hussein

Chapter One

Introduction and Literatures Review

Chapter Two

Materials and Methods

Chapter Three

Results and Discussion

Conclusions and Recommendations

References

Appendices

Supervision Certificate

We certify that this thesis was prepared under our supervision at the Department of College of Medicine/Babylon University, as a partial requirements for the degree of **Master of Science in Medical Microbiology (Bacteriology)** and this work has never been published anywhere.

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Introduction and Literature Review

1.1. Introduction:

Male urogenital tract infection is one of the most important causes of male infertility, worldwide since genital tract infection and inflammation have been associated with 8-35% of male infertility cases (Keck *et al.*, 1998; Elbhar, 2005). Bacteriospermia is defined as the presence of bacteria in seminal fluid samples (Onemu and Ibeh, 2001). Bacteriospermia may play a major role in infertility (Li and Liu, 2005; Bukharin *et al.*, 2003). Male accessory sex glands infection is a major risk factor in infertility (Diemer *et al.*, 2000). The significance of pathophysiology of bacteriospermia has been seriously discussed in recent years. Some possible pathomechanisms of the development of infertility linked with infection are considered: direct effect on sperm function (motility, morphology), deterioration of spermatogenesis, autoimmune processes induced by inflammation and dysfunction of accessory sex glands (Keck *et al.*, 1998; Bukharin *et al.*, 2003). Hence, microbiological investigation of male partners in infertile couple can be useful to detect the male urogenital tract infection, especially asymptomatic infections.

The isolation of microorganisms from seminal fluid especially of infertile men had been widely reported (Mogra *et al.*, 1981; Villanueva-Diaz *et al.*, 1999; Orji *et al.*, 2007; Gdoura *et al.*, 2008). It is always recommended that microbiological study of semen can be performed in asymptomatic infertile men with leukocytospermia. Aerobic and anaerobic culture of semen can detect a wide range of urogenital pathogens (Palayekar *et al.*, 2000). The most widely studied genital microorganism in relation to male infertility is *Escherichia coli*, which is also the principal microorganism that causes prostatitis and epididymitis (Bartoov *et al.*, 1991; Diemer *et al.*, 1996). Infections in the

reproductive tract of infertile men have been acknowledged for decades (Nikkanen *et al.*, 1979). Until recently, the condition of leukocytospermia was used as an indicator of genital tract infection (Behre *et al.*, 1997). However, a relatively large number of men who attend fertility clinics exhibit leukocytospermia without symptoms of genital infections, indicating that there is not a necessary relationship between infections in the genital tract and the amount of leukocytes or antisperm antibodies in semen (Eggert-Kruse *et al.*, 1998; Micic *et al.*, 1990; Wolff, 1995; Trum *et al.*, 1998). Since little or no attention has been paid to the role of bacterial infection of seminal fluid in asthenospermia and male infertility in Iraq by many researchers and postgraduate students, hence this study was suggested and designated to fulfill the following goals:

- 1-Investigation of the relationship between bacteriospermia and leukocytospermia in infertile male with asthenospermia.
- 2-Isolation and identification of the bacterial isolates by some biochemical test and rapid identification systems.
- 3-Studying some of the virulence factors and antimicrobial susceptibility patterns of the isolated bacteria.

1.2. Literature review

1.2.1. Bacteriospermia:

1.2.1.1. Definition and etiology:

Genital tract infection can be caused by many types of bacteria e.g. *Neisseria gonorrhoeae*, *Chlamydia trachomatis*, *Mycoplasma hominis*, *Ureaplasma urealyticum*, *Escherichia coli* and other gram negative (G-ve) bacilli (Shefi and Turek, 2006). Genital ureaplasmas (*U. urealyticum* and *U. parvum*) and genital mycoplasmas (*M. genitalium* and *M. hominis*) are natural inhabitants of male urethra contaminating the semen during ejaculation. However, these microorganisms particularly *U. urealyticum* are potentially pathogenic species playing an etiologic role in both genital infections and male infertility (Andrade-Rocha, 2003; Wang *et al.*, 2006). During the past decade, evidences for damage caused by *U. urealyticum* to the development and vitality of human embryos had accumulated. In human *in vitro* fertilization systems, the presence of *U. urealyticum* in either semen or female genital tract resulted in a decline in pregnancy rate per embryo transfer (Montagut *et al.*, 1991; Reichart *et al.*, 2000). Bacteriospermia was caused by both gram positive (G+ve) bacteria and G-ve bacteria as well as by *Chlamydia* spp. and *Mycoplasma* spp. (Chimura and Saito, 1990; Villanueva-Diaz *et al.*, 1999; Lackner *et al.*, 2006). Chimura and Saito (1990) stated that the rate of detection of G+ve bacteria in semen was high (40/51), while the rate of G-ve bacteria accounted for (11/51). Rodin and his colleagues (2003) found that staphylococci were the most common bacteria detected in semen of infected patients followed by viridans Streptococci and *Enterococcus faecalis*. Lackner and his colleagues (2006) found that the most bacterial pathogens that caused bacteriospermia were *U. urealyticum*, *Enterococcus faecalis* and *Escherichia coli* which constituted 23.8%, 16.8%,

and 7.0% of respectively. Other study revealed that, the most common bacterial types isolated from patients with bacteriospermia were *C. trachomatis* (41.4%), *U. urealyticum* (15.5%) and *M. hominis* (10.3%) (Gdoura *et al.*, 2008). The Presence of pathogenic microorganisms in semen, which may be related to a breach in the integrity of the blood-testes barrier, may provide early warning signals of impairment of male fertility (Onemu and Ibeh, 2001). Also bacteriospermia may be resulted from recent seminal tract infection such as orchitis, epididymitis and prostatitis (Keck *et al.*, 1998; Weinder *et al.*, 1999). Swenson and his colleagues (1980) affirmed that the presence of organisms in the semen may also be related to gynecologic infections.

1.2.1.2. Effect of bacteriospermia on spermatozoa:

The harmful effect of bacteria on spermatozoa depends on the type and species of microorganisms invading, colonizing, or infecting the male genital tract and is associated with the accompanying oxidative stress (Fraczek *et al.*, 2007). Genital infections may affect the secretory function in seminal vesicles and prostate. Male accessory gland inflammation (MAGI) may also lead to decreased epididymal secretion of alpha-glucosidase (Depuydt *et al.*, 1998), which has been shown to have a positive effect on spermatozoa binding capacity and intrauterine insemination (Ben Ali *et al.*, 1994; Milingos *et al.*, 1996). Microbial infection has been linked with infertility problem in a number of studies (Swenson *et al.*, 1980; Osegbe and Amaku, 1985; Rodin *et al.*, 2003). While, the exact role of microbial infection in the aetiology of infertility is not very certain owing to the limitations in diagnostic criteria and asymptomatic nature of infection (Purvis and Christiansen, 1993). Some possible effect on the properties of seminal fluid associated with fertility had been suggested (Bukharin *et al.*, 2003; Rodin *et al.*, 2003).

Bacterial inhibitory effect on sperm motility parameter had been documented. *E. coli* had been shown to have a significant negative effect on sperm motility (Diemer *et al.*, 1996; Huwe *et al.*, 1998; Philip and Folstad, 2003). Golshani and his colleagues (2006) noted that the rate of non-motile and morphologically abnormal sperms was higher in positive cases of bacteriospermia, especially in *E. coli* and enterococci positive samples. *S. aureus* had an inhibitory effect on human sperm motility *in vitro* (Ji-Hong *et al.*, 2002). In fact, such inhibitory effects on sperm motility were not found with other pathogens such as *S. saprophyticus*, *Pseudomonas aeruginosa*, and *Enterococcus* (Huwe *et al.*, 1998; Kohn *et al.*, 1998).

Another effect of pathogenic bacterium on spermatozoa was the impairment of spermatozoal membrane. The functional and structural integrity of sperm membrane are crucial for the viability of spermatozoa. The Effect on spermatozoal membrane was studied *in vitro* by Qiang and his colleagues (2007), the results revealed that, when sperm treated with β -hemolytic strains, the membranes of their heads were swollen, deformed, obscured and even broken off. The acrosomal membrane and nuclear membrane could be seen injured too, which was curled, distorted and broken off. The membrane in the neck and the middle piece of the tail was defective. Mitochondria were disorderly arranged, and some components were released from the cytoplasm, but the membrane in the end piece of the tail were less damaged and its membranes were comparatively intact. This indicated that hemolytic bacterium impaired significantly spermatozoa membrane. *U. urealyticum* affected sperm quality, but the mechanism had not been yet elucidated. Some investigators did not find any correlation between the presence of *U. urealyticum* and semen alteration (Bornman *et al.*, 1990; Wang *et al.*, 2006), other workers reported that the presence of *U. urealyticum* in semen was related to a decrease in sperm

concentration (Wang *et al.*, 2006), motility (De Jong *et al.*, 1990), and/or morphology (Xu *et al.*, 1997). The dual effect of *U. urealyticum* on the sperm activity (inhibition of sperm motility at low pH and increase of sperm velocity at higher pH, depending on sperm metabolism) has been recently demonstrated (Reichart *et al.*, 2001).

1.2.1.3. Spermagglutination by bacteria:

The importance of the receptor-ligand interaction in the pathogenesis of urinary tract infection was well documented (Roberts, 1992). Certain *E. coli* strains are pathogenic in the urinary tract because they possess fimbriae, rigid filamentous proteinaceous appendages that are attached to specific uroepithelial receptors. These receptors may be glycoproteins as with type 1 fimbriae or glycolipids as with P-fimbriae. The essential receptor component in glycoproteins for type 1 fimbriae is an mannose group (mannose). The essential minimal active moiety in glycolipids for P-fimbriae is an α -D-galp-1-4-9-D-galp (gal gal). Fimbriae-dependent interactions can be confirmed if they are competitively inhibited by addition of the specific receptor component. Because the surface of spermatozoa is rich in glycoproteins, even asymptomatic colonization of the male or female genitalia with Enterobacteriaceae may result in similar interactions. Isolation and characterization of the receptors may allow therapy aimed for prevention of colonization with spermagglutinating microorganisms, or directed for inhibiting the receptor-ligand interaction. The corollary would be development of monoclonally derived receptor clones capable of spermagglutination as a biological contraceptive (Monga and Roberts, 1994).

1.2.2. Male infertility

1.2.2.1. Overview:

Infertility is defined as the lack of conception after 12 months of unprotected intercourse. On evaluation, roughly 50% of affected couples have causal or associated male factors as a cause of infertility (Greenspan and Gardener, 2001; Shefi and Turek, 2006). Evaluation of the infertile men requires a complete medical history, physical examination and laboratory investigation. Usually 80% of couples are able to conceive within the first year of marriage (McClure, 1992). Male infertility has several different possible causes which are primary or secondary testicular failure, infection and obstruction, but the most common diagnosis is idiopathic infertility, which accounts for about 60-70% of the patients (Nieschlag and Behre, 1997).

Primary infertility is a term used for those couples who have never conceived while; secondary infertility is a term that refers to those couples who have at least one conception but currently unable to achieve pregnancy. Greenspan and Gardner (2001) reported that male factors were responsible for about 40% of cases, female factors for about 40% and couple factors for 20%. The male needed normal spermatogenesis, normal reproductive system anatomy and normal sexual function to deposit an adequate number of morphologically normal, motile spermatozoa in the upper part of vagina (Jaffe and Jewelewicz, 1991).

1.2.2.2. Seminal fluid nomenclatures:

1.2.2.2.1. Azoospermia:

Azoospermia means the absence of sperms in the seminal fluid and this is either due to testicular dysfunction or due to bilateral obstruction in ducts

system of male reproductive tract (WHO, 1999). Defect in spermatogenesis can be classified into maturation arrest, hypoplasia involving germ cells and disorganization and sloughing of the germinal epithelium (Paulsen, 1974). Maturation arrest may be resulted from trauma or torsion of testis associated with a damage to the spermatogenic epithelium including sertoli cells, while leydig cells are resistant and remain functionally normal (Steinberger and Steinberger, 1972).

1.2.2.2.2. Asthenospermia:

Asthenospermia is a term that indicates a significant reduction in sperm motility. It is an important parameter for evaluating the fertility potential of sperm, so sperm count is meaningless unless sperm motility is also taken into account (Macleod and Wang, 1979). The ejaculate may be considered abnormal if more than 50% of spermatozoa showed a decrease in motility within one hour after ejaculation (Al-barazanchi *et al.*, 1992). Asthenospermia can be either moderate asthenospermia when motility 20% - 40% or severe asthenospermia when motility < 20% (Pardo *et al.*, 1988).

The increase of abnormalities in the asthenospermia was of testicular origin during spermatogenesis, and the spermatozoa of asthenospermic patients were characterized not only by their defective concentration, morphology and motility but also by their relative nuclear immaturity (Pardo *et al.*, 1988). Sometimes drug therapy may result in decreased sperm motility and this can be improved by the removal of the drug, e.g. cimitidine, or introduction of specific culture media to the sperm washing procedures (Ng *et al.*, 1990).

High viscosity of semen impaired spermatozoal motility and this was due to seminal fluid infection and agglutination. The improvement of spermatozoal motility occurred after treatment of patients with antibiotics or after washing the spermatozoa and *in vitro* activation (Fakhrildin, 2000). Some studies reported

that the abnormalities in the seminal fluid osmolality had an adverse effect on sperm viability and motility, also its affect *in vitro* fertilization rate (Al-Anssari *et al.*, 1997; Al-Anssari, 2000).

1.2.2.2.3. Teratospermia:

Teratospermia is defined as the presence of more than 70% of abnormal sperm morphology (WHO, 1999). These abnormal sperms were unable to fertilize the ovum *in vitro* and the fertilization rate was markedly diminished (Oehninger and Alexander, 1991). It had been shown that sperm morphology was the most significant seminal parameter which correlated with sperm fertilizing ability *in vitro* (Kruger *et al.*, 1988). Morphologically normal spermatozoa were more likely to be motile and had significantly higher velocity than abnormal ones, and they were more advantaged in transport through female reproductive tract (Morales *et al.*, 1988).

1.2.2.2.4. Oligospermia:

Oligospermia is defined as a reduction in the sperm count as well as the seminal plasma volume of the ejaculate. The normal sperm count for fertile male ranges from 20 to 60 million /ml of semen (WHO, 1999), this value does not represent the minimum number of sperms which are necessary to achieve fertility (Dana and Alan, 1996).

1.2.2.2.5. Leukocytospermia:

Leukocytospermia or pyospermia, an increase in leukocytes in the ejaculate, is defined as > 1 million leukocytes/ml semen and is a significant cause of male infertility (Shefi and Turek, 2006). The prevalence of pyospermia ranges from 3% to 23% of infertile men. Seminal fluid infection is regarded as one of the semen abnormalities which affect male fertility due to urogenital tract infections. This case is manifested by the presence of leukocytes in semen.

Semen parameters including sperm count, sperm motility, sperm velocity and total number of motile sperm were significantly reduced in the presence of leukocytes (Wolff *et al.*, 1990). Leukocytospermia male are consequently of interest as they may show symptoms of heightened systemic immune activity that is not caused by genital tract infections (Purvis and Christiansen, 1993; Anderson, 1995). Infections outside the genital tract may be asymptomatic but could still contribute to an increase in somatic immune activity and increased influx of leukocytes to the genital tract. Various mechanisms had been proposed on how various inflammatory conditions of the genital tract may lead to male infertility. Many of these notions, however, are still under debate (Eggert-Kruse *et al.*, 1998; Michelmann, 1998; Wolff, 1998; Hales *et al.*, 1999). Leukocyte products such as lymphokines, monokines, and reactive oxygen species had been shown to reduce sperm fertilizing ability (Henkel and Schill, 1998). Leukospermia had been associated with abnormal spermatozoal morphology, including elongated and small heads, tail and neck abnormalities, retention of cytoplasmic droplets, and abnormal acrosomal morphology (Menkveld and Kruger, 1998). Leukocytospermia might also affect hyperactivation of spermatozoa during capacitation (Chan *et al.*, 1994). Elevated leukocytes and granulocytes were believed to release various proinflammatory/bioactive cytokines, hydrogen peroxide, and other reactive oxygen species (ROS) (Aitken *et al.*, 1994; Rajasekaran *et al.*, 1995). Lamirande and Gagnon (1992) declared that lipid peroxidation of sperm membrane is considered to be the key mechanism of this ROS-induced sperm damage leading to infertility.

1.2.2.2.6. Immunological infertility:

Serological studies found that spermatozoa had a considerable number of autoantigens, sperm specific proteins, subsurface antigen in the acrosome, which is the major antigen in the spermatozoa; other antigens were found on the

head and tail (Rose, 1978). It had been suggested that antibodies were present in the accessory fluid bound to sperm at the time of ejaculation (Kay *et al.*, 1993). Other researchers had shown that antibodies on the female genital tract can interfere with sperm motility and potentially, with sperm- ovum interaction by interfering with the dispersion of cumulus mass and sperm binding, penetration of the sperm into the zona pellucida, and sperm- ovum fusion (Marshburn, 1997; Zavos *et al.*, 1998). Antisperm antibodies may be detected in 8%-21% of infertile males. Autoimmunity was firstly enhanced after the initiation of spermatogenesis and sperm-specific antigens first appear at the time of puberty. Since such antigens were not present during the development of immunological tolerance, these proteins are potential targets for an immune response and therefore generation of antisperm antibodies (ASA) (Dana & Alan, 1996).

The roles of most of these antigens in sperm function are currently unknown, some suggested that antibodies to sperm antigens may inactivate their functions and therefore lead to infertility, others suggested that agglutination of sperm leads to their inability to move through the female reproductive tract and sperm cytotoxicity may result. Sperm with bound antibodies may be unable to penetrate through cervical mucus (Mazumdar and Levine, 1998). Antisperm antibodies on the sperm head might impair the development of the acrosome reaction and this will decrease *in vitro* fertilization rate (Al-barazanchi *et al.*, 1992; Ford *et al.*, 1996).

1.2.3. Bacteriospermia and male infertility:

Elbhar (2005) reported that the male fertility was greatly reduced by infections of the urogenital tract. Therefore, infection, which is regarded as one of the abnormalities in the semen because it can contribute to infertility, was manifested by the presence of leucocytes, so it is called leukocytospermia or pyospermia (Gonzales *et al.*, 1992; Shimoya *et al.*, 1993). Wolff and associates (1990) suggested that the identification and quantification of leukocytes in the

semen should be an integral part of every male infertility workup since leukocyte can adversely affect sperm quality *in vitro* or *in vivo*. Munoz and Witkin (1995) declared that the mechanism that results in infertility through *C. trachomatis* infection is not clear. It was assumed that bacterial infections of the genital tract, in particular with *C. trachomatis*, may stimulate the immune system, perhaps via vasoeppididymitis with unilateral obstruction or exposure of the spermatozoa to immunologically competent cells in inflammatory conditions.

Wentz (1988) reported that more than the normal 2-5 leukocytes per high power field (HPF) may suggest prostatitis or another significant infection, and also reported that greater than one million WBCs/ml of semen was considered abnormal. Donovan and Lipshultz (1988) presumed mechanism of infection causing infertility as follows (1) bacterial attachment to sperm, (2) an immobilizing factor produced by bacteria, especially *E. coli*, (3) immune system recruitment, and (4) alterations of glandular function.

1.2.4. Bacterial agents:

1.2.4.1. Gram positive bacteria:

Gram positive bacteria represent a large constituent of bacteriospermia (Golshani *et al.*, 2006). The most important G+ve bacterial types associated with bacteriospermia are described below:

1.2.4.1.1. Coagulase positive staphylococci(*Staphylococcus aureus*):

The pathogenicity of *S. aureus* contributes to hemolysis of the blood, coagulation of the plasma and production of extracellular enzymes and toxins which act on host cell membrane and mediate the cell destruction (Mims *et al.*, 2004). *S. aureus* is often β -hemolytic on blood agar (Murray *et al.*, 2003). *S. aureus* is considered as one of the probable seminal tract pathogens and their pathogenesis is attributed to combined effects of extracellular factors and toxins

together with invasive properties such as adherence and biofilm formation that substantiate their resistance to most available antimicrobial agents and phagocytosis (Goran, 2001; Eiichi *et al.*, 2004). Other virulence factors of *S. aureus* include colonization factor antigen CFA/I, CFA/III, protein A which binds IgG molecules by the Fc region, in serum, bacteria will bind IgG molecules the wrong way round by non immune mechanism; in principle this will disrupt opsonization and phagocytosis (Todar, 1998) indicated that they are important virulence factors, polysaccharide capsule and cell wall that protect it from lysis by osmotic condition and aid the bacteria to attach to mucosal surfaces (Al-Saigh, 2005). Also *S. aureus* possesses both siderophore-mediated and non siderophore iron uptake systems. Each system plays a role during pathogenesis. Several *S. aureus* strains produce siderophores, two of these siderophores, staphyloferrin A and staphyloferrin B are of the polycarboxylate class, while the third is aureochelin which is chemically uncharacterized (Dale *et al.*, 2004). *S. aureus* may be able to scavenge different various sources of host iron (e.g. heme and hemoglobin) during the establishment of an infection, and indeed, *S. aureus* does possess the ability to bind heme and hemoglobin involved in the transport of staphylobactin (Mazmanian *et al.*, 2003).

Methicillin resistant *S. aureus* (MRSA) developed resistance to the antibiotic methicillin and other penicillins (Elshafie and Bernardo, 2001). Staphylococci are carried by healthy people in a variety of body sites without disease being present. Most people do not get sick from staphylococcal bacteria, even MRSA (Infectious Diseases and Immunization Committee, 1999). MRSA become increasingly problematic due to the emergence of resistant strain (Murray *et al.*, 2003). MRSA generally remained an uncommon finding even in hospital settings until the 1990s when there was an explosion in MRSA prevalence in

hospitals where it is now endemic (Johnson *et al.*, 2001). Since first described in 1961 (Jevons, 1961), MRSA has become an increasingly common cause of nosocomial infection and thus a problem of increasing importance. These organisms are frequently associated with infections at the sites of indwelling catheters or in patients who are hospitalized for prolonged periods of time (Romero-Vivas *et al.*, 1995). MRSA infections have become increasingly common over the last several decades and are now present or endemic world wide, more recently, an increasing proportion of MRSA isolates were from hospitalized patients admitted from the community (Moline and Hadler, 2001). MRSA infections in both the hospital and community setting are commonly treated with non- β -lactam antibiotics such as clindamycin (a lincosamine) and co-trimoxazole (also commonly known as trimethoprim/sulfamethoxazole). Resistance to these antibiotics has also lead to the use of new, broad-spectrum anti-gram positive antibiotics such as linezolid because of its availability as an oral drug. First-line treatment for serious invasive infections due to MRSA is currently glycopeptide antibiotics (vancomycin and teicoplanin). There are some problems with these antibiotics, mainly centered around the need for intravenous administration (there is no oral preparation available), toxicity and the need to monitor drug levels regularly by means of blood tests. Glycopeptides must not be used to treat methicillin-sensitive *S. aureus* as outcomes are inferior (Blot *et al.*, 2002).

Vancomycin-resistant *S. aureus* (VRSA) is a strain of *S. aureus* that has become resistant to the glycopeptides. The first case of vancomycin-intermediate *S. aureus* (VISA) was reported in Japan in 1996 (Hiramatsu *et al.*, 1997) but the first case of *S. aureus* was truly resistant to glycopeptide antibiotics was only reported in 2002 (Chang *et al.*, 2003). Three cases of VRSA infection had been reported in the United States in 2005 (Menichetti, 2005).

1.2.4.1.2. Coagulase negative staphylococci (CoNS):

Coagulase-negative staphylococci (CoNS) are ubiquitous microorganisms and predominant in the normal bacterial flora of the skin (Meskin, 1998). They are commonly detected in clinical specimens and several species are recognized as important agents of nosocomial infections, especially in neonates, immunocompromised individuals and patients with internal prosthetic devices (Jarlov, 1999). The origin of these infections can be endogenous or exogenous, coming from the hospital environment or from personnel hands. One critical factor for transmission of microorganisms from a person (patients or health care workers) to the environment and then to another person is the ability of these agents to survive on environmental surfaces (Neely and Maley, 2000). At the present time, CoNS, especially *S. epidermidis* strains, represent the most frequent cause of nosocomial sepsis and they are the most common agents of infections associated with implanted medical devices (Mack *et al.*, 2000). The most important CoNS members to human are *S. epidermidis* and *S. saprophyticus*. They constitute major component of the normal flora of humans, causing nosocomial infection that cause infection in debilitated or compromised patients (Kloss and Bannerman, 1994; Brook *et al.*, 2004). The main focus on mechanisms of pathogenesis has been with foreign body infections and the role of specific adhesions and slime produced by *S. epidermidis*. Slime can reduce the immune response and opsonophagocytosis (Kloos and Bannerman, 1994), and it needs to be pointed out that *S. epidermidis* and other CoNS can cause sepsis, particularly in preterm infants, immunosuppressed patients and patients with intravascular devices (Raad, 2000; Haimi *et al.*, 2002).

1.2.4.2. Gram negative bacteria:**1.2.4.2.1. *Escherichia coli*:**

One of the most important Enterobacteriaceae species and it is the most common cause of urinary tract infections (UTIs), predominantly, strains of *E. coli* that have been identified in 65% to 80% of chronic bacterial prostatitis as the cause of cultural prostatitis (Lipsky, 2003). It is G-ve rods, usually motile, produce polysaccharide capsule, positive tests for indole, lysine decarboxylase and mannitol fermentation and produces gas from glucose. Typical morphology with a metallic sheen on differential media such as EMB agar (Smith and Scotland, 1993; Brook *et al.*, 2004). The majority of community acquired urinary tract infections are caused by uropathogenic *E. coli* and which caused recurrent infection (Ad Dhhan *et al.*, 2005; Joel *et al.*, 2002; Al-Amedi, 2003).

Many strains of *E. coli* uropathogens belong to limited number of O, K, and H serogroups mainly O2, O4, O6, O7, O8 and O75, also production of CFA/I, CFA/II and CFA/III, they increased adherence properties to uroepithelial cells (Blance *et al.*, 1996). Uro-virulence factors play a significant role in the pathogenesis of bacterial prostatitis, for instance, bacterial P-fimbriae binds to the uroepithelial receptors, and this subsequently facilitates ascent into the urinary tract as well as establishing deep infections in the prostate gland itself (Robery *et al.*, 1997). These are observed in 90% of *E. coli* strains causing pyelonephritis but less than 20% of the strains causing lower urinary tract infection (Svenson *et al.*, 1983).

Colonization of the lower urinary tract by *E. coli* is also facilitated by the presence of the type 1 fimbriae, also known as mannose-sensitive fimbriae which bind to glycolipids or glycoproteins receptors on the surface membrane of uroepithelial cells, help bacteria to adhere to bladder and prostatic mucosa and to be important in the development of cystitis and prostatitis in humans,

and its presence in prostatitis has also been documented (Marty *et al.*, 2000).

Most uropathogenic *E. coli* strains produce hemolysin, which initiates tissue invasion and makes iron available for infecting pathogens (Huges, 1996). The presence of K antigen on the invading bacteria protects them from phagocytosis by neutrophil. These factors allow the infecting pathogens to escape the various host defenses (Svanborg *et al.*, 1996). Some strains are urease-producing *E. coli* and they are commonly present in the complicated UTI (Falkow and Collinins, 1990).

1.2.4.2.2. *Enterobacter aerogenes*:

It is a member of Enterobacteriaceae, mucoid on sheep blood agar, some of which are encapsulated, they also possess flagella. In contrast to *Klebsiella*, organisms are motile. *E. aerogenes* is important nosocomial pathogen responsible for a variety of UTIs especially in patients having anatomical defects or indwelling catheters also isolated from patients with chronic prostatitis (Lipsky, 2003). It has been recognized as a nosocomial pathogen, and sometimes as a primary pathogen mainly due to its ability to develop resistance to antibiotics (Neto *et al.*, 2003). It rarely causes disease in a healthy individual (Alhambra *et al.*, 2004). Patients most susceptible to acquire infections with this opportunistic pathogen are those who stay in the hospital, especially the intensive care units for prolonged periods, those using foreign devices such as intravenous catheter and those with serious underlying conditions including burns and immunosuppressant (Clark *et al.*, 2003). Most isolates involved in nosocomial infections are resistant to multiple antibiotics (Arpin *et al.*, 1996). The important virulence factors seem to be largely due to an endotoxin that it produces community-acquired infections which are sometimes observed. The *Enterobacter* species are resistant to cephalothin due to β -lactamase enzymes production, so, strains that cause

hospital-acquired infection are more frequently antibiotic resistant than other strains due to β -lactamase enzymes production (Pitout *et al.*, 1998).

1.2.4.2.3. *Acinetobacter* spp.:

This genus often capsulated, oxidase negative, nonmotile G-ve bacilli or coccobacilli (often diplococco-bacilli), some strains have gelatin liquefaction (Brooks *et al.*, 2001). *Acinetobacter* species are generally considered nonpathogenic to healthy individuals. However, several species persist in hospital environments and cause severe, life-threatening infections in compromised patients. The spectrum of antibiotic resistances of these organisms together with their survival capabilities make them a threat to hospitals as documented by recurring outbreaks both in highly developed countries and elsewhere (Gerischer, 2008). They are one of acknowledged prostate pathogens with other gram-negative uropathogens (Goran, 2001). *A. baumannii* causes 2-10% of all G-ve infections in the U.S. and Europe, poses little risk to healthy individuals, but generally causes infections to those with weakened immune systems (Fournier *et al.*, 2006). The virulence factors of it include production of hemolysin or siderophore, some species produced lipase, and they have no adhesive agents like that of *E. coli* (Bonnet, 2004). In addition, Al-shukri (2003) mentioned that *Acinetobacter* spp. produced CFA/III, siderophore and extracellular protease enzymes but did not produce CFA/I, CFA/II and hemolysin. *A. baumannii* is capable of forming biofilm on glass and plastic surfaces via pili formation (Tomaras *et al.*, 2003). The production of biofilm may explain how *A. baumannii* can survive in different types of conditions in the hospitals, including static conditions such as bed sheets and furniture, while also capable of living in harsh conditions such as catheters and respiratory tubes. *A. baumannii* also produces exopolysaccharides which strengthens the biofilm (Tomaras *et al.*, 2003).

Acinetobacter species are innately resistant to many classes of antibiotics, including penicillin, chloramphenicol, and often aminoglycosides. Resistance to fluoroquinolones has been reported during therapy and this has also resulted in increased resistance to other drug classes mediated through active drug efflux. Efflux pumps located in the cell membrane are used to pump chemicals and antibiotics out of the cell. Efflux pumps in *A. baumannii* include resistance to tetracycline called Tet (A) and Tet (B), part of the major facilitator superfamily (MFS) and functions in the exchange of protons and tetracycline (Vila *et al.*, 2007). Reduced outer-membrane permeability and increased AmpC beta-lactamase production are known as important factors leading to carbapenems resistance in *Acinetobacter* (Quale *et al.*, 2003; Urban *et al.*, 2003). Some strains of *A. baumannii* have become resistant to almost all currently available antibacterial agents (Van Looveren and Goossens, 2004) mostly through the acquisition of plasmids (Seifert *et al.*, 1994), transposons or integrons (Segal *et al.*, 2003; Poirel *et al.*, 2003) carrying clusters of genes encoding resistance to several antibiotic families (Devaud *et al.*, 1982; Poirel *et al.*, 2003) at once.

A dramatic increase in antibiotic resistance in *Acinetobacter* strains has been reported by the center of disease control and prevention (CDC) and the carbapenems are recognized as the gold-standard and/or treatment of last resort. Rather worryingly is an increase in resistance to the carbapenems which leaves very little treatment option although there some success reported with polymyxin B as well as the use of novel combinations of antibiotics (Rahal, 2006). *Acinetobacter* species are unusual in that they are sensitive to sulbactam; sulbactam is most commonly used to inhibit bacterial beta-lactamase, but this is an example of the antibacterial property of sulbactam itself (Wood *et al.*, 2002). As summarized by Go and Cunha (1999), medications to which *Acinetobacter*

is usually sensitive include Meropenem, Colistin, Polymyxin B, Amikacin, Rifampin, Minocycline and Tigecycline.

1.2.4.2.4. *Moraxella* spp.:

The genus *Moraxella* is a member of the family Nisseriaceae *M. catarrhalis* was previously named *Branhamella catarrhalis* and before that *Neisseria catarrhalis* (Brook *et al.*, 2004). The organism is characterized as G-ve, aerobic, oxidase positive, catalase positive, diplococci. They are commensals of mucosal surfaces of upper respiratory tract and occasionally give rise to opportunistic infection. *Moraxella* spp. have loose capsule, relatively unstructured network of polymers that covers the surface of an organism. The capsular polysaccharides are essential virulence factors (Rubens and Wessels, 1987). They inhibit phagocytosis and causes complement inactivation in the absence of specific antibody (Bliss and Silver, 1996). Some pyogenic intracellular cocci have the capacity to resist phagocytosis (Gray *et al.*, 1999). Ahmed and his coworkers (1991) stated that the *M. catarrhalis* strains contain capsular polysaccharide but this capsule can not be differentiated. Other researchers declared that the *M. catarrhalis* strains don't have capsule (Mellenkvist *et al.*, 2003). Many studies (Compagnari *et al.*, 1994) had mentioned that *M. catarrhalis* have high affinity to lactoferrin, transferrin and hemoglobin receptors as a source of iron in the body. The bacterial hemolysin is one of virulence factors but *M. catarrhalis* strains do not produce the hemolysin (Catlin, 1990). Many strains of *M. catarrhalis* can produce amino peptidase (Proteases) (Perez *et al.*, 1990).

1.2.5. Virulence factors of bacteria:

The most common virulence factors of bacteria are:

1.2.5.1. Capsule formation:

Capsule is a discrete detectable layer of polysaccharide deposited outside the cell wall of bacteria. The production of extracellular polysaccharide molecules is a common feature of many bacteria (Whitfield and Valvano, 1993; Roberts, 1996). These molecules may be linked to the cell surface and organized into a discrete structure termed the capsule or, alternatively, may comprise an amorphous slime layer that is easily sloughed from the cell surface. It is known to protect bacteria from engulfment by phagocytes and from attack by antimicrobial agents. Since capsular substances are antigenic they can stimulate B-cells and produce antibodies (Abs) that can neutralize the effect of capsular substances and make the bacteria susceptible to phagocytic cells, this phenomenon does not found in immunocompromised patient because of decrease activity of B-cell to produce Abs required for the opsonization (Rajesh and Rutten, 2004). In the absence of specific antibody, a capsule offers protection against the nonspecific arm of the host's immune system by conferring increased resistance to complement-mediated killing and complement-mediated opsonophagocytosis (Michalek *et al.*, 1988; Moxon and Kroll, 1990). In addition to mediating interactions with the host, it has been suggested that the expression of a hydrated capsule around the cell surface may protect the bacteria from the harmful effects of desiccation and aid in the transmission of encapsulated pathogens from one host to the next (Ophir and Gutnick, 1994). This may be particularly important in highly host adapted pathogens for which there are no alternative hosts and which are unable to survive in the environment. Encapsulated strains of many bacteria are more virulent and more resistant to phagocytosis and intracellular killing than are non

encapsulated strains (Oksuz *et al.*, 2005). *S. aureus* isolates can produce one of 11 different capsular serotypes.

E. coli synthesize at least 80 distinct capsular polysaccharides on the cell surface (Orskov and Orskov, 1992). These capsules have been classified into three groups based on biochemical and genetic criteria (Jann and Jann, 1990; Pearce and Roberts, 1995). Group I capsules are heat-stable, high-molecular weight polysaccharides with a low charge density. Group II capsules are heat labile, have a high charge density, and have a lower molecular weight than those of group I (Jann and Jann, 1990). Group III capsules (formerly group I/II) are also located on the *E. coli* chromosome and have the same general characteristics as those of group II.

1.2.5.2. Hemolysin production:

Many bacteria produce substances that dissolve red blood cells (RBCs) and called hemolysins. There are three types of hemolysis, alpha (α) hemolysis that is characterized by incomplete hemolysis and appears as greenish-darkening of the agar that contain RBCs, beta (β) hemolysis, that is a complete lyses of RBCs in the media, the area around and under the colonies are lightened and transparent, and gamma (γ) hemolysis, the RBCs in the media is unchanged and this called non-hemolysis (Forbes *et al.*, 2007; Ryan and Ray, 2004). In addition to destroyed RBCs, hemolysins destroy other cells (phagocytes) to protect bacteria from phagocytosis and facilitate the spreading in blood stream (Rajesh and Rutten, 2004).

1.2.5.3. Siderophore production:

Iron is an absolute requirement for the growth of most microorganisms, with the possible exceptions of lactobacilli and *Borrelia burgdorferi* (Archibald, 1983; Posey and Gherardini, 2000). Despite being the fourth most abundant element in the Earth's crust, iron is frequently a growth-limiting nutrient. In

aerobic environments and at physiological pH, iron is present in the ferric (Fe^{3+}) state and forms insoluble hydroxide and oxyhydroxide precipitates. Mammals overcome iron restriction by possessing high-affinity iron-binding glycoproteins such as transferrin and lactoferrin that serve to solubilize and deliver iron to host cells (Weinberg, 1999). This results in a further restriction of free extracellular iron and, accordingly, the concentration of free iron in the human body is estimated to be 10^{-18} M, a concentration that is several orders lower than that is required to support a productive bacterial infection (Braun *et al.*, 1998).

To overcome iron restriction, bacteria have evolved several different mechanisms to acquire this essential nutrient. One of the most common iron acquisition mechanisms, though, is the use of low molecular weight, high affinity iron chelators, termed siderophores, and cognate cell envelope receptors that serve to actively internalize ferric-siderophore complexes. The ability of the bacteria to acquire iron during *in vivo* growth is also likely important to its pathogenesis, and several research groups have characterized several different genes whose products are involved in the binding and/or transport of host iron compounds (Mazmanian *et al.*, 2003 ; Modun *et al.*, 1998; Taylor and Heinrichs, 2002).

Several members of the staphylococci, including numerous CoNS and *S. aureus* strains produce siderophores. Two of these siderophores, staphyloferrin A (Konetschny-Rapp *et al.*, 1990; Meiwes *et al.*, 1990) and staphyloferrin B (Dreschel *et al.*, 1993; Haag *et al.*, 1994), are of the polycarboxylate class, while the third, aureochelin (Courcol *et al.*, 1997), is chemically uncharacterized. Iron starvation is one of the major barriers that virulent bacteria must overcome in order to proliferate in the host. There are two types of siderophores:

1.2.5.3.1. Phenolate-type siderophores:

The most common group and their best known enterobactin representative, (also known as enterochelin), is a cyclic trimmer of 2, 3-dihydroxy-benzoyl-serine. This siderophore appears to comprise the main iron uptake systems of Enterobacteriaceae and is synthesized by almost all clinical isolates of *E. coli* and *Salmonella* spp. (Griffiths *et al.*, 1988).

1.2.5.3.2. Hydroxamate-type siderophores:

The ferrichromes; which are synthesized only by fungi, the ferrioxamines, and aerobactin are most important. In contrast to enterobactin, the contribution of aerobactin to bacterial virulence has been clearly demonstrated (De Lorenzo and Martinez, 1988). The observations of Martinze and his coworkers (1987) indicate that the enterobacterial genera can be divided into two groups according to their incidence of aerobactin synthesis. The group with a low rate of aerobactin producing strains (<20%) comprises genera such as *Serratia*, *Proteus* and *Salmonella*, the second group which includes the genus *E. coli* shows a high incidence of aerobactin synthesis (>40%). Recently, it was found that there are three types of siderophore systems for Enterobacteriaceae. Their most prevalent are: enterobactin, aerobactin, and yersiniabactin (Raymond *et al.*, 2003; Mokracka *et al.*, 2004). Phenolate siderophore (yersiniabactin) is a siderophore system which is first described in *Yersinia* species, but it can be found among some isolates of other enterobacterial species and is believed to be acquired via horizontal gene transfer (Bach *et al.*, 2000).

1.2.5.4. Coagulase production:

Coagulase is a cell-associated and diffusible enzyme that convert fibrinogen to fibrin which causes clotting around bacteria lesions, which helps them persist in tissues (Green wood *et al.*, 2002). Coagulase also causes deposition of fibrin

on the surfaces of individual staphylococci, which may protect them from phagocytosis (Brooks *et al.*, 2004, Ryan and Ray, 2004).

1.2.5.5. Protease production:

Proteases are enzymes that break down protein to primary elements (amino acids); gelatin is a protein derivative of animal collagen. Protease is a proteolytic enzyme which is often important in the invasiveness of microorganisms into the host tissues and considers as virulence factor as a result of ability to break down immunoglobulins and complement components (Barrett *et al.*, 2003; Al-Rassam, 2004). Proteases play a role in the transition of *S. aureus* cells from an adhesive to an invasive phenotype by degrading bacterial cell surface proteins, such as fibronectin binding protein and protein A (Karlsson *et al.*, 2001). *S. aureus* produces four major extracellular proteases: serine protease, a cysteine protease, metalloprotease and a second cysteine protease (also named staphopain) (Karlsson and Arvidson, 2002).

1.2.5.6. Lipase production:

Lipases are enzymes that catalysis the hydrolysis of triglycerides and diglycerides to fatty acids and glycerol. Epithelial cells surface in human contain lipids, which hydrolyzed by lipase from many organisms that help in spreading of organisms through cutaneous and subcutaneous tissues and enhance colonization of the skin (MacFaddin, 2000).

1.2.5.7. Colonization factors:

The first stage of microbial infection is the colonization that is the mean establishment of pathogen at the appropriate portal of entry (Maria *et al.*, 2007). Colonization factors include: the first type is the type-I fimbriae enables the bacteria to bind to D- mannose residues on eukaryotic cell surfaces. Type 1- fimbriae are said to be mannose -sensitive since exogenous mannose blocks

binding to receptors on red blood cells. The second type is the type III fimbriae, which are mannose-resistant fimbriae. This type of fimbriae is associated with their ability to hemagglutinate at presence of tannic acid-treated erythrocytes from several animal species (Old and Adegbola, 1985). Colonization factors (CFs) and putative colonization factors (PCF) are proteins exposed on the surface of bacteria and are fimbrial (or fibrillar if they are especially thin). They promote attachment of the Enterotoxogenic *E. coli* (ETEC) to epithelial cells of the small intestine and therefore serve as virulence factors (Cassels and Wolf, 1995). Both epidemiological and challenge experiments in humans suggest that CFA are protective antigens such that immunity to a colonization factor antigen (CFA) protects against challenge by other ETEC strains expressing the same CFA (Cravioto *et al.*, 1990). *S. aureus* expresses fibronectin-binding adhesions. Two genes encoding for fibronectin-binding proteins have been identified in *S. aureus*-fnbA and fnbB. Fibronectin binding activity is critical in pathogenesis because it allows the bacteria to adhere to extracellular matrix components including fibronectin and collagen. This can result in cutaneous infections and in life-threatening bacteremia and endocarditis (Schennings *et al.*, 1993).

1.2.5.8. Bacteriocin production:

Bacteriocins are antibacterial proteins produced by bacteria. They differ from traditional antibiotics in having a relatively narrow spectrum of action and being lethal only for bacteria which are closely related to the producing strains (Riley and Gordon, 1992). Based on their chemical structures, stability, and mode of action, bacteriocins have been classified as: (i) lantibiotics; (ii) small heat-stable peptides; (iii) large heat-labile proteins; and (iv) complex proteins that require carbohydrate or lipid moieties for activity (Klaenhammer, 1993). The mechanisms of action of peptide antibiotics are diverse, but the bacterial membrane is the target for most bacteriocins (Klaenhammer, 1993). The most

extensively studied the colicins produced by *E. coli* (Braun *et al.*, 1994; Cramer *et al.*, 1995; Gouaux, 1997). *E. coli* is known to produce two types of bacteriocins. One class, colicins, is diverse. This diversity and the evolutionary forces creating it are well known, as are the molecular and biochemical characteristics of these compounds (Riley and Wertz, 2002). The colicin gene cluster is plasmid-encoded and always consists of two tightly linked genes: a gene that encodes the toxin, and a constitutively expressed immunity gene, whose product protects the cell from the colicin. Many colicin determinants also encode a stress-induced lysis protein. This protein ruptures the cell, releasing the colicin into the environment. If a lysis protein is not produced, the colicin is actively transported across the cell membrane into the external environment. Once released, colicin molecules bind to specific cell surface receptors on target bacteria, from which they are transported into the cell. Colicins typically exploit receptors involved in nutrient uptake, such as vitamin B12. Once the colicin has entered the target cell it will, depending on the type of colicin, kill the cell in one of three ways: by forming channels in the cytoplasmic membrane, by non-specific DNA degradation, or by inhibiting protein synthesis (David and Claire, 2006). Colicin M is unique among these toxins in that it acts in the periplasm and specifically inhibits murein biosynthesis by hydrolyzing the pyrophosphate linkage between bactoprenol and the murein precursor (Kornelius *et al.*, 2008).

The second class of bacteriocins produced by *E. coli*, the microcins, is less well understood (Braun *et al.*, 2002). The gene cluster may be chromosomally or plasmid encoded and comprises two genes: the microcin gene, which encodes the bactericidal protein, and the immunity gene. Cells are induced to produce the microcin protein under specific conditions, such as iron limitation. Most microcin are thought to bind to surface receptors on target cells involved

in iron uptake. The manner in which microcins kill cells is not generally known, but some disrupt the target cell's membrane potential (David and Claire, 2006). Although colicins are representatives of gram-negative bacteriocins, there are differences found within this subgroup of bacteriocin family. *E. coli* encodes its colicins exclusively on plasmid replicons (Pugsley, 1984; James *et al.*, 1996). The bacteriocins (klebocins) of *Klebsiella pneumoniae* are found exclusively on plasmids (Al-Charrakh, 2005). The nuclease pyocins of *Pseudomonas aeruginosa* are found exclusively on chromosomes (Sano *et al.*, 1990).

1.2.6. Resistance of bacteria to antibiotics:

Resistance to antibiotic is considered as a virulence factor for the pathogenic microorganisms to cause the infections. Generally there are two major mechanisms of antibiotic resistance, genetic and biological mechanisms of antibiotic resistance (Alanis, 2005).

1.2.6.1. Genetic mechanisms of antibiotic resistance:

The development of antibiotic resistance tends to be related to the degree of simplicity of the DNA present in the microorganism becoming resistant and to the ease with which it can acquire DNA from other microorganisms. For antibiotic resistance to develop, it is necessary that two key elements combine: the presence of an antibiotic capable of inhibiting the majority of bacteria present in a colony and a heterogeneous colony of bacteria where at least one of this bacterium carries the genetic determinant capable of expressing resistance to the antibiotic (Levy and Marshall, 2004). Resistance to antibiotics can be natural (intrinsic) or acquired and can be transmitted horizontally or vertically. Whereas the natural form of antibiotic resistance is caused by a spontaneous gene mutation in the lack of selective pressure due to the presence of antibiotics and is far much less common than the acquired one, it can also play a role in

the development of resistance. For the most part, however, the micro-ecological pressure exerted by the presence of an antibiotic is a potent stimulus to elicit a bacterial adaptation response and is the most common cause of bacterial resistance to antibiotics (Sefton, 2002). Susceptible bacteria can acquire resistance to antimicrobial agents by either genetic mutation or by accepting antimicrobial resistance genes from other bacteria. The genes that codify this resistance (the “resistant genes”) are normally located in specialized fragments of DNA known as transposons (sections of DNA containing “sticky endings”), which allow the resistance genes to easily move from one plasmid to another (Sefton, 2002). Some transposons may contain a special, more complex DNA fragment called “integron”, a site capable of integrating different antibiotic resistance genes and thus able to confer multiple antibiotic resistance to a bacteria. Integrons have been identified in both gram-negative and gram-positive bacteria, and they seem to confer high-level multiple drug resistance to the bacteria that carry and express them (Levy and Marshall, 2004).

1.2.6.2. Biological mechanisms of antibiotic resistance:

Whichever way a gene is transferred to a bacterium, the development of antibiotic resistance occurs when the gene is able to express itself and produce a tangible biological effect resulting in the loss of activity of the antibiotic. These biological mechanisms are many and varied but they can be summarized as follows.

1.2.6.2.1. Antibiotic destruction or antibiotic transformation:

This destruction or transformation occurs when the bacteria produces one or more enzymes that chemically degrade or modify the antimicrobial making them inactive against the bacteria. This is a common mechanism of resistance and probably one of the oldest ones affecting several antibiotics but especially

β -lactam antibiotics via the bacterial production of β -lactamases (Jacoby and Munoz-Price, 2005).

1.2.6.2.2. Impermeability:

In order for antibiotics to exert their bacteriostatic or bactericidal actions on bacteria they must access intracellular targets. This necessitates, in G^{-ve} bacteria, that they cross the outer membrane, a substantial permeability barrier and thus, a major determinant of antimicrobial resistance in these bacteria. Indeed, the outer membrane barrier explains, at least in part, the enhanced resistance of G^{-ve} and G^{+ve} organisms to many antimicrobials. The intrinsic resistance of many G^{-ve} organisms to macrolides, for example, is probably explained by the limited permeability of this membrane to macrolides (Dowson and Coffey, 2000).

1.2.6.2.3. Receptor modification:

Receptor modification occurs when the intracellular target or receptor of the antibiotic drug is altered by the bacteria, resulting in the lack of binding and consequently the lack of antibacterial effect. Examples of this mechanism include modifications in the structural conformation of penicillin-binding proteins (PBPs) observed in certain types of penicillin resistance, ribosomal alterations that can render aminoglycosides, macrolides or tetracyclines inactive, and DNA-gyrase modifications resulting in resistance to fluoroquinolones (Levy and Marshall, 2004; Sefton, 2002). It is likely that more and newer biological mechanisms of resistance will develop in the future. One can only hope that as these appear, we will be able to use these new mechanisms as new targets for the development of newer, effective antibiotics (Alanis, 2005).

1.2.6.2.4. Antibiotic active efflux:

Antibiotic active efflux is relevant for antibiotics that act inside the bacteria and takes place when the microorganism is capable of developing an active transport mechanism that pumps the antibiotic molecules that penetrated into the cell to the outside milieu until it reaches a concentration below that necessary for the antibiotic to have antibacterial activity. This means that the efflux transport mechanism must be stronger than the influx mechanism in order to be effective (Hooper, 2005). Efflux was first described for tetracycline (e.g. TetA, TetB, TetK pumps) and the fluoroquinolones in both Gram-positive and G-ve bacteria (Jarlier *et al.*, 1996; Roberts, 1996a; Leclercq, 2002) but is now common for many other antibiotics such as fluoroquinolones (Sefton, 2002; Hooper, 2005). These pumps contribute to both intrinsic and acquired resistance, the latter arising from mutational hyperexpression of these chromosomally encoded efflux systems. Many of these and related efflux systems also provide for efflux of and thus, resistance to macrolides, β -lactams, aminoglycosides and tetracycline. Efflux-mediated resistance to macrolides has also been described in G+ve bacteria. Chloramphenicol resistance can also be afforded by efflux (Brooks *et al.*, 2004).

1.2.6.2.5. Alteration of metabolic pathway:

Some sulfonamide-resistant bacteria do not require para-aminobenzoic acid (PABA) an important precursor for the synthesis of folic acid and nucleic acids in bacteria inhibited by sulfonamides. Instead, like mammalian cells, they turn to utilizing preformed folic acid (Hall, 1997; Murry *et al.*, 2003).

Materials and Methods

2.1. Materials

2.1.1. Patients:

Asthenospermic seminal fluid specimens were collected from (100) infertile males who referred to infertility center in Babylon maternity and children hospital through a period of six months (from November 2007 to April 2008). The asthenospermic specimens were divided into two groups according to the presence of leukocytes in their specimens (leukocytospermia) :

1-First group: this group included 70 asthenospermic specimens with leukocytospermia ($>1 \times 10^6$ pus cell/ml of seminal fluid).

2-Second group: this group included 30 asthenospermic specimens without leukocytospermia ($<1 \times 10^6$ pus cell/ml of seminal fluid).

Infertile male age range from (25-44) years with mean age of (32.11) years. Abstinence time range from (72-120 hrs.). The specimens of patients who treated with antibiotic were excluded.

2.1.2.Laboratory equipments

Table (2-1) Laboratory equipments

Equipments	Company/ Origin
Sensitive Electronic Balance	Kern /Germany
Autoclave	Herayama/Japan.
Incubator, Oven, Shaker water Bath	Memmert/Germany
Distillator	GFL/ Germany
Centrifuge	Gemmy/ Taiwan
Refrigerator	Concord/ Italy
Millipore Filter	Proway /China
Light Microscope	Olympus/ Japan
Micropipette	Slamid / England

2.1.3.Chemical materials

Table (2-2) Chemical materials

Materials	Company/ Origin
Tannic acid, HCL, KOH, D-mannose	BDH / England.
K ₂ HPO ₄ , KH ₂ PO ₄ , Na ₂ HPO ₄ , NaCl, MgSO ₄ , CaCl ₂ , CuSO ₄ , NH ₄ Cl	
Urea, Methyl red, α -naphthol, gelatin	Sigma /Germany
P-dimethylamine benzylaldehyde, Trichloroacetic acid	
Phenol red, Glucose, 2,2-dipyridyle	GCC /England
Amyle-alchole, ethanol (99%) glycerol, H ₂ O ₂ ,	
Oxidase disk	Himedia /India.
Gram stain set	Crescent /KSA

2.1.4. Biological materials

Table (2-3) Biological materials

Materials	Company/ Origin
<u>Culture media:</u> Blood agar base, MacConkey agar, Agar-agar, Muller-Hinton agar, Nutrient agar, Nutrient broth, Mannitol salt agar, Urea base agar, Brain heart infusion agar, Brain heart infusion broth, EMB agar.	Himedia /India.
<u>Rapid identification system kit:</u> Hi 25 Enterobacteriaceae identification kit	Himedia / India
HiStph identification kit	

2.1.5. Antibiotic disks (Bioanalyse /Turkey)

Table (2-4) Antibiotic disks

Group	Antimicrobial agents	Disk potency (µg)	Symbol
Penicillins	Penicillin G	10 unites	P
	Oxacillin	1	OX
	Amoxicillin	10	AM
	Methicillin	5	ME
B- lactam / β -lactamase inhibitor combinations	Amoxicillin–clavulanic acid	30	AMC
Cephems (cephalosporins)	Cefamandole	30	MA
	Cefepime	30	FEP
	Ceftizoxime	30	ZOX
	Ceftazidime	30	CAZ

Continued table (2-4) Antibiotic disks

Carbapenems	Imipenem	10	IPM
	Meropenem	10	MEM
Glycopeptides	Vancomycin	30	VA
Aminoglycosides	Gentamycin	10	CN
	Amikacin	30	AK
	Tobramycin	10	TOB
Tetracyclines	Doxycycline	30	DO
Fluoroquinolones	Ciprofloxacin	5	CIP
	Norfloxacin	10	NOR
Folate pathway inhibitors	Trimethoprim-sulfamethoxazol	25	TMP-SMX

2.2.Methods

2.2.1. Specimens collection:

Seminal fluid specimens were collected from infertile patients by masturbation, under aseptically conditions. They were also asked to pass urine first and then wash and rinse hands and penis before the specimens were collected (WHO, 1999). The specimens were collected into clean wide-mouthed 15ml sterile plastic vials and incubated at 37°C for 30 minutes for liquefaction and then seminal fluid analysis (SFA) was done to diagnose asthenospermia and leukocytospermia. Swabs were inserted into the specimens and then directly inoculated on blood agar, chocolate agar and MacConkey agar. All plates were incubated aerobically at 37°C for 24-48 hrs.

2.2.1.1. Seminal fluid analysis (SFA)

In this experiment SFA method was used to investigate leukocytospermia and asthenospermia. According to World Health Organization (WHO, 1999) criteria asthenospermia defined as less than 50% of spermatozoa with forward progression or less than 25% of spermatozoa with rapid progression within 60 min after semen collection. Leukocytospermia was defined as more than 1×10^6 pus cell/ml of seminal fluid (WHO, 1999).

2.2.2. Preparation of the reagents and solutions

2.2.2.1. Catalase reagent

This reagent was used at a concentration (3%) using H_2O_2 in D.W and stored in a dark container. It was used for identification of catalase producing bacteria (Forbes *et al.*, 2007).

2.2.2.2. Ready made reagents (Himedia /India)

These reagents were brought with the rapid identification system kits:

1-NaOH (40%): it was used in alkaline phosphatase production test to detect the ability of organism to produce sufficient phosphatase enzyme.

2-TDA reagent: it was used in phenylalanine deamination test to detect phenylalanine deamination activity by bacteria.

3-Nitrate reduction reagent: it was used to detect nitrate reduction. This reagent composed of two reagents, **A** and **B** as follows: Reagent A: sulphanilic acid.

Reagent B: N-dimethyl-naphthylamine.

4-Vogus-Proskauer's reagent: it was used in Vogus-Proskauer's test to detect acetoin production. This reagent was composed of two reagents, **Baritt reagent A** and **Baritt reagent B**.

5- Methyl red reagent: it was used in methyl red test to detect acid production.

6- Kovac's reagent: it was used in indole test to detect deamination of tryptophan.

7- Oxidase disk: it was used in oxidase test to detect oxidase positive bacteria.

2.2.2.3. Phosphate buffer solution (PBS) (pH=7.3)

Eighteen gm of NaCl, 0.34 gm of KH_2PO_4 and 1.12 gm of K_2HPO_4 were all dissolved in 1000 ml of D.W. The pH was adjusted at 7.3, then the solution was autoclaved .It was used in washing and preserving human and chicken RBCs used in the haemagglutination test (Forbes *et al.*, 2007).

2.2.2.4. Coppric sulphate solution (20%)

It was prepared by dissolving 20 gm of CuSO_4 in small volume of D.W. and completed up to 100 ml. It was used in capsule staining (Forbes *et al.*, 2007).

2.2.2.5. Tannic acid solution (1%)

It was prepared by dissolving 1gm of tannic acid in small volume of D.W. and completed up to 100 ml D.W. and then sterilized by Millipore filter paper. It was used in haemagglutination test for detection colonization factor antigen-III (Sambrook and Rusell, 2001).

2.2.2.6. D- mannose solution preparation (0.1 M)

It was prepared by dissolving 1.8 gm of D-mannose in 100 ml D.W. and then sterilized by Millipore filter paper. It was used in haemagglutination test for detection colonization factor antigen-I (Sambrook and Rusell, 2001).

2.2.2.7. Urea solution (20%)

It was prepared by dissolving 20 gm of urea in small volume of D.W. and completed up to 100 ml D.W. and then sterilized by Millipore filter paper .It was used in urease test for detection of urease positive bacteria (MacFaddin, 2000).

2.2.2.8. Trichloroacetic acid (TCA) solution (5%)

It was prepared by dissolving 5 gm of TCA in small volume of D.W. and completed up to 100 ml D.W. It was used in the extracellular protease production test for precipitation of unlysed protein (Piret *et al.*, 1983).

2.2.3. Preparation of culture media

The general culture media described below were prepared using the routine methods and used in appropriate experiments:-

2.2.3.1. Blood agar medium

Blood agar medium was prepared according to manufacturer by dissolving 40 gm blood agar base in 1000 ml D.W. The medium was autoclaved at 121°C for 15 min, cold to 50 C° and 5% of fresh human blood was added. This medium was used as enrichment medium for cultivation of the bacterial isolates and to determine their ability of blood hemolysis (Forbes *et al*, 2007).

2.2.3.2. Chocolate agar medium

Chocolate agar medium was prepared by dissolving 40 gm of blood agar base in 1000 ml D.W. and sterilized by autoclaving, and then 8% of human blood was added to the medium after cooling to 80°C. This medium was especially used for isolation and cultivation of bacterial isolates that need 5-10% CO₂ tension (Forbes *et al*, 2007).

2.2.3.3. MacConkey agar medium

MacConkey agar medium was prepared according to the method recommended by the manufacturing company and it was used for the primary isolation of G-ve bacteria and differentiation of lactose fermentative from the non lactose fermentative bacteria (Collee *et al*, 1996).

2.2.3.4. Nutrient agar medium

Nutrient agar medium was prepared according to the manufacturing company. It used for general experiments, cultivation and activation of bacterial isolates when it is necessary (MacFaddin, 2000).

2.2.3.5. Mannitol salt agar medium

This medium was prepared according to the manufacturing company .It was used as a selective medium for the isolation and differentiation of staphylococci (MacFaddin, 2000).

2.2.3.6. Muller- Hinton agar

Muller- Hinton agar was prepared according to the manufacturing company. It was used in anti-bacterial susceptibility testing (MacFaddin, 2000).

2.2.3.7. M9 medium

Six gm of Na_2HPO_4 , 3 gm of KH_2PO_4 , 0.5 gm of NaCl , and 1 gm of NH_4Cl were dissolved in 950 ml of D.W. with 2% agar, and then sterilized by autoclave. After cooling, 2 ml of 1M of MgSO_4 , 10 ml of 20% glucose and 0.1 ml of 1M of CaCl_2 (sterilized separately by filtration) were added, then the volume was completed to 1000 ml. This media was used for the detection of the siderophore and extracellular proteases production (Sambrook and Rusell, 2001).

2.2.3.8. Brain heart infusion (BHI) broth–glycerol medium

This medium was prepared by mixing 5 ml of glycerol with 95 ml of BHI broth (sterilized by autoclave) .It was used for preservation of bacterial isolates as stock for long time (Forbes *et al.*, 2007).

2.2.3.9. Egg- yolk agar medium:

This medium was used to detect the ability of bacteria to produce lipase enzyme. It was prepared by suspending 7.4 gm of blood agar base in 200 ml D.W heating and sterilizing by autoclave and then supplemented with 20 ml of yolk-normal saline mixture after cooling to 45°C (Collee *et al.*, 1996).

2.2.3.10. Urea agar medium:

It was prepared by adding 10 ml of urea solution (20% sterilized by Millipore filter paper) in volume of autoclaved urea agar base and completed up to 100 ml and cooling to 50°C, the pH was adjusted to 7.1 and the medium was

distributed into sterilized test tubes and allowed to solidify in a slant form. It was used to test the ability of bacteria to produce urease enzyme (MacFaddin, 2000).

2.2.4. Laboratory diagnosis

According to the diagnostic procedures recommended by Collee and his colleagues (1996); Maccfaddin (2000) and Forbes and his colleagues (2007). the isolation and identification of G+ve and G-ve bacteria associated with bacteriospermia in asthenospermic patients were performed as follows :

2.2.4.1. Microscopic examination and colonial morphology

A single colony was taken from each primary positive culture and its identification was depending on the morphology properties (colony size, shape, color and natural of pigments, translucency, edge, and elevation, and texture). Colonies were investigated by gram stain to observe the specific shape, the gram reaction staining, the cells arrangement and the specific intracellular compounds. Bacterial isolates were identified to the level of species using traditional biochemical tests and then confirmed using the rapid identification systems as recommended by (Himedia/India)

2.2.4.2. Physiological and biochemical tests

2.2.4.2.1. Oxidase Test

Oxidase disks (included in Hi 25 Enterobacteriaceae identification kit) were used to detect oxidase production using small portion of the colony to be tested .It was removed and rubbed on the oxidase disk changing in the color to blue or purple within 10 seconds indicated for a positive result (Forbes *et al*, 2007).

2.2.4.2.2. Catalase test

Nutrient agar medium was streaked with the selected bacterial colonies and incubated at 37°C for 24 hrs then transfer the growth by the wooden steak and put it

on the surface of a clean slide and add a drop of (3% H₂O₂). Formation of gas bubbles indicates for positive results (Forbes *et al.*, 2007).

2.2.4.2.3. Coagulase test

This test was used to differentiate coagulase producing pathogenic staphylococci (*S. aureus*) from other CoNS. In this test the tube method was used as it is reliable method as follow: Half ml of human plasma was placed in a glass tube and equal volume of the bacterial suspension or bacterial filtrate was added to the glass tube contained human plasma, then the suspension was incubated for 1-4 hrs at 37°C and observed each 30 minutes; the presence of clot that cannot be resuspended by gentle shaking was recorded as a positive result. The organism that fails to clot the plasma within 24 hrs is considered as coagulase negative (Forbes *et al.*, 2007).

2.2.4.2.4. Mannitol fermentation test

The colony of staphylococci under test was cultivated mannitol salt agar and incubated at 37°C for 24 hrs. Colonies surrounded by a yellow halo indicated mannitol fermentation, a character closely related with *S. aureus* (MacFaddin, 2000).

2.2.4.2.5. Urease (Christensen's) test

This test was used to detect the ability of an organism to split urea into two molecules of ammonia by the action of the urease enzyme. Urea agar tube was inoculated with single colony of tested bacteria and incubated at 37°C for 24 hrs. Conversion of the medium to pink color indicated a positive result (MacFaddin, 2000).

2.2.4.2.6. Motility test

The tubes that contained semisolid motility medium stabbed with the specific bacterial culture which was incubated at 37°C for 24hrs. The distribution of growth outer of stabbing region means positive result (MacFaddin, 2000).

2.2.4.3. Rapid identification system

The present study used two types of rapid identification systems:

1-Hi 25 Enterobacteriaceae identification kit(Himedia/India):

This kit consists of 24 wells containing dehydrated substrates. It is a standardized colorimetric identification system utilizing 13 biochemical test and 11 carbohydrate utilization tests. On incubation, organisms undergo metabolic changes which were indicated by a color change in the media that was either visible spontaneously or after addition of a reagent. Oxidase test was performed separately using oxidase disk. The results were read according to the reading result interpretation chart (Index 1) and the final identification was reordered according to the identification index (Index 2).

2- HiStaph identification kit(Himedia /India)

This system consists of 12 wells containing dehydrated substrates .The results were read according to the reading result interpretation chart (Index 3) and the final identification was reordered according to the identification index (Index4).

2.2.5.Virulence factors tests

2.2.5.1.Capsule stain test (Hiss's Method)

This test was performed as mentioned in Forbes and his colleagues (2007), as fellows:

- a- A smear was prepared from bacterial suspension on glass slide without fixing and allowed to dry.
- b- Slide was flooded gently with 1% solution of crystal violet and left for about 4 minutes.
- c- The smear was washed with a solution of 20% copper sulfate and allowed to dry in air, and examined under the microscope.

The organism should be appeared as deep purple color while the capsule appeared in a faint blue against a light purple background.

2.2.5.2. Hemolysin production test

Blood agar medium was streaked with a pure culture of bacterial isolate to be tested and incubated at 37°C for 24-48 hrs. The appearance of a clear zone surrounding the colony is an indicator of β - hemolysis while the greenish zone is an indicator of α - hemolysis (Forbes *et al.*, 2007).

2.2.5.3. Siderophores production test

The medium required for this test was prepared by addition of 200 μ mol/L of 2,2-dipyridyl (M.W.=156.2) to M9 medium and incubated at 37° C for 24 hrs. Growth in the presence of 2,2,-dipyridil indicated a positive result (Sambrook and Rusell ,2001)

2.2.5.4. Extracellular protease production test

This test was carried out by using M9 medium supported by 1% gelatin. After the inoculation of this medium with bacterial isolates and incubation for 24 – 48 hrs. at 37°C, 3 ml of trichloroacetic acid (5%) was added to precipitate the protein. The positive result was read by observing a transparent area around the colony (Piret *et al.*, 1983).

2.2.5.5. Haemagglutination test (HA)

It was performed to show the ability of bacterial isolates to produce colonization factors antigen (CFA). Colonization factor antigen I (CFA-I) production can be detected as follows:

a- RBCs suspension was prepared by placing blood specimens of human (group A) in phosphate buffer solution in proportion 1:1 and centrifuged at 3000 rpm for 15 minutes . The supernatant was discarded and the sediment of RBCs was washed three times with PBS and then RBCs were resuspended up to 3%.

b- One volume of bacterial growth was placed on glass slide and mix with same volume of D-mannose solution (0.1 M) ,and same volume of the above RBCs

suspension was added and allowed two minutes to observe the agglutination .Absence of agglutination indicates positive result and vice versa.

Colonization factor antigen III (CFA/ III) production was detected using same procedure as described above ,except using tannic acid solution (1%) instead of D-mannose solution (Sambrook and Rusell ,2001).

2.2.5.6. Bacteriocin production test

This test was performed using cup assay method as described by (Al-Qassab and Al-Khafaji, 1992) as follows:

- a- All isolates were grown in BHI broth with 5% glycerol at 37°C for 18-24 hrs.
- b- The growing bacterial isolates were heavily streaked on BHI agar with 5% glycerol and then incubated at 37°C for 18 hrs.
- c- An *E. coli* isolates (obtained from department of microbiology–collage of medicine/Babylon university) was used as an indicator (sensitive isolates) for detection of bacteriocin production by G-ve bacterial isolates.
- d- An *S. aureus* isolates (obtained from department of microbiology–collage of medicine/Babylon university) was used as an indicator (sensitive isolates) for detection of bacteriocin production by G+ve bacterial isolates.
- e- Sterile cork borer (5mm diameter) was used to cut agar disks from the cultured agar layer of tested bacteria (bacteriocin producers).
- f- The indicator isolates was allowed to grow in nutrient broth for 2-3 hrs. in a shaker water bath at 37°C (to obtain cell density up to 1×10^6 - 1×10^7 cells/ ml).
- g- A volume of 0.1 ml of indicator growth was spread on nutrient agar plates and left to dry, then transfer agar disks of tested bacteria to the agar surface seeded with indicator isolates and incubated for overnight at 37°C.
- h- Presence of inhibition zone around the agar disk of tested bacteria indicates a positive result.

2.2.5.7. Lipase production test

Lipase test was carried out in egg-yolk agar medium to determine the ability of microorganisms to produce lipase enzyme. After inoculation of the medium agar, plates were incubated for overnight at 37°C. The appearance of opaque pearly layer around the colonies indicated for a positive result (Collee *et al.*, 1996).

2.2.6. Antimicrobial susceptibility test

It was performed by using a pure culture of previously identified bacterial isolate. The inoculum to be used in this test was prepared by adding growth from 5 isolated colonies grown on blood agar plate to 5 ml of Nutrient broth and incubated at 37°C for 18 hrs. A sterile swab was used to obtain an inoculum from the bacterial suspension. This inoculum was streaked on a Muller-Hinton agar plate and left to dry. The antibiotic discs were placed on the surface of the medium at evenly spaced intervals with flamed forceps or a disc applicator and incubated for 24 hrs. at 37°C (NCCLS ,2002). Inhibition zones were measured using a ruler and compared with the zones of inhibition determined by the National Committee for Clinical Laboratory Standards (NCCLS) .The most effective antibiotic for each bacterial isolate was determined as recommended by NCCLS (2002).

2.2.7. Preservation of bacterial isolates

The bacterial isolates were preserved in BHI broth supplemented with 5% glycerol at -20°C for 6-8 months (Collee *et al.*, 1996).

2.2.8. Statistical analysis:

The χ^2 (Chi-square) test was used for statistical analysis. $P < 0.01$ was considered to be statistically significant.

Results and Discussion

3.1. Laboratory investigation:

3.1.1. Asthenospermia and leukocytospermia:

One hundred asthenospermic seminal fluid specimens were diagnosed using seminal fluid analysis (SFA). Motile spermatozoa in all specimens were ranged 10-40% with mean motile spermatozoa (25%) and this result revealed asthenospermia according to world health organization criteria (WHO, 1999). Asthenospermic seminal fluid specimens were divided into two groups according to leukocytospermia, 70 specimens, first group, who had leukocytospermia and 30 specimens, second group, who had no leukocytospermia. White blood cells (WBCs) in seminal fluid specimens were counted and the results showed that, all specimens of first group had more than 1×10^6 pus cell/ml of seminal fluid revealed to leukocytospermia which indicates an infection (WHO, 1999), while all specimens of second group had no leukocytospermia as shown in table (3-1).

Table (3-1) Illustration of asthenospermia, leukocytospermia and bacteriospermia.

Cases		Patients	
		First group <i>n</i> (%) <i>n</i> =70	Second group <i>n</i> (%) <i>n</i> =30
Asthenospermia		70(100%)	30(100%)
Leukocytospermia	Positive	70(100%)	0.0
	Negative	0.0	30(100%)
Bacteriospermia	Positive	61(87.1%)	0.0
	Negative	9(12.9%)	30(100%)

3.1.2. Bacterial isolates from asthenospermic specimens:

The results of this experiment showed that 61(87.1%) specimens of first group revealed positive bacterial culture as shown in table (3-1), whereas 9(12.9%) specimens of first group showed no bacterial growth even after 48

hours, which may be due to the presence of another type of causative agents that might need special technique for their detection such as viruses, *Chlamydia* or *Mycoplasma*. These results were corresponding to those results being reported by Mogra and his colleagues (1981) and Shefi and Turek (2006). However the results were higher than those reported by Jiao and his colleagues (2002), who found that (5-15%) of samples, gave positive culture. All specimens of second group gave negative bacterial culture. The results in table (3-1) were statistically analyzed by using χ^2 test showed that there was a strong relationship between the bacteriospermia and asthenospermia ($P < 0.01$). This result agreed with that result being reported by Golshani and his colleagues (2006) who declared that semen specimens of infertile men, especially those contain high number of *E. coli* and *Enterococci* isolates, had high rate of non-motile and morphologically abnormal sperms. Philip and Folstad (2003) confirmed that there was a significant positive effect of antibiotic treatment for the following sperm parameters: sperm volume, sperm concentration, sperm motility, and sperm morphology. Antibiotic treatment also significantly reduced the number of leukocytes in ejaculates of male infertility patients. Thus, in general, males treated with antibiotics were relieved from leukocytospermia and produced ejaculates of high quality. Also there was a strong relationship between bacteriospermia and leukocytospermia ($P < 0.01$). This result was in accordance with Jedrzejczak and his colleagues (1996) who found that seminal white blood cells counts ($> 1 \times 10^5$ /ml) correlated well with bacteriospermia (more than 1000 cells/ml). A significant correlation was found between bacteriospermia and leukocytospermia at the cut-off level of $\geq 0.275 \times 10^6$ leukocytes per ml of semen specimens (Gdoura *et al.*, 2008).

A total of (70) bacterial isolates were obtained from the (61) seminal fluid specimens in which gram positive bacteria constituted 44(62.9%) of the total

isolates and were considered as the largest etiological agent of bacteriospermia compared with gram negative bacteria which constituted 26(37.1%) as indicated in table (3-2) and this might be due to the fact that gram positive bacteria are commensals of mucosal surfaces of urogenital tract and these results were similar to those results being reported by Chimura and Saito (1990) who found that G+ve bacterial strains constituted (78.4%), while G-ve bacterial strains constituted (21.6%). Also these results were in line with those declared by Riegel and his colleagues (1995).

Table (3-2) Distribution of bacterial isolates from patients with asthenospermia according to the isolates.

Bacterial species		Single isolates <i>n</i>	Mixed isolates <i>n</i>	Total isolates <i>n</i> (%)	Total <i>n</i> (%)
CoNS	<i>S. saprophyticus</i>	14	*4	25 (35.7)	44(62.9)
	<i>S. epidermides</i>	7	-		
<i>S. aureus</i>		14	5	19(27.2)	
<i>Escherichia coli</i>		9	**3	12(17.1)	26(37.1)
<i>Enterobacter aerogenes</i>		6	2	8(11.4)	
<i>Acinetobacter</i> spp.		4	-	4(5.7)	
<i>Moraxella</i> spp.		2	-	2(2.9)	
Total		56	14	70 (100)	100%

*Four isolates of *S. saprophyticus* were mixed with Four isolated of *S. aureus*

**Three isolates of *E. coli* were mixed with one isolate of *S. aureus* and two isolates of *E. aerogenes*

3.2. Pathogenicity of bacteria in asthenospermic patients:

The present study showed that asthenospermia were caused by 70 bacterial isolates (Table 3-2). Coagulase negative staphylococci (CoNS) represented by *S. epidermides* and *S. saprophyticus* which constituted 25(35.7%), *S. aureus* constituted 19(27.2%) were predominant in causative microorganism of bacteriospermia followed by *E. coli* 12(17.1%). However, each of the following bacteria *E. aerogenes*, *Acinetobacter* spp. and *Moraxella* spp. constituted 8(11.4); 4(5.7) and 2(2.9) respectively.

CoNS organisms were the most common bacterial group isolated from seminal fluid infections (35.7%); CoNS infections in the present study were less than those reported by other researchers (Sharon *et al.*, 1990; Riegel *et al.*, 1995) who found that these infections constituted (50-89%), but they were more than those reported by Virecoulon and his colleagues (2005), who reported that seminal fluid infections caused by CoNS were constituted (15.7%).

The high percentage of CoNS infections may be due to that they are common contaminant of skin and urethral meatus, and also their ability to resist antibiotics commonly used in medical therapy (Mogra *et al.*, 1981). These commensals bacteria may have a role as opportunistic pathogens in the presence of weakened local tissue defence when immunosuppressive agents were used, and also the antibiotics had been associated with emergence of opportunistic infection by microorganisms not previously regarded as pathogenic bacteria (El-Shamy, 1993).

S. aureus was the second in occurrence in seminal fluid specimens which constituted 19(27.2%). This was in line with reports from other studies (Merino *et al.*, 1995; Rodin *et al.*, 2003 and Ikechukwu *et al.*, 2007). However, the percentage of *S. aureus* was more than those reported in similar studies (Mogra *et al.*, 1981) it compile (14.3%) and (Alwash, 2006) reported (12.2%). *S. aureus*

had detrimental effect of spermatozoa resulted from damage of sperm membrane lipids (Fraczek *et al.*, 2007). The pathogenesis of *S. aureus* was attributed to the combined effects of extracellular factors and toxins, together with invasive properties such as adherence, biofilm formation, and resistance to phagocytosis (Eiichi *et al.*, 2004). *S. aureus* may inherent nature of developing resistant strains for antibiotics. *S. aureus* also contains teichoic acid and lipoteichoic acid, capsular material which facilitated the adherence of these bacteria to epithelium of urogenital tract. This agreed with result mentioned by Yassin (1990) and Brook and his colleagues (1998).

The detection of staphylococci from seminal fluid specimens was documented. It was found that staphylococci involved in the pathogenesis of chronic pelvic pain syndrome (CPPS) (Stimac *et al.*, 2001). They were identified in focal colonies adherent to the prostatic duct walls (Lee, 2000). Bergman (1994) demonstrated that staphylococci were found in significant numbers in 43% of patients with symptoms of prostatitis.

Results of this study also found that (37.1%) of bacteriospermia were caused by gram negative bacteria. *E. coli* represented the common gram negative bacteria isolated from seminal fluid specimens. They accounted for (17.1%) of total bacterial isolates of asthenospermic patients. This result was close to the finding by other researchers (Rehewy *et al.*, 1979; Mogra *et al.*, 1981; Merino *et al.*, 1995 and Rodin *et al.*, 2003). In other studies *E. coli* isolates were found to be less than 10% (Riegel *et al.*, 1995; Lackner *et al.*, 2006; Ikechukwu *et al.*, 2007). However, Alberto and his colleagues (2006) reported that, *E. coli* isolates compile (70.4%) of total isolates. Of particular significance *E. coli* was reported to possess *in vitro* spermicidal activity (Swenson *et al.*, 1980). Immobilizing effect of certain bacteria, particularly *E. coli* on spermatozoa had been demonstrated, and this was the mechanism responsible for the

asthenospermia resulted from bacteriospermia (Teague *et al.*, 1971). Also, *E. coli* has the ability to cause sperm membrane lipid damage (Fraczek *et al.*, 2007).

The other group of gram negative bacteria isolated from seminal fluid specimens were *E. aerogenes* (11.4%), *Acinetobacter spp.* (5.7) and *Moraxella spp.* (2.9%). This result was the highest of those reported by other studies as in Alwash (2006). *E. aerogenes* possesses many factors that facilitate their pathogenicity as endotoxin, which have deleterious effect on seminal fluid; capsules and adhesion proteins that support their attachment to mucosal surfaces of urogenital and also have the ability of resistance to multiple antimicrobial agents (Forbes *et al.*, 2007).

3.3. Identification of bacterial isolates:

3.3.1 Gram positive bacteria:

The identification of G+ve bacteria was accomplished using rapid identification system and conventional method according to (MacFaddin, 2000) as shown in table (3-3).

Table (3-3): conventional and rapid identification system (HiStph identification kit (Himedia /India) for gram positive bacteria.

Tests	<i>S. aureus</i>	<i>S. epidermides</i>	<i>S. saprophyticus</i>
Gram stain	G+ve cocci (clusters)	G+ve cocci (clusters)	G+ve cocci (clusters)
Catalase	+	+	+
Coagulase	+	—	—
*Novobiocin resistance	—	—	+
Voges Proskauer's	+	+	+
Alkaline phosphatase	+	+	—
ONPG	—	—	+
Urease	+	+	+
Arginine utilization	+	+	—
Mannitol fermentation	+	—	+
Sucrose fermentation	+	+	+
Lactose fermentation	+	+	+
Arabinose fermentation	—	—	—
Raffinose fermentation	—	—	—
Trehalose fermentation	+	—	—
Maltose fermentation	+	+	—

*Novobiocin resistance (+); growth inhibition zone ≤ 16 mm with 5 μ g novobiocin disk (Skov *et al.*, 2001).

3.3.2 Gram negative bacteria:

The identification of G-ve bacteria was accomplished using rapid identification system and conventional method according to (MacFaddin, 2000) as shown in table (3-4).

Table (3-4): conventional and rapid identification system (Hi 25

Enterobacteriaceae identification kit Himedia /India) for gram negative bacteria.

Tests	<i>E.coli</i>	<i>E. aerogenes</i>	<i>Acinetobacter</i> spp.	<i>Moraxella</i> spp.
Gram stain	G-ve, short rods	G-ve, short rods	G-ve, coccobacilli or diplococci	G-ve, short rods arranged in pairs
Catalase	+	+	+	+
Oxidase	—	—	—	+
Motility	+	+	—	—
ONPG	+	+	non	non
Lysine decarboxylase	+	+	—	non
Ornithine decarboxylase	+	+	+	non
Urease	—	—	non	—
Phenylalanine deamination	—	—	+	—
Nitrate reduction	+	+	—	—
H ₂ S production	—	—	non	—
Citrate utilization	—	+	+	—
Voges Proskauer's	—	+	non	non
Methyl red	+	—	non	non

Indole	+	—	non	—
Malonate	—	+	+	non
Esculin hydrolysis	+	+	—	—
Arabinose fermentation	+	+	non	non
Xylose ferm.	+	+	non	non
Adonitol ferm.	*V	+	non	non
Rhamanose ferm.	*V	+	non	non
Cellobiose ferm.	—	+	non	non
Melibiose ferm.	V	+	non	non
Saccharose ferm.	+	+	non	non
Raffinose ferm.	V	+	non	non
Trehalose ferm.	+	+	non	non
Glucose ferm.	+	+	non	non
Lactose ferm.	+	+	—	—

* V=variable, usually positive 11-89%.

3.4. Virulence factors of the bacterial isolates:

The factors that determine the initiation, development, and outcome of an infection involve a series of complex and shifting interaction between the host and the parasite, which can vary with different infecting microorganisms (Brogden *et al.*, 2000). Virulence factors of the bacterial isolates demonstrated in this work included coagulase, hemolysin, capsule, siderophore, bacteriocin, lipase and extracellular protease production as well as colonization factor antigens (CFA/I, and CFA/III).

3.4.1. Coagulase production:

All isolates of *S. aureus* bacteria were able to produce coagulase enzyme (Table 3-5). It was considered as a virulence factor for pathogenicity of these bacteria by clumping the fibrin around the bacteria (Hall, 1991 ; Todar, 2005). Possibly coagulase could provide an antigenic disguise if it clotted fibrin on the cell surface or could make the bacterial cells resistant to phagocytes or tissue bacterial target (Humphreys, 2004).

Table (3-5) virulence factor of gram positive bacterial isolate.

Virulence factors	Type of bacteria	
	<i>S. aureus</i> n(%) n=19	CoNS n(%) n=25
Coagulase production	19(100%)	0.0
Hemolysin production	19(100%) β -hemolysis	18(72%) α -hemolysis
Capsule production	5(26.3%)	0.0
Siderophore Production	3(15.8%)	17(68%)
Bacteriocin production	6(31.6%)	0.0
Lipase production	15(78.9%)	7(28%)
Extracellular protease production	7(36.8%)	0.0
*CFA I	10(52.6%)	8(32%)
**CFA III	19(100%)	25(100%)

* CFA I, colonization factor antigen 1

** CFA III, colonization factor antigen 3

3.4.2. Capsule production:

In this study, among G+ve bacterial isolates all CoNS isolates were non-capsule producers. Only 5(26.3%) of *S. aureus* isolates were polysaccharide capsule producers (Table 3-5). Polysaccharide capsule was an important

component in the pathogenesis, and enhances bacterial virulence by modulate *S. aureus* adherence to endothelial surface in vitro, animal studies suggest that it also promotes bacterial colonization and persistence on mucosal surfaces, this agrees with the result mentioned by Nair and his colleagues (2000). Also all G-ve bacterial isolates were non-capsule producers except *E. coli* in which only two isolates were exhibited clear capsule when examined by Hiss's method (Table 3-6). Capsules are known to mediate specific or nonspecific adherence of bacteria to particular surfaces (Tamura *et al.*, 1994; Hyde, 2000; Tyrrell *et al.*, 2000; Brooks *et al.*, 2004). The role of capsules in microbial virulence is to protect the organism from complement activation and phagocyte-mediated destruction. Although the host will normally make antibodies directed against the bacterial capsule, some bacteria are able to weaken this response by having capsules that resemble host polysaccharide (Brogden, *et al.* 2000).

3.4.3. Hemolysin production:

Microorganisms evolve a number of mechanisms for the acquisition of iron from their environments (Litwin and Calderwood, 1993). One of them is the production of hemolysins, which acts to release iron complexed to intracellular heme and hemoglobin. Another mechanism for iron acquisition is to produce siderophores which chelate iron with a very high affinity and which compete effectively with transferrin and lactoferrin to mobilize iron for microbial use (Neilands, 1995).

The results of this study revealed that all isolates of *S. aureus* were able to expressed β -hemolytic mode on blood agar. Among CoNS isolates only 18(72%) exhibited α -hemolytic pattern, while the rest CoNS isolates were γ -hemolytic

(non hemolytic) pattern, which no color change around the bacterial colonies (Table 3-5). This agreed with the result mentioned by Dinges and his colleagues (2000). The production of hemolysin by *S. aureus* is well known and considered as a main virulence factor for these bacteria and it associated with increased severity of infections (Vergis *et al.*, 2002). In G-ve bacterial isolates five isolates of *E. coli* and four isolates of *E. aerogenes* displayed β -hemolytic pattern. The other G-ve isolates demonstrated γ -hemolytic pattern (Table 3-6). Iron can increase disease risk by functioning as a readily available essential nutrient for invading microbial and neoplastic cell. To survive and replicate in hosts, microbial pathogens must acquire host iron. Highly virulent strains possess exceptionally powerful mechanisms for obtaining host iron from health hosts (Weinberg, 1998).

3.4.4. Siderophore production:

One of the most common iron acquisition mechanisms, though, is the use of low molecular weight, high affinity iron chelators, termed siderophores, and cognate cell envelope receptors that serve to actively internalize ferric-siderophore complexes. Many siderophores are able to successfully compete with transferrin and lactoferrin for host iron (Suzanne *et al.*, 2004). In the present study the siderophore synthesis was investigated among bacterial isolates and the results showed that 3(15.8%) of *S. aureus* isolates were able to produce siderophore. Among CoNS isolates only 17(68%) of isolates were siderophore producers. *S. aureus* and *S. epidermidis* transferring receptors exhibited significant transferrin species specificity (Modun *et al.*, 1994). These results were in accordance with other observations (Modun *et al.*, 1994) that staphylococci grown *in vitro* express the 42 kDa transferrin binding proteins (TBP) and were coated with surface-associated transferrin. Given that these TBPs were involved in iron-scavenging (Williams and Griffiths, 1992), their

expression *in vivo* was likely to confer a significant survival advantage in the severely iron-restricted environment. Among G+ve bacterial isolates all *E. coli* and *Moraxella* spp. and (6/8) of *E. aerogenes* and (3/4) of *Acinetobacter* spp. isolates were able to synthesis siderophore.

3.4.5. Bacteriocin production:

Cup assay method was used for detection of bacteriocin production. Among G+ve bacterial isolates only 6(31.6%) *S. aureus* isolates were able to produce bacteriocin and form a clear inhibition zones (9-15mm) on solid medium (Table 3-5). All CoNS isolates were unable to produce bacteriocin. In G-ve isolates the production of bacteriocin among bacterial isolates was variable according to bacterial species. Except *Moraxella* spp., 10(83.3%) of *E. coli*, 2(25%) of *E. aerogenes*, 1(25%) of *Acinetobacter* spp. isolates were capable of bacteriocin production. These findings are in agreement with the results obtained by many researchers (Al-Qassab and Al-Khafaji, 1992; Al-Dulami, 1999; Al-Charrakh, 2005) who found that cup assay method was the best method used for detection of bacteriocin-producers *Lactobacilli*, *E. coli*, and *K. pneumoniae* strains, respectively. The importance of bacteriocin for virulence and pathogenicity of bacteria was controversial. Bacteriocin is essential for virulence and pathogenicity of the of *Enterococcus* in septicemia (Hancock and Gilmore, 2000; Al-barazanchi, 2001), because it was found that cytolysin of *E. faecalis* (possess both hemolysin and bacteriocin activities) promotes the appearance of this bacteria in blood indicating that the bacteriocin is essential for virulence of these bacteria in blood stream infections. By contrast several researches revealed that the bacteriocin activity is not essential for virulence and pathogenicity of the producing isolates (Opal *et al.*, 1988; Vidotto *et al.*, 1991). It was found that bacteriocin activity of *E. coli* isolates is not essential for virulence and pathogenicity of the producing isolates, but it aids them in their

competition (Vidotto *et al.*, 1991). Moreover, the ability of bacteriocin production in *E. coli* strains, isolated from urine of patients suffering of UTIs and from stool of healthy individuals, was tested. It was found that there was non significant difference in ability of these strains to produce bacteriocin, between those isolated from urine or stool samples, which indicates that the bacteriocin is not a virulence factor (Opal *et al.*, 1988). It was also found that in a mixed fermentation environment, production of bacteriocin may prove advantageous for a producer organism to dominate the microbial population (Graciela *et al.*, 1995).

3.4.6. Lipase production:

Production of lipase were detected among bacterial isolates and the results showed that 15(78.9%) of *S. aureus* and 7(28%) of CoNS isolates were capable of lipase production (Table 3-5). Results of lipase production test in G-ve bacterial isolates revealed that 9(75%) of *E. coli*, 7(87.5%) of *E. aerogenes* and 1(50%) isolate of *Moraxella* spp. were lipase producer (Table 3-6). Host cell membranes contain lipids in their components; lipase enzyme will destroy these elements and aids the pathogen to penetrate the host tissue to develop the infections (Lisa *et al.*, 1994; Bartels *et al.*, 2007).

3.4.7. Extracellular protease production:

The ability of the bacterial isolates to produce extracellular protease by using M9 media supported by 1% gelatin was investigated and it was found that 7(36.8%) of *S. aureus* isolates were able to produce extracellular protease after 24 hrs. of incubation and gave transparent area around the colony after the addition of 3ml (5%) of trichloroacetic acid (TCA). All CoNS isolates were unable to produce extracellular protease enzyme (Table 3-5). Extracellular protease production results among G-ve isolates revealed that all isolates were not capable of extracellular protease production except *E. coli* isolates in which

5(41.7%) of isolates were protease producers (Table 3-6). Extracellular protease was one potential virulence factors of many microorganisms because of its ability to breakdown immunoglobulins and complement components that make up the host defenses against microbial infections, and therefore enable the pathogen to invade the host tissues (Travis *et al.*, 1995; Poeta *et al.*, 2006). Extracellular protease enzymes were able to realize many functions such as inactivates the human phagocyte chemotaxin C5a and in the evasion of opsonophagocytosis (Cheng *et al.*, 2002; Harris *et al.*, 2003). Also this enzyme may associate with bacterial nutrition or metabolism (Brooks *et al.*, 2004) .

3.4.8. Colonization factor antigen (CFA):

All isolates were tested for their ability to produce colonization factor antigens type (CFA/I) and (CFA/III). The results revealed that all G+ve isolates were able to produce (CFA/III) and 10(52.6%) of *S. aureus*, 8(32%) of CoNS isolates were capable to produce (CFA/I) as shown in table (3-5). These factors are considered primary factors which cause adhesion of bacteria to the target host cell, and their presence indicates that the bacteria contain cell surface fimbrial antigens. Detection of CFA in G-ve bacterial isolates were done and the results indicated presence of (CFA/III) in all G-ve isolates, while (CFA/I) were found in 8(66.7%) of *E. coli*, 6(75%) of *E. aerogenes*, 2(50%) of *Acinetobacter* spp. and 1(50%) of *Moraxella* spp. isolates (Table 3-6). The (CFA/I) contributed and aided the bacteria to adhere and multiply within eukaryotic cells. Bacterial adherence to host tissues is a complex process that, in many cases, involves the participation of several distinct adhesions, all of which may act at the same time or at different stages during infection (Ofek *et al.*, 2002). Many pathogenic bacteria displayed polymeric adhesive fibers termed "pili" or "fimbriae" that facilitated the initial attachment to epithelial cells and subsequent successful colonization of the host (Ofek *et al.*, 2002). Pili are

virulence factors that mediate interbacterial aggregation and biofilm formation, or mediate specific recognition of host-cell receptors (Jonson *et al.*, 2005). It is clear that pili play similar biological roles for commensal bacteria because they also have to colonize specific niches and overcome the host's natural clearing mechanisms. It is thought that commensal and some pathogenic *Escherichia coli* strains use type I pili or curli to colonize human and animal tissues (Maria *et al.*, 2007).

3.5. Effect of some antibiotics on bacterial isolates:

The effect of some antibiotics on isolated bacteria was assessed using Kirby-Bauer disk diffusion method (Bauer *et al.*, 1966). Resistance of *S. aureus* and CoNS to penicillin and methicillin, oxacillin and vancomycin was presented in figure (3-1). The results revealed that 16(84.2%) of *S. aureus* and 20(80%) of CoNS isolates were resistant to penicillin. Staphylococcal resistance to penicillin is mediated by penicillinase (a form of β -lactamase) production: an enzyme which broke down the β -lactam ring of the penicillin and its derivatives giving rise to inactivate products, can not bind to penicillin binding proteins (PBPs) on cell wall (Humphreys, 2004; Forbes *et al.*, 2007). This result was in agreement with those displayed by other studies (Bayram and Balci, 2006; Joseph and Alexander, 2007). Also, Amyes (1983) expressed that *S. epidermidis* exhibited resistance to many types of antibiotics and this resistance was attributed to the R-plasmid acquired from pathogenic bacteria present in the site of infection. Results also showed that *S. epidermidis* bacteria had the highest number of multiresistant isolates and these findings are in agreement with those isolated from clinical specimens (Brooks *et al.*, 2004). *S. epidermidis* may act as a reservoir for resistance which can be transferred to *S. aureus*. The transfer of resistance among different genera of Gram-positive and Gram-negative bacteria was reported by many researchers (Schaberg and Zervos,

1986; Mazodier and Davies, 1991; Courvalin, 1994). Penicillinase-resistant penicillins such as methicillin, oxacillin, cloxacillin, dicloxacillin and flucloxacillin were able to resist degradation by staphylococcal penicillinase (Blot *et al.*, 2002; Cook *et al.*, 2007). All isolates of *S. aureus* and CoNS were (100%) resistant to methicillin and this isolates defined as methicillin resistant *S. aureus* (MRSA) and methicillin resistant coagulase negative staphylococci (MRCoNS) as shown in figure(3-1). Methicillin sensitive *S. aureus* (MSSA) and methicillin sensitive coagulase negative (MSCoNS) were not isolated in this study. MRSA was able to resist certain antibiotics which include methicillin, oxacillin, penicillin, and amoxicillin. It is defined as an *S. aureus* possessing the *mecA* gene that confers methicillin resistance (Araki *et al.*, 2002; Mongkolrattanothai *et al.*, 2003).

In the present study resistance to oxacillin was reported in 12(63.2%) of *S. aureus* and 14(56%) of CoNS isolates. This result was like those reported by Lucia and here group (2003). There are two mechanisms of resistance in methicillin resistant staphylococci (MRS) organisms: the first one is concerned with penicillin-binding proteins. All penicillins and cephalosporins require binding to a penicillin-binding protein (PBP) located in the bacterial cell wall to initiate their activity. MRS produced a defective low-affinity PBP (**PBP2a**) due to the presence and activation of the *mecA* gene which was borne on plasmid DNA.

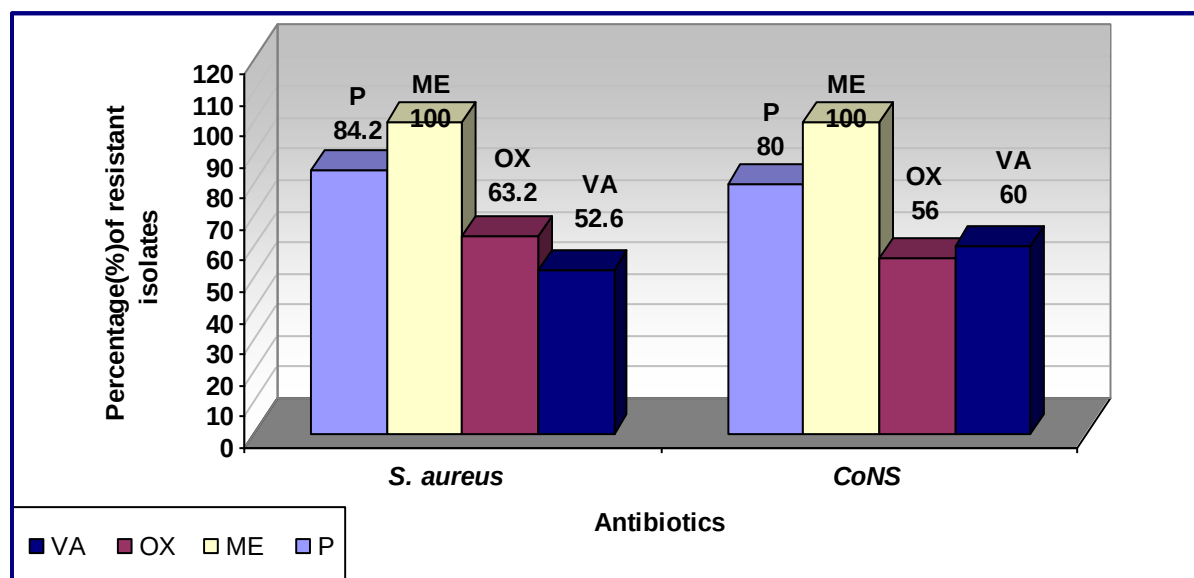


Figure (3-1) Antibiotics resistance of gram positive isolates to penicillin, methicillin, oxacillin and vancomycin.

P: Penicillin, ME: methicillin, OX: oxacillin, VA: vancomycin

Penicillins and cephalosporins binding affinity to PBP2a is very low disabling its ability to disrupt cell wall synthesis and rendering the drug ineffective. Expression of PBP2a on a *Staphylococcus* organism confers resistance to all penicillins and cephalosporins (Gillespie *et al.*, 1985). Other mechanisms were regarded to cell wall thickening. MRSA also possessed a thick cell wall that makes penetration by antibiotics difficult, conferring resistance to multiple antibiotics not just β -lactams (Mongkolrattanothai *et al.*, 2004; Noto and Archer, 2006; Prakash *et al.*, 2007; Shorman *et al.*, 2008). This confers resistance to all β -lactam antibiotics and obviates their clinical use during MRSA infections. Resistance to oxacillin in CoNS has become a problem, as CoNS express resistance to all β -lactam antibiotics, and leads to a significant limitation in therapeutic options (York *et al.*, 1996). Oxacillin resistance may not be mediated by *mecA* gene. York and his colleagues (1996) and Bogado his colleagues (2001) observed this trend, and suggested that a mechanism other than the production (PBP2a) may be involved in methicillin resistance in CoNS.

The hyperproduction of β -lactamase was suggested as a major factor in oxacillin resistance in CoNS (Ghoshal *et al.*, 2004).

Resistance of G+ve isolates to vancomycin was investigated and the results showed that 10(52.6%) of *S. aureus* and 15(60%) of CoNS isolates were vancomycin resistant and called vancomycin resistant *S. aureus* (VRSA) and vancomycin resistant CoNS (VRCoNS) respectively. Specific mechanism for vancomycin resistance was hypothesized by Forbes and his colleagues (2007) who stated that alteration in the molecular structure of cell wall precursor components which decreases binding of vancomycin so that allowing the cell wall synthesis to be continued. Glycopeptide resistance have been emerged in *S. aureus* either by interspecies transfer of resistance genes or by selection of resistant mutants as a result of prolonged antimicrobial therapy (Forbes *et al.*, 2007). The ability of G+ve bacteria to acquire glycopeptide-resistance genes became a matter of concern with the emergence of vancomycin-resistant enterococci, and vancomycin- resistance genes have been transferred from vancomycin-resistant enterococci to *S. aureus* in vitro (Noble *et al.*, 1992). This result was in line with those proved by Theresa and her group (1999). Other studies found that, none of the *S. aureus* isolates with glycopeptide resistance have had *vanA*, *vanB*, *vanC1*, *vanC2*, or *vanC3* genes (Tenover *et al.*, 1998), suggesting that interspecies transfer of resistant genes from vancomycin-resistant enterococci is not the mechanism by which glycopeptide resistance developed in the *S. aureus* isolates (Clark *et al.*, 1993). Other researchers noted that *S. aureus* isolates with vancomycin resistance had increased extracellular material associated with the cell wall a finding similar to that observed in *S. aureus* organisms with intermediate glycopeptide resistance induced in vitro (Daum *et al.*, 1992; Shlaes *et al.*, 1993; Sieradzki and Tomasz, 1997).

Figure(3-2) displays the resistance of all G+ve and G-ve bacterial isolates to amoxicillin and amoxicillin-clavulanic acid .The results revealed that all bacterial isolates showed high resistance (75% - 100%) to amoxicillin , but less resistance to amoxicillin-clavulanic acid (47.4% - 75%).Among G+ve bacterial isolates the resistance of *S. aureus* and CoNS isolates to amoxicillin were (100%) for both. These results are agreeable with results obtained by Dan, (2005) and Humphreys and his colleagues (2004) who confirmed that the resistance of CoNS isolates to β -lactams was mediated by β -lactamase enzymes production under chromosomal control. Both *S. aureus* and CoNS isolates exhibited low level of resistance toward amoxicillin-clavulanic acid 9(47.4%), 13(52%) respectively. Addition of clavulanic acid can inhibit the action of β -lactamases enzyme (Dulawa *et al.*, 2003; Aggarwal *et al.*, 2003). These results matched those obtained by Romolo and his colleagues (2004) who pointed out that the uropathogens resistance to amoxicillin was as high as to amoxicillin-clavulanic acid. The use of clavulanic acid decreased the resistance of bacteria to β -lactame antibiotics .The mechanism of this resistance is mostly due to either production of β -lactamases that hydrolyze β -lactame ring which was controlled by plasmid or chromosomal regulation, or lack of penicillin receptors on cell wall and/or alteration in their permeability to β -lactam antibiotics and preventing the uptaking of antibiotics (Thomson and Amyes, 1993; Ang *et al.*,2004). In addition, Gupta and his colleagues (2001) explained that over the past decade the utility of this class of antibiotics for treatment of UTIs and chronic prostatitis decreased due to increasing levels of resistance to β -lactam antibiotics.

Among G-ve bacterial isolates the resistance of *E. coli* to amoxicillin was 12(100%) which was higher than to amoxicillin-clavulanic acid 8(66.7%). This result was in line with other results reported by Wiedeman and his group (1991) who observed an upward trend in the resistance of *E. coli* to amoxillin/ampcillin and this resistance is predominantly caused by plasmid-encoded β -lactamase TEM-1; these enzymes preferentially hydrolyze penicillin, which was sensitive to β -lactamase inhibitors such as clavulanic acid. So, addition of clavulanic acid can inhibit the action of these enzymes and only 70% were resistant to amoxicillin-clavulanic acid (Dulawa *et al.*, 2003; Aggarwal *et al.*, 2003). Generally resistance to beta-lactam antibiotics in G-ve bacteria can be due to four mechanisms: Decreased permeability of the drug into the cell, hydrolysis of the drug by β -lactamase, decreased affinity of the target penicillin-binding proteins (PBPs), or by pump-mediated resistance (Piddock *et al.*, 1997; Forbes *et al.*, 2007).

The resistance of *Acinetobacter* to amoxicillin was (100%) and this result was higher than those reported by Al-Shukri (2003) and Alwash (2006) who clarified that the resistance rate of uropathogenic *Acinetobacter* to amoxicillin was (63.6%) and (80%) respectively. Enzyme resistance was resulted from the ability of *Acinetobacter* to produce β -lactamase (Al-Shukri, 2003; Forbes *et al.*, 2007). Charrel and his colleagues (1996) and Dumarche and his colleagues (2002) pointed that most of *E. aerogenes* isolates were able to produce extend spectrum β -lactamase (ESBL). Only three isolates of *E. aerogenes* were resisted to amoxicillin and this results in agreement with those results being reported by other researcher (Jalaluddin *et al.*, 1998; Mallea *et al.*, 1998; Tzelepi *et al.*, 2000) but it was disagreed with those obtained by Conceicao and his colleagues (2000) who pointed that all isolates of *E. aerogenes* were fully resistance to amoxicillin. Also Dumarche and his colleagues (2002) reported that all

E. aerogenes isolates which produce (ESBL) had one or more of plasmids which carry multiresistance genes. Two isolates of *Moraxella* spp. were resistant to amoxicillin and amoxicillin-clavulanic acid. Mechanism of resistance exhibited by *Moraxella* was similar to those of *Acinetobacter*. Varon and his researchers (2000) found that *M. catarrhalis* were fully sensitive to amoxillin.

Resistance of bacterial isolates to the cephalosporins was studied. Figure (3-3) reveals variable levels of resistance to different generations of cephalosporins. *S. aureus* resistance to cefamandole (2nd generation), ceftizoxime, ceftazidime (3rd generation) and cefepime (4th generation) were 73.7%, 84.2%, 100% and 68.4% respectively. This result revealed that *S. aureus* exhibited low level of resistance to 4th generation cephalosporin than other cephalosporins. This result agreed with Brooks and his colleagues (2004). It was in line with reports of Pankuch and Applebaum (2006). CoNS isolates displayed low level of resistance to cephalosporins (56%-80%) than those exhibited by *S. aureus*. Resistance to cephalosporins mediated by cephalosporinase production (Forbes *et al.*, 2007)

All G-ve bacterial isolates were fully (100%) resistance to Cefamandole (second-generation cephalosporin) except *E. coli* and *E. aerogenes* (91.7%, 75%) respectively. *S. aureus* and CoNS isolates exhibited less level of resistance to cefamandole than G-ve isolates. All isolates of G-ve bacteria exhibited nearly similar levels of resistance to cephalosporins. *Acinetobacter* spp. isolates were fully resistance to cephalosporins, also six isolates of *E. aerogenes* were resistant to all cephalosporins. This resistance may be resulted from combination of unusually restricted outer membrane permeability and chromosomally encoded β -lactamase. This agreed with results mentioned by Hankok and Speert (2000) and Bisiklis and his workers (2005).

Figure (3-4) showed that all bacterial isolates exhibited high sensitivity to imipenem and meropenem (carbapenems) except in *Moraxella* spp. which displayed resistance to both of these antibiotics which might be due to the low number of *Moraxella* isolates in the present study. However, the result was in accordance with those reported by Tsuji and his colleagues (1998); Yamaguchi and his group (1998); Watanabe and his colleagues (2000) and Nomura and Nagayama (2002). Imipenem and meropenem are broad-spectrum carbapenems antibiotics. Beta-lactam rings of these antibiotics are resistant to hydrolysis by most beta-lactamases (Kropp *et al.*, 1985; Barry *et al.*, 1985) and the activity of meropenem against most clinical isolates was comparable with imipenem (Murray *et al.*, 1990). These antibiotics pass through the outer membrane of G-ve bacteria via the water filled porin channels to reach their targets, penicillin binding proteins (Cornaglia *et al.*, 1995; Forbes *et al.*, 2007). Deletion or diminished production of these outer membrane proteins (porins) decreases outer membrane permeability of some G-ve bacteria for diffusion of these antibiotics and decreases susceptibility to imipenem and meropenem (Cornaglia *et al.*, 1995; Brooks *et al.*, 2004; Forbes *et al.*, 2007). Generally a distinct difference was present between β -lactamase production by G+ve and G-ve bacterial isolates, for example β -lactamase produced by staphylococci were excreted into the surrounding environment where the hydrolysis of β -lactams takes place before the drug can bind to PBPs in the cell membrane. In contrast, β -lactamase produced by G-ve bacteria remained intracellular in the periplasmic space where they were strategically positioned to hydrolyze β -lactams as they transverse the outer membrane through water filled, protein lined porin channels (Forbes *et al.*, 2007).

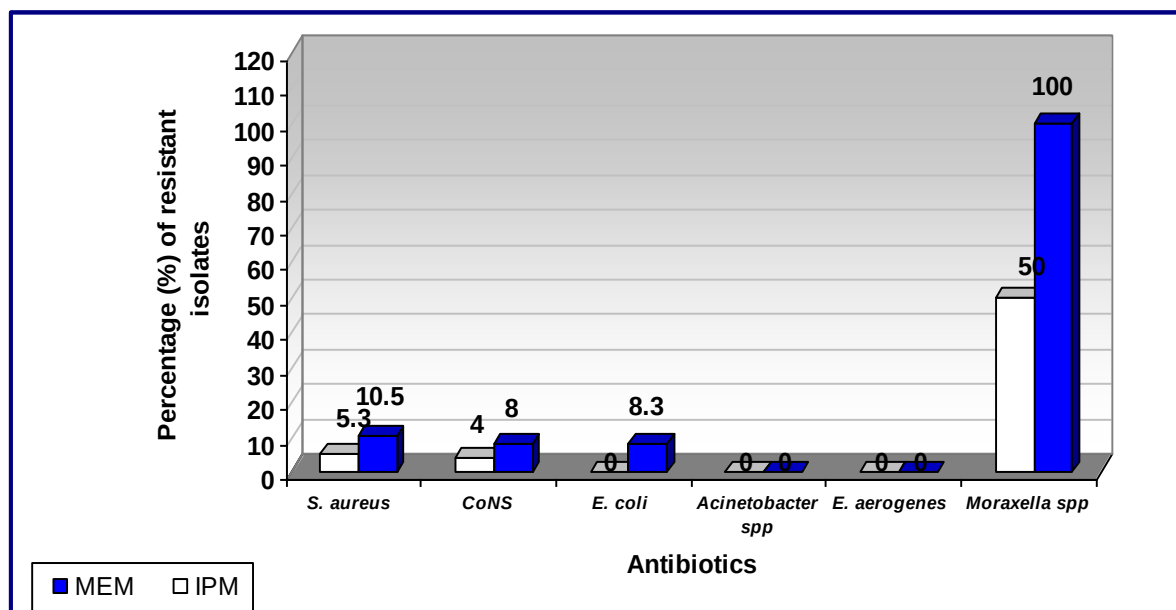


Figure (3-4) Resistance of bacterial isolates to carbapenems.

MEM: meropenem, IPM: imipenem

Resistance of the bacterial isolates to aminoglycosides were established in figure (3-5). The results revealed that *S. aureus* and CoNS isolates showed similar status of resistance to gentamycin (84.2%, 88%) respectively. The mechanism of aminoglycosides resistance by staphylococcal isolates is enzymatic modification, in which modifying enzymes alter various sites on the aminoglycosides molecule so that the ability of drug to bind the ribosome and halt protein synthesis was greatly diminished or lost (Hogg, 2005; Forbes *et al.*, 2007). This result was agreed with Al-Nuaimi (2002) and Alwash (2006), who found that (80%) of *Staphylococcus* spp. isolates were exhibited resistance to gentamycin. However, Khorshed (2005) reported that *Staphylococcus* spp. isolated from UTI were very sensitive to gentamycin (low level of resistance 15%). *S. aureus* and CoNS gave low level of resistance to amikacin, (36.3%, and 32% respectively) and also to tobramycin (57.9%, 36% respectively) when compared with their resistance to gentamycin.

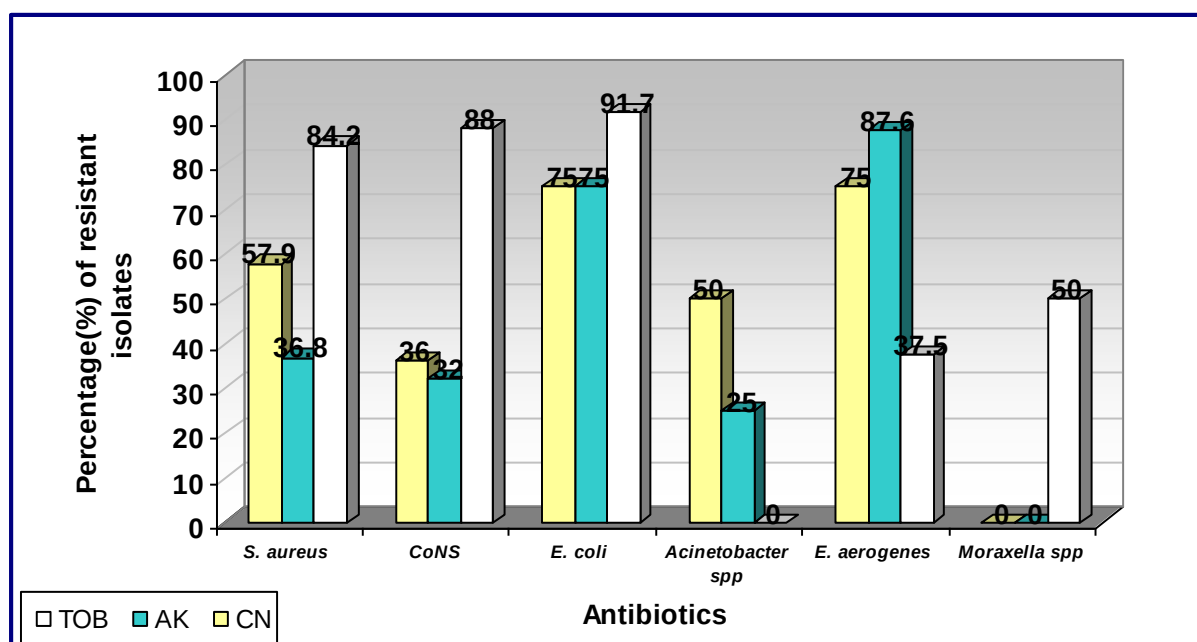


Figure (3-5) Resistance of bacterial isolates to aminoglycosides.

TOB: tobramycin, AK: amikacin, CN: gentamycin.

These results were high in relative with those of Jukka and his workers (1995) who pointed that uropathogens *S. epidermidis* resistance to gentamycin were 46% also they noticed that multiresistant in *Staphylococcus* species in bacterial prostatitis was due to the distribution of the *mecA* gene among the *S. epidermidis*. Similarly, Konino and his group (1995) showed that the endemic aminoglycoside-resistant MRSA strain persisted while new clones became endemic in hospitals, perhaps after changes in the use of aminoglycosides (decrease of gentamycin and increase of amikacin consumption). The antibiotic resistance in MRS was due to the presence of plasmid DNA and those separate plasmids encode resistance to gentamycin and chloramphenicol. (Gillespie *et al.*, 1985). Resistance to gentamycin had been identified in CoNS isolates (Archer and Johnston, 1983). Moreover, CoNS may function as a reservoir for antibiotic resistant genes to *S. aureus* (Archer and Climo, 1994). Among G-ve bacterial isolates, 91.7% of *E. coli* isolates were resistant to tobramycin. Only (75%) of *E. coli* isolates were

resistant to amikacin and gentamycin. These results agreed with those reported with Al-Muhanna (2001) and Al-Nuaimi (2002), who found that *E. coli* was fully resistant to amikacin. However, this result disagreed with other local studies such as Khorshed (2005) and Alwash (2006) who found that *E. coli* isolated from patients with urinary tract infections (UTI) and from those with prostatitis exhibited low level of resistance to amikacin (7.7%-25%). This resistance could be interpreted depending on the fact that many strains of *E. coli* have acquired plasmids conferring resistance to one or more than one type of antibiotics, therefore antimicrobial therapy should be guided by laboratory result test of sensitivity (Chart, 2004; Al-Hamawandi, 2005). *Acinetobacter* spp. isolates were fully sensitive to tobramycin, but they showed low resistance to amikacin (1/4) and (2/2) of them were resist gentamycin. This result was in line with those documented by Khorshed (2005) and Al-Shukri, (2003) who observed that *Acinetobacter* was resistant to gentamycin and this resistance was produced through alteration of the ribosomal target site, and production of aminoglyside-modifying enzyme. Moreover, Hpa (2003) established that resistance of uropathogenic *Acinetobacter* to gentamycin and amikacin were 43% and 5% respectively. Concerning *E. aerogenes* resistance of aminoglycosides, the results revealed that (6/8) of *E. aerogenes* isolates were resistant to gentamycin (7/8) were resistant to amikacin and (3/8) of them were resisted tobramycin. This results agreed with Alwash (2006).

Enterobacter spp. resistance to gentamycin was (75%). These results agreed with Zahac and his colleagues (2003) who revealed that *Enterobacter* was resistant to gentamycin. This result was in contrast in relative with other studies such as Carapeti and his colleagues (1996) who found that *E. aerogenes* isolates showed partial resistance to gentamycin (58%) while they were completely sensitive

to amikacin (100%). Park and his colleagues (2003) had stated that the resistance rate of *Enterobacter* spp. to gentamycin was (33.3%) while it was (54%) for amikacin and that differ from the results in the present study. Moreover, Al-Mashriky (2003) found that (20%, 40%, and 50%) of *E. aerogenes* isolates were resistant to tobramycin, amikacin and gentamycin respectively.

The mechanism of *E. aerogenes* resistance to aminoglycosides was mediated by the production of more than one type of aminoglycosidases located on the R plasmid (Maes and Vanhoof, 1992). Other mechanism was post transcriptional modification of 16S rRNA which can confer high level resistance to all aminoglycosides except streptomycin in G-ve human pathogens including *E. aerogenes* (Galimand *et al.*, 2005). *Moraxella* spp. isolates were fully sensitive to gentamycin and amikacin. Only (1/2) of *Moraxella* spp. isolates were resist to tobramycin.

In the present study the results of fluoroquinolones (ciprofloxacin and norfloxacin) resistance are displayed in figure (3-6). G+ve isolates exhibited low resistance to both ciprofloxacin and norfloxacin, (42.1%) of *S. aureus* and (12%) of CoNS isolates were resist to ciprofloxacin, while resistance to norfloxacin was (36.8%, 32%) respectively.

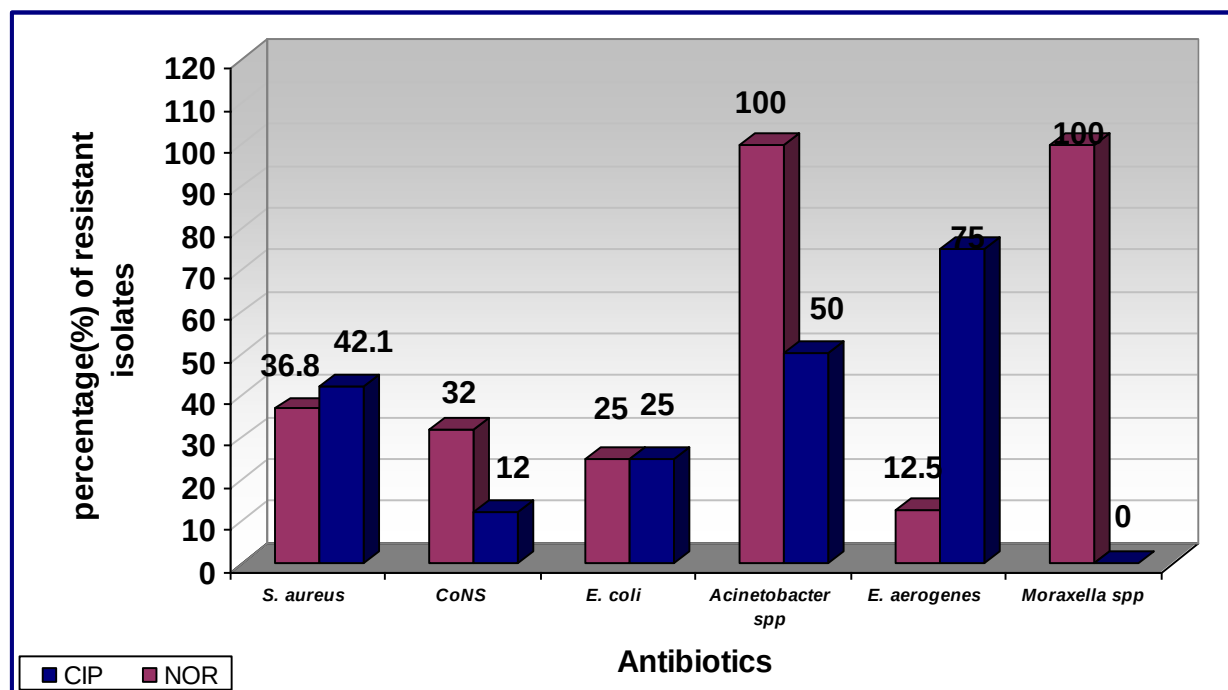


Figure (3-6) Resistance of bacterial isolates to Fluoroquinolones.

CIP: Ciprofloxacin, NOR: norfloxacin

This result agreed with other local studies such as Khorshed (2005) who found that only (20%) of *staphylococcus* spp. isolated from patients with UTI were resistant to ciprofloxacin. Also, Alwash (2006) found that (33.3%) of *S. aureus* and (11.1%) of CoNS isolates were resisted ciprofloxacin. Similarly, Rachid and his group (2000) observed that there were an increased number of strains resistant to ofloxacin and ciprofloxacin. In addition, Jukka *et. al.*, (1995) noticed that 23% of CoNS isolates were resistant to ciprofloxacin among staphylococci isolates. Kurt and Naber (2001) document that the ciprofloxacin was the first choice for seminal fluid tract infection. Bach and his colleagues (1995) establish that, ciprofloxacin had the ability to cure most uropathogens that cause UTI with very low side effects. Moreover, Donnell and Gelone, (2000) reported that the resistance to flouroquinolones was through chromosomal mutations or alterations affecting the ability of fluoroquinolones

to permeate the bacterial cell wall. Fortunately, separate isomerases were required to produce this form of resistance (Romolo *et al.*, 2004). Forbes and his colleagues (2007) stated that staphylococci had two mechanisms to resist flouroquinolones; the first one was efflux mechanism in which an activation of efflux pump that removes flouroquinolones before intracellular concentration sufficient for inhibiting DNA metabolism can be achieved (Mallea *et al.*, 1998). The other mechanism (target alteration) included changes in DNA gyrase subunits decrease ability of flouroquinolones to bind this enzyme and interfere with DNA processes.

Flouroquinolones resistance among G-ve bacterial isolates were also studied. (25%) of *E. coli* isolates were resistant to both ciprofloxacin and norfloxacin. This result was in line with results obtained by Khorshed (2005) and Alwash (2006) who found that, the resistance rate of *E. coli* to ciprofloxacin was (36.4%), and differed from Donnell and his group (2000) and Klligore and his colleagues (2004) who demonstrated that the resistance rate of *uropathogenic E. coli* to ciprofloxacin was (0.4%, 13%) respectively. (4/4) and (2/4) of *Acinetobacter* spp. isolates were resistant to norfloxacin respectively. This result was agreed with those documented by some local studies such as Al-Nuaimi (2002) and Alwash (2006) who found that some of *Acinetobacter* isolate exhibited resistance to ciprofloxacin, while other studies, Khorshed (2005) and Al-Shukri (2003) stated that, all *uropathogenic Acinetobacter* isolates were fully sensitive to ciprofloxacin. Among *E. aerogenes* isolates, only one isolate was resistant to norfloxacin, while (6/8) of it was resisted ciprofloxacin. This result disagreed with other studies (Brisse *et al.*, 1999; Bornet *et al.*, 2000; Karlowsky *et al.*, 2003; Al-Mashriky, 2003; Al-Shukri, 2003; Khorshed, 2005) who stated that, *E. aerogenes* isolates were fully sensitive to ciprofloxacin. Resistance of G-ve

isolates to flouroquinolones occurred by one of the two strategies, either by alteration in the outer membrane led to diminishes uptake of drug, or by changes in DNA gyrase subunits which decreases ability of flouroquinolones to bind this enzyme and interfere with DNA processes (Hogg, 2005; Forbes *et al.*, 2007). In addition to that, Jacoby and his collageus (2006) stated that *Enterobacter* had plasmid-mediated quinolones resistance gene which confer their resistance to the flouroquinolones.

Resistance of the bacterial isolates to doxycycline and trimethoprim-sulfamethoxazole were also studied (Figure 3-7). Regarding doxycycline resistance the results revealed that, *S. aureus* and CoNS isolates exhibited similar level of resistance to doxycycline (57.9% , 52%) respectively. This result was in line with Kolar and his colleagues (2002) who expressed that, in Staphylococci there were increased in number of strains resistant to tetracyclines.

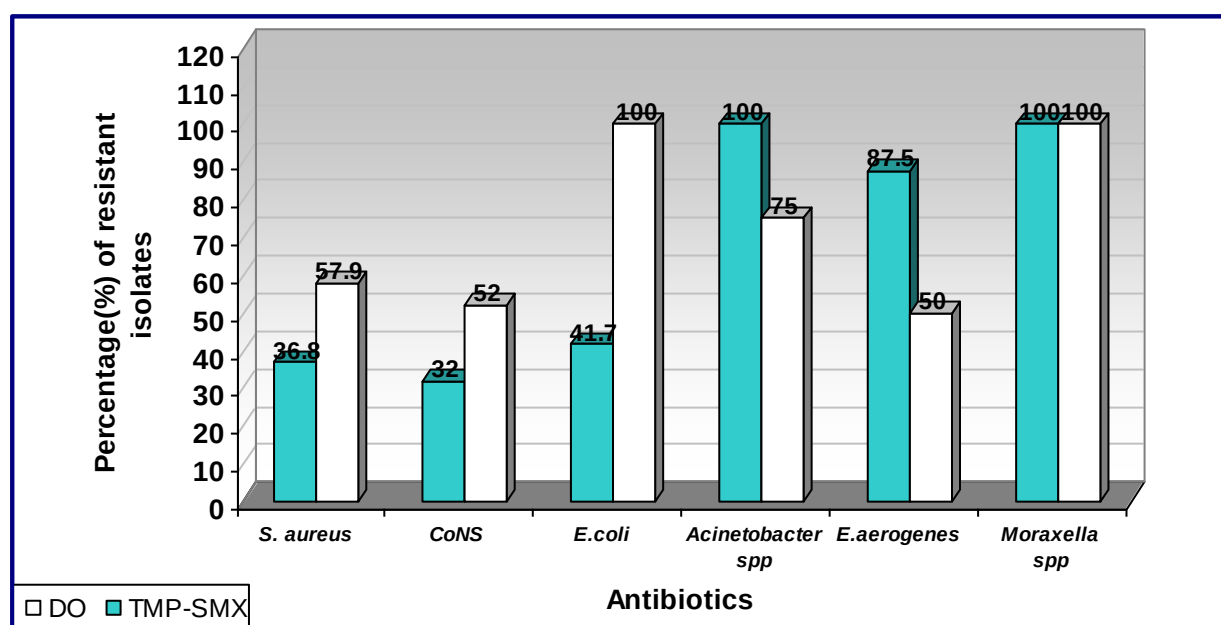


Figure (3-7) Resistance of bacterial isolates to doxycycline and trimethoprim-sulfamethoxazole.

DO: doxycycline, TMP-SMX : trimethoprim-sulfamethoxazole

E. coli isolates were fully resistance to doxycycline. These results were in contrast with those reported by Alwash (2006) who found that (40%) of *E. coli* isolates were resist doxycycline. The last five-year period demonstrated significant increases in the resistance of *E. coli* to tetracycline from 29-40% (Romolo *et al.*, 2004). Only (4/8) of *E. aerogenes* isolates were doxycycline resistant. This result was correlated to those recorded by some local studies such as Alwash (2006) who expressed that resistance of *Enterobacter* to doxycycline was (50%). This result was correlated with Marco and Parker (1997) result who declared that bacteria became resistant to tetracyclines by transferring of DNA from a resistant cell to another. However, Korshed (2005) and Salman (2006) declared that all isolates of *Enterobacter* exhibited fully resistance to doxycycline (100%). Three isolates of *Acinetobacter* and all *Moraxella* isolates were resistant to doxycycline and this result disagreed with Al-Sukri (2003) who reported that most of uropathogenic *Acinetobacter* were sensitive to tetracycline, so this resistance might be due to the low number of *Acinetobacter* and *Moraxella* isolates in the present study.

Regarding trimethoprim-sulfamethoxasole resistance among bacterial isolates , figure (3-7) showed that *S. aureus*, CoNS and *E. coli* isolates displayed low level of resistance (36.8%, 32%, 41.7%) respectively. This result was lower than those reported by other studies. Khorshed (2005) and Alwash (2006) stated that 50%-70% of *Staphylococcus* spp. isolates exhibited resistant to trimethoprim-sulfamethoxasole and this agreed with Al-Salay (2002) who found that, most of the bacterial isolates were resisted trimethoprim-sulfamethoxasole. Jukka and his colleagues (1995) declared that, half of *S. epidermidis* isolates were resistant to TMP-SMX. *S. epidermidis* exhibit resistance to many types of antibiotics attributed to R-plasmid acquired from

pathogenic bacteria present at the site of infection (Rachid *et al.*, 2000). *E. coli* isolates resistance to trimethoprim-sulfamethoxazole was (41.7%). This result was similar to those reported by Alwash (2006) who stated that, *E. coli* isolates resistance rate to trimethoprim-sulfamethoxazole was (40.9%). This result was agreed with those declared by Huda and his colleagues (2001) and disagreed with Khorshed (2005). Also results in the present study revealed that, *Acinetobacter* and *Moraxella* isolates were fully resisted trimethoprim-sulfamethoxazole. This result was higher than those obtained by other studies, Khorshed (2005) and Alwash (2006) who stated that resistance of *Acinetobacter* isolates to trimethoprim-sulfamethoxazole was (75% , 67%) respectively. This result might be due to low number of *Acinetobacter* and *Moraxella* isolates in the present study. Concerning *E. aerogenes* resistance to trimethoprim-sulfamethoxazole, the results revealed that (87.5%) of *E. aerogenes* isolates were resistant to trimethoprim-sulfamethoxazole. This result was similar to those documented by Salman (2006) who found that, resistance rate of *Enterobacter* spp. to trimethoprim-sulfamethoxazole was (85.7%) and this was in agreeable with those of Bell and his colleagues (2003) who had pointed that the percentage of *Enterobacter* spp. resistance strains in Singapore was (83%) while in the same survey the resistance rate to *Enterobacter* it was (100%) in Hong Kong and (28%) in Japan. Also Bonnet, (2004) asserted that *Enterobacter* was completely resistant to trimethoprim-sulfamethoxazole. The mechanism of trimethoprim-sulfamethoxazole resistance was either by changing the target site of this antibiotic (in both G+ve and G-ve bacterial isolates) or changing its permeability through the outer membrane (Cohen *et al.*, 1993).

4.1.Conclusions:

1. There is a significant relationship between asthenospermia and bacteriospermia.
2. *Staphylococcus aureus* (CoNS) represented by *Staphylococcus epidermidis* and *Staphylococcus saprophyticus*, *Escherichia coli*, *Enterobacter aerogenes*, *Acinetobacter* spp. and *Moraxella* spp. seem to be the most common bacteria associated with bacteriospermia.
3. There is a significant relationship between leukocytospermia and bacteriospermia and leukocytospermia can be used as predictor of bacteriospermia.
4. The bacterial isolates associated with bacteriospermia showed resistance to many antibiotics but they were highly susceptible to imipenem, meropenem and ciprofloxacin.
5. All bacterial isolates in this study have the ability to possess more than one virulence factors such as coagulase, capsule, siderophore, hemolysin, extracellular protease, lipase and adherence factors to produce asthenospermia.

4.2.Recommendations:

1. It is necessary to perform seminal fluid culture in all asthenospermic patients with leukocytospermia to investigate presence of bacteria and prescribe the reliable antimicrobial agent.
2. Current studies need to be performed depending on the polymerase chain reaction (PCR) which helps to detect microorganisms that are not diagnosed by standard means.
3. It is necessary to perform flow up study for bacteriospermic patients to asses their treatment status depending on the duration and the dose.
4. Study the effect of bacteria on other semen parameters.
5. In spite of the result revealed that imipenem, meropenem and ciprofloxacin highly affect the bacterial isolates than other antibiotics used in this study but the choice of the drug depend on antibiotics susceptibility test.

References

References:

- Ad Dahhan, H. A., Naher H. S. and Shubber E.K. (2005). Isolation and identification of bacterial chronic suppurative otitis media. Kufa governorate Journal. College of medicine, Al-Qadisia University, Iraq; 1(2):62-67.
- Aggarwal, A., Kanna S. and Arova U. (2003). Characterization biotyping antibiogram and klebocin typing of *Klebsiella*. Indian J. Med.; 57(2): 68-70.
- Ahmed, K., Rikitomi N., Ichinose A. and Mastsumoto K. (1991). Possible presence of capsule in *Branhamella catarrhalis*. Microbiol. Immunol.; 35:361-366.
- Aitken, R. J., West K. M. and Buckingham D. W. (1994). Leukocyte infiltration into the human ejaculate and its association with semen quality, oxidative stress, and sperm function. J. Androl.; 15:343-352.
- Al-Amedi, B. H. (2003). Comparative study of mucosal immune responses for the persistent pyuria patients. Ph.D. thesis College of Science, Babylon University. Iraq.
- Alanis, A. J. (2005). Resistance to antibiotics: are we in the post-antibiotic era? Archives of Medical Research; 36:697–705.
- AL-Anssari, S. A. M. (2000). Seminal fluid osmotic pressure measurement and sperm function tests and in vitro fertilization rate of human eggs. Kufa med. J.; 4: 66- 69.
- Al-Anssari, S. A. M., Albarzanchi R. M. T. and Khunda S. S. (1997). Seminal fluid osmolarity and in vitro fertilization of human eggs. 3rd Asian symposium on animal biotechnology. Seoul, Korea; pp. 232-236.
- Al-barazanchi, S. I. (2001). A genetic study on bacteriocin producer *Enterococcus faecalis*. M.Sc. thesis. College of Science, Baghdad University, Iraq.

References

- Al-barazanchi, R., Khunda M. T., Al-Fayad R. S. F., Al-Anssari S. A. M. and Jassim M. M. (1992). Human pregnancy following sperm antibody separation technique and sperm intrauterine transfer. J. comm. Med. Baghdad.; 80: 1-10.
- Al-barzanchi, R., Al-Naeib Z. T. H., Al- Anssari S. A. M., Khunda S. S., Zakria M. R., Al-Fayad R. S. F., and Azat L. N. (1991). Successful live birth following human ovarian stimulation and in vitro sperm cell hyperactivation and application of sperm intrauterine transfer technique. J. Fac. Med. Baghdad; 33: 327-336.
- Alberto, B., Palmero J. L., Broseta E., Tejadillos S., Benedicto A., Queipo J. A., Gobernado M. and Cruz J. F. (2006). Value of semen culture in the diagnosis of chronic bacterial prostatitis: A simplified method. J. Medic. Microbiol.; 40(4):326-331.
- Al-Charrakh, A. H. (2005). Bacteriological and genetic study on extended-spectrum beta lactamases and bacteriocins of *Klebsiella* isolated from Hilla city. Ph.D. Thesis College of Science. Baghdad University, Iraq.
- Al-Dulami, H. H. (1999). Effect of crude colicin extracted from *Escherichia coli* on immune cells. M.Sc. thesis. College of Science. Al-Mustansiriya University, Iraq.
- Al-Hamawandi, J. A. (2005). Bacteriological and immunological study on infants pneumonia at Babylon governorate. M.Sc. thesis. College of Science. Al-Mustansiriya University, Iraq.
- Alhambra, A., Cuadros J. A. and Cacho J. (2004). In vitro susceptibility of recent antibiotic resistant urinary pathogens to ertapenem and 12 other antibiotics. J. Antimicrob. Chemother.; 53(6):1090-1094.

References

- Al-Mashriky, J. H. (2003). A study of identification and pathogenicity on *Enterobacter aerogenes* isolated from infantile diarrhoeal cases in Mosul. Msc. thesis. College of science. Al-Mosul university, Iraq.
- Al-Muhanna, A. S. (2001). Identification of bacteria causing pneumonia in three medical centers in Najaf. M.Sc. thesis. Kufa University, Iraq.
- Al-Nuaimi, E. M.(2002). Urinary tract infection in pregnant women .M.Sc. thesis. College of science. Al-Mustansiriya university, Iraq.
- Al-Qassab, A. O. and Al-Khafaji Z. M. (1992). Effect of different conditions on inhibition activity of enteric lactobacilli against diarrhea causing enteric bacteria. J. Agric. Sci.; 3(1):18-20.
- Al-Rassam, Z. T. D. (2004). Urinary tract infections caused by *Alcaligenes* spp. and its relation to immune suppression in diabetic patients. M.Sc. thesis. College of Science. Al-Mosul University, Iraq.
- Al-Saigh, R. J. (2005). A study of some clinical and bacteriological aspects of patients with chronic pyelonephritis in Hilla-teaching Hospital. Thesis of M.Sc. College of medicine. Babylon University, Iraq.
- Al-Salay, T. S. (2002). Common bacterial causes of urinary tract infections in children below five years of age in maternity and pediatrics Hospital-Erbil . MSc. thesis. University of Salahaddin , Iraq.
- Al-Shukri, M. S. (2003). A study of some bacteriological and genital aspects of *Acinetobacter* isolated from patients in Hilla-city. Msc. thesis. College of Science. Babylon University, Iraq.
- Alwash, B. H. (2006). A bacteriological and clinical study of patients with benign prostatic hyperplasia and or chronic prostatitis .Msc. thesis . College of medicine. Babylon university, Iraq.
- Amelar, D.A., Dubin L. and Schoenfeld C. (1980). Sperm motility. Fertil. Steril.; 34:197-215.

References

- Amyes, S. (1983). The detection and incidence and transferable trimethoprim resistance. *Health Bull*; 41:100-107.
- Anderson, D. J. (1995). Should male infertility patients be tested for leukocytospermia? *Fertil. Steril.*; 63:246-248.
- Andrade-Rocha, F. T. (2003). *Ureaplasma urealyticum* and *Mycoplasma hominis* in men attending for routine semen analysis. Prevalence, incidence by age and clinical settings, influence on sperm characteristics, relationship with the leukocyte count and clinical value. *Urol. Int.*; 71:377-381.
- Ang, J. Y., Ezike E. and Asmar B. (2004). Antibacterial resistant. *Indian. J. pediatrics.*; 71(3):229-239.
- Araki, M., Kariyama R., Monden K., Tsugawa M. and Kumon. H. (2002). Molecular epidemiological studies of *staphylococcus aureus* in urinary tract infection .*J. infect. Chemother.*; 8:167-174
- Archer, G. L. and Johnston L. J. (1983). Self-transmissible plasmids in staphylococci that encode resistance to aminoglycosides. *Antimicrob. Agents Chemother.*; 24:70-77.
- Archer, G. L., and Climo M. W. (1994). Antimicrobial susceptibility of coagulase negative staphylococci. *Antimicrob. Agents Chemother.*; 38:2231-2237.
- Archibald, F. (1983). *Lactobacillus plantarum*, an organism not requiring iron. *FEMS Microbiol. Lett.*; 19:29-32.
- Arpin, C., Coze C., Rogues A. M., Gachie J.P., Babear C. and Quentin C. (1996). Epidemiological study of an outbreak due to multidrug resistant *Enterobacter aerogenes* in a medical intensive care unit. *J.Clin .Microbiol.*; 34: 2163-2169.
- Bach , D., Annelis B., Andreade H., Gerard B., Marina C. and Yannick P. (1995). Rufloxacin once daily versus Ciprofloxacin twice daily in

References

- the treatment of patients with acute uncomplicated pyelonephritis, J. Urol.; 154 : 19-24 .
- Bach, S., De Almeida A. and Carniel E. (2000). The *Yersinia* high-pathogenicity island is present in different members of the family Enterobacteriaceae. FEMS Microbiol. Lett.; 1:83-28
- Barrett, A. J., Rowling N. D. and Woessner J. F. (2003). The Handbook of proteolytic enzymes. 2nd ed. Academic press. ISBN.
- Barry, A. L., Jones R. N., Thornsberry C., Ayers L. W. and Kundargi R. (1985). Imipenem (N. forimidoly thienamycin): in vitro antimicrobial activity on β -lactamase stability. Diagn. Microbiol. and Infect. Dis.; 3:93-104,
- Bartels, E. D., Nielsen J. E., Lindgaard M. L. and Hulten L. M. (2007). Endothelial lipase is highly expressed in macrophages in advanced human atherosclerotic lesions Atherosclerosis. J. Appl. Microbiol.; 12:65-73.
- Bartoov, B., Ozbonfil D., Maayan M. C., Ohad E. and Nitzan Y. (1991). Virulence characteristics of male genital tract *Escherichia coli* isolated from semen of suspected infertile men. Andrologia; 23: 387-394.
- Bauer, A.W.; Kirby W.M.; Sherris, J.C. and Turck, M. (1966). Antibiotic susceptibility testing by a standardized single disk method. Am. J. Clin. Pathol.; 45:493-496.
- Bayram, A. and Balci I. (2006). Patterns of antimicrobial resistance in a surgical intensive care unit of a university hospital in Turkey. BMC Infect. Dis.; 6:155.
- Behre, H. M., Yeung C. H. and Nieschlag E. (1997). Diagnosis of male infertility and hypogonadism. In: Andrology: male reproductive

References

- health and dysfunction (Nieschlag E., Behre H. M., eds.). Berlin: Springer-Verlag; 87:110.
- Bell, J. M., Turniding J. D., Jones R. N. and Sentry Asia-Pacific Participant. (2003). Prevalence of extended –spectrum β -lactamases producing *Enterobacter cloacae* in the Asia-Pasific region: results from the Sentry. Antimicrob. Agent. Chemother.; 47(12):3989-3993.
- Ben Ali, H., Guerin J. F., Pinatel M. C., Mathieu C., Boulieu D. and Tritar B. (1994). Relationship between semen characteristics, alpha-glucosidase and the capacity of spermatozoa to bind to the human zona pellucida. Int. J .Androl.; 17:121-126.
- Bergman, B. (1994). On the prevalence of gram positive bacteria in prostatitis. J. Infect. Urol.; 22:22-30.
- Bisiklis, A., Tsiakiri E. and Alexiou-Danieel S. (2005). Phenotypic detection of *Pseudomonas aeruginosa* producing metallo- β -lactamases. European Society of clinical microbiology and infectious disease, 15th European congress of clinical microbiology and infectious disease.; 1134:1-11.
- Blance, S. K., Andro M. B. and Jone L. V. (1996). Virulence factors and O groups of *Escherichia coli* isolates in patients with UTIs. J. Eur. Epidemiol.; 12(2):191.
- Bliss, J. M. and Silver R. P. (1996). Coating the surface: a model for expression of capsular polysialic acid in *Escherichia coli* K1. Microbiology; 21:221-231.
- Blot, S. I., Vandewoude K. H., Hoste E. A. and Colardyn F. A. (2002). Outcome and attributable mortality in critically Ill patients with bacteremia involving methicillin susceptible and methicillin resistant *Staphylococcus aureus*. Arch. Intern. Med.; 162(19): 2229-2235.

References

- Bogado, I., Sutich E., Krapp A., Marchiaro P., Marzi M., Putero J. and Carrillo N. (2001). Methicillin resistance study in clinical isolates of coagulase negative staphylococci and determination of their susceptibility to alternative antimicrobial agents. *J. Appl. Microbiol.*; 91:344-350.
- Bonnet, R. K. (2004). *Enterobacter* infections. *J. Mind. Med.*; 48:142.
- Bornet, C., David R., Bosi C., Pages J. and Bollet C. (2000). Imipenem resistance of *Enterobacter aerogenes* mediated by outer membrane permeability, *J. Clin. Microbiol.*; 38(3): 1048-1052.
- Bornman, M. S., Mohamed M. F., Boomker D., Schulenburg G. W., Reif S. and Crewe-Brown H. H. (1990). Microbial flora in semen of infertile African men at Garankuwa hospital. *Andrologia.*; 22:118-121.
- Braun, V., Pilsl H. and Grob P. (1994). Colicins :structures, mode of action, transfer through membranes, and evolution. *Arch. Microbiol.*; 161: 199-206.
- Braun, V., Hantke K. and Köster W. (1998). Bacterial iron transport: mechanisms, genetics, and regulation. *Metal Ions Biol. Syst.*; 35:67-145.
- Braun, V., Patzer S. I. and Hantke K. (2002). Ton-dependent colicins and microcins: modular design and evolution. *Biochimie.*; 84:365-380.
- Brisse, S., Milatovic D., Fluit A. C., Verhoff J., Martin N., Scheuring S., Kohrer K. and Schemitz F. J. (1999). Comparative *in vitro* activities of ciprofloxacin, clindoxacin, gatifloxacin against *Klebsiella pneumoniae*, *Klebsiella oxytoca* and *Enterobacter aerogenes*. clinical isolates with alteration in Gyr A and Par C proteins, *Antimicrob. Agent. Chemother.*; 43(8): 2051-2055.

References

- Brogden, K. A., Roth J. A., Stanton T. B., Bolin C. A., Minion C. F. and Wannemuehler M. J. (2000). Virulence mechanism of Bacterial Pathogens. ASM Press, Washington; D.C.
- Brook, I., Yocum P. and Shah K. (1998). Aerobic and anaerobic bacteriological of otorrhea associated with tympanostomy tubes in children. Acta. Otolaryngology.; 118:206-210.
- Brooks, G. F., Butel J. S. and Morse S. A. (2001). Jawetz, Melnick and Aldelberg's Medical Microbiology. 22nd edi. McGraw-McGraw-Hill.; PP:156-170.
- Brooks, G. F., Butel J. S. and Morse S. A. (2004). Medical microbiology. 23rd ed. Lange Medical books .McGraw-Hill Companies.USA.
- Bukharin, O. V., Kuzimin M. D. and Ivanov I. B. (2003). The role of the microbial factor in the pathogenesis of male in fertility. Zh. Microbial. Epidemiol. Immunobiol.; 2:106-110.
- Carapeti, E. A., Andrews S. M. and Bentley P. G. (1996). Randomized study of sterile versus non sterile urethral catheterization. Ann. R. Coll. Surg. Engl.; 78:59-60.
- Cassels, F. J. and Wolf M. K. (1995). Colonization factors of diarrheagenic *E. coli* and their intestinal receptors. J. Ind. Microbiol.; 15:214-226.
- Catlin, B. W. (1990). *Branhamella catarrhalis* An organism gaining respect as a pathogen. Clin. Microbiol. Rev.; 3:293-320.
- Chan, P., Su B. C., Tredway D. R., Whitney E. A., Pang S. C., Corselli J. and Jacobson J. D. (1994). White blood cells in semen affect hyperactivation but not sperm membrane integrity in the head and tail regions. Fertil. Steril.; 61:986-989.
- Chang, S., Sievert D. M., Hageman J. C., Boulton M. L., Tenover F. C., Downes F. P., Shah S., Rudrik J. T., Pupp G. R., Brown W. J., Cardo D. and Fridkin S. K. (2003). Infection with vancomycin-resistant

References

- Staphylococcus aureus* containing the vanA resistance gene. N. Engl. J. Med.; 348 (14):1342-1347.
- Charrel, N. R., Pages J. M., Demicco P. and Mallea M. (1996). Prevalence of outer membrane porin alteration in β -lactam antibiotic resistant *Enterobacter aerogenes*. Antimicrobiol. Agent Chemother.; 40(12):2854-2858.
- Chart, H. (2004). *Escherichia coli* In Greenwood, D., Slack, R. and Penethrer, J. Medical microbiology a guide to microbial infections: pathogenesis, immunity, laboratory diagnosis and control.; PP: 265-274 .
- Cheng, Q., Stafslie D., Purushoth S. S. and Cleary P. (2002). The Group B Streptococcal C5a peptidase IS Both a specific protease and an Invasive. J. Infect. Immun.; 70(5):2408-2413.
- Chimura, T. and Saito H. (1990). A clinical study of asymptomatic bacteriospermia. Jpn. J. Antibiot.; 43(1):139-146.
- Clark, N. C., Cooksey R. C., Hill B. C., Swenson J. M. and Tenover F. C. (1993). Characterization of glycopeptide-resistant enterococci from U.S. hospitals. Antimicrob Agents Chemother.; 37:2311-2317.
- Clark, N. M., Patterson J. and Lynch J. P. (2003). Antimicrobial resistance among Gram negative organisms in the intensive care unit. Curr. Opin. Crit. Care.; 9(5):413-423.
- Cohen, S. P., Yan W. and Levy S. B. (1993). A multidrug resistance regulatory chromosomal locus is widespread among enteric bacteria . J. Infect. Dis.; 168:484-488.
- Collee, J. G., Fraser A. G., Marmino B. P. and Simons A. (1996). Mackin and McCartney Practical Medical Microbiology. 14th ed., The Churchill Livingstone, Inc. USA.
- Compagnari, A. A., Shanks K. L. and Dyer D. W. E. (1994). Growth of *Moraxella catarrhalis* with human transferrin and

References

- lactoferrin expression of iron repressible proteins without. Siderophore production. Infect. Immun.; 62: 4906-4914.
- Conceicao, T., Faria N., Pimentel M., Soveral G. and Duarte A. (2004). New chromosomal AmpC β -lactamase in *Enterobacter cloacae*. Antimicrob. Agents and Chemoth.; 48(4):1437-1445.
- Cook, H., Furuya E., Larson E., Vasquez G. and Lowy F. (2007). Heterosexual transmission of community-associated methicillin-resistant *Staphylococcus aureus*. J. Infect. Dis.; 44 (3):410-413.
- Cornaglia, G., Russell K., Satta G., and Fontana R. (1995). Relative importances of outer membrane permeability and group 1 β -lactamase as determinants of meropenem and imipenem activities against *Enterobacter cloacae*. Antimicrob. Agents Chemother.; 39(2):350-355.
- Courvalin, P. (1994). Transfer of antibiotic resistance between Gram positive and Gram negative bacteria. Antimicrob. Agents Chemother.; 38: 1447-1451.
- Courcol, R. J., Trivier D., Bissinger M. C., Martin G. R. and Brown M. R. W. (1997). Siderophore production by *Staphylococcus aureus* and identification of iron-regulated proteins. Infect. Immun.; 65:1944-1948.
- Cramer, W. A. , Heymann J. B. , Schendel S. L., Deriy B. N., Cohen F. S., Elkins P. A. and Stauffacher C. V. (1995). Structure-function of the channel forming colicin. Ann. Rev. Biophys. Biomol. Struct.; 24 :611-641.
- Cravioto, A. R., Reyes F., Trujillo F., Uribe A., Navarro J. M. and Vazques V. (1990). Risk of diarrhea during the first year of life associated with initial and subsequent colonization by specific enteropathogens. Am. J. Epidemiol.; 131: 886-904.

References

- Dale, S. E., Doherty-Kirby A., Lajoie G. and Heinrichs D. E. (2004). Role of siderophore biosynthesis in virulence of *Staphylococcus aureus*: Identification and characterization of genes involved in production of a siderophore. *Infect. Immun.*; 72 (1):29-37.
- Dan, P. N. (2005). Treatment of acute and chronic bacterial prostatitis caused by *Staphylococcus epidermidis*. *J. Clin. Infect. Dis.*; 43:147.
- Dana, A. and Alan C. M. (1996). Assessment of sperm function and clinical aspects of impaired sperm function. *Fertil. Steril.*; 60:33-36.
- Daum, R. S., Gupta S., Sabbagh R. and Milewski W. M. (1992). Characterization of *Staphylococcus aureus* isolates with decreased susceptibility to vancomycin and teicoplanin: isolation and purification of a constitutively produced protein associated with decreased susceptibility. *J. Infect. Dis.*; 166:1066-1072.
- David, M. G. and Claire L. O. (2006). Bacteriocin diversity and the frequency of multiple bacteriocin production in *Escherichia coli*. *Microbiology*; 152:3239-3244
- De Jong, Z., Pontonnier F., Plante P., Perie N., Talazac N., Mansat A. and Chabanon G. (1990). Comparison of the incidence of *Ureaplasma urealyticum* infertile men and in donors of semen. *Eur. Urol.*; 18:127-131.
- Depuydt, C., Zalata A., Christophe A., Mahmoud A. and Comhaire F. (1998). Mechanisms of sperm deficiency in male accessory gland infection. *Andrologia.*; 30(1):29-33.
- Diemer, T., Weidner W., Michelmann H. W., Schiefer H. G., Rován E. and Mayer F. (1996). Influence of *Escherichia coli* on motility parameters of human spermatozoa in vitro. *Int. J. Androl.*; 19:271-277.

References

- Diemer, T., Ludwig M., Huwe P., Haler D. B. and Weidner W. (2000). Influence of genital urogenital infection on sperm function. *Curr. Opin. Urol.*; 1(1):39-44.
- Dinges, M. M., Orwin P. M. and Schlievert P. M. (2000). Exotoxins of *Staphylococcus aureus*. *J. Clin. Microbiol.*; 13(1):16-34.
- Donnell, J. A. and Gelone S. P. (2000). Antimicrobial therapy: fluoroquinolones. *J. Clin. Infect. Dis.*; 4:488-513.
- Donovan, J. F. and Lipshultz L. I. (1988). Infection and infertility. In: Contemporary management of importance and infertility. Tanago EA ,Lue TF and McClure RD .(eds.). Williams and Wilkins, Baltimore, USA; 268-276.
- Dowson, C. G. and Coffey T. J. (2000). β -lactam resistance mediated by changes in penicillin-binding proteins. In: Woodford, N. and Johnson A.P.(ed.). *Molecular bacteriology: protocols and clinical applications*. Humana press. Inc., Totowa, N. J. USA.; 15:537-553
- Dreschel, H., Freund S., Nicholson G., Haag H., Jung O., Zähler H., and Jung G. (1993). Purification and chemical characterization of staphyloferrin B, a hydrophilic siderophore from staphylococci. *BioMetals*; 6:185-192.
- Dulawa, J., Poland K. and Katowice J. (2003). Urinary tract infection and metabolic diseases. *Ann. Academia Medicae Bialotocensis*; 10(3):45-50.
- Dumarche, P., Dechamps C., Sirot D., Chanal C., Bonnet R. and Sirot J. (2002). TEM derivative-producing *Enterobacter aerogenes* Strains: dissemination of a prevalent clone. *Antimicrobiol. Agent Chemother.*; 46(4):1128-1131.

References

- Eggert-Kruse, W., Rohr G. and Probst S. (1998). Antisperm antibodies and microorganisms in genital secretions a clinically significant relationship. *Andrologia*.; 30(1):61-71.
- Eiichi, A., Monden K., Mitsuata R., Kariyama R. and Kumon H. (2004). Biofilm formation among methicillin resistant *staphylococcus aureus* isolates from patients with urinary tract infection .*Acta. Medica. Okayama*.; 58(4):207-214.
- Elbhar, A. (2005). Male genital tract infection: the point of view of the bacteriologist. *Gynecol. Obstet. Fertil.*; 33(9):691-697.
- El-Shamy, H. A. (1993). Bacteriology of chronic secretory otitis media in children. *J. Egypt public health association*, Lxv III; 495-505.
- Elshafie, S. S. and Bernardo A. R. (2001). An outbreak of methicillin resistant *Staphylococcus aureus*. *Saudi Med. J.*; 22(9):815-816.
- Fakhrildin, M. B. (2000). Fertilization potential of fertile and infertile human spermatozoa in vitro by application of sperm penetration assay and seminal fluid analysis. PhD thesis. College of science. AL-Mustansiriya University; pp.154-163.
- Falkow, S. A. and Collinins C. M. (1990). Genetic analysis of *Escherichia coli* urease genes. *J. Bact.*; 127(12): 7138-7144.
- Forbes, B. A., Daniel F. S. and Alice S. W. (2007). *Bailey and Scott's Diagnostic microbiology* . 12th ed. ,Mosby Elsevier company , USA .
- Ford, W. C. L., Williams K. M., Mclaughlin E. A., Harrison S., Ray B. and Hull M. G. R. (1996). The indirect immuno bead test for seminal antisperm antibodies and fertilization rates at in vitro fertilization. *Hum. Reprod.*; 11: 1418-1422.
- Fournier, P. E., Vallenet D., Barbe V., Audic S., Ogata H., Poirel L., Richet H., Robert C., Mangenot S., Abergel C., Nordmann P., Weissenbach J.,

References

- Raoult D. and Claverie J. M. (2006). Comparative genomics of multidrug resistance in *Acinetobacter baumannii*. PLOS Genet.; 2:7.
- Fraczek, M., Szumala A. K., Jedrzejczak P., Kamieniczna M. and Kurpisz M. (2007). Bacteria trigger oxygen radical release and sperm lipid peroxidation in vitro model of semen inflammation. Fertil. Steril.; 88(4):1076-1085.
- Freund, M. J. and Peterson R. N. (1976). Semen evaluation and fertility. In: Human Semen and Fertility Regulation in Men. Hafez, E. S. E. (ed.), The C. V. Mosby company, Saint Louis.; pp.344- 354.
- Galimand, M., Lambert T. and Courvalin P. (2005). Emergence and dissemination of a new mechanism of resistance to aminoglycosides in gram- negative bacteria: 16s rRNA methylation. J. Biol. Chem. ; 10(1):243-331.
- Gdoura, R., Kchaou W., Znazen A., Chakroun N., Fourati M., Ammar-Keskes L. and Hammami A. (2008). Screening for bacterial pathogens in semen samples from infertile men with and without leukocytospermia.; 40(4):209-218.
- Gerischer, U. (2008). *Acinetobacter* Molecular Biology, 1st edi., Caister Academic Press.
- Ghoshal, U., Prasad K. N., Singh M., Tiwari D. P. and Ayyagari A. (2004). A comparative evaluation of phenotypic and molecular methods for the detection of oxacillin resistance in coagulase negative staphylococci. J. Infect. Chemother.; 10:86-89.
- Gillespie, M. T., May J. W. and Skurray R. A. (1985). Antibiotic susceptibilities and plasmid profiles of nosocomial MRSA. A retrospective study. J. Med. Microbiol.; 17: 295-310.
- Go, J. and Cunha B. A. (1999). *Acinetobacter baumannii*: Infection control implications. Infect. Dis. Pract.; 23:65-68.

References

- Golshani, M., Taheri S., Eslami G., Suleimani A. A., Fallah F. and Goudarzi H. (2006). Genital tract infection in asymptomatic infertile men and its effect on semen quality. *Iranian J. Publ. Health*; 35(3):81-84.
- Gonsales G. F., Kortebarani G. and Mazzoli A. B. (1992). Leukocytospermia and function of the seminal vesicles on seminal quality. *Fertile. Sterile.*; 57:1058-1065.
- Goran, S. D. (2001). New prospect for chronic prostatitis. *J. Acta. Clin. Croat. Chinese.*; 40:109-16.
- Gouaux, E. (1997). The long and short of colicin action; the molecular basis for the biological activity of channel-forming colicins. *Structure*; 5:13-77.
- Graciela, M., Vignolo M., Kairuz M., Aida A. P., Ruiz H., and Oliver G. (1995). Influence of growth conditions on the production of lactocin 705, a bacteriocin produced by *L. casei* CRL 705, *J. Appl. Bacteriol.*; 78:5-10.
- Gray, G. L., Smith D. H., Baldrige J. S., Harkins R. N., Vasil M. L., Chen E. Y. and Heyneker H. L. (1999). Cloning, nucleotide sequence, and expression in *Escherichia coli* of the exotoxin A structural gene of *Pseudomonas aeruginosa*. *Proc. Nat. Acad. Sci. USA*; 81:2635-2649.
- Greenspan, F. S. and Gardner D. G. (2001). Pancreatic Hormones and Diabetes Mellitus by Masharani, U., and Karam, J. H. In: *Basic and Clinical Endocrinology*. 6th edition.; pp.692.
- Greenwood, D., Slack R. C. and Pentherer J. F. (2002). *Medical Microbiology, A guide to microbial infections, pathogenesis, immunity, laboratory diagnosis and control* 16th edi. Churchill Livingstone.

References

- Griffiths, E., Chart M. and Stevenson P. (1988). High affinity iron uptake systems and bacterial virulence. p. 121-137. In. Roth , J.A. (ed.), Virulence mechanisms of bacterial pathogens. American Society for Microbiology, Washington, D. C.
- Gupta, K., Hooton M. and Stamm W. (2001). Increasing antimicrobial resistance and the management of community acquired urinary tract infections. J. Ann. Int. Med.; 135:41-50.
- Haag, H., Fiedler H. P., Meiwes J., Drechsel H., Jung G., and Zähler H. (1994). Isolation and biological characterization of staphyloferrin B, a compound with siderophore activity from staphylococci. FEMS Microbiol. Lett.; 115:125-130.
- Haimi, Y., Vellozzi E. M. and Rubin L. G. (2002). Initial concentration of *Staphylococcus epidermidis* in simulated pediatric blood cultures correlates with time to positive results with the automated, continuously monitored BACTEC blood culture system. J. Clin. Microbiol.; 40:898-901.
- Hales, D. B., Diemer T. and Hales K. H. (1999). Role of cytokines in testicular function. Endocrine.; 10:201-217.
- Hall, S. L. (1991). Coagulase-negative staphylococcal infections in neonates. J. Infect. Dis.; 10:50-67 .
- Hall, R. M. (1997). Mobile gene cassettes and integrons: moving antibiotic resistance genes in gram-negative bacteria. Ciba. Found. Symp.; 207:192-202.
- Hancock, I. E. and Gilmore M. S. (2000). Pathogenicity of enterococci. In : Fischetti, V., Novick R., Ferretti J., Poruoy D. and Rood J. gram positive pathogens. ASM publications, Washington; pp.751-758.
- Hancock, R. E. and Spreet D. P. (2000). Antibiotic resistance in *Pseudomonas aeruginosa*: mechanism and impact on treatment.

References

- Drug Resistance updates. Antimicrob Agents Chemother.; 3:247-255.
- Harris, O., Shelver D. W., Bohnsack J. F. and Rubens C. E. (2003). A novel Streptococcal protease promotes virulence, resistance to opsonophagocytosis, and cleavage of human fibrinogen. J.Clin. Invest.; 111(1):1061-1071.
- Henkel, R. and Schill W-B. (1998). Sperm separation in patients with urogenital infections. Andrologia; 30(1):91-97.
- Hiramatsu, K., Hanaki H., Ino T., Yabuta K., Oguri T. and Tenover F. C. (1997). Methicillin resistant *Staphylococcus aureus* clinical strain with reduced vancomycin susceptibility. J. Antimicrob. Chemother.; 40 (1):135-136.
- Hogg, H. (2005). Essential microbiology John Wiley and Sons Ltd, 1st ed., England; pp.353-364.
- Hooper, D. C. (2005). Efflux pumps and nosocomial antibiotic resistance: a primer for hospital epidemiologists. Clin. Infect. Dis.; 40:1811-1817.
- Hpa, S. V. (2003). *Acinetobacter* species in bacteremia in North Ireland. J. Infect. Dis.; 23: 456-60.
- Huda, A. R., Burkarie M. D. and Ibrahiem M. S. (2001). Antimicrobial resistance among pathogens causing complicated UTIs. Saudi Arabia. J. Infect. Med.; 18(7):358-362.
- Huges, C. F. (1996). Maturation and export of E.coli hemolysin. J. Med. Microbiol. Immun.; 185(6):1165.
- Humphreys, H. (2004). *Staphylococcus*. In Greenwood, D. ,Slack, R. and Penethrer, J. (eds.)(2004). Medical Microbiology, a guide to microbial infections: pathogenesis, immunity ,laboratory diagnosis and control. 6th ed.,Churchill Livingstone , London.; pp.174-188 .

References

- Huwe, P., Diemer T., Ludwig M., Lui J., Schiefer H.G. and Weidner W.(1998).Influence of different uropathogenic microorganisms on human sperm motility parameters in an in vitro experiment. *Andrologia*.; 30(1):55-59.
- Hyde, R. M. (2000). Immunology. 4th edi. Lippincott-Williams and Wilkins, Awlterklumer company, USA .
- Ikechukwu, O., E. George, A. E. Sabinus and Florence O. (2007). Role of enriched media in bacterial isolation from semen and effect of microbial infection on semen quality. *Pak. J. Medic. Sci.*; 23(6):885-888 .
- Infectious Diseases and Immunization Committee, Canadian Pediatric Society (CPS).(1999). *Pediatrics &Child Health*; 4(5):337-341.
- Jack, R. W., Tagg J. R. and Ray B. (1995). Bacteriocins of gram positive bacteria. *Microbiol. Rev.*; 59:171-200.
- Jacoby, G. A., Walsh K. E., Mills D. M., Walker J., Oh H., Robicsek A. and Hooper D. C. (2006). A qnrB, another plasmid mediated gene for quinolone resistance. *Antimicrob. Agents and Chemother.*; 50(4):1178-1182.
- Jacoby, G. A. and Munoz-Price L. S. (2005). The new b-lactamases. *N. Engl. J. Med.*; 352:380-391.
- Jaffe, S. B. and Jewelewicz R. (1991). The basic infertility investigation. *Fertil. Steril.*, 56: 599-613.
- Jalaluddin, S., Devaster J. M., Scheen R., Gerard M. and Butzler J. (1998). Molecular epidemiological study of nosocomial *Enterobacter aerogenes* isolation a Belgian Hospital .*J. Clin. Microbiol.*; 36(7): 1846-1852.

References

- James, R., Kleanthous C. and Moor G. R. (1996). The biology of *Escherichia coli* colicins : paradigms and paradoxes. J. Microbiol.; 142:1569-1580.
- Jann B., and Jann K. (1990). Structure and biosynthesis of the capsular antigens of *Escherichia coli*. Curr. Top. Microbiol. Immunol.; 150:19-42.
- Jarlier, V., Fosse T. and Philipon A. (1996). Antibiotic susceptibility in aerobic gram negative bacilli isolated in intensive care units in 39 French teaching hospitals (ICU study).Intensive Care Med.; 22:1057-1065.
- Jarlov, J. O. (1999). Phenotypic characteristics of CoNS typing and antibiotic susceptibility. APMIS; 91:1842.
- Jedrzejczak, P., Szumata A., Dydowicz P. and Pisarski T. (1996). Usefulness of counting leukocytes and round cells in determination of bacterial infection of semen in infertile men. Ginekol. Pol., 67(11):569-573.
- Jevons, M. P. (1961). Celbenin resistant Staphylococci. Br. Med. J.; 14(1):124-125.
- Jiao, Y., Long S. D. and Grange W. X. (2002). The observation of chronic prostatitis using national institutes of health. J. Hosp. Chin. Acad. Trad. Med.; 8(2):127-129.
- Ji-Hong, L., Hao-Yong L., Zheng-Guo C., Yong-Fang D. , Yang L. and Zhang-Qun Y. (2002). Influence of several uropathogenic microorganisms on human sperm motility parameters in vitro. Asian J. Androl.; 4: 179-182.
- Joel, D., Schilling A. E., Scott F. G. and Hutgren S. N.(2002).Recent advances into the pathogenesis of recurrent urinary tract infection caused by uropathogenic *Escherichia coli*. J. Coligi. Int. Antimicrob. Agents.; 19: 454-60.
- Johnson, A. P., Aucken H. M., Cavendish S., Ganner M., Wale M. C., Warner M., Livermore D. M. and Cookson B. D. (2001). "Dominance of

References

- EMRSA-15 and -16 among MRSA causing nosocomial bacteraemia in the UK: analysis of isolates from the European Antimicrobial Resistance Surveillance System (EARSS)". *J. Antimicrob. Chemother.*; 48 (1):143-144.
- Jonson , A. B., Normak S. and Rhen M. (2005). Concepts in bacterial virulence. *J. Karger Basel*; 12:67-89.
- Joseph, F. J. and Alexander M. H. (2007). History and evolution of antibiotic resistance in coagulase negative staphylococci: Susceptibility profiles of new anti staphylococcal agents. *Ther. Clin. Risk. Manag.*; 3(6): 1143-1152.
- Jukka, A., Flower S. and Susan J.(1995). Multiresistant in *Staphylococcus* species in bacterial prostatitis. *J. Drugs.*; 30:123-128.
- Karlowsky, A. J., Dragh D. C. and Jones M. E. (2003). Antimicrobial susceptibility among clinical isolates *Pseudomonas aeruginosa* and *Acinetobacter baumannii* from hospitalized patients in the United States. *J. Antimicrob. Agents Chemother.*; 47:1681-88.
- Karlsson, A. and Arvidson S. (2002). Variation in extracellular protease production among clinical isolates of *Staphylococcus aureus* due to different levels of expression of the protease repressor SarA. *Infect. Immun.*; 70 (8):4239-4246.
- Karlsson, A., Saravia-Otten P., Tegmark K., Morfeldt E. and Arvidson S. (2001). Decreased amounts of cell wall-associated protein A and fibronectin-binding proteins in *Staphylococcus aureus* SarA mutants due to up-regulation of extracellular protease. *Infect. Immun.*; 69: 4742-4748.
- Kay, D. J., Clifton V. and Taylor J. S. (1993). Anti sperm antibodies and semen profiles in reanastomosed men. *Reprod. Fertil. Dev.*; 5: 135-139.

References

- Keck, C., Gerber-Schafer C., Clad A., Wilhelm C. and Breckwoldt M. (1998). Seminal tract infections: impact on male fertility and treatment options. *Hum. Reprod. Update.*; 4(6): 891-903.
- Klaenhammer, T. (1993). Genetics of bacteriocins produce by lactic acid bacteria. *FEMS Microbiol. Rev.*; 12:39-85.
- Klligore, D., March K. L. and Blance B. J. (2004). Risk factors for community acquired ciprofloxacin resistant *Escherichia coli* urinary tract infection. *J. Ann. Pharmacol.*; 38(7): 1148-1152.
- Kloss, W. E. and Bannerman T. L. (1994). Up date on clinical significance of coagulase negative Staphylococci. *Clin. Microbiol. Rev.*; 7(11):117-120
- Kohn , F-M., Erdmann I., Oeda T. , El-Mulla K. F., Schiefer H. G. and Schill W-B. (1998). Influence of urogenital infections on sperm functions. *Andrologia.*; 30(1):73-80.
- Konetschny-Rapp, S., Jung G., Meiwes J. and Zähler H. (1990). Staphyloferrin A: a structurally new siderophore from staphylococci. *Eur. J. Biochem.*; 191:65-74
- Konino, M. H. (1995). Nosocomial infections caused by MRSA in Japan. *J. Infect. Dis.*; 1:30-39.
- Kornelius, Z., Christin R., Silke I. P. and Volkmar B. (2008). Crystal structure of colicin M, a novel phosphatase specifically imported by *Escherichia coli*. *J. Biol. Chem.*; 10:1074.
- Khorshed, P. A. (2005). Bacteriological study of some pathogens causing urinary tract infection in pateints attending Azadi general hospital in Kirkuk city. Msc thesis ,College of Education – Tikret Univercity.Iraq.
- Kropp, H., Gerckens L., Sundelof J. G., Kahan F. M. (1985). Antibacterial activity of imipenem; the first thienamycin antibiotic. *Rev. Infect. Dis.*; 7(3): 389-410.

References

- Kruger, T. F., Acosta A. A., Simmons K. F., Swanson R. J., Matta J. F. and Oehninger S. (1988). Predictive value of abnormal sperm morphology in vitro fertilization. *Fertil. Steril.*; 49:112-116.
- Kurt , G. and Naber I. (2001). Which fluoroquinolones are suitable for the treatment of urinary tract infection . *Intern. .J. Antimicrob. Agent.*; 17: 331-341 .
- Lackner , J., Schatzl G., Horvath S., Kratzik C. and Marberger M. (2006). Value of counting white blood cells in semen samples to predict the presence of bacteria. *Eur Urol.*; 49(1):148-152.
- Lamirande, E. and Gagnon C. (1992). Reactive oxygen species and human spermatozoa. In Effects on the motility of intact spermatozoa and on sperm axonemes; and II. Depletion of adenosine triphosphate plays an important role in the inhibition of sperm motility. *J. Androl.*; 13:368-386.
- Leclercq, R. (2002). Mechanisms of resistance to macrolides and lincosamides: nature of the resistance elements and their clinical implications. *Clin. Infect. Dis.*; 34:482-492.
- Lee, J. C. (2000). Microbiology of prostate. *J. Clin. Dis.*; 1:159-163.
- Levy, S. B. and Marshall B. (2004). Antibacterial resistance worldwide: causes, challenges and responses. *Nat. Med.*; 10:122-129.
- Li, H. Y. and Liu J. H. (2005). Influence of male genital bacterial infection on sperm function. *Zhonghoa. Nan. Ke. Xue.*; 8(6):442-444.
- Lipsky, B. X. (2003). Prostatitis and urinary tract infection in men. *J. Am. Med. Urol.*; 106(3):327-34.
- Lisa, T. A., Lucchesi G. I. and Domenech C. E. (1994). Pathogenicity of *Pseudomonas aeruginosa* and its relationship to the cholin metabolism through the action of cholinesterase, acid phosphatase and phospholipase. *J. Curr. Microbiol.*; 29:193-199.

References

- Litwin, C. M. and Calderwood S. B. (1993). Role of iron in regulation of virulence genes. Clin. Microbiol. Rev.; 6:137-149.
- Lucia, E., Satorres S., Lucero M. and Olga N. (2003). Species identification ,slim production ,slim production and oxacillin susceptibility in CoNS isolated from nosocomial specimens. Braz. J. Microbiol.; 34:45-51.
- MacFaddin, J. F. (2000). Biochemical tests for the identification of medical bacteria. 3rd ed., The Williams. and Wilkins-Baltimor,USA.
- Macleod, J., and Wang Y.(1979). Male fertility potential in term of semen quality; A review of the past, a study of the present. Fertil. Steril.; 31:103-116.
- Mack, D., Rohde H., Dobinsky S., Riedewald J., Nedelmann M., Knobloch J. K. M., Elsner H. A. and Feuchet H. H. (2000). Identification of three essential regulatory gene loci governing expression of *Staphylococcus epidermidis* polysaccharides intercellular adhesion and biofilm formation. Infect. Immun.; 8:3799-3807.
- Maes, P. and Vanhoof R. (1992). A 56-month prospective surveillance study on the epidemiology of aminoglycosides resistance in a Belgian general hospital . Scand. J. Infect. Dis.; 24:495-501.
- Mallea, M. , Chevalier J., Bornet C., Eyraud A., Davin A., Bollet C., and Pages J. M. (1998). Porin alteration and active efflux: two in-vivo drug resistance strategies used by *Enterobacter aerogenes*. J. Microbial.; 144:3003-3004.
- Marco, C. A. and Parker K. G. (1997). Antimicrobial resistance among organisms causing urinary tract infections. Acad. Emerg. Med.; 4: 154-160.

References

- Maria, A. R., Zeus S., Aysen L. E., and Valerio M. N. (2007). Commensal and pathogenic *Escherichia coli* use a common pilus adherence factor for epithelial cell colonization. Proc. National Acad. Sci. USA.
- Marshburn, P. B. (1997). Antisperm antibodies and infertility. Infer.& Reprod. Medicine. Clin of North America.; 8:243-286.
- Martinz, J. K., Cercenado E., Baquero F., Perez J. C., Diaz and Delgado-Iribarren A. (1987). Incidence of aerobactin production in gram-negative hospital isolates. FEMS Microbiol. Lett.; 43:351-353.
- Marty, J. J, Robert R. S. and Jone F. A. (2000). Type 1 pilus-mediated bacterial invasion of bladder cells. J. Embo. Bact.; 19(3):2803.
- Mazmanian, S. K., Skaar E. P., Gaspar A. H., Humayun M., Gornicki P., Jelenska J., Joachmiak A., Missiakas D. M., and Schneewind O. (2003). Passage of heme iron across the envelope of *Staphylococcus aureus*. Science; 299:906-909
- Mazodier, P. and Davies J.(1991). Gene transfer between distantly related bacteria. Ann. Rev. Genet.; 25:147-171.
- Mazumdar, S. and Levine A. S. (1998). Antisperm antibodies: Etiology pathogenesis, diagnosis and treatment. Fertil. Steril.; 70:799-810.
- McClure, R. D. (1992). Male infertility. In: Smith's General Urology. Tanagho, E.A., and Mc Aninch, J.W. (eds.) Lange Medical Book, California ; pp. 669-695.
- Meiwes, J., Fiedler H. P., Haag H., Zähler H., Konetschny-Rapp S. and Jung G.(1990). Isolation and characterization of staphyloferrin A, a compound with siderophore activity from *Staphylococcus* DSM 20459. FEMS Microbiol. Lett.; 67:201-206.
- Mellenkvist, A., Nordst T. , Hallden C. and Christenses J. J. (2003). The *Moraxella catarrhalis* Immunoglobulin D-Binding protein MID has

References

- conserved sequences and is regulated by a mechanism corresponding to phase variation. *J. Bacteriol.*; 185(7):2285-2295.
- Menichetti, F. (2005). Current and emerging serious gram positive infections". *Clin. Microbiol. Infect.*; 11(3):22-28.
- Menkveld, R. and Kruger T. F. (1998). Sperm morphology and male urogenital infection. *Andrologia.*; 30(1):49 -53.
- Merino, G., Carranza L. S., Murrieta S., Rodriguez L., Cnevas E., and Moran C. (1995). Bacterial infection and semen characteristics in infertile men. *Arch. Androl.*; 35(1):43-47.
- Meskin, I. (1998). *Staphylococcus epidermidis*. *Pediatrics in Review*. American Academy of Pediatrics; 19(3):12-15.
- Michalek, M., Mold C. and Brenner E. (1988). Inhibition of the alternative pathway of human complement by structural analogues of sialic acid. *J. Immun.*; 140:1588-1599.
- Michelmann, H. W. (1998). Influence of bacteria and leukocytes on the outcome of in vitro fertilization (IVF) or intracytoplasmic sperm injection (ICSI). *Andrologia.*; 30(1):99-101.
- Micic, S., Petrovic S. and Dotlic R. (1990). Seminal antisperm antibodies and genitourinary infection. *Urology*; 35:54-56.
- Milingos, S., Comhaire F. H., Liapi A. and Aravantinos D. (1996). The value of semen characteristics and tests of sperm function in selecting couples for intra-uterine insemination. *Eur. J. Obstet. Gynecol. Reprod. Biol.*; 64:115-118.
- Mims, C., Docknell H. M., Goering R. V. and Roitt I. (2004). *Medical microbiology*. 3rd ed, Elsevier Limited.

References

- Modun, B., Kendall D. and Williams P. (1994). Staphylococci express a receptor for human transferrin : identification of a 42-kilodalton cell wall transferrin-binding protein. *Infect. Immun.*; 62:3850-3858.
- Modun, B., Evans R. W., Joannou C. L., and Williams P. (1998). Receptor-mediated recognition and uptake of iron and human transferrin by *Staphylococcus aureus* and *Staphylococcus epidermidis*. *Infect. Immun.*; 66:3591-3596.
- Mogra, N. N., Dhruva A. A., and kothari L. K. (1981). Non specific seminal tract infection and male infertility. *J. Postg. Med.*; 27(2):99-104.
- Mokracka, J., Koczura R. and Kazaonwski I. (2004). Yersiniabactin and other siderophores produced by clinical isolates of *Enterobacter* spp. . and *Citrobacter* spp. .FEMS. *Immunol. Med . Microbiol.*; 40:51-55.
- Monga, M. and Roberts J. A. (1994). Spermagglutination by bacteria: Receptor-Specific Interactions. *J. Androl.*; 15:151-156
- Mongkolrattanothai, K., Boyle S. and Murphy T. V. (2004). Novel non-*mecA* containing staphylococcal chromosomal cassette composite island containing *pbp4* and *tagf* genes in a commensal staphylococcal species: a possible reservoir for antibiotic resistance islands in *Staphylococcus aureus*. *Antimicrob. Agents Chemother.*; 48:1823-1836.
- Montagut, J. M., Lepretre S., Degoy J. and Rousseau M. (1991). *Ureaplasma* in semen and IVF. *Hum. Reprod.*; 6:727-729.
- Morales, P. Katz D. F., Overstreet J. W., Samueles S. J. and Chang R. J. (1988). The relationship between the motility and morphology of spermatozoa in human semen. *J. Androl.*; 9:241- 247.
- Morine, C. A. and Hadler J. L. (2001). Population-Based Incidence and Characteristics of Community Onset *Staphylococcus aureus*

References

- Infections with bacteremia in 4 Metropolitan Connecticut Areas, 1998. J. Infect. Dis.; 184:1029-1034.
- Moxon, E. R. and Kroll J. S. (1990). The role of bacterial polysaccharide capsules as virulence factors. Curr. Topics Microbiol. Immun.; 150:65-86.
- Munoz, M. G. and Witkin S. S. (1995). Autoimmunity to spermatozoa, asymptomatic *Chlamydia trachomatis* genital tract infection and ST lymphocytes in seminal fluid from the male partners of couples with unexplained infertility. Hum. Reprod.; 10:1070-1074.
- Murray, P. R., Niles A. C. (1990). In vitro activity of meropenem (SM.7338), Imipenem, and five other antibiotics against anaerobic clinical isolates. J. Diag. Microbiol. Infect. Dis.; 13:57-61.
- Murray, P. R., Baron E. J., Jorgensen J. H., Pfaller M. A. and Tenover F. C. (2003). Manual of clinical Microbiology. 8th ed., Washington, D.C., Vol (1) .
- Nair, S. P., Williams R. J. and Henderson B. (2000). Advance in our understanding of the bone and joint pathology caused by *Staphylococcus aureus* infection. Rheumatology; 39:821-834
- National committee of clinical laboratory standards (NCCLS) .(2002). Performance standards for antimicrobial susceptibility testing. 12th informational supplement. M 100-S12., Wayne, Pannsylvania; 22(1) .
- Neely, A. N. and Maley M. P. (2000). Survival of enterococci and staphylococci on hospital fabrics and plastics. J. Clin. Microbiol.; 38:724-726.
- Neilands, J. B. (1995). Siderophores: Structure and function of microbial Iron transport compounds. J. Biolog. Chem.; 270(45):26723-26726.
- Neto, J. R., Yano T., Beriam L. O. S. and Destefano S. A. L. (2003). Comparative RFLP-ITS analysis between *Enterobacter cloacae*

References

- strains isolated from plants and clinical origin. *Arq. Inst. Biol.* ; 70(3):367-372.
- Ng, S., Bongso A., and Rothan S. S. (1990). Micro manipulation. Its relevance to human in vitro fertilization. *Fertil. Steril.*; 53: 203-219.
- Nieschlag, E., and Behre H. M. (1997). Cited by Leijonhufvud, P.(1998) Aspects of the Regulation of Human sperm Motility. Published on the Web by the Library of karloinska instituted 171 76 Stockholm.
- Nikkanen, V., Gronroos M., Suominen J. and Multamaki S. (1979). Silent infection in male accessory genital organs and male infertility. *Andrologia.*; 11:236-241.
- Noble, W. C., Virani Z. and Cree R. G. (1992). Co-transfer of vancomycin and other resistance genes from *Enterococcus faecalis* NCTC 12201 to *Staphylococcus aureus*. *FEMS Microbiol Lett*; 93:195-198.
- Nomura, S. and Nagayama A. (2002). In vitro antibacterial activity of S-4661, a new parenteral carbapenems, against urological pathogens isolated from patients with complicated urinary tract infections. *J. Chemother.*; 14:155-160.
- Noto, M. J. and Archer G. L. (2006). A subset of *Staphylococcus aureus* strains harboring staphylococcal cassette chromosome mecA (SCCmec) type IV is deficient in CcrAB-mediated SCCmec excision. *J. Antimicrob. Agents Chemother.*; 50:2782-2800.
- Oehninger, S. and Alexander N. J. (1991). Male infertility; the focus shifts to sperm manipulation. *Curr. Opin. Obstet. Gyneco.*; 3:182-190.
- Ofek, I., Hasty D. L. and Doly R. J. (2002). Bacterial Adhesion. *Am. Soc. Microbiol. Washington, DC*; pp.63-69.
- Oksuz, S., Ozturk E., Sahin I., Ertor O. and Kaya D. (2005). Biliary infection and bacteremia caused by β -lactamase-positive, ampicillin resistant

References

- Haemophilus influenzae* in diabetic patients. J. Infect. Dis., 58:34-35.
- Old, D. C. and Adegbola R. A. (1985). Antigenic relationships among type-3 fimbriae of Enterobacteriaceae revealed by immuno electron microscopy. J. Med. Microbiol.; 20:113-121.
- Onemu, S. O. and Ibeh I. N. (2001). Studies on the significance of positive bacterial semen cultures in male fertility in Nigeria. Int. J. Fertil. Women Med.; 46(4):210-214.
- Opal, S. M., Cross A. S., Gemski P. and Lyhte L. W. (1988). Survey of purported virulence factors of *Escherichia coli* isolated from blood, urine and stool. Euro. J. Clin. Microbiol. Infect. Dis.; 7:425-427.
- Ophir, T. and Gutnick D. (1994). A role for exopolysaccharides in the protection of micro-organisms from desiccation. Appli. Environ. Microbiol.; 60:740-745.
- Orji, I., Ezeifeaka G., Amadi E. S. and Okafor F. (2007). Role of enriched media in bacterial isolation from semen and effect of microbial infection on semen quality: A study on 100 infertile men. Pak. J. Med. Sci.; 23(6):885-888.
- Orskov, F., and Orskov I. (1992). *Escherichia coli* serotyping and disease in man and animals. Can. J. Microbiol.; 38:699-704.
- Osegbe, D. N. and Amaku E. O. (1985). The causes of male infertility in 504 consecutive Nigerian patients. Int. Urol. Nephrol., 17:349-358.
- Pal, R. B., Rebello J. S., Banker D. D. and Jain P. K. (1997). Klebocin typing of *Klebsiella* species isolated from intensive care unit. J. Postgrad. Med.; 43(4):98-101.
- Palayekar, V. V., Joshi J. V., Hazari K. T., Shah R. S. and Chitlange S. M. (2000). Comparison of four nonculture diagnostic tests for

References

- Chlamydia trachomatis* infection. J. Assoc. Physicians .India.; 48:481-483.
- Pankuch, G. A. and Appelbaum P. C. (2006). Postantibiotic effect of ceftibiprole against 12 gram positive organisms. J. Antimicrob. Agents Chemother.; 50:3956-3958.
- Pardo, M., Barri P. N., Bancells N., Coroleu B., buxaderas C., Pomerol J. M. and Shabater J. (1988). Spermatozoa selection in discontinuous percoll gradients for use in artificial insemination. Fertil. Steril.; 49: 505-509.
- Park ,Y. J., Lee S., Park J. J., Park K. G., Kim B. K. and Kang C.S. (2003). Antimicrobial susceptibility of inducible AmpC β -lactamase-producing *Enterobacter Cloacae* , *Citrobacter freundii* and *Serratia marcescense*. Korean survey department of clinical pathology.; 51(4):265-269.
- Paulsen, C. V. (1974). The testes In: Text Book of Endocrinology. Williams, R. (ed.). W. B. Saunders Company. Philadelphia., pp.323-367.
- Pearce, R. and Roberts I. S. (1995). Cloning and analysis of gene clusters for production of the *Escherichia coli* K10 and K54 antigens: identification of a new group of serA-linked capsule gene clusters. J. Bacteriol.; 177:3992-3997.
- Perez, J. L., Pulido A., Gomez E., Sauca G. and Martin R. (1990). Superoxol and amino peptidase tests for identification of pathogenic *Neisseria* species and *Moraxella (Branhamella) catarrhalis* .Eur. J. Clin. Microbiol. Infect. Dis.; 9(6):421-423
- Philip, A. S. and Folstad I. (2003). Do bacterial infections cause reduced ejaculate quality? A meta-analysis of antibiotic treatment of male infertility. Behav. Ecolo.; 14(1):40-47.

References

- Piddock, L. J., Walters R.N., Jin Y. F., Turner H. L., Gascoyne-Binski D. M. and Hawkey P. M. (1997). Prevalence and mechanism of resistance to third-generation cephalosporins in clinically relevant isolates of Enterobacteriaceae from 43 hospitals in the UK, 1990-1991. J. Antimicrob. Chemother.; 39:177-187.
- Piret, J., Millet J. and Demain A. (1983). Production of intracellular protease during sporulation of *Bacillus brevis*. Eur. J. Appl. Microbiol. Biotechnol.; 17: 227-230.
- Pitout, J. D., Thomson K. S., Sandra C. C. and Hanson N. D. (1998). Plasmid-mediated resistance to extended spectrum cephalosporins among *Enterobacter aerogenes* strains. J. Antimicrob. Agents Chemother.; 42:596-600.
- Poeta, P., Costa D., Klibi N., Rodrigues J. and Torres C. (2006). Phenotypic and genotypic study of gelatinase and beta haemolysis activities in faecal enterococci of poultry in Portugal. J. Vet. Med. Infect. Dis.; 53(5):203- 208.
- Poirel, L., Menuteau O., Agoli N., Cattoen C. and Nordmann P. (2003). Outbreak of extended-spectrum beta-lactamase VEB-1-producing isolates of *Acinetobacter baumannii* in a French hospital. J. Clin. Microbiol.; 41:3542–3547.
- Posey, J. E. and Gherardini F. C. (2000). Lack of a role for iron in the Lyme disease pathogen. Science; 288:1651-1653.
- Prakash, M., Rajasekaran K. and Karmegam N. (2007). Prevalence of methicillin resistant *Staphylococcus aureus* in clinical samples collected from Kanchipuram Town, Tamil Nadu, South India. J. Appl. Sci. Research; 3(12):1705-1709.
- Pugsley, A. P. (1984). The ins and outs of colicins. J. Microbiol. Sci., 1:168-175.

References

- Purvis, K. and Christiansen E. (1993). Infection in the male reproductive tract: impact, diagnosis and treatment in relation to male infertility. *Int. J. Androl.*; 16:1-13.
- Qiang , H., Jiang M., Lin J. and He W. (2007). Influence of enterococci on human sperm membrane in vitro. *Asian J. Androl.*, 9 (1): 77-81.
- Quale, J., Bratu S., Landman D. and Heddurshetti R. (2003). Molecular epidemiology and mechanisms of Carbapenems resistance in *Acinetobacter baumannii* endemic in New York City. *Clin. Infect. Dis.*; 37 :214-220.
- Raad, I. (2000). Management of intravascular catheter- related infections. *J. Antimicrob. Chemother.*; 45: 267-270.
- Rachid, S. A., Witte V. M. and Hacker J. Z. (2000). Effect of subinhibitory antibiotic concentration on Intracellular adhesion expression in biofilm-forming *Staphylococcus epidermidis*. *J. Antimicrob. Agents Chemother.*; 32: 755-760.
- Rahal, J. (2006). Novel antibiotic combinations against infections with almost completely resistant *Pseudomonas aeruginosa* and *Acinetobacter* species. *Clin. Infect. Dis.*; 43(2):95–99.
- Rajasekaran, M., Hellstrom W. J., Naz R. K., and Sikka S.C.(1995). Oxidative stress and interleukins in seminal plasma during leukocytospermia. *Fertil. Steril.*; 64:166-171.
- Rajesh, B. and Rutten I. (2004). *Essential medical microbiology*. 3rd ed. New Delhi–Indian.
- Raymond, K. N., Dertz E. A. and Kim S. S. (2003). Enterobactin: an archetype for microbial iron transport. *Proc. Natl. Acad. Sci. USA.*; 100: 3584-3588.

References

- Rehewy, M. S. E., Hafez E. S. E., Thomas A. and Brown W. J. (1979). Aerobic and anaerobic bacterial flora in semen from fertile and infertile groups of men. *Arch. Andrology*; (2): 238-263.
- Reichart, M., Kahane I. and Bartoov B. (2000). In Vivo and In Vitro Impairment of Human and Ram Sperm Nuclear Chromatin Integrity by Sexually Transmitted *Ureaplasma urealyticum*. *Infect. Biol. Reprod.*; 63:1041-1048.
- Reichart, M., Kahane I. and Bartoov B. (2001). Dual energy metabolism-dependent effect of *Ureaplasma urealyticum* infection on sperm activity. *J. Androl.*; 22:404-412.
- Riegel, P., Ruimy R., Briel D., Prevost G., Jehl F., Bimet F., Christen R. and Monteil H. (1995). *Corynebacterium seminali* sp. Nov., new species associated with genital infection in male patients. *J. Clin. Microbiol.*, 33(9) .p. 2244-2249.
- Riley, M. A. and Gordon D. M. (1992). A survey of col plasmids in natural isolates of *Escherichia coli* and an investigation into the stability of col plasmid lineages. *J. General Microbiol.*; 138:1345-1352.
- Riley, M. A. and Wertz J. E. (2002). Bacteriocins: Evolution, ecology, and applications. *Ann. Rev. Microbiol.*; 56:117-137.
- Roberry, J. A., Scott K. L. and Joe E. T. (1997). Epitopes of the P-fimbrial adhesion of E.coli causes of urinary tract infection. *J. Urol.*; 158(9):1610.
- Roberts, J. A. (1992). Role of bacterial adherence in urinary tract infection. *Curr. Opin. Urol.*; 2:33-36.
- Roberts, I. S. (1996). The biochemistry and genetics of capsular polysaccharide production in bacteria. *Ann. Rev. Microbiol.*; 50:285–315.

References

- Roberts, M. C. (1996a). Tetracycline resistance determinants: mechanism of action, regulation of expression, genetic mobility, and distribution. *FEMS Microbiol. Rev.*; 19:1–24.
- Rodin, D. M. , Larone D. and Goldstein M. (2003). Relationship between semen culture, leukospermia and semen analysis in men undergoing fertility evaluation. *Fertil. Steril.*; 79:(3)1555-1558.
- Romero-Vivas, J., Rubio M., Fernandez C. and Picazo J. J. (1995). Mortality associated with nosocomial bacteremia due to methicillin resistant *Staphylococcus aureus*. *J. Clin. Inf. Dis.*; 21:1417-1423.
- Romolo, G., Facep L., Warcester M. A. and Gideon B. (2004). Complicated urinary tract infection: Risk stratification, clinical evaluation and evidence-based antibiotic therapy for out management. Update. *J. American Health Consultants.*; 68(30): 230-274.
- Rose, N. R. (1978). Endocrine diseases. In: Basic and clinical Immunology: Fudenberg, H.H. , Stites D.P. , Caldwell J.L. and Wells J.V. (eds.) Lange Medical Publication. California; pp. 628- 641.
- Rubens, C. E., Wessels M. R. (1987). Transposon mutagenesis of type III GBS, correlation of capsule expression with virulence. *Proc Natl. Acad. Sci USA*; 84:7208-7212.
- Ryan, K. J. and Ray C. G. (2004). Sherri's Microbiology. 4th ed. McGraw Hill. ISBN .
- Salman, H. D. (2006). A Bacteriological Study of Local Strains of *Enterobacter cloacae* Isolated from Urine Samples.Msc. Thesis .Babylon university. Collage of Medicine Iraq.
- Sambrook, J. and Rusell D. W. (2001). Molecular cloning .A laboratory manual .3rd edi. Cold spring .Harber laboratory press .New York.
- Sano, Y., Matsui H., Kobayashi M. and Kageyama M. (1990). Pyocins S1 and S2, bacteriocins of *Pseudomonas aeruginosa*. In: *Pseudomonas*

References

- Biotransformation pathogenesis and evolving biotechnology .Silver, S. , Chakrabarty A.M., Iglewski B. and Kaolan, S.,(eds.), American Society for Microbiology, Washington; pp.352-358
- Schaberg, D. R., and Zervos M. J. (1986). Intergeneric and interspecies gene exchange in gram positive cocci. J. Antimicrob. Agents Chemother.; 30:817-822.
- Schennings, T., Heimdahl A., Coster K. and Flock J. (1993). Immunization with fibronectin binding protein from *Staphylococcus aureus* protects against experimental endocarditis in rats. J. Microb. Pathol.; 15:227-236.
- Sefton, A. M. (2002). Mechanisms of antimicrobial resistance. Drugs; 62:557-566.
- Segal, H., Thomas R. and Gay E. B. (2003). Characterization of class 1 integron resistance gene cassettes and the identification of a novel IS-like element in *Acinetobacter baumannii*. Plasmid.; 49:169-178.
- Seifert, H., Boullion B., Schulze A. and Pulverer G. (1994). Plasmid DNA profiles of *Acinetobacter baumannii*: Clinical application in a complex endemic setting. Infect. Control. Hosp. Epidemiol.; 15:520-528.
- Sharon, L.H., Rabe K. L., Muller C. H., Zarutskie P., Kuzan F. B. and Stenchever M. A. (1990). Relationship of bacteriologic characteristics to semen indices in men attending an infertility clinic. Obstetrics & Gynecology; 75:800-804.
- Shefi ,S. and Turek P.J.(2006).Definition and current evaluation of subfertile men. Int. Braz. J. Urol. Rio de Janeiro; 23(4):212-222.
- Shimoya, K., Matsuzaki N., Tsutsui T., Taniguchi T., Saji F. and Tanizawa O. (1993). Detection of interleukin-8 (IL-8) in seminal plasma and

References

- elevated IL-8 in seminal plasma of infertile patients with leukocytospermia . *Fertile. Sterile.*; 59:885-888.
- Shlaes, D. M., Shlaes J. H., Vincent S., Etter L., Fey P. D. and Goering R. V. (1993). Teicoplanin-resistant *Staphylococcus aureus* expresses a novel membrane protein and increases expression of penicillin-binding protein 2 complex. *J. Antimicrob Agents Chemother*; 37:2432-2437.
- Shorman, M. A., Atoom A. M., Abuharfeil N. M. and Al-Majali A. M. (2008). Identification of methicillin resistant *Staphylococcus aureus* (MRSA) and methicillin resistant coagulase negative Staphylococci in Clinical Settings. *Amer. J. Infec. Dis.*; 4 (2):156-161.
- Sieradzki, K. and Tomasz A. (1997). Inhibition of cell wall turnover and autolysis by vancomycin in a highly vancomycin resistant mutant of *Staphylococcus aureus*. *J. Bacteriol.*; 179:2557-2566.
- Skov, R., Frimodt-Moller N. and Espersen F. (2001). Correlation of MIC methods and tentative interpretive criteria for disk diffusion susceptibility testing using NCCLS methodology for fusidic acid. *J. Diag. Microbiol. Infect. Dis.*; 40:111-116.
- Smith, H. R. and Scotland S. M. (1993). Isolation and identification methods for *Escherichia coli* O157 and other verocytotoxic producing strain. *J. Clin. Pathol.*; 46:10-17.
- Steinberger, A., and Steinberger E. (1976). Secretion of FSH inhibiting factor by cultured Sertoli cells. *Endocrinology.*; 99: 918-924.
- Stimac, G. D., Agreb A. N. and Gorica M. A. (2001). New prospect for chronic prostatitis. *J. Acta. Clin. Croat. Urol.*; 40(2):109-116.
- Suzanne, E. D., Kirby A. D., Lajoie G. and Heinrichs D. E. (2004). Role of siderophore biosynthesis in virulence of *Staphylococcus aureus*:

References

- identification and characterization of genes involved in production of a siderophore .J. Infect. Immun.; 72(1):429-437.
- Svanborg, A. D., Alin B. G. and Plotti F. H. (1996). Bacterial adherence and mucosal cytokine responses: Responses and transmembrane signaling. J. Ann. N. Acadia.; 7(10):377.
- Svenson, S. B, Moris K. M. and Brunner V. A. (1983). P-fimbriae of pyelonephritis genic *Escherichia coli*. J. Infect. Dis.; 123(11):1161.
- Swenson, C. E., Toth A., Toth C., Wolfgruber L. and O'Leary W. M. (1980). Asymptomatic bacteriospermia in fertile men. Andrologia; 12:7-11.
- Tamura, G. S., Kuypers J. M., Smith S., Raff H. and Rubens C. E. (1994). Adherence of group B streptococci to cultured epithelia cells: roles of environmental factors and bacterial surface components. J. Infect. Immun.; 62:2450-2458.
- Taylor, J. M. and Heinrichs D. E. (2002). Transferrin binding in *Staphylococcus aureus*: Involvement of a cell wall anchored protein. Mol. Microbiol.; 43:1603-1614
- Teague, N. S., Boyarsky S., and Glenn J. F. (1971). Interference of human spermatozoa motility by *Escherichia coli*. Fertil. Steril.; 22:281-285.
- Tenover, F. C., Lancaster M. V. and Hill B. C. (1998). Characterization of staphylococci with reduced susceptibilities to vancomycin and other glycopeptides. J. Clin. Microbiol.; 36:1020-1027.
- Theresa, L., Michele L. and Kenneth R. (1999). Emergence of vancomycin resistance in *Staphylococcus aureus* . The New England Journal of Medicine; 340(7):493-501.
- Thomson, C. J. and Amyes S. G. (1993). Selection of variants of TEM-1 β -lactamase encoded by a plasmid of clinical origin with increased resistance to β -lactamase inhibitors. J. Antimicrob. Agents Chemother.; 31: 655-664.

References

- Todar , K. (2005). Staphylococci . Todar's online textbook of bacteriology.
- Tomaras, A., Dorsey C., Edelmann R. and Actis L. (2003). Attachment to and biofilm formation on a biotic surfaces by *Acinetobacter baumannii*: involvement of a novel chaperone-usher pili assembly system. Microbiology.; 149:3473-3484.
- Travis, J., Potempa J. and Maeda H. (1995). Are bacterial proteinases pathogenic factors? Trends in Microbiol., 3:405-407.
- Trum, J. W., Mol B. W. J., Pannekoek Y., Spanjaard L., Wertheim P., Bleker O. P., Veen van der F.(1998). Value of detecting leukocytospermia in the diagnosis of genital tract infection in subfertile men. Fertil. Steril.; 70:315-319.
- Tsuji, M., Ishii Y., Ohno A., Miyazaki S., and Yamaguchi K. (1998). In vitro and in vivo antibacterial activities of S-4661, a new carbapenems. J. Antimicrob. Agents Chemother.; 42:94-99.
- Tyrrell, G. J., Senzilet L. D., Spika J. S., Kertesz D. A., Alagaratnam M., Lovgren M. and Talbot J. A.(2000). Invasive disease due to group B streptococcal infection in adults: results from Canadian, population based, active laboratory surveillance study. J. Infect. Dis.; 182:168-173.
- Tzelepi, E., Giakkoupi P., Ofianou D., Loukova S. V., kemeroglou A., and Tsakris A. (2000). Detection of extended-spectrum β -lactamases in clinical isolates of *Enterobacter cloacae* and *Enterobacter aerogenes*. J. clin. Micorbiol.; 38(3):542-546.
- Urban, C., Segal-Maurer S. and Rahal J. J. (2003). Consideration in control and treatment of nosocomial infections due to multidrug resistant *Acinetobacter baumannii* . Clin. Infect. Dis.; 36 :1268-1274.
- Van Looveren, M. and Goossens H. (2004). Antimicrobial resistance of *Acinetobacter* spp. in Europe. Clin. Microbiol. Infect.; 10:684-704

References

- Varon, E., Levy C., Dela R. and Boucherat M. (2000). Impact of antimicrobial therapy on nasopharyngeal carriage of *Streptococcus pneumoniae*, *Haemophilus influenzae* and *Branhamella catarrhalis* in children with respiratory tract infections. J. Clin. Infect. Dis.; 31:477-481.
- Vergis, E. N., Shankar N., Chow J. W. and Hayden M. K.(2002). Association between the presence of enterococcal virulence factors gelatinase, hemolysin and enterococcal surface protein and mortality among patients with bacteremia due to *Enterococcus faecalis*. J. Clin. Infect. Dis.; 35(5):570-575.
- Vidotto, M. C., Furlaneto M. C. and Perugini M. R. E. (1991). Virulence factors of *Escherichia coli* in urinary isolates. Braze. J. Med. Biol. Res.; 24:365-373.
- Vila, J., Marti S. and Sanchez-Cespedes J. (2007). Porins, efflux pumps and multidrug resistance in *Acinetobacter baumannii*. J. Antimicrob. Chemother.; 59(6):1210-1215.
- Villanueva-Diaz, C. A., Flores-Rejes G. A., Beltran-Zuniga M., Echavarria-Sauche M., Ortiz-Ibarra F. J. and Arredondo-Garcia J. L. (1999). Bacteriospermia and male infertility: A method for increasing the sensitivity of semen culture. Int. J. Fertile Women Med.; 44(4):198-203.
- Virecoulon, F., Wallet F., Fruchart A. F., Rigot J. M., Peers M. C., Mitchell V. and Courcol R. J. (2005). Bacterial flora of the low male genital tract in patients consulting for infertility .Andrologia; 37(5):160-165.
- Wang, Y., Liang C. L., Wu J. Q., Xu C., Qin S. X. and Gao E. S. (2006). Do *Ureaplasma urealyticum* infections in the genital tract affect semen quality? Asian J. Androl.; 8:562-568.

References

- Watanabe, A., Takahashi H., Kikuchi T., Kobayashi T., Gomi K., Fujimura S., Tokue Y. and Nukiwa T. (2000). Comparative in vitro activity of S-4661, a new parenteral carbapenems, and other antimicrobial agents against respiratory pathogens. *J. Antimicrob. Agents Chemother.*; 46:184-187.
- Weinberg, E. D. (1998). Patho-ecological implication of microbial acquisition of host iron. *Review in Medical Microbiology.*; 9:171-178.
- Weinberg, E. D. (1999). Iron loading and disease surveillance. *Emerg. Infect. Dis.*; 5:346-352.
- Weinder, W., Krause W. and Ludwig M. (1999). Relevance of male accessory gland infection for subsequent fertility with special focus on prostatitis. *Hum. Reprod. Update*; 5(5):421-432.
- Whitfield, C. and Valvano M. A. (1993). Biosynthesis and expression of cell surface polysaccharides in gram negative bacteria. *Adv. Microbiol. Physiol.*; 35:135-246.
- Williams, P. and Griffiths E. (1992). Bacterial transferrin receptors, structure, function and contribution to virulence. *Med. Microbiol. Immunol.*; 181: 301-322.
- Wolff, H. (1998). Methods for the detection of male genital tract inflammation. *Andrologia.*; 30(1):35-39.
- Wolff, H. (1995). The biologic significance of white blood cells in semen. *Fertil. Steril.*; 63:1143-1157.
- Wolff, H., Politch J. A., Martinez A., Haimovici F., Hill J. A. and Anderson D. J. (1990). Leukocytospermia is associated with poor semen quality. *Fertil. Steril.*; 53:528-536.
- Wood, G. C., Hanes S. D., Croce M. A., Fabian T. C. and Bougher B. A. (2002). Comparison of ampicillin sulbactam and imipenem-cilastin for the

References

- treatment of *Acinetobacter* ventilator-associated pneumonia. Clin. Infect. Dis.; 34: 1425–1430.
- World Health Organization (WHO). (1999). WHO laboratory manual for the examination of human semen and sperm- cervical mucus interaction, 4th edition; Cambridge, Cambridge University Press. UK.
- Xu, C., Sun G. F., Zhu Y. F. and Wang Y. F. (1997). The correlation of *Ureaplasma urealyticum* infection with infertility. Andrologia.; 29:219-226.
- Yamaguchi, K., Domon H., Miyazaki S., Tateda K., Ohno A., Ihii K., Matsumoto T. and Furuya N. (1998). In vitro and in vivo antibacterial activities of CS-834, a new oral carbapenems. J. Antimicrob. Agents Chemother.; 42:555-563.
- Yassin, H. (1990). Chronic otitis media microbiological and epidemiological study. Msc. thesis. College of medicine. University of Basrah , Iraq.
- York, M., Gibbs L., Chehab F. and Brooks G. (1996). Comparison of PCR detection of *mecA* gene with standard susceptibility testing methods to determine methicillin resistance in coagulase negative staphylococci. J. Clin. Microbiol.; 34:249-253.
- Zahac, L., Mady U., Lig Y. and Yu Y. (2003). The states of drug resistance and amp C gene expression in *Enterobacter cloacae*. J. Chine. Med.; 116: 1244-1247.
- Zavos, P. M., Correa J. R. and Zarmakoupis-Zavos P. N. (1998). Antisperm antibody treatment mode, levels of antisperm antibodies after incubation with TEST- Yolk buffer and filtration using the sperm prep. II method. Fertil. Steril.; 69: 517-521.

Index (1) Result interpretation chart of Hi 25 Enterobacteriaceae identification kit (Himedia/India).

Strip I	Result Interpretation chart
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Index (2) Identification index of Hi 25 Enterobacteriaceae identification kit(Himedia/India).



KB003 : Hi24 TM Enterobacteriaceae Identification Kit												
Identification Index of various Enterobacteriaceae species												
Tests	ONPG	Lysine	Ornithine	Urease	TDA	Nitrate	H ₂ S	Citrate Utilization	Voges Proskauer's	Methyl Red	Indole	Malonate
<i>Budvicia aquatica</i>	+	—	—	V	—	+	V	—	—	+	—	—
<i>Buttiauxella agrestis</i>	+	—	+	—	—	+	—	+	—	+	—	V
<i>Cedecea davisae</i>	+	—	+	—	—	+	—	+	V	+	—	+
<i>Cedecea lapagei</i>	+	—	—	—	—	+	—	+	V	V	—	+
<i>Cedecea neteri</i>	+	—	—	—	—	+	—	+	V	+	—	+
<i>Citrobacter amalonaticus</i>	+	—	+	V	—	+	—	V	—	+	+	—
<i>Citrobacter diversus</i>	+	—	+	V	—	+	—	+	—	+	+	+
<i>Citrobacter freundii</i>	+	—	V	V	—	+	V	+	—	+	—	V
<i>Enterobacter aerogenes</i>	+	+	+	—	—	+	—	+	+	—	—	+
<i>Enterobacter amnigenus</i> (Biogroup I)	+	—	V	—	—	+	—	V	+	—	—	+
<i>Enterobacter amnigenus</i> (Biogroup II)	+	—	+	—	—	+	—	+	+	V	—	+
<i>Enterobacter taylorae</i> (E. cancerogenus)	+	—	+	—	—	+	—	+	+	—	—	+
<i>Enterobacter cloacae</i>	+	—	+	V	—	+	—	+	+	—	—	V
<i>Enterobacter gergoviae</i>	+	+	+	+	—	+	—	+	+	—	—	+
<i>Enterobacter sakazakii</i>	+	—	+	—	V	+	—	+	+	—	V	V
<i>Escherichia coli</i>	+	+	V	—	—	+	—	—	—	+	+	—
<i>Escherichia coli, inactive</i>	V	V	V	—	—	+	—	—	—	+	V	—
<i>Escherichia blattae</i>	—	+	+	—	—	+	—	V	—	+	—	+
<i>Escherichia fergusonii</i>	V	+	+	—	—	+	—	V	—	+	+	V
<i>Escherichia hermannii</i>	+	—	+	—	—	+	—	—	—	+	+	—
<i>Escherichia vulneris</i>	+	V	—	—	—	+	—	—	—	+	—	V
<i>Ewingella americana</i>	V	—	—	—	—	+	—	+	+	V	—	—
<i>Hafnia alvei</i>	+	+	+	—	—	+	—	—	V	V	—	V
<i>Klebsiella oxytoca</i>	+	+	—	+	—	+	—	+	+	V	+	+
<i>Klebsiella pneumoniae subspecies ozaenae</i>	V	V	—	—	—	V	—	V	—	+	—	—
<i>Klebsiella pneumoniae subspecies pneumoniae</i>	+	+	—	+	—	+	—	+	+	V	—	+
<i>Klebsiella pneumoniae subspecies rhinoscleromatis</i>	—	—	—	—	—	+	—	—	—	+	—	+
<i>Klebsiella terrigena</i>	+	+	V	—	—	+	—	V	+	V	—	+
<i>Kluyvera ascorbata</i>	+	+	+	—	—	+	—	+	—	+	+	+
<i>Leclercia adecarboxylata</i> (<i>Escherichia adecarboxylata</i>)	+	—	—	V	—	+	—	—	—	+	+	+
<i>Morganella morganii subspecies morganii</i>	—	—	+	+	+	+	—	—	—	+	+	—
<i>Morganella morganii subspecies sibonii</i>	—	V	V	+	+	+	—	—	—	V	V	—
<i>Pantoea agglomerans</i>	+	—	V	—	V	+	—	+	+	V	—	+
<i>Pantoea dispersa</i>	+	—	—	—	—	V	—	+	+	V	—	—
<i>Proteus mirabilis</i>	—	—	+	+	+	+	+	V	V	+	—	—
<i>Proteus myxofaciens</i>	—	—	—	+	+	+	—	+	+	+	—	—
<i>Proteus penneri</i>	—	—	—	+	+	+	V	—	—	+	—	—
<i>Proteus vulgaris</i>	—	—	—	+	+	+	+	V	—	+	+	—
<i>Providencia alcalifaciens</i>	—	—	—	—	+	+	—	+	—	+	+	—
<i>Providencia rettgeri</i>	—	—	—	+	+	+	—	+	—	+	+	—
<i>Providencia rustigianii</i>	—	—	—	—	+	+	—	V	—	V	+	—
<i>Rahnella aquatilis</i>	+	—	—	—	+	+	—	+	+	V	—	+
<i>Salmonella bongori</i>	+	+	+	—	—	+	+	+	—	+	—	—
<i>Salmonella choleraesuis subspecies arizonae</i>	+	+	+	—	—	+	+	+	—	+	—	+
<i>Salmonella choleraesuis subspecies choleraesuis</i>	—	+	+	—	—	+	+	+	—	+	—	—
<i>Salmonella choleraesuis subspecies diarizonae</i>	+	+	+	—	—	+	+	+	—	+	—	+
<i>Salmonella choleraesuis subspecies houtenae</i>	—	+	+	—	—	+	+	+	—	+	—	—
<i>Salmonella choleraesuis subspecies indica</i>	V	+	+	—	—	+	+	V	—	+	—	—
<i>Salmonella choleraesuis subspecies salamae</i>	V	+	+	—	—	+	+	+	—	+	—	+
<i>Salmonella enteritidis</i>	—	+	+	—	—	+	+	+	—	+	—	—
<i>Salmonella typhi</i>	—	+	—	—	—	+	+	—	—	+	—	—
<i>Salmonella typhimurium</i>	—	+	+	—	—	+	+	+	—	+	—	—
<i>Serratia entomophila</i>	+	—	—	—	—	+	—	+	+	V	—	—
<i>Serratia ficaria</i>	+	—	—	—	—	+	—	+	V	V	—	—
<i>Serratia fonticola</i>	+	+	+	V	—	+	—	+	—	+	—	V
<i>Serratia marcescens</i>	+	+	+	V	—	+	—	+	+	V	—	—
<i>Serratia odorifera</i> (Biogroup I)	+	+	+	—	—	+	—	+	V	+	V	—
<i>Serratia odorifera</i> (Biogroup II)	+	+	—	—	—	+	—	+	+	V	V	—
<i>Serratia plymuthica</i>	V	—	—	—	—	+	—	V	V	+	—	—
<i>Serratia proteamaculans</i>	+	+	+	—	—	+	—	+	V	V	—	—
<i>Serratia rubidaea</i>	+	V	—	—	—	+	—	+	+	V	—	+
<i>Shigella boydii</i> , <i>Shigella flexneri</i> , <i>Shigella dysenteriae</i>	—	—	—	—	—	+	—	—	—	+	V	—
<i>Shigella sonnei</i>	+	—	+	—	—	+	—	—	—	+	—	—

RAPID

Identification Index of various *Enterobacteriaceae* species

126

Index (3) Result interpretation chart of HiStaph identification kit(Himedia /India)

Result interpretation chart						
No.	Test	Reagents to be added after incubation	Principle	Original colour of the medium	Positive reaction	Negative reaction
1	Voges Proskauer's	1-2 drops of Barritt reagent A and 1-2 drops of Barritt reagent B	Detects acetoin production	Colourless	pinkish red	Colourless/ slight copper
2.	Alkaline phosphatase	1-2 drops of 40% NaOH	Detects ability of organism to produce sufficient phosphatase enzyme	Cream	Pink	Cream
3	ONPG	—	Detects β -galactosidase activity	Colourless	Yellow	Colourless
4	Urease	—	Detects Urease activity	Orangish-yellow	Pink	Orangish-yellow
5.	Arginine utilization	—	Detects Arginine decarboxylation	Olive green	Purple	Yellow
6	Mannitol	—	Carbohydrate utilization	Red	Yellow	Red / Pink
7	Sucrose	—	Carbohydrate utilization	Red	Yellow	Red / Pink
8	Lactose	—	Carbohydrate utilization	Red	Yellow	Red / Pink
9	Arabinose	—	Carbohydrate utilization	Red	Yellow	Red / Pink
10	Raffinose	—	Carbohydrate utilization	Red	Yellow	Red / Pink
11	Trehalose	—	Carbohydrate utilization	Red	Yellow	Red / Pink
12	Maltose	—	Carbohydrate utilization	Red	Yellow	Red / Pink

Index (4) Identification index of HiStaph identification kit(Himedia /India)



KB004 : HiStaph™ Biochemical Test Kit (used for differentiation of Staphylococcus species)

Biochemical Identification Test Kits

Identification Index of various *Staphylococcus* species

Tests	Voges Proskauer's	Alkaline phosphatase	ONPG	Urease	Arginine utilization	Mannitol	Sucrose	Lactose	Arabinose	Raffinose	Trehalose	Maltose
<i>S. aureus</i> subsp. <i>aureus</i>	+	+	-	+W	+W	+	+	+	-	-	+	+
<i>S. epidermidis</i>	+	+	-	+	+W	-	+	V	-	-	-	+
<i>S. haemolyticus</i>	V	-	-	-	+	V	+	V	-	-	+	+
<i>S. lugdunensis</i>	+	-	-	V	-	-	+	+	-	-	+	+
<i>S. saprophyticus</i>	+	-	V	+	-W	V	+	V	-	-	+	+
<i>S. schleiferi</i> subsp. <i>coagulans</i>	+	+	nd	+	+	V	V	V	-	-	-	-
<i>S. schleiferi</i> subsp. <i>schleiferi</i>	+	+	V	-	+	-	-	-	-	-	V	-
<i>S. arlettae</i>	-	+	V	-	-	+	+	+	+	+	+	+
<i>S. auricularis</i>	V	-	V	-	V	-	V	-	-	-	+	+
<i>S. capitis</i> subsp. <i>capitis</i>	V	-	-	-	V	+	+	-	-	-	-	-
<i>S. capitis</i> subsp. <i>ureolyticus</i>	V	-	-	+	+	+	+	V	-	-	-	+
<i>S. caprae</i>	+	+	-	+	+	V	-	+	-	-	+	V

<i>S. cohnii</i> subsp. <i>cohnii</i>	V	-	-	-	-	V	-	-	-	-	+	V
<i>S. cohnii</i> subsp. <i>urealyticum</i>	V	+W	+	+	-W	V	-	+	-	-	+	V
<i>S. hominis</i>	V	-	-	+	V	-	+	V	-	-	V	+
<i>S. pasteurii</i>	V	-	-	+	V	V	+	V	-	-	+	V
<i>S. simulans</i>	-W	+	+	+	+	+	+	-	-	-	V	-W
<i>S. warneri</i>	+	-	-	+	V	V	+	+	-	-	+	+
<i>S. xylosus</i>	V	V	+	+	-	V	+	V	-	-	+	+
<i>S. caseolyticus</i>	-	-	-	-	V	-	V	+	-	nd	V	+
<i>S. carnosus</i>	+	+	+	-	+	+	-	V	-	-	V	-
<i>S. chromogens</i>	-	+	-	V	+	+	+	+	-	-	+	V
<i>S. delphini</i>	-	+	nd	+	+	+	+	+	-	nd	-	+
<i>S. equorum</i>	-	+	V	+	-	+	+	V	+	-	+	V

<i>S. felis</i>	-	+	+	+	+	V	V	+	-	-	+	-
<i>S. gallinarum</i>	-	+	-W	+	-	+	+	V	+	+	+	+
<i>S. hyicus</i>	-	+	-	V	+	-	+	+	-	-	+	-
<i>S. intermedius</i>	-	+	V	+	V	V	+	V	-	-	+	+
<i>S. kloosii</i>	V	+	V	V	-	+	-	V	V	-W	+	V
<i>S. lentus</i>	-	+W	-	-	-	+	+	V	V	+	+	V
<i>S. muscae</i>	-	+	-	-	-	-	+	-	-	-	+	-
<i>S. piscifermentans</i>	-	+	V	+	+	V	V	V	-	-	+	V
<i>S. sciuri</i>	-	+W	-	-	-	+	+	-W	V	-	+	V
<i>S. vitulus</i>	-	-	-	-	-	+	+	-	-	-	V	-

<i>S. hominis</i> subsp. <i>novobiisepticus</i>	V	-	-	+	-	-	+	V	-	-	-	+
<i>S. saprophyticus</i> subsp. <i>bovis</i>	V	-	V	+	-	+	+	-	-	-	+	+
<i>S. succinus</i>	-	+	nd	+	-	nd	nd	+	nd	V	+	nd
<i>S. carnosus</i> subsp. <i>utilis</i>	nd	-	-	-	+	-	-	-	-	-	V	-
<i>S. condimentii</i>	nd	+	+	+	+	+	+W	+	-	-	+	-
<i>S. lutrae</i>	-	+	+	+	-	V	nd	+	nd	nd	+	+
<i>S. sciuri</i> subsp. <i>carnaticus</i>	-	V	-	-	-	+	+	V	V	-	+	V
<i>S. sciuri</i> subsp. <i>rodentium</i>	-	V	-	-	-	+	+	-	V	-	+	V
<i>S. fleurettii</i>	V	V	-	-	-	nd	+	-	V	-	+	+

Note : Based on % strains showing reactions following symbols have been assigned from laboratory results and standard references.

+W = positive to weak reaction
 -W = negative to weak reaction
 Nd = not detected
 V = variable, usually positive 11-89%

+ = Positive (more than 90%)
 - = Negative (more than 90%)

References

- Acosta, A.A. ,Vem J.V. ,Ackerman S.B. ,Mayer J.F. and Ftecker J.F. (1986).Estimation of male infertility by examination and testing of spermatozoa. In: In vitro Fertilization. Norfolk. Jones, H. W.; Jones, G. S.; Hodgen, G. D. and Rosenmarks, Z. (eds.). Williams and Wilkins. Baltimore, USA.; 126- 161.
- Adam, F.H., Morici R.J. and Wynn J.L.(2002).A study of Coagulase-negative Staphylococci, adherence, slime product and resistance patterns. J. Clin. Hospt. , 22: 217-27.
- Ad Dahhan, H.A. , Naher H.S. and Shubber E.K.(2005).Isolation and Identification of Bacterial chronic suppurative otitis media, Kufa covernorate .J. college of medicine Al-Qadisia University Diwaniah ,Iraq. ,1(2),62-67.
- Adebayo, S. ,Johnson L. ,Morrison D and Kolawole D.(2006). Identification and molecular characterization of mannitol salt positive, coagulase-negative staphylococci from nasal samples of medical personnel and students. J. Medic. Microbiol., 55: 317–324.
- Aggarwal, A. ,Kanna S. and Arova U.(2003).Characterization biotyping antibiogram and klebocin typing of Klebsiella. Indian. J. Med. ,57(2): 68-70.
- Aguilar, G.M. , Hammerman W.A. , Mason E.O. and Kaplan S.L. (2003). Clindamycin treatment of invasive infections caused by community-acquired, Methicillin-resistant and Methicillin-susceptible Staphylococcus aureus in children. Pediatr. Infect. Dis. J., 22(7):593-598.

References

- Ahmed, K., Rikitomi N., Ichinose A. and Mastsumoto K. (1991). Possible Precence of capsule in Branhamella catarrhalis. Microbiol. Immunol. , 35:361-366.
- Aitken, R.J. ,West K.M. and Buckingham D.W.(1994).Leukocyte infiltration into the human ejaculate and its association with semen quality, oxidative stress, and sperm function. *J. Androl.* 15:343- 352.
- Al-Amedi, B.H.(2003). Comparative study of mucosal immune responses for the persistent pyuria patients. Ph.D. thesis College of Science, Babylon University. Iraq.
- Alanis, A.J.(2005). Resistance to Antibiotics: Are We in the Post-Antibiotic Era? Archives of Medical Research, 36:697–705
- AL-Anssari, S.A.M.(2000).Seminal fluid osmotic pressure measurement and sperm function tests and in vitro fertilization rate of human eggs. Kufa med. J. , 4: 66- 69.
- Al-Anssari,S.A.M. , Albarzanchi R.M.T. and Khunda S.S.(1997).Seminal fluid osmolarity and in vitro fertilization of human eggs. 3rd Asian Symposium on Animal Biotechnology. Seoul, Korea.; PP: 232- 236.
- Al-barazanchi , S.I.(2001).A genetic study on bacteriocin producer Enterococcus faecalis. M.Sc. thesis College of Science, Baghdad University, Iraq.
- Al-barazanchi , R. , Khunda M.T. , Al-Fayad R.S.F., Al-Anssari S.A.M. and Jassim M.M.(1992).Human pregnancy following sperm

References

- antibody separation technique and sperm intrauterine transfer. J. comm. Med. Baghdad. , 80: 1-10.
- Al-barzanchi R. , AL- Naeib Z.T.H. , AL- Anssari S.A.M. , Khunda S. S. , Zakria M.R. , AL- Fayad R.S.F., and Azat L.N.(1991). Successful live birth following human ovarian stimulation and in vitro sperm cell hyperactivation and application of sperm intrauterine transfer technique. J. Fac. Med. Baghdad, 33: 327-336.
- Alberto, B. , Palmero J.L. ,Broseta E. , Tejadillos S. , Benedicto A. , Queipo J.A. ,Gobernado M. and Cruz J.F.(2006).Value of semen culture in the diagnosis of chronic bacterial prostatitis: A simplified method . J. Medic. Microbiol., 40 (4) :326 – 331.
- Al-Charrakh, A.H.(2005).Bacteriological and genetic study on extended-spectrum beta-lactamases and bacteriocins of Klebsiella isolated from Hilla city. Ph.D. Thesis College of Science. Baghdad University, Iraq.
- Al-Dulami, H.H.(1999).Effect of crude colicin extracted from Escherichia coli on immune cells. M.Sc. Thesis College of Science. Al-Mustansiriya University, Iraq.
- Alexander, N.J.(1989).Natural and induced immunological infertility. Curr. Opin. Immunol., 1: 1125- 1130.
- Al-Hamawandi, J.A.(2005).Bacteriological and immunological study on infants pneumonia at Babylon Governorate . M.Sc. thesis. College of Science. Al-Mustansiriya University, Iraq.

References

- Alhambra, A., Cuadros J.A. and Cacho J. (2004). In vitro susceptibility of recent antibiotic resistant urinary pathogens to ertapenem and 12 other antibiotics. *J. Antimicrob. Chemother.*, 53(6):1090-4.
- Al-Mashriky ,J.H.(2003).A Study of identification and pathogenicity on *Enterobacter aerogenes* isolated from infantile diarrhoeal cases in Mosul. Msc. thesis , college of science ,Al-Mosul university ,Iraq.
- Al-Muhanna, A.S.(2001). Identification of bacteria causing pneumonia in three medical centers in Najaf. M.Sc. thesis. Kufa University, Iraq.
- Al-Nuaimi ,E.M.(2002).Urinary tract infection in pregnant women .M.Sc. thesis college of science. Al-Mustansiriya university, Iraq.
- Al-Qassab, A.O. and Al-Khafaji Z.M.(1992).Effect of different conditions on inhibition activity of enteric lactobacilli against diarrhea causing enteric bacteria. *J. Agric. Sci.*, 3(1): 18-20.
- Al-Rassam, Z.T.D.(2004). Urinary tract infections caused by *Alcaligenes* spp. and its relation to immune suppression in diabetic patients. M.Sc. Thesis. College of Science. Mosul University, Iraq.
- Al-Sahllawi, Z.S. (2002). A comparative study on local isolates of Methicillin resistant and Methicillin sensitive *Staphylococcus aureus*. M.Sc. Thesis, College of science, kufa university. Iraq. (In Arabic)

References

- Al-Saigh, R.J.(2005). A study of some clinical and bacteriological aspects of patients with chronic pyelonephritis in Hilla-teaching Hospital. Thesis of M.Sc. College of Medicine, Babylon University. Iraq.
- AL-Salayi , T.S.(2002). Common bacterial causes of urinary tract infections in children below five years of age in Maternity and pediatrics Hospital – Erbil . MSc. thesis. University of Salahaddin ,Iraq.
- Al-Shukri, M.S.(2003). A study of some bacteriological and genital aspects of Acinetobacter isolated from patients in Hilla-city. Msc. thesis. College of Science, Babylon University. Iraq.
- Alwash, B.H.(2006).A bacteriological and clinical study of patients with benign prostatic hyperplasia and or chronic prostatitis .Msc. thesis . College of Medicine ,Babylon university .Iraq.
- Amelar, D.A. , Dubin L. and Schoenfeld C. (1980). Sperm motility. Fertil. Steril. ,34: 197- 215.
- Amelar, R.D. (1966). Infertility, in: Infertility of Men. Amelar R.D. (ed.), Davis comp., Philadelphia., PP: 1-17.
- Amsden, G.W.(2004).Pneumococcal resistance in perspective: how well are we combating it? Pediatr. Infect. Dis. J., 23:125–128.
- Amyes, S.(1983).The detection and incidence and transferable trimethoprim resistance. Health Bull ,41 : 100-107 .

References

- Anderson , D.J.(1995).Should male infertility patients be tested for leukocytospermia? *Fertil. Steril.* ; 63:246-248.
- Andrade-Rocha, F.T.(2003).Ureaplasma urealyticum and Mycoplasma hominis in men attending for routine semen analysis. Prevalence, incidence by age and clinical settings, influence on sperm characteristics, relationship with the leukocyte count and clinical value. *Urol. Int.*, 71:377-381.
- Ang, J.Y. , Ezike E. and Asmar B.(2004). Antibacterial resistant. *Indian. J. pediatrics.*, 71 (3):229-239.
- Araki,M. , Kariyama R. , Monden K. , Tsugawa M. and Kumon. H.(2002). Molecular epidemiological studies of staphylococcus aureus in urinary tract infection .*J. infect. Chemother.*, 8:167-174
- Arbeit, R.D., Karakawa W.W. , Vann W. F. and Robbins J.B.(1984). Predominance of two newly described capsular polysaccharide types among clinical isolates of Staphylococcus aureus. *Diag. Microbiol. Infect. Dis.* 2:85-91.
- Archer, G.L., and Johnston L.J.(1983).Self-transmissible plasmids in staphylococci that encode resistance to aminoglycosides. *Antimicrob. Agents Chemother.*, 24:70–77.
- Archer, G.L., and Climo M.W.(1994).Antimicrobial susceptibility of coagulase-negative staphylococci. *Antimicrob. Agents Chemother.* 38:2231–2237.

References

- Archer, G.L. ,Coughter J.P. and Johnston L.J.(1986).Plasmid encoded trimethoprim resistance in staphylococci. Antimicrob.Agents Chemother., 29:733–740.
- Archibald, F.(1983).Lactobacillus plantarum, an organism not requiring iron. FEMS Microbiol. Lett., 19:29-32.
- Arpi, B.M. and Rennerg D.S.(1984). Staphylococcus bacteriuria. J. FMC. Clin. Micorbiol., 50: 879-880.
- Arpin, C., Coze C. ,Rogues A. M. ,Gachie J. P. ,Babear C. and Quentin C. (1996).Epidemiological study of an outbreak due to multidrug resistant Enterobacter aerogenes in a medical intensive care unit. J.Clin .Microbiol. 34: 2163 -9.
- Bach , D. , Annelis B. , Andreade H. , Gerard B. , Marina C. and Yannick P.(1995). Rufloxacin once daily versus Ciprofloxacin twice daily in the treatment of patients with acute uncomplicated pylonephritis , J. Urol . , 154 : 19-24 .
- Bach, S., De Almeida A. and Carniel E. (2000). The Yersinia high-pathogenicity island is present in different members of the family Enterobacteriaceae. FEMS Microbiol. Lett. , 1:83-28
- Barrett, A.J. , Rowling N.D. and Woessner JF.(2003).The Handbook of proteolytic enzymes. 2nd ed. Academic press. ISBN.
- Barry, A.L. , Jones R.N. , Thornsberry C. , Ayers L.W. and Kundargi R. (1985).Imipenem(N. forimidoly thienamycin): in vitro antimicrobial activity on β -lactamase stability. Diagno. Microbiolo. and Infect. Dis., 3:93-104,

References

- Bartels,E.D. , Nielsen J.E. , Lindgaard M.L. and Hulten L.M. (2007). Endothelial lipase is highly expressed in macrophages in advanced human atherosclerotic lesions *Atherosclerosis. J. Appl. Microbiol.*, 12:65-73.
- Bartoov, B. , Ozbonfil D. , Maayan M.C. , Ohad E. and Nitzan.Y. (1991). Virulence characteristics of male genital tract *Escherichia coli* isolated from semen of suspected infertile men. *Andrologia* , 23: 387-394.
- Bayram ,A. and Balci I.(2006). Patterns of antimicrobial resistance in a surgical intensive care unit of a university hospital in Turkey. *BMC Infect. Dis.*; 6:155.
- Behre, H.M., Yeung C.H. and Nieschlag E.(1997).Diagnosis of male infertility and hypogonadism. In: *Andrology: male reproductive health and dysfunction* (Nieschlag E, Behre HM, eds). Berlin: Springer-Verlag; 87–110.
- Bell, J.M. , Turniding J.D. , Jones R.N. and Sentry Asia-Pacific Participant.(2003).Prevalence of extended –spectrum β -lactamases-producing *Enterobacter cloacae* in the Asia-Pasific region: results from the Sentry. *Antimicrob. Agent. Chemother.*,47(12):3989-3993.
- Ben Ali , H. , Guerin J.F. , Pinatel M.C. , Mathieu C. , Boulieu D. and Tritar B.(1994).Relationship between semen characteristics, alpha-glucosidase and the capacity of spermatozoa to bind to the human zona pellucida. *Int. J .Androl.* , 17:121 -126.

References

- Berger, R.E. , Karp L.E. , Williamson R.A. , Koehler J. , Moore D.E. and Holmes K.K.(1982).The relationship of pyospermia and seminal fluid bacteriology to sperm function as reflected in the sperm penetration assay. *Fertil. Steril.*; 37: 557- 564.
- Bergman, B.(1994).On the prevalence of gram-positive bacteria in prostatitis. *J. Infect. Urol.* 22: 22-30.
- Bisiklis, A. , Tsiakiri E. and Alexiou-Danieel S.(2005). Phenotypic detection of *Pseudomonas aeruginosa* producing metallo- β -lactamases. *European Society of clinical microbiology and infectiousdisease,15th European congress of clinical microbiology and infectious disease.* ,1134:1-11.
- Blance, S.K. Andro M.B. and Jone L.V.(1996). Virulence factors and O groups of *Escherichia coli* isolates in patients with UTIs. *J. Eur. Epidemiol.* , 12(2):191.
- Blandow, R.J. and Rumery R.E.(1964).The relationship of swimming movement of epididymal spermatozoa to their fertilizing capacity. *Fertil. Steril.*; 15: 571- 573.
- Bliss, J.M. and Silver R.P. (1996).Coating the surface: a model for expression of capsular polysialic acid in *E. coli* K1. *Microbiology* , 21:221-231.
- Blot, S.I. , Vandewoude K.H. , Hoste E.A. and Colardyn F.A.(2002). Outcome and attributable mortality in critically Ill patients with bacteremia involving methicillin-susceptible and methicillin-

References

- resistant *Staphylococcus aureus*. *Arch. Intern. Med.* , 162(19): 2229–2235.
- Bogado, I., Sutich E. , Krapp A. , Marchiaro P. , Marzi M. , Putero J. and Carrillo N.(2001).Methicillin resistance study in clinical isolates of coagulase-negative staphylococci and determination of their susceptibility to alternative antimicrobial agents. *J. Appl. Microbiol* 91:344–350.
- Bonnet, R.K.(2004). Enterobacter infections. *J. Med. Microbiol.*, 48:142.
- Bornet, C. , David R. , Bosi C. , Pages J. and Bollet C. (2000). Imipenem resistance of *Enterobacter aerogenes* mediated by outer membrane permeability, *J. Clin. Microbiol.*, 38(3): 1048–1052.
- Bornman, M.S. , Mahomed M.F. , Boomker D. , Schulenburg G.W. , Reif S. and Crewe-Brown H.H.(1990).Microbial flora in semen of infertile African men at Garankuwa hospital. *Andrologia.*, 22:118-121.
- Bostofte, E. (1987).Prognostic parameters in predicting pregnancy. *Acta Obst. Gynecol. Scand.*; 66: 617- 624.
- Braun, V. , Pilsl H. and Grob P.(1994). Colicins :structures, mode of action, transfer through membranes, and evolution. *Arch. Microbiol.*, 161: 199-206.
- Braun, V., Hantke K. and Köster W.(1998).Bacterial iron transport: mechanisms, genetics, and regulation. *Metal Ions Biol. Syst.* 35:67-145.

References

- Braun, V., Patzer S.I. and Hantke K.(2002). Ton-dependent colicins and microcins: modular design and evolution. *Biochimie.*, 84:365–380.
- Brisse, S. , Milatovic D., Fluit A.C. , Verhoff J. , Martin N. , Scheuring S., Kohrer K. and Schemitz F.J.(1999).Comparative in-vitro activities of ciprofloxacin, clindoxacin, gatifloxacin against *Klebsiella pneumoniae*, *Klebsiella oxytoca* and *Enterobacter aerogenes*. clinical isolates with alteration in Gyr A and Par C proteins, *Antimicrob. Agent. Chemother.*, 43(8): 2051–2055.
- Brogden, K.A., Roth J.A. , Stanton T.B. , Bolin C.A. , Minion C.F. and Wannemuehler M.J.(2000).Virulence mechanism of Bacterial Pathogens. ASM Press, Washington, D.C.
- Brook ,I., Yocum P. and Shah K.(1998).Aerobic and Anaerobic Bacteriological of otorrhea associated with tympanostomy tubes in children. *Acta. Otolaryngology.*, 118: 206-210.
- Brooks, G.F., Butel J.S. and Morse S.A.(2001).Jawetz, Melnick and Aldelberg's Medical Microbiology. 22nd edi. McGraw-McGraw-Hill. Pp. 156-170.
- Brooks,G.F. , Butel J.S. and Morse S.A. (2004). Medical microbiology. 23rd ed. Lange Medical books .McGraw-Hill Companies.USA.
- Bukharin ,O.V. , Kuzimin M.D. and Ivanov I.B.(2003).The role of the microbial factor in the pathogenesis of male in fertility. *Zh. Microbial. Epidemiol. Immunobiol.* ; 2:106-110.

References

- Busolo ,F. , Zanchetta R. , Lanzone E. and . Cusinato R.(1984). Microbial Flora in semen of asymptomatic infertile men. *Andrologia* , 16:269-275.
- Busolo, F. , Zanchetta R. and Bertoloni G.(1984a).Mycoplasmic localization patterns on spermatozoa from infertile men. *Fertil. Steril.* , 42:412-417.
- Bussen, S. , Zimmermann M. , Schlever M. and Steck T.(1997). Relationship of bacteriological characteristics to semen indices and its influence on fertilization and pregnancy rates after IVF. *Acta Obst. Gynecol. Scand.*;76(10):964-968.
- Carapeti, E.A. , Andrews S.M. and Bentley P.G.(1996). Randomized study of sterile versus non- sterile urethral catheterization . *Ann. R. Coll. Surg. Engl.*, 78:59-60.
- Carrera, A. and Mendoza C.(1990).Zinc levels in seminal plasma of fertile and infertile men. *Andrologia.*; 22: 279- 283.
- Cassels, F.J. and Wolf M.K.(1995).Colonization factors of diarrheagenic *E. coli* and their intestinal receptors. *J. Ind. Microbiol.* , 15:214–226.
- Catlin, B.W.(1990). *Branhamella catarrhalis* .An organism gaining respect as a pathogen . *Clin . Microbiol. Rev.*, 3:293-320.
- Chan, P., Su B.C., Tredway D.R. , Whitney E.A., Pang S.C., Corselli J. and Jacobson J.D.(1994).White blood cells in semen affect hyperactivation but not sperm membrane integrity in the head and tail regions. *Fertil. Steril.*;61:986 -989.

References

- Chang, F.Y. , Peacock J.E. and Musher D.M.(2003).Staphylococcus aureus bacteremia: recurrence and the impact of antibiotic treatment in a prospective multicenter study.Medicine (Baltimore), 82:333-339.
- Chang, S., Sievert D.M., Hageman J.C., Boulton M.L., Tenover F.C., Downes F.P., Shah S., Rudrik J.T., Pupp G.R., Brown W.J., Cardo D. and Fridkin S.K.(2003).Infection with vancomycin-resistant Staphylococcus aureus containing the vanA resistance gene. *N. Engl. J. Med.*, 348 (14): 1342-1347.
- Charrel, N.R. , Pages J.M. , Demicco P. and Mallea M.(1996). Prevalence of outer membrane porin alteration in β -lactam antibiotic resistant Enterobacter aerogenes. *Antimicrobiol. Agent, Chemother.*; 40(12):2854– 2858.
- Chart, H.(2004). Escherichia. In Greenwood, D.; Slack, R. ;and Penethrer, J. Medical microbiology a guide to microbial infections: pathogenesis, immunity, laboratory diagnosis and control.pp : 265-274 .
- Cheng, Q. , Stafslie D., Purushoth S.S. and Cleary P.(2002).The Group B Streptococcal C5a peptidase IS Both a specific protease and an Invasive. *J. Infect. Immun.*,70(5):2408-2413.
- Chimura , T. and Saito H.(1990).A clinical study of asymptomatic bacteriospermia. *Jpn. J. Antibiot.* ,43(1):139-146.
- Choi, C.H., Lee E., Lee Y.C., Park T.I., Kim H.J., Hyun S.H., Kim S.A., Lee S.K. and Lee J.C.(2005).Outer membrane protein 38 of Acinetobacter baumannii localizes to the mitochondria and

References

- induces apoptosis of epithelial cells. *Cellul. Microbiol.* , 7(8):1127-1138.
- Clark ,N.C., Cooksey R.C. , Hill B.C. , Swenson J.M. and Tenover F.C.(1993).Characterization of glycopeptide-resistant enterococci from U.S. hospitals. *Antimicrob Agents Chemother.* , 37:2311-2317.
- Clark, N.M., Patterson J. and Lynch J.P. (2003). Antimicrobial resistance among Gram negative organisms in the intensive care unit. *Curr. Opin. Crit. Care.* , 9(5): 413-423.
- Cohen ,S.P. , Yan W. and Levy S.B.(1993).A multidrug resistance regulatory chromosomal locus is widespread among enteric bacteria . *J. Infect. Dis.*, 168:484-488.
- Collee, J.G. , Fraser A.G. , Marmino B.P. and Simons A. (1996).Mackin and McCartney Practical Medical Microbiology.14thed., The Churchill Livingstone, Inc. USA.
- Compagnari, A.A., Shanks K.L. and Dyer D.W.E.(1994).Growth of *Moraxella catarrhalis* with human transferrin and lactoferrin expression of iron repressible proteins without siderophore. production. *Infect. Immun.* , 62: 4906-4914.

References

- Conceicao , T. , Faria N. , Pimentel M. , Soveral G. and Duarte A.(2004).New chromosomal AmpC β -lactamase in *E. cloacae* .
Antimicrob. Agents and Chemoth.,48(4):1437-1445.
- Cook, H. , Furuya E. , Larson E. , Vasquez G. and Lowy F.(2007).
Heterosexual transmission of community-associated methicillin-resistant *Staphylococcus aureus*. *J. Infect. Dis.* , 44 (3): 410-413.
- Cornaglia,G. , Russell K., Satta G., and Fontana R.(1995). Relative importances of outer membrane permeability and group 1 beta-lactamase as determinants of meropenem and imipenem activities against *Enterobacter cloacae*. *Antimicrob. Agents Chemother.* , 39(2): 350–355.
- Cosgrove, S.E. ,Sakoulas G. ,Perencevich E.N. , Schwaber M.J. , Karchmer A.W. and Carmeli Y.(2003).Comparison of mortality associated with Methicillin-resistant and Methicillin-susceptible *Staphylococcus aureus* bacteremia: A meta-analysis. *J. Clin. Infect. Dis.* , 36:53-59
- Courvalin, P.(1994).Transfer of antibiotic resistance between Gram-positive and Gram-negative bacteria. *Antimicrob. Agents Chemother.*, 38: 1447-1451.
- Courcol, R.J., Trivier D., Bissinger M.C., Martin G.R. and Brown M.R.W.(1997).Siderophore production by *Staphylococcus aureus* and identification of iron-regulated proteins. *Infect. Immun.* 65:1944-1948.

References

- Cramer, W.A. , Heymann J.B. , Schendel S.L. , Deriy B.N. , Cohen F.S. , Elkins P.A. and Stauffacher C.V.(1995).Structure-function of the channel-forming colicin. *Ann. Rev. Biophys. Biomol. Struct.*, 24:611-641.
- Cravioto, A.R. ,Reyes F. ,Trujillo F. ,Uribe A. ,Navarro J.M. and Vazques V.(1990). Risk of diarrhea during the first year of life associated with initial and subsequent colonization by specific enteropathogens. *Am. J. Epidemiol.* , 131: 886-904.
- Crosa, J.H., Hodges L.L. and Schiewe M.H.(1980). Curing of a plasmid is correlated with an attenuation of virulence in the marine fish pathogen *Vibrio anguillarum*. *Infect. Immun.* 27:897-902.
- Dale, S.E., Doherty-Kirby A., Lajoie G. and Heinrichs D. E.(2004).Role of siderophore biosynthesis in virulence of *Staphylococcus aureus*:Identification and characterization of genes involved in production of a siderophore *Infect. Immun.*, 72 (1): 29 - 37.
- Dan, P.N.(2005). Treatment of acute and chronic bacterial prostatitis caused by *Staphylococcus epidermidis*. *J. Clin. Infect. Dis.* 43:147.
- Dana, A. and Alan C.M.(1996).Assessment of sperm function and clinical aspects of impaired sperm function. *Fertil. Steril.*; 60: 33-36.
- Daum, R.S. , Gupta S. , Sabbagh R. and Milewski W.M. (1992).Characterization of *Staphylococcus aureus* isolates with

References

- decreased susceptibility to vancomycin and teicoplanin: isolation and purification of a constitutively produced protein associated with decreased susceptibility. *J. Infect. Dis.* , 166:1066-1072.
- David, M.G. and Claire L.O.(2006).Bacteriocin diversity and the frequency of multiple bacteriocin production in *Escherichia coli*. *Microbiology* , 152:3239–3244
- Davies, A.L. and Stone J.W.(1986).Current problems of chemotherapy of infections with coagulase–negative staphylococci. *Europ. J. Clin. Microbiol.*, 5 : 277- 281.
- De Jong, Z., Pontonnier F., Plante P., Perie N., Talazac N., Mansat A. and Chabanon G.(1990).Comparison of the incidence of *Ureaplasma urealyticum* in infertile men and in donors of semen. *Eur. Urol.*, 18:127-131.
- De Lisle, S. and Perl T.M.(2003).Vancomycin-resistant enterococci. A road map on how to prevent the emergence and transmission of antimicrobial resistance. *Chest* , 123:504–518.
- Del Porto, G.B. , Derrick F.C. and Bannister E.R.(1975).Bacterial effect on sperm motility. *Urology* , 5:638-639.
- De Lorenzo, V. and Martinez J.L.(1988).Aerobactin production as a virulence factor: A re-evaluation. *Eur. J. Clin. Microbiol. Infect. Dis.*, 7: 621-629.
- Depuydt ,C., Zalata A., Christophe A., Mahmoud A. and Comhaire F.(1998). Mechanisms of sperm deficiency in male accessory gland infection. *Andrologia.*; 30(1):29 -33.

References

- Deresinski, S.(2005).Methicillin-resistant *Staphylococcus aureus*: an evolutionary, epidemiologic, and therapeutic odyssey. *Clin. Infect. Dis.* , 40:562–573.
- Devaud , M., Kayser F.H. and Bachi B.(1982).Transposon-mediated multiple antibiotic resistance in *Acinetobacter* strains. *J. Antimicrob. Agents Chemother.* ,22:323–329
- Diemer, T., Weidner W., Michelmann H.W., Schiefer H.G., Rován E. and Mayer F.(1996). Influence of *Escherichia coli* on motility parameters of human spermatozoa in vitro. *Int. J. Androl.*, 19:271 -277.
- Diemer,T., Ludwig M., Huwe P., Haler D.B. and Weidner W.(2000). Influence of genital urogenital infection on sperm function. *Curr. Opin. Urol.*;1(1):39-44.
- Dinges, M.M. , Orwin P.M. and Schlievert P.M.(2000).Exotoxins of *Staphylococcus aureus* .*J.Clin. Microbiol.* , 13(1):16–34.
- Donnell, J.A. and Gelone S.P.(2000).Antimicrobial therapy: Fluoroquinolones. *J. Clin. Infect. Dis.* , 4: 488-513.
- Donovan , J.F. and Lipshultz L.I.(1988).Infection and infertility .In: Contemporary management of importance and infertility .Tanago EA ,Lue TF and McClure RD .(eds.). Williams and Wilkins , Baltimore , USA ,268-276.
- Dowson, C.G. and Coffey T.J.(2000). β -lactam resistance mediated by changes in penicillin-binding proteins. In: Woodford, N. and

References

- Johnson A.P.(eds). Molecular bacteriology: protocols and clinical applications. Humana press.Ic., vol. 15 ,pp537-553,Totowa,N.J. USA.
- Dreschel, H., Freund S., Nicholson G., Haag H., Jung O., Zähler H., and Jung G.(1993). Purification and chemical characterization of staphyloferrin B, a hydrophilic siderophore from staphylococci. *BioMetals* , 6:185-192.
- Dulawa, J., Poland K. and Katowice J.(2003). Urinary tract infection and metabolic diseases. *Ann. Academia Medicae Bialotocensis*. 10(3):45-50.
- Dumarche, P. , Dechamps C. , Sirot D. , Chanal C. , Bonnet R. and Sirot J.(2002).TEM derivative–producing *Enterobacter aerogenes* Strains: dissemination of a prevalent clone. *Antimicrobiol. Agent Chemother.* , 46(4): 1128 – 1131.
- Edmondson, A.S. and Cooke E.M.(1979).The development and assessment of a bacteriocin typing method for *Klebsiella*. *J. Hyg. Camb.*, 82 :207-233.
- Eggert-Kruse ,W. , Rohr G. and Probst S.(1998). Antisperm antibodies and microorganisms in genital secretions—a clinically significant relationship. *Andrologia*.; 30(1):61 -71.
- Eiff, A., Peters G. and Heilmannng C.(2002). Pathogenesis due Coagulase-negative *Staphylococci*. *J. Infect. Dis.*, 2: 677-85.

References

- Eiichi,A. , Monden K. , Mitsuata R. , Kariyama R. and Kumon H. .(2004).Biofilm formation among methicillin –resistant staphylococcus aureus isolates from patients with urinary tract infection .Acta. Medica. Okayama. , 58(4):207-214.
- Elbhar,A.(2005).Male genital tract infection: the point of view of the bacteriologist. Gynecol. Obstet. Fertil. , 33(9):691-697.
- El-Shamy, H.A.(1993). Bacteriology of chronic secretory otitis media in children. J. Egypt public health association, Lxv III , 495-505.
- Elshafie, S.S. and Bernardo A.R.(2001).An outbreak of Methicillin Resistant Staphylococcus aureus. Saudi Med. J., 22(9): 815-816.
- Enard, C., Diolez A. and Expert D.(1988). Systemic virulence of *Erwinia chrysanthemi* 3937 requires a functional iron assimilation system. J. Bacteriol. , 170:2419-2426.
- Fakhouri, S. (1980) .Semen characters of infertile patients. In: Infertility of Men and Women its causes and Treatment. Fakhouri , S. (ed.). Beirut, Lebanon, pp 117- 124.
- Fakhrildin, M.B. (2000). Fertilization potential of fertile and infertile human spermatozoa in vitro by application of sperm penetration assay and seminal fluid analysis. PhD Thesis. College of science AL- Mustansiriya University, PP: 154-163.
- Falkow, S.A. and Collinins C.M.(1990). Genetic analysis of *Escherichia coli* urease genes. J. Bact. , 127(12): 7138-44.
- Fauser, B.C. , Bogers J.W. , Hop W.C. and De Jong F.H.(1990). Bioactive and immunoreactive FSH in serum of normal and oligospermic men. Clin. Endocrin. , 32: 433- 442.

References

- File, T.M.(2004).Streptococcus pneumoniae and community-acquired pneumonia: a cause for concern. Am. J. Med. ,117(3):39-50.
- Finlay, B.B. and Falkow S.(1989).Common themes in microbial pathogenicity. Microbiol. Rev. , 53:210-230.
- Folstad, I. and Skarstein F.(1997). Is male germ line control creating avenues for female choice? Behav. Ecol. , 8:109-112.
- Forbes ,B.A. , Daniel F.S. and Alice S.W.(2007).Bailey and Scott's Diagnostic microbiology . 12th ed. ,Mosby Elsevier company , USA .
- Ford, W.C.L. , Williams K.M. , Mclaughlin E.A. , Harrison S., Ray B. and Hull M.G.R.(1996).The indirect immuno bead test for seminal antisperm antibodies and fertilization rates at in vitro fertilization. Hum. Reprod. , 11: 1418- 1422.
- Foster, T.J.(2004).The Staphylococcus aureus “superbug”. J. Clin. Invest. , 114:1693–1696.
- Fournier, P.E., Vallenet D., Barbe V., Audic S., Ogata H., Poirel L., Richet H., Robert C., Mangenot S., Abergel C., Nordmann P., Weissenbach J., Raoult D. and Claverie J.M.(2006). Comparative genomics of multidrug resistance in Acinetobacter baumannii. PLOS Genet. , 2:7.
- Fraczek ,M. , Szumala A.K., Jedrzejczak P. , Kamieniczna M. and Kurpisz M.(2007).Bacteria trigger oxygen radical release and

References

- sperm lipid peroxidation in vitro model of semen inflammation .
Fertil. Steril., 88(40):1076-1085.
- Freund, M.J. and Peterson R.N.(1976).Semen evaluation and fertility. In:
Human Semen and Fertility Regulation in Men. Hafez, E. S. E.
(eds.), The C. V. Mosby company, Saint Louis.; PP: 344- 354.
- Freund, M.J.(1968).Semen and Lysis. In: Progress in Fertility. Behrman,
S. J. and Kistner, R. W. (eds.), Boston, Little Brown.; P. 593.
- Galetto, D.W. , Johnston L.J. and Archer G.L.(1987).Molecular
epidemiology of trimethoprim resistance among coagulase-
negative staphylococci. Antimicrob. Agents Chemother.
31:1683–1688.
- Galimand, M. , Lambert T. and Courvalin P.(2005).Emergence and
dissemination of a new mechanism of resistance to
aminoglycosides in gram- negative bacteria: 16s rRNA
methylation. J. Biol. Chem. ,10(1):243-331.
- Ganong, W.F., (1981): Pulmonary function. In: Review of medical
physiology. Ganong, W. F. (eds.), Appleton and Lange.
California.; PP: 507-519.
- Gdoura, R. , Kchaou W. , Znazen A., Chakroun N., Fourati M.,
Ammar-Keskes L. and Hammami A.(2008).Screening for
bacterial pathogens in semen samples from infertile men with
and without leukocytospermia.;40(4):209-218.
- Gerischer, U.(2008).Acinetobacter Molecular Biology, 1st edi., *Caister
Academic Press*.

References

- Ghoshal, U. , Prasad K.N. , Singh M. , Tiwari D.P. and Ayyagari A.(2004).A comparative evaluation of phenotypic and molecular methods for the detection of oxacillin resistance in coagulase negative staphylococci. *J. Infect. Chemother.* 10:86–89.
- Gillespie, M.T. , May J.W. and Skurray R.A.(1985).Antibiotic susceptibilities and plasmid profiles of nosocomial MRSA. A retrospective study. *J. Med. Microbiol.* , 17: 295-310.
- Go, J. and Cunha B.A.(1999).Acinetobacter baumannii: Infection control implications. *Infect. Dis. Pract.* , 23:65-68.
- Goel, U.K. and Kapil A.(2001).Monoclonal antibodies against the iron regulated outer membrane proteins of Acinetobacter baumannii are bactericidal. *B.M.C. Microbiol* 1:16.
- Golshani, M., Taheri S., Eslami G., Suleimani A.A. , Fallah F. and Goudarzi H.(2006).Genital tract infection in asymptomatic infertile men and its effect on semen quality. *Iranian J. Publ. Health* , 35(3):81-84.
- Gonsales G.F., Kortebani G. and Mazzoli A.B.(1992).Leukocytospermia and function of the seminal vesicles on seminal quality .*Fertile. Sterile.*, 57:1058-1065.
- Goran, S.D.(2001).New prospect for chronic prostatitis. *J. Acta. Clin. Croat. Chinese.*, 40: 109-16.

References

- Gouaux, E. (1997).The long and short of colicin action; the molecular basis for the biological activity of channel-forming colicins. *Structure* , 5: 13-77.
- Graciela, M. , Vignolo M., Kairuz M., Aida A.P., Ruiz H., and Oliver G. .(1995).Influence of growth conditions on the production of lactocin 705, a bacteriocin produced by *L. casei* CRL 705, *J. Appl. Bacteriol.*, 78:5-10.
- Gray, G.L., Smith D.H., Baldrige J.S., Harkins R.N., Vasil M.L. , Chen E.Y. and Heyneker H.L. (1999).Cloning, nucleotide sequence, and expression in *Escherichia coli* of the exotoxin A structural gene of *Pseudomonas aeruginosa*. *Proc. Nat. Acad. Sci. USA* 81:2635-2649.
- Greenspan, F.S and Gardner D.G.(2001).Pancreatic Hormones and Diabetes Mellitus by Masharani, U., and Karam, J. H. In: *Basic and Clinical Endocrinology*. 6th edition. , p. 692.
- Green wood, D., Slack R.C. and Pentherer J.F.(2002).Medical Microbiology, A guide to microbial infections, pathogenesis, immunity, laboratory diagnosis and control 16th edi. Churchill Livingstone.
- Griffiths, E. , Chart M. and Stevenson P.(1988).High affinity iron uptake systems and bacterial virulence. p. 121-137. In. Roth , J.A. (ed.),*Virulence mechanisms of bacterial pathogens*. American Society for Microbiology, Washington, D. C.

References

- Gupta, K. , Hooton M. and Stamm W.(2001). Increasing antimicrobial resistance and the management of community-acquired urinary tract infections. *J. Ann. Int. Med.* 135: 41-50.
- Hacker, J. and Goebel W.(1987).Mechanism and methods for analyzing pathogenicity. *Swiss Biotechnol.* , 5:21-31.
- Hafez, E.S.E. and Prasad M.R.N.(1976): Functional aspects of the epididymis. In: Human semen and Fertility Regulation in Men. Hafez, E. S. E. (ed.), The C. V. Mosby Company. Saint Louis; pp. 31-43.07904396024
- Hafez, E.S.E.(1977).Introduction to andrology .physico-anatomical parameters of andrology .In: Techniques of human andrology. Hafez E.S.E. (eds.).Elsevier –North-Holland Biochemical Press ,USA ,3-79.
- Haag, H., Fiedler H.P., Meiwes J., Drechsel H., Jung G., and Zähler H.(1994).Isolation and biological characterization of staphyloferrin B, a compound with siderophore activity from staphylococci. *FEMS Microbiol. Lett.* , 115:125-130.
- Hagberg, L. ,Jodal U. , Korhonen T. , Lidin G. ,Lindberg U. and Eden C.(1981).Adhesion haemagglutination and virulence of *Escherichia coli* causing urinary tract infections. *J. Infect. Immuno.* , 31(2) :564-570 .
- Haimi, Y. , Vellozzi E.M. and Rubin L.G. (2002). Initial concentration of *Staphylococcus epidermidis* in simulated pediatric blood cultures correlates with time to positive results with the

References

- automated, continuously monitored BACTEC blood culture system. *J. Clin. Microbiol.*, 40:898-901.
- Hales, D.B., Diemer T. and Hales K.H.(1999). Role of cytokines in testicular function. *Endocrine.* , 10:201 -217.
- Hall ,S.L.(1991).Coagulase-negative staphylococcal infections in neonates. *J. Infect. Dis.* ,10 : 50-67 .
- Hall, R.M.(1997).Mobile gene cassettes and integrons: moving antibiotic resistance genes in gram-negative bacteria. *Ciba. Found. Symp.* 207:192-202.
- Hanberger, H. , Diekema D. , Fluit A. , Jones R. , Struelens M. , Spencer R. and Wolff M.(2001). Surveillance of antibiotic resistance in European ICUs. *J. Hosp. Infect.* , 48:161–176.
- Hancock, I.E. and Gilmore M.S.(2000). Pathogenicity of enterococci. In : Fischetti, V.; Novick,R.; Ferretti, J.; Poruoy, D.; and Rood, J. Gram positive pathogens. ASM publications, Washington, USA. pp. 751-758.
- Hancock, R.E. and Spreet D.P.(2000).Antibiotic resistance in *P.aeruginosa*: mechanism and impact on treatment. *Drug Resistance updates. Antimicrob Agents Chemother.*, 3:247-255.
- Harris, O., Shelper D.W. , Bohnsack J.F. and Rubens C.E.(2003).A novel Streptococcal protease promotes virulence, resistance to opsonophagocytosis, and cleavage of human fibrinogen. *J.Clin. Invest.*, 111(1):61-1071.

References

- Heikens, E.A., Fler A.H., Flor D.A. and Pavw N.B.(2005). Comparison of genotypic and phenotypic methods for identification of Coagulase-negative Staphylococci . J. Clin. Microbiol., 43:2286-2290.
- Henkel, R. and Schill W-B.(1998). Sperm separation in patients with urogenital infections. Andrologia. , 30(1):91 -97.
- Hill, J.A. , Haimovici F. , Politch J.A. and Anderson D.J.(1987).Effects of soluble Products of activated lymphocytes and macrophages (Lymphokines and monokines) on human sperm motion parameters. Fertil. Steril., 47: 460-465
- Hill, J.A., Cohen J. and Anderson D.J.(1989).The effects of lymphokines and monokines on sperm fertilizing ability in the zona-free hamster egg penetration test. Am. J. Obstet. Gynecol.; 160:1154 -1159.
- Hiramatsu, K. , Hanaki H. , Ino T. , Yabuta K. , Oguri T. and Tenover F.C. (1997).Methicillin-resistant Staphylococcus aureus clinical strain with reduced vancomycin susceptibility. *J. Antimicrob. Chemother.* , 40 (1): 135-136.
- Hogg ,H.(2005).Essential microbiology John Wiley & Sons Ltd, 1st ed. , England ,p353-364.
- Hooper, D.C.(2005).Efflux pumps and nosocomial antibiotic resistance: a primer for hospital epidemiologists. Clin. Infect. Dis. ,40: 1811–1817.
- Hpa, S.V.(2003). Acinetobacter species in bacteremia in North Ireland. J. Infect. Dis. 23: 456-60.

References

- Huda, A.R., Burkarie M.D. and Ibrahiem M.S.(2001).Antimicrobial resistance among pathogens causing complicated UTIs. Saudi Arabia . J. Infect. Med., 18(7): 358-362.
- Huges, C.F.(1996). Maturation and export of E.coli hemolysin. J. Med. Microbiol. Immun. , 185 (6):65.
- Hull, M.G.R. , Glazener C.M.A., Kelly N.J., Coway D.I., Faster P.A., Hinton R.A., Coulson C., Lambert P.A., Watt E.M. and Desai K.M.(1985).Population study of causes, treatment and outcome of infertility. Br. Med. J. ,291: 1693- 1697.
- Humphreys, H.(2004). Staphylococcus. In Greenwood, D. , Slack, R. and Penethrer, J. (eds.)(2004). Medical Microbiology, a guide to microbial infections: pathogenesis, immunity ,laboratory diagnosis and control. 6th ed. , Churchill Livingstone , London. pp. 174-188 .
- Huwe, P., Diemer T., Ludwig M., Lui J., Schiefer H.G. and Weidner W.(1998).Influence of different uropathogenic microorganisms on human sperm motility parameters in an in vitro experiment. Andrologia. , 30(1):55 -59.
- Hyde, R.M.(2000). Immunology. 4th ed. Lippincott-Williams and Wilkins , Awlterklumer company , USA .
- Ikechukwu ,O. ,E. George , A.E. Sabinus and O. Florence .(2007). Role of enriched media in bacterial isolation from semen and effect of microbial infection on semen quality. Pak. J. Medic. Sci. , 23(6): 885-888 .

References

- Infectious Diseases and Immunization Committee, Canadian Pediatric Society (CPS). (1999). *Pediatrics & Child Health*, 4(5):337-341.
- Irvine, D.S. (1998). Epidemiology and etiology of male infertility. *Hum. Reprod.*, 13:33- 44.
- Ito, T., Katayama Y., Asada K., Mori N., Tsutsumimoto K. , Tiensasitorn C. and Hiramatsu K.(2001). Structural comparison of three types of Staphylococcal cassette chromosome mec integrated in the chromosome in Methicillin-resistant *Staphylococcus aureus*. *J. Antimicrob. Agents Chemother.*, 45:1323-1336.
- Jack, R.W. , Tagg J.R. and Ray B.(1995). Bacteriocins of Gram-positive bacteria. *Microbiol. Rev.*, 59: 171-200.
- Jacobs, M.R.(2004). *Streptococcus pneumoniae*: epidemiology and patterns of resistance. *Am. J. Med.*, 117(3):3-15.
- Jacoby ,G.A. , Walsh K.E. , Mills D.M. , Walker J. , Oh H. , Robicsek A. and Hooper D.C.(2006). A qnrB ,another plasmid –mediated gene for quinolone resistance. *Antimicrob. Agents and Chemother.*,50(4):1178-1182.
- Jacoby, G.A. and Munoz-Price L.S.(2005).The new b-lactamases. *N. Engl. J. Med.* ,352:380–391.
- Jaffe, S. B. and Jewelewicz R.(1991).The basic infertility investigation. *Fertil. Steril.* , 56: 599- 613.
- Jalaluddin, S. , Devaster J.M. , Scheen R. , Gerard M. and Butzler J. (1998).Molecular Epidemiological study of nosocomial *Enterobacter aerogenes* isolation a Belgian Hospital .*J. Clin. Microbiol.* , 36(7): 1846 – 1852.

References

- James, R. , Kleanthous C. and Moor G.R.(1996). The biology of *E. coli* colicins : paradigms and paradoxes. *J. Microbiol.*, 142:1569-80.
- James-Holmquest, A.N. , Swanson J., Buchanan T.M. , Wend R.D. and Williams R.P.(1974).Differential attachment by piliated and nonpiliated *Neisseria gonorrhoeae* to human sperm. *Infect. Immun.* , 9:897-902.
- Jann B., and Jann K.(1990). Structure and biosynthesis of the capsular antigens of *Escherichia coli*. *Curr. Top. Microbiol. Immunol.* ,150:19–42.
- Jarlier, V. , Fosse T. and Philipon A.(1996). Antibiotic susceptibility in aerobic Gram-negative bacilli isolated in intensive care units in 39 French teaching hospitals (ICU study). *Intensive Care Med.*, 22:1057-1065.
- Jarlov, J.O.(1999).Phenotypic characteristics of CoNS typing and antibiotic susceptibility. *APMIS* , 91:1-42.
- Jedrzejczak ,P. , Szumata A. , Dydowicz P. and Pisarski T.(1996). Usefulness of counting leukocytes and round cells in determination of bacterial infection of semen in infertile men. *Ginekol. Pol.* , 67(11):569-573.
- Jevons, M.P.(1961).Celbenin resistant *Staphylococci*.*Br. Med. J.* 14(1):124-125.
- Jiao, Y., Long S.D. and Grange W.X.(2002).The observation of chronic prostatitis using national institutes of health. *J. Hosp. Chin. Acad. Trad. Med.*, 8 (2): 127-129.

- Ji-Hong, L., Hao-Yong L. , Zheng-Guo C., Yong-Fang D. , Yang L. and Zhang-Qun Y.(2002).Influence of several uropathogenic microorganisms on human sperm motility parameters in vitro. *Asian J. Androl.*, 4: 179-182.
- Joe, S. , Downey J. , Nickel C. and Hunter D.(2000). Prostatitis selected abstracts. *J. Jacob Rochester.* , 14: 345-350.
- Joel, D., Schilling A.E., Scott F.G. and Hutgren S.N.(2002).Recent advances into the pathogenesis of recurrent urinary tract infection caused by uropathogenic E.coli. *J. Coligi. Int. Antimicrob. Agents.* ,19: 454-60.
- Johnson, J.R.(1991). Virulence factors in *Escherichia coli* urinary tract infection. *Clin. Microbiol. Rev.* , 4:80-121.
- Johnson, A.P., Aucken H.M. , Cavendish S., Ganner M., Wale M.C., Warner M., Livermore D.M. and Cookson B.D.(2001). "Dominance of EMRSA-15 and -16 among MRSA causing nosocomial bacteraemia in the UK: analysis of isolates from the European Antimicrobial Resistance Surveillance System (EARSS)". *J. Antimicrob. Chemother.* , 48 (1): 143-144.
- Jones, T.F. , Kellum M.E. , Porter S.S. , Bell M. and Schaffner W. (2002).An outbreak of community-acquired food borne illness caused by Methicillin-resistant *Staphylococcus aureus*. *Emerg. Infect. Dis.*, 8:82-84.
- Jonson , A.B. , Normak S. and Rhen M.(2005).Concepts in bacterial virulence. *J. Karger Basel* , 12:67–89.

- Joseph, F.J. and Alexander M.H.(2007).History and evolution of antibiotic resistance in coagulase-negative staphylococci: Susceptibility profiles of new anti-staphylococcal agents. *Ther. Clin. Risk. Manag.* , 3(6): 1143–1152.
- Jukka, A., Flower S. and Susan J.(1995).Multiresistant in *Staphylococcus* species in bacterial prostatitis. *J. Drugs.* , 30: 123-128.
- Karlowsky, A.J., Dragh D.C. and Jones M.E.(2003).Antimicrobial susceptibility among clinical isolates *Pseudomonas aeruginosa* and *Acinetobacter baumannii* from hospitalized patients in the United States. *J. Antimicrob. Agents Chemother.*, 47: 1681-88.
- Karlsson, A. and Arvidson S.(2002).Variation in extracellular protease production among clinical isolates of *S.aureus* due to different levels of expression of the protease repressor SarA. *Infect. Immun.* 70 (8): 4239 – 4246.
- Karlsson, A. , Saravia-Otten P., Tegmark K., Morfeldt E. and Arvidson S.(2001)Decreased amounts of cell wall-associated protein A and fibronectin-binding proteins in *Staphylococcus aureus* SarA mutants due to up-regulation of extracellularprotease. *Infect. Immun.* 69: 4742 – 4748.

References

- Kay, D.J. , Clifton V. and Taylor J.S.(1993).Anti- sperm antibodies and semen profiles in reanastomosed men. *Reprod. Fertil. Dev.* ,5: 135- 139.
- Keck, C., Gerber-Schafer C., Clad A., Wilhelm C. and Breckwoldt M.(1998).Seminal tract infections: impact on male fertility and treatment options. *Hum. Reprod. Update.* , 4(6): 891-903.
- Klaenhammer, T.(1993) Genetics of bacteriocins produce by lactic acid bacteria. *FEMS Microbiol. Rev.*, 12:39-85.
- Klligore, D., March K.L. and Blance B.J.(2004).Risk factors for community–acquired ciprofloxacin resistant E.coli urinary tract infection. *J. Ann. Pharmacol.* , 38(7): 1148-1152.
- Kloss, W.E. and Bannerman T.L.(1994).Up date on clinical significance of coagulase negative Staphylococci. *Clin. Microbiol. Rev.* , 7(11):117-120
- Kohn , F-M. , Erdmann I., Oeda T. , El-Mulla K.F. , Schiefer H.G. and Schill W-B.(1998).Influence of urogenital infections on sperm functions. *Andrologia.* , 30(1):73 -80.
- Kolettis ,P.N. (2003). Evaluation of the Subfertile Man . *Amr. Fam. Physician J.* , 67(10): 2165-2173.
- Kollef, M.H. and Fraser V.J. (2001).Antibiotic resistance in the intensive care unit. *Ann. Intern. Med.* , 134:298–314.
- Konetschny-Rapp, S., Jung G., Meiwes J. and Zähler H.(1990). Staphyloferrin A: a structurally new siderophore from staphylococci. *Eur. J. Biochem.*, 191:65-74

References

- Konino, M.H.(1995). Nosocomial infections caused by MRSA in Japan. *J. Infect. Dis.* , 1: 30-39.
- Kornelius, Z., Christin R., Silke I.P. and Volkmar B.(2008). Crystal structure of colicin M, a novel phosphatase specifically imported by *Escherichia coli*. *J. Biol. Chem.* ,10:1074.
- Khorshed ,P.A.(2005).Bacteriological study of some pathogens causing urinary tract infection in pateints attending Azadi general hospital in Kirkuk city.Msc thesis ,College of Education – Tikret Univercity.Iraq.
- Kropp, H. , Gerckens L., Sundelof J.G., Kahan F.M.(1985). Antibacterial activity of imipenem; the first thienamycin antibiotic. *Rev. Infect. Dis.* , 7(3): 389-410.
- Kruger, T.F. , Acosta A.A. , Simmons K.F. , Swanson R.J. , Matta J.F. and Oehninger S.(1988).Predictive value of abnormal sperm morphology in vitro fertilization. *Fertil. Steril.* , 49:112- 116.
- Kurt , G . and Naber I.(2001).Which fluoroquinolones are suitable for the treatment of urinary tract infection . *Intern. .J. Antimicrob. Agent.* , 17: 331-341 .
- Lackner , J. , Schatzl G. , Horvath S. , Kratzik C. and Marberger M.(2006).value of counting white blood cells in semen samples to predict the presence of bacteria . *Eur Urol.* , 49(1) :148-152 .

- Lamirande, E. and Gagnon C.(1992).Reactive oxygen species and human spermatozoa. I. Effects on the motility of intact spermatozoa and on sperm axonemes; and II. Depletion of adenosine triphosphate plays an important role in the inhibition of sperm motility. *J. Androl.*, 13:368-386.
- Leclercq, R.(2002).Mechanisms of resistance to macrolides and Lincosamides: nature of the resistance elements and their clinical implications. *Clin. Infect. Dis.* ,34:482–492.
- Lee, J.C.(2000). Microbiology of prostate. *J. Clin. Dis.* , 1:159- 163.
- Levy, S.B.(2002)From tragedy the antibiotic era is born. In: Levy, S.B., (ed.) *The Antibiotic Paradox: How the Misuse of Antibiotics Destroys Their Curative Powers*, 2nd edi. Cambridge, MA: Perseus Publishing , pp. 1–14.
- Levy, S.B. and Marshall B.(2004).Antibacterial resistance worldwide: causes, challenges and responses. *Nat. Med.* , 10:122-129.
- Li, H.Y. and Liu J.H.(2002).Influence of male genital bacterial infection on sperm function. *Zhonghoa. Nan. Ke. Xue.* , 8(6): 442-444.
- Lind, I.(1990)Epidemiology of antibiotic resistant *Neisseria gonorrhoeae* in industrialized and developing countries. *Scand. J. Infect. Dis.*, 69:77–82.
- Lipsky, B.X.(2003). Prostatitis and urinary tract infection in men. *J. Am. Med. Urol.* ,106 (3): 327-34.

References

- Lisa, T.A. , Lucchesi G.I., and Domenech C.E.(1994).Pathogenicity of *Pseudomonas aeruginosa* and its relationship to the cholin metabolism through the action of cholinesterase, acid phosphatase and phospholipase. *J. Curr. Microbiol.*, 29:193-199.
- Litwin, C.M. and Calderwood S.B.(1993).Role of iron in regulation of virulence genes. *Clin. Microbiol. Rev.*, 6:137-149.
- Liu ,G.Y., Essex A. , Buchanan J.T. , Datta V. , Hoffman H.M. , Bastian J.F. , Fierer J and Nizet V.(2005).Staphylococcus aureus golden pigment impairs neutrophil killing and promotes virulence through its antioxidant activity. *J. Exp. Med.*, 202 (2): 209-215.
- Lowy, F.D.(2003).Antimicrobial resistance: the example of *Staphylococcus aureus*. *J. Clin. Invest.*,111:1265–1273.
- Lu, P.L., Chin L.C., Peng C.F., Chiang Y.H., Chen T.P., Ma L. and Siu L.K.(2005).Risk Factors and Molecular Analysis of Community Methicillin-Resistant *Staphylococcus aureus* Carriage. *J. Clin. Microbiol.* , 43(1): 132-139.
- Lucia ,E. ,Satorres S. , Lucero M. and Olga N.(2003).Species identification ,slim production ,slim production and oxacillin susceptibility in CoNS isolated from nosocomial specimens. *Braz. J. Microbiol.*, 34:45-51.
- MacFaddin ,J.F.(2000).Biochemical tests for the identification of medical bacteria. 3rd ed., The Williams. and Wilkins-Baltimor,USA.

References

- Macleod, J., and Wang Y.(1979). Male fertility potential in term of semen quality; A review of the past, a study of the present. *Fertil. Steril.*, 31: 103-116.
- Mack ,D. , Rohde H. , Dobinsky S. , Riedewald J. , Nedelmann M. , Knobloch J.K.M. , Elsner H.A. and Feuchet H.H. (2000).Identification of three essential regulatory gene loci governing expression of *S. epidermidis* polysaccharides intercellular adhesion and biofilm formation. *Infect. Immun.* , 8:3799- 3807.
- Madani, T.A., Al Abdullah N.A. , Al Sanous A.A., Ghabrah T.M., Afandi S.Z. and Bajunid H.A.(2001). Methicillin-resistant *Staphylococcus aureus* in two tertiary-care centers in Jeddah, Saudi Arabia. *Infect. Control Hosp. Epidemiol.*, 22:211-216.
- Maes ,P. and Vanhoof R.(1992). A 56-month prospective surveillance study on the epidemiology of aminoglycosides resistance in a Belgian general hospital . *Scand. J. Infect. Dis.* , 24: 495-501.
- Maguire, G.P., Arthur A.D. , Boustead P.J., Dwyer B. and Currie B.J. (1998).Clinical experience and outcomes of community-acquired and nosocomial Methicillin-resistant *Staphylococcus aureus* in a northern Australian hospital. *J. Hosp. Infect.*, 38:273-281.
- Mallea, M. , Chevalier J., Bornet C., Eyraud A., Davin A., Bollet C., and Pages J.M.(1998).Porin alteration and active efflux: two in-vivo drug resistance strategies used by *Enterobacter aerogenes*. *J. Microbial.*, 144: 3003 – 3004.

References

- Marco, C.A. and Parker K.G. (1997). Antimicrobial resistance among organisms causing urinary tract infections. *Acad. Emerg. Med.* 4: 154-160.
- Maria, A.R. , Zeus S., Aysen L.E., and Valerio M.N. (2007). Commensal and pathogenic *Escherichia coli* use a common pilus adherence factor for epithelial cell colonization. *Proc. National Acad. Sci. U S A.*
- Marshburn, P.B. (1997). Antisperm antibodies and infertility. *Infer. & Reprod. Medicine. Clin of North America.*, 8: 243- 286.
- Martinez, J.K. , Cercenado E. , Baquero F. , Perez J.C. , Diaz and Delgado-Iribarren A. (1987). Incidence of aerobactin production in gram-negative hospital isolates. *FEMS Microbiol. Lett.* 43:351-353.
- Marty, J.J, Robert R.S. and Jone F.A. (2000). Type 1 pilus-mediated bacterial invasion of bladder cells. *J. Embo. Bact.* , 19(3): 2803.
- Mazmanian, S.K., Skaar E.P., Gaspar A.H., Humayun M., Gornicki P., Jelenska J., Joachmiak A., Missiakas D.M., and Schneewind O. (2003). Passage of heme-iron across the envelope of *Staphylococcus aureus*. *Science* , 299:906-909
- Mazodier, P. and Davies J. (1991). Gene transfer between distantly related bacteria. *Ann. Rev. Genet.*, 25:147-171.
- Mazumdar, S. and Levine A.S. (1998). Antisperm antibodies: Etiology pathogenesis, diagnosis and treatment. *Fertil. Steril.*, 70:799- 810.

References

- McClure, R.D.(1992).Male infertility. In: Smith's General Urology. Tanagho, E.A., and Mc Aninch, J.W. (eds.) Lange Medical Book, California , pp. 669- 695.
- Meiwes, J., Fiedler H.P., Haag H., Zähler H., Konetschny-Rapp S. and Jung G.(1990). Isolation and characterization of staphyloferrin A, a compound with siderophore activity from *Staphylococcus hyicus* DSM 20459. FEMS Microbiol. Lett. , 67:201-206.
- Mellenkvist, A., Nordst T. , Hallden C. and Christenses J.J. (2003).The *Moraxella catarrhalis* Immunoglobulin D-Binding protein MID has conserved sequences and is regulated by a mechanism corresponding to phase variation. J. Bacteriol. , 185(7):2285-2295.
- Menichetti, F.(2005).Current and emerging serious Gram-positive infections". *Clin. Microbiol. Infect.* , 11(3): 22-28.
- Menkveld, R. and Kruger T.F.(1998).Sperm morphology and male urogenital infection. *Andrologia.* , 30(1):49 -53.
- Merino,G. , Carranza L.S. , Murrieta S. , Rodriguez L. , Cnevas E., and Moran C.(1995). Bacterial infection and semen characteristics in infertile men. *Arch. Androl.* , 35(1) :43-47.
- Meskin, I. (1998). *Staphylococcus epidermidis*, Pediatrics in Review. American Academy of Pediatrics, 19(3):12-15.
- Meyer, J.M., Neely A., Stintzi A., Georges C. and Holder I.A.(1996). Pyoverdine is essential for virulence of *Pseudomonas aeruginosa*. *Infect. Immun.* , 64:518-523

- Michalek, M., Mold C. and Brenner E.(1988).Inhibition of the alternative pathway of human complement by structural analogues of sialic acid. *J. Immun.*, 140:1588–1599.
- Michelmann, H.W.(1998).Influence of bacteria and leukocytes on the outcome of in vitro fertilization (IVF) or intracytoplasmic sperm injection (ICSI). *Andrologia.*, 30(1):99-101.
- Micic, S., Petrovic S. and Dotlic R.(1990). Seminal antisperm antibodies and genitourinary infection. *Urology* , 35:54-56.
- Milingos ,S. , Comhaire F.H., Liapi A. and Aravantinos D.(1996). The value of semen characteristics and tests of sperm function in selecting couples for intra-uterine insemination. *Eur. J. Obstet. Gynecol. Reprod. Biol.*, 64:115 -118.
- Mims, C., Docknell H.M., Goering R.V. and Roitt I.(2004). *Medical microbiology*. 3rd ed, Elsevier Limited.
- Miniatis, T., Frtsch E. and Sambrook J. (1982).Laboratory Manual. Cold Spring . Harber laboratory press .New York.
- Modun, B. , Kendall D. and Williams P.(1994). Staphylococci express a receptor for human transferrin : identification of a 42-kilodalton cell wall transferrin-binding protein. *Infect. Immun.*, 62:3850-3858.
- Modun, B., Evans R.W., Joannou C.L., and Williams P.(1998). Receptor-mediated recognition and uptake of iron and human transferrin by *Staphylococcus aureus* and *Staphylococcus epidermidis*. *Infect. Immun.* , 66:3591-3596.

References

- Moellering, G.F., Green D.J. and Stare H.L.(2002). Staphylococci as uropathogens-frequency of isolation in hospitalized patients and sensitivity to antimicrobial agents. J. Ann. Urol. Paris. 36(5): 341-7.
- Mogra, N.N. , Dhruva A.A., and kothari L.K.(1981).Non-specific seminal tract infection and male infertility. J. Postg. Med. , 27(2):99-104.
- Mokracka, J., Koczura R. and Kazaonwski I.(2004). Yersiniabactin and other siderophores produced by clinical isolates of *Enterobacter* spp . and *Citrobacter* spp .FEMS. Immunol. Med . Microbiol., 40:51-55.
- Monga, M. and Roberts J.A.(1994).Spermagglutination by Bacteria: Receptor-Specific Interactions. J. Androl.,15:151-156
- Mongkolrattanothai ,K. , Boyle S. and Murphy T.V.(2004). Novel non-*mecA*-containing staphylococcal chromosomal cassette composite island containing *pbp4* and *tagf* genes in a commensal staphylococcal species: a possible reservoir for antibiotic resistance islands in *Staphylococcus aureus*. *Antimicrob. Agents Chemother.* , 48:1823–1836.
- Montagut, J.M. , Lepretre S., Degoy J. and Rousseau M.(1991). Ureaplasma in semen and IVF. Hum. Reprod., 6:727-729.
- Morales, P. Katz D.F. , Overstreet J.W., Samueles S.J. and Chang R.J.(1988).The relationship between the motility and morphology of spermatozoa in human semen. J. Androl. , 9: 241- 247.

References

- Morine, C.A. and Hadler J.L. (2001). Population-Based Incidence and Characteristics of Community-Onset *Staphylococcus aureus* Infections with Bacteremia in 4 Metropolitan Connecticut Areas, 1998. *J. Infect. Dis.* , 184:1029-1034.
- Mota-Meira, M. , Lapointe G. , Lacroix C. and Lavoie M.C.(2000). MICs of Mutacin B-Ny266, Nisin A, Vancomycin, and Oxacillin against bacterial pathogens. *Antimicrob. Agents Chemother.*, 44:24-29.
- Moxon, E.R. and Kroll J.S. (1990). The role of bacterial polysaccharide capsules as virulence factors. *Curr. Topics Microbiol. Immun.* , 150:65–86.
- Munoz, M.G. and Witkin S.S.(1995).Autoimmunity to spermatozoa, asymptomatic *Chlamydia trachomatis* genital tract infection and ST lymphocytes in seminal fluid from the male partners of couples with unexplained infertility. *Hum. Reprod.*,10 : 1070 - 1074.
- Murray, P.R., Niles A.C.(1990). In vitro activity of meropenem (SM.7338), Imipenem, and five other antibiotics against anaerobic clinical isolates. *J. Diag. Microbiol. Infect. Dis.* , 13: 57-61.
- Murray, P.R. , Baron E.J., Jorgensen J.H. ,Pfaller M.A. and Tenover R.H.(2003).Manual of clinical Microbiology. 8th edi., Washington, D.C. ,Vol (1) .

References

- Nair, S.P. Williams R.J. and Henderson B.(2000). Advance in our understanding of the bone and joint pathology caused by *Staphylococcus aureus* infection. *Rheumatology*. 39: 821 – 834
- National Committee for Clinical Laboratory Standards.(1997). Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically. 3rd ed. Approved Standard. Document M7-A4.Natl. Committee Clin. Lab. Stand., Wayne, PA.
- National Committee for Clinical Laboratory Standards.(1999). Performance standards for antimicrobial susceptibility testing; Ninth Informational Supplement. Document M100-S9. Natl. Committee Clin. Lab. Stand., Wayne, PA.
- National committee of clinical laboratory standards (NCCLS).(2002).Performance standards for antimicrobial susceptibility testing. 12th informational supplement .M 100-S12., Wayne, Pannsylvania .22(1) .
- National Nosocomial Infections Surveillance System. (2003). National Nosocomial Infections (NNIS) System report, data summary from January 1992 through June 2003. *Am. J. Infect. Control.*, 31:481-498.
- Neely, A.N. and Maley M.P.(2000).Survival of enterococci and staphylococci on hospital fabrics and plastics . *J. Clin. Microbiol.* ,38:724-726.
- Neilands, J.B. (1995). Siderophores: Structure and function of microbial Iron transport compounds. *J. Biolog. Chem.*, 270(45):26723-26726.

References

- Neto, J.R., Yano T., Beriam L.O.S. and Destefano S.A.L.(2003). Comparative RFLP - ITS analysis between *Enterobacter cloacae* strains isolated from plants and clinical origin. *Arq. Inst. Biol.* , 70(3): 367-372.
- Ng, S. , Bongso A., and Rothan S.S.(1990): Micro manipulation. Its relevance to human in vitro fertilization. *Fertil. Steril.* , 53: 203-219.
- Nickel, J.C. and Costeron J.W.(1992).Coagulase-negative *Staphylococcus* in chronic prostatitis. *J. Urol.* , 147:398-400.
- Nieschlag, E., and Behre H.M.(1997).Cited by Leijonhufvud, P.(1998) Aspects of the Regulation of Human sperm Motility. Published on the Web by the Library of karloinska instituted 171 76 Stockholm.
- Nikkanen ,V., Gronroos M., Suominen J. and Multamaki S.(1979). Silent infection in male accessory genital organs and male infertility. *Andrologia.* , 11:236-241.
- Noble ,W.C. , Virani Z. and Cree R.G. (1992). Co-transfer of vancomycin and other resistance genes from *Enterococcus faecalis* NCTC 12201 to *Staphylococcus aureus*. *FEMS Microbiol Lett* ; 93:195-198.
- Nomura, S. and Nagayama A.(2002). In vitro antibacterial activity of S-4661, a new parenteral carbapenems, against urological

References

- pathogens isolated from patients with complicated urinary tract infections. *J. Chemother.*, 14:155-160.
- Noto, M.J. and Archer G.L.(2006). A subset of *Staphylococcus aureus* strains harboring staphylococcal cassette chromosome *mecA* (SCC*mec*) type IV is deficient in CcrAB-mediated SCC*mec* excision. *J. Antimicrob. Agents Chemother.* , 50:2782–2800.
- Novick, R.P.(2003). Autoinduction and signal transduction in the regulation of *Staphylococcus* virulence. *J. Mol. Microbiol.*, 36: 1429-1449.
- Nser, S., Di Pompeo C., Soubrier S., Delour P., Lenci H., Roussel-Delvallez M., Onimus T., Saulnier F., Mathieu D. and Durocher A.(2005).First generation fluoroquinolone use and subsequent emergence of multiple drug-resistant bacteria in the intensive care unit. *Crit. Care Med.*, 33:283–289.
- Odd, G.B. and Maeland J.A.(1997).Mechanisms of methicillin resistance in staphylococci. *AMPIS.* 105:264–276.
- Oehninger, S. and Alexander N.J.(1991).Male infertility; the focus shifts to sperm manipulation.*Curr. Opin. Obstet. Gyneco.*,3: 182-190.
- Ofek ,I. , Mrelman D. and Sharon N.(1977).Adherence of *E. coli* to human mucosal cells mediated by mannose receptor .*Nature*, 265:623-625.

References

- Ofek, I. , Hasty D.L. and Doly R.J.(2002). Bacterial Adhesion. Am. Soc. Microbiol. Washington, DC. pp.63-69.
- Oksuz, S. , Ozturk E. , Sahin I., Ertor O. and Kaya D.(2005). Biliary infection and bacteremia caused by β -lactamase-positive, Ampicillin-resistant *Haemophilus influenzae* in diabetic patients. J. Infect. Dis. 58 : 34-35.
- Old, D.C. and Adegbola R.A.(1985).Antigenic relationships among type-3 fimbriae of Enterobacteriaceae revealed by immunoelectronmicroscopy. J. Med. Microbiol., 20:113-121.
- Onemu , S.O. and Ibeh I.N.(2001). Studies on the significance of positive bacterial semen cultures in male fertility in Nigeria. Int. J. Fertil. Women Med. , 46(4):210-214.
- Opal, S.M. , Cross A.S. , Gemski P. and Lyhte L.W.(1988). Survey of purported virulence factors of *Escherichia coli* isolated from blood, urine and stool. Euro. J. Clin. Microbiol. Infect. Dis., 7:425-427.
- Ophir, T. and Gutnick D. (1994). A role for exopolysaccharides in the protection of micro-organisms from desiccation. Appli. Environ. Microbiol., 60:740–745.
- Oppenheim, B.A.(1998).The changing pattern of infections in neutropenic patients. J. Antimicrob. Chemother. , 41(4):7–11.
- Orji, I. , Ezeifeaka G. , Amadi E.S. and Okafor F.(2007). Role of enriched media in bacterial isolation from semen and effect of microbial infection on semen quality: A study on 100 infertile men. Pak. J. Med. Sci. ; 23(6):885-888.

References

- Orskov, F., and Orskov I.(1992). *Escherichia coli* serotyping and disease in man and animals. *Can. J. Microbiol.*, 38:699–704.
- Osegbe, D.N. and Amaku E.O.(1985).The causes of male infertility in 504 consecutive Nigerian patients. *Int. Urol. Nephrol.*, 17:349-58.
- Pal, R.B., Rebello J.S. , Banker D.D. and Jain P.K.(1997).Klebsiella typing of *Klebsiella* species isolated from intensive care unit. *J. Postgrad. Med.*, 43(4):98-101.
- Palayekar, V.V., Joshi J.V., Hazari K.T., Shah R.S. and Chitlange S.M.(2000). Comparison of four nonculture diagnostic tests for *Chlamydia trachomatis* infection. *J. Assoc. Physicians India.*, 48:481 -483.
- Pankuch, G.A. and Appelbaum P.C.(2006). Postantibiotic effect of ceftibiprole against 12 Gram-positive organisms. *J. Antimicrob. Agents Chemother.* , 50:3956–3958.
- Pardo, M., Barri P.N. , Bancells N. , Coroleu B. , buxaderas C. , Pomerol J.M. and Shabater J.(1988).Spermatozoa selection in discontinuous percoll gradients for use in artificial insemination. *Fertil. Steril.* , 49: 505-509.

References

- Park ,Y.J. , Lee S. , Park J.J. , Park K.G. , Kim B.K. and Kang C.S. .(2003).antimicrobial susceptibility of inducible AmpC β -lactamase-producing *E. Cloacae* , *citrobacter freundii* and *serratia marcescense*. Korean survey department of clinical pathology . 51(4): 265-269.
- Paulsen, C.V.(1974).The testes In: Text Book of Endocrinology. Williams, R. (ed.). W. B. Saunders Company. Philadelphia.; PP 323-367.
- Paulson, J.D. and Polakoski K.L.(1977).Isolation of a spermatozoal immobilization factor from *Escherichia coli* filterates.Fertil. Steril., 28: 182-185.
- Pearce, R. and Roberts I.S.(1995).Cloning and analysis of gene clusters for production of the *Escherichia coli* K10 and K54 antigens: identification of a new group of serA-linked capsule gene clusters. J. Bacteriol., 177:3992–3997.
- Perez, J.L. , Pulido A. , Gomez E. , Sauca G. and Martin R.(1990). Superoxol and anino peptidase tests for identification of pathogenic *Neisseria* species and *Moraxella* (*Branhamella*) *catarrhalis* .Eur. J. Clin. Microbiol. Infect. Dis. ,9(6): 421-423
- Philip, A.S. and Folstad I. (2003). Do bacterial infections cause reduced ejaculate quality? A meta-analysis of antibiotic treatment of male infertility. Behav. Ecolo. ,14(1): 40-47.
- Picazo, J.J.(2004).Management of the febrile neutropenic patient: a consensus conference. Clin. Infect. Dis. ,39(1):1-6.

- Piddock, L.J. , Walters R.N. , Jin Y.F., Turner H.L. , Gascoyne-Binski D.M. and Hawkey P.M.(1997).Prevalence and mechanism of resistance to third-generation cephalosporins in clinically relevant isolates of Enterobacteriaceae from 43 hospitals in the UK, 1990-1991. J. Antimicrob. Chemother., 39:177-187.
- Piret, J. , Millet J. and Demain A.(1983).Production of intracellular protease during sporulation of *Bacillus brevis* . Eur. J. Appl. Microbiol. Biotechnol. , 17: 227-230.
- Pitout, J.D., Thomson K.S., Sandra C.C. and Hanson N.D.(1998). Plasmid-mediated resistance to extended spectrum cephalosporins among *Enterobacter aerogenes* strains. J. Antimicrob. Agents Chemother. 42: 596-600.
- Podschun, R. and Ullmann U.(1998).Klebsiella species. as nosocomial pathogens, epidemiology, taxonomy, typing methods, and pathogenicity factors. Clin. Microbiol. Rev. , 11(4). 589-603.
- Poeta, P., Costa D. , Klibi N. , Rodrigues J. and Torres C.(2006). Phenotypic and genotypic study of gelatinase and beta haemolysis activities in faecal enterococci of poultry in Portugal. J. Vet. Med. Infect. Dis. , 53(5):203- 208.
- Poirel, L., Menuteau O., Agoli N., Cattoen C. and Nordmann P. (2003).Outbreak of extended-spectrum beta-lactamase VEB-1-producing isolates of *Acinetobacter baumannii* in a French hospital. J. Clin. Microbiol. ,41:3542–3547.

References

- Posey, J.E. and Gherardini F.C.(2000). Lack of a role for iron in the Lyme disease pathogen. *Science* , 288:1651-1653.
- Prakash , M. , Rajasekaran K. and Karmegam N.(2007).Prevalence of Methicillin Resistant *Staphylococcus aureus* in Clinical Samples Collected from Kanchipuram Town, Tamil Nadu, South India . *J. Appl. Sci. Research*, 3(12): 1705-1709.
- Pugsley, A.P.(1984).The ins and outs of colicins. *J. Microbiol. Sci.*,1:168-175.
- Purvis, K. and Christiansen E.(1993).Infection in the male reproductive tract: impact, diagnosis and treatment in relation to male infertility. *Int. J. Androl.* , 16:1-13.
- Qiang , H. , Jiang M. , Lin J. and He W.(2007). Influence of enterococci on human sperm membrane in vitro. *Asian J. Androl.*, 9 (1): 77-81.
- Quale, J., Bratu S., Landman D. and Heddurshetti R.(2003).Molecular epidemiology and mechanisms of Carbapenems resistance in *Acinetobacter baumannii* endemic in New York City. *Clin. Infect. Dis.* , 37 :214-220.
- Raad, I. (2000). Management of intravascular catheter- related infections. *J. Antimicrob. Chemother.*, 45: 267-270
- Rachid, S.A., Witte V.M. and Hacker J.Z.(2000). Effect of subinhibitory antibiotic concentration on Intracellular adhesion expression in

References

- biofilm-forming *Staphylococcus epidermidis*. *J. Antimicrob. Agents Chemother.* , 32: 755-760.
- Rahal, J. (2006). Novel antibiotic combinations against infections with almost completely resistant *Pseudomonas aeruginosa* and *Acinetobacter* species. *Clin. Infect. Dis.*, 43(2):95–99.
- Rajasekaran, M., W.J. Hellstrom, R.K. Naz, and S.C. Sikka.(1995). Oxidative stress and interleukins in seminal plasma during leukocytospermia. *Fertil. Steril.*, 64:166-171.
- Rajesh, B. and Rutten I.(2004). Essential medical microbiology. 3rd ed. New Delhi–Indian.
- Raymond, K.N., Dertz E.A and Kim S.S. (2003). Enterobactin: an archetype for microbial iron transport. *Proc. Natl. Acad. Sci. USA.* , 100: 3584-3588.
- Raz, R., Colodner R. and Kunin C.M. (2005). Who are you - *Staphylococcus saprophyticus*? *CID [serial online]*; 40:896-898.
- Rehewy, M.S.E., Hafez E.S.E., Thomas A. and Brown W.J. (1979). Aerobic and anaerobic bacterial flora in semen from fertile and infertile groups of men. *Arch. Andrology*, (2): 238-263.
- Reichart, M., Kahane I. and Bartoov B. (2000). In Vivo and In Vitro Impairment of Human and Ram Sperm Nuclear Chromatin Integrity by Sexually Transmitted *Ureaplasma urealyticum*. *Infect. Biol .Reprod.* 63:1041-1048.

References

- Reichart, M. , Kahane I. and Bartoov B.(2001).Dual energy metabolism-dependent effect of *Ureaplasma urealyticum* infection on sperm activity. *J. Androl.* , 22:404-412.
- Riegel ,P. , Ruimy R. , Briel D., Prevost G. , Jehl F. , Bimet F. , Christen R. and Monteil H.(1995). *Corynebacterium seminali* sp. Nov., new species associated with genital infection in male patients. *J. Clinic. Microbiol.*, 33(9) .p. 2244-2249.
- Riley, M.A. and Gordon D.M.(1992).A survey of col plasmids in natural isolates of *Escherichia coli* and an investigation into the stability of col plasmid lineages. *J. General Microbiol.*, 138:1345-1352.
- Riley, M.A. and Wertz J.E. (2002). Bacteriocins: Evolution, ecology, and applications. *Ann. Rev. Microbiol.* , 56:117-137.
- Rings, T. , Findlay R. and Lang S.(1998). Ethnicity and Methicillin-resistant *S.aureus* in South Auckland. *N. Z. Med. J.*, 111:151.
- Robery, J.A., Scott K.L. and Joe E.T.(1997). Epitopes of the P-fimbrial adhesion of *E.coli* causes of urinary tract infection. *J. Urol.* ,158(9):1610.
- Roberts, J.A.(1992).Role of bacterial adherence in urinary tract infection. *Curr. Opin. Urol.*,2:33-36.
- Roberts, I.S.(1996).The biochemistry and genetics of capsular polysaccharide production in bacteria. *Ann. Rev. Microbiol.*, 50:285–315.
- Roberts, M.C.(1996a).Tetracycline resistance determinants: mechanism of action, regulation of expression, genetic mobility, and distribution. *FEMS Microbiol. Rev.* ,19:1–24.

- Rodin ,D.M. , Larone D. and Goldstein M.(2003).Relationship between semen culture, leukospermia and semen analysis in men undergoing fertility evaluation. *Fertil. Steril.* , 79:(3)1555-1558.
- Rojas, F.J. , Bruzzone E.M. and Moretti- Rojas L.(1991).Regulation of Cyclic AMP (cAMP) synthesis in human ejaculated spermatozoa. (Abstract), *Fertil. Steril.* , 1: 512-513.
- Romero-Vivas, J. ,Rubio M. ,Fernandez C. and Picazo J.J.(1995). Mortality associated with nosocomial bacteremia due to Methicillin resistant *Staphylococcus aureus*. *J. Clin. Inf. Dis.*, 21:1417-1423.
- Romolo, G. , Facep L. , Warcester M.A. and Gideon B.(2004). Complicated urinary tract infection: Risk stratification, clinical evaluation and evidence-based antibiotic therapy for out management. Update. *J. American Health Consultants.*, 68(30): 230-274.
- Rose, N.R.(1978).Endocrine diseases. In: *Basic and clinical Immunology*: Fudenberg, H.H. , Stites D.P. , Caldwell J.L. and Wells J.V. (eds.) Lange Medical Publication. California , pp. 628- 641.
- Rubens, C.E., Wessels M.R.(1987).Transposon mutagenesis of typeIII GBS, correlation of capsule expression with virulence. *Proc Natl Acad Sci USA.* 84:7208-7212.
- Rupp, M.E. and Fey P.D.(2003).Extended spectrum b-lactamase (ESBL)-producing *Enterobacteriaceae*: considerations for diagnosis, prevention and drug treatment. *Drugs* , 63:353–365.

References

- Ryan, K.J. and Ray C.G.(2004). Sherri's Microbiology. 4th ed. McGraw Hill. ISBN .
- Salman,H.D.(2006). A Bacteriological Study of Local Strains of *Enterobacter cloacae* Isolated from Urine Samples.Msc. Thesis .Babylon university .Collage of Medicine Iraq..
- Salmenlinna, S. , Lyytikainen O. and Vuopio-Varkila J. (2002). Community-acquired Methicillin-resistant *Staphylococcus aureus*. Finland. Emerg. Infect. Dis., 8:602-607.
- Sambrook ,J. and Rusell D.W.(2001).Molecular cloning .A laboratory manual .3rd edi. Cold spring .Harber laboratory press .New York.
- Sano, Y. , Matsui H. , Kobayashi M. and Kageyama M. (1990). Pyocins S1 and S2, bacteriocins of *Pseudomonas aeruginosa*. In: *Pseudomonas Biotransformation pathogenesis and evolving biotechnology* .Silver, S. , Chakrabarty A.M., Iglewski B. and Kaolan, S.,(eds.), pp.352-358, American Society for Microbiology, Washington.
- Schaberg, D.R., and Zervos M.J.(1986). Intergeneric and interspecies gene exchange in Gram-positive cocci. J. Antimicrob. Agents Chemother., 30: 817-822.
- Schennings, T., Heimdahl A., Coster K. and Flock J. (1993). Immunization with fibronectin binding protein from

References

- Staphylococcus aureus protects against experimental endocarditis in rats. J. Microb. Pathol. , 15: 227 – 236.
- Sefton, A.M.(2002).Mechanisms of antimicrobial resistance. Drugs , 62: 557–566.
- Segal, H., Thomas R. and Gay E.B.(2003).Characterization of class 1 integron resistance gene cassettes and the identification of a novel IS-like element in *Acinetobacter baumannii*. Plasmid., 49:169–178.
- Seifert, H., Boullion B., Schulze A. and Pulverer G.(1994).Plasmid DNA profiles of *Acinetobacter baumannii*: Clinical application in a complex endemic setting. Infect. Control. Hosp. Epidemiol.,15:520–528.
- Sharon , L.H. , Rabe K.L., Muller C.H., Zarutskie P., Kuzan F.B. and Stenchever M.A.(1990).Relationship of bacteriologic characteristics to semen indices in men attending an infertility clinic. Obstetrics & Gynecology , 75:800-804.
- Shefi ,S. and Turek P.J.(2006).Definition and current evaluation of subfertile men. Int. Braz. J. Urol. Rio de Janeiro ., 23(4) :212-222.
- Sherins, R.J., Brighwell D. and Sternthal P.M.(1977).Longitudinal analysis of semen of fertile and infertile men. In: The Testes in Normal and Infertile Men. Torean P. and Nankin H.R. (eds.), Raven Press, New York; PP. 473-488.

References

- Shimoya ,K. , Matsuzaki N. , Tsutsui T. , Taniguchi T. , Saji F. and Tanizawa O.(1993).Detection of interleukin-8 (IL-8) in seminal plasma and elevated IL-8 in seminal plasma of infertile patients with leukocytospermia . *Fertile. Sterile.*,59:885-888.
- Shlaes, D.M., Shlaes J.H., Vincent S. , Etter L., Fey P.D. and Goering R.V.(1993).Teicoplanin-resistant *Staphylococcus aureus* expresses a novel membrane protein and increases expression of penicillin-binding protein 2 complex. *J. Antimicrob Agents Chemother* ; 37:2432-2437.
- Shorman, M.A., Atoom A.M., Abuharfeil N.M. and Al-Majali A.M.(2008).Identification of Methicillin Resistant *Staphylococcus aureus* (MRSA) and Methicillin Resistant Coagulase-Negative *Staphylococcus* (CoNS) in Clinical Settings. *Amer. J. Infec. Dis.*, 4 (2): 156-161.
- Sieradzki , K. and Tomasz A.(1997). Inhibition of cell wall turnover and autolysis by vancomycin in a highly vancomycin-resistant mutant of *Staphylococcus aureus*. *J. Bacteriol.*,179:2557-2566.
- Sipsas, N.V., Bodey G.P. and Kontoyiannis D.P.(2005).Perspectives for the management of febrile neutropenic patients with cancer in the 21st century. *Cancer* ,103:1103–1113.
- Skov, R. , Frimodt-Moller N. and Espersen F.(2001). Correlation of MIC methods and tentative interpretive criteria for disk diffusion susceptibility testing using NCCLS methodology for fusidic acid. *J. Diag. Microbiol. Infect. Dis.* , 40: 111–116.

References

- Smith ,H.R. and Scotland S.M.(1993).Isolation and identification methods for E.coli O157 and other verocytotoxic producing strain. *J. Clin. Pathol.*, 46:10-17.
- Souli, M., and Giamarellou H.(1998). Effects of slime produced by clinical isolates of coagulase-negative staphylococci on activities of various antimicrobial agents. *J. Antimicrob. Agents Chemother.* , 42:939–941.
- Stacey, A.R., Endersby K.E. , Chan P.C. and Marples R.R.(1998).An outbreak of Methicillin resistant *Staphylococcus aureus* infection in a rugby football team. *Br. J. Sports Med.*, 32:153-154.
- Stegmayer, B. and Ronquist G.(1982).Stimulation of sperm progressive, motility by organelles in human seminal plasma. *Scand. J. Urol. Nephrol.* , 16: 85- 94.
- Steinberger, A., and Steinberger, E.(1976).Secretion of FSH inhibiting factor by cultured Sertoli cells. *Endocrinology.* , 99: 918- 924.
- Stimac ,G.D. , Agreb A.N. and Gorica M.A.(2001).New prospect for chronic prostatitis. *J. Acta. Clin. Croat. Urol.* , 40(2):109-116.
- Suzanne, E.D. , Kirby A.D. , Lajoie G. and Heinrichs D.E.(2004). Role of Siderophore Biosynthesis in Virulence of *Staphylococcus aureus*: Identification and Characterization of Genes Involved in Production of a Siderophore .*J. Infect. Immun.* 72(1):29-37.
- Svanborg, A.D., Alin B.G. and Plotti F.H.(1996). Bacterial adherence and mucosal cytokine responses: Responses and transmembrane signaling. *J. Ann. N. Acadsia.*, 797(10):177.

References

- Svenson, S.B, Moris K.M. and Brunner V.A.(1983). P-fimbriae of pyelonephritis genic *Escherichia coli*. *J. Infect. Dis.* ,123(11):61.
- Swenson,C.E. , Toth A. , Toth C. , Wolfgruber L. and O'Leary W.M.(1980).Asymptomatic bacteriospermia in fertile men. *Andrologia.* , 12: 7-11.
- Tagg, J.R., Dajani A.S. and Wannamaker L.W.(1976). Bacteriocins of Gram-positive bacteria. *Bacteriol. Rev.* 40 : 722-756.
- Tamura, G.S., Kuypers J.M., Smith S., Raff H. and Rubens C.E.(1994). Adherence of Group B Streptococci to cultured epithelial cells: roles of environmental factors and bacterial surface components. *J. Infect. Immun.* 62:2450-2458.
- Taylor-Robinson, D. and Manchee R.J.(1967).Spermadsorption and spermagglutination by mycoplasmas. *Nature*,215:484-487.
- Taylor, J.M. and Heinrichs D.E.(2002).Transferrin binding in *Staphylococcus aureus*: Involvement of a cell wall anchored protein. *Mol. Microbiol.*, 43:1603-1614
- Teague, N.S. , Boyarsky S., and Glenn J.F.(1971). Interference of human spermatozoa motility by *Escherichia coli*. *Fertil. Steril.*, 22: 281-285.
- Tenover ,F.C., Lancaster M.V. and Hill B.C.(1998). Characterization of staphylococci with reduced susceptibilities to vancomycin and other glycopeptides. *J. Clin. Microbiol.* ,36:1020-1027.
- Tenover, F.C., Jame S.T. and Gaynes R.P.(2005). The epidemiology of *Staphylococcus* infections. *J. Am. Sc. Microbiol.* ,34:414-21.

References

- Theresa , L. , Michele L. and Kenneth R.(1999).Emergence of vancomycin resistance in *S. aureus* . The New England Journal of Medicine , 340(7):493-501.
- Thomson, C.J. and Amyes S.G.(1993). Selection of variants of TEM-1 β -lactamase encoded by a plasmid of clinical origin with increased resistance to β -lactamase inhibitors. J. Antimicrob. Agents Chemother. , 31: 655-664.
- Todar , K.(2005). Staphylococci . Todar's online textbook of bacteriology.
- Todar , K.(2006). Mechanisms of bacterial pathogenicity:Colonization and invasion. Todar's online textbook of bacteriology.
- Tomaras, A., Dorsey C., Edelmann R. and Actis L.(2003).Attachment to and biofilm formation on abiotic surfaces by *Acinetobacter baumannii*: involvement of a novel chaperone-usher pili assembly system. Microbiology. ,149:3473-3484.
- Travis, J. , Potempa J. and Maeda H. (1995). Are bacterial proteinases pathogenic factors? Trends in Microbiol. , 3:405-407.
- Trum, J.W., Mol B.W.J. , Pannekoek Y., Spanjaard L., Wertheim P., Bleker O.P., Veen van der F.(1998).Value of detecting leukocytospermia in the diagnosis of genital tract infection in subfertile men. Fertil. Steril. , 70:315–319.
- Tsuji, M., Ishii Y., Ohno A., Miyazaki S., and Yamaguchi K.(1998). In vitro and in vivo antibacterial activities of S-4661, a new carbapenem. J. Antimicrob. Agents Chemother. , 42:94-99.
- Tyrrell, G.J., Senzilet L.D., Spika J.S., Kertesz D.A., Alagaratnam M., Lovgren M. and Talbot J.A.(2000). Invasive disease due to

References

- group B Streptococcal infection in adults: results from Canadian, population based, active laboratory surveillance study. *J. Infect. Dis.* 182:168-173.
- Tzelepi, E. , Giakkoupi P. , Ofianou D., Loukova S.V. , kemeroglou A., and Tsakris A.(2000).Detection of extended-spectrum β -lactamases in clinical isolates of *Enterobacter cloacae* and *Enterobacter aerogenes*. *J. clin. Micorbiol.*, 38(3): 542 – 546.
- Umeyama, T. , Ishikawa H. , Takeshima H. , Yoshii S. and Koiso K. (1986).A comparative study of seminal trace elements in fertile and infertile men. *Fertil. Steril.* , 46: 494-499.
- Urban, C., Segal-Maurer S. and Rahal J.J.(2003).Consideration in control and treatment of nosocomial infections due to multidrug resistant *Acinetobacter baumannii* . *Clin. Infect. Dis.* , 36 :1268-1274.
- Vanderkooi, O.G., Low D.E., Green K., Powis J.E. and McGeer A.(2005).Predicting antimicrobial resistance in invasive pneumococcal infections. *Clin. Infect. Dis.*,40:1288–1297.
- Van Looveren , M. and Goossens H.(2004).Antimicrobial resistance of *Acinetobacter* spp. in Europe. *Clin. Microbiol. Infect.*,10:684–704
- Varon, E. , Levy C. , Dela R. and Boucherat M.(2000). Impact of antimicrobial therapy on nasopharyngeal carriage of *Streptococcus pneumoniae*, *Haemophilus influenzae*, and *Branhamella catarrhalis* in children with respiratory tract infections. *J. Clin. Infect. Dis.* , 31:477-481.

References

- Vergis , E.N. , Shankar N. , Chow J.W. and Hayden M.K.(2002). Association between the presence of enterococcal virulence factors gelatinase, hemolysin and enterococcal surface protein and mortality among patients with bacteremia due to *Enterococcus faecalis*. *J. Clin. Infect. Dis.* , 35(5) :570-575.
- Vidotto, M.C., Furlaneto M.C. and Perugini M.R.E.(1991). Virulence factors of *Escherichia coli* in urinary isolates. *Braz. J. Med. Biol. Res.*, 24:365-373.
- Vila, J., Marti S. and Sanchez-Cespedes J.(2007). Porins, efflux pumps and multidrug resistance in *Acinetobacter baumannii*. *J. Antimicrob. Chemother.* , 59(6):1210-1215.
- Villanueva-Diaz, C.A., Flores-Rejes G.A., Beltran-Zuniga M., Echavarria-Sauche M., Ortiz-Ibarra F.J. and Arredondo-Garcia J.L.(1999). Bacteriospermia and Male infertility: A method for increasing the sensitivity of semen culture. *Int. J. Fertile Women Med.* , 44(4):198-203.
- Virecoulon ,F. , Wallet F. , Fruchart A.F. , Rigot J.M., Peers M.C., Mitchell V. and Courcol R.J.(2005). Bacterial flora of the low male genital tract in patients consulting for infertility. *Andrologia* ,37(5):160-165.
- Wang, Y. , Liang C.L. , Wu J.Q. , Xu C., Qin S.X. and Gao E.S.(2006). Do *Ureaplasma urealyticum* infections in the genital tract affect semen quality? *Asian J. Androl.*, 8:562-568.
- Watanabe, A., Takahashi H., Kikuchi T., Kobayashi T., Gomi K., Fujimura S., Tokue Y. and Nukiwa T.(2000). Comparative in

References

- vitro activity of S-4661, a new parenteral carbapenems, and other antimicrobial agents against respiratory pathogens. . J. Antimicrob. Agents Chemother. , 46:184-187.
- Waterer, G.W. and Wunderink R.G.(2001).Increasing threat of gram-negative bacteria. Crit. Care Med. , 29:75-81.
- Weinberg, E.D.(1998).Patho-ecological implication of microbial acquisition of host iron. Review in Medical Microbiology. 9:171-8.
- Weinberg, E.D.(1999).Iron loading and disease surveillance. Emerg. Infect. Dis., 5:346-352.
- Weinder ,W. , Krause W. and Ludwig M.(1999).Relevance of male accessory gland infection for subsequent fertility with special focus on prostatitis. Hum. Reprod. Update ,5(5):421-432.
- Wentz, A.C. (1988): Infertility. In: Novak's Text Book of Gynecology. Jones, H.W., Wentz A.C. and Burnett L.S. (eds.), Williams and Wilkins, Baltimore. , pp.263-302.
- White, M.M. and Warren N.(1953).The significance of pus cells in human semen. In, "Proceedings of the Society for the Study of Fertility. No. V, Liverpool conference, Blackwell Scientific Publications Ltd., Oxford, England, pp. 66.
- White, D.G., Zhao S., Sudler R., Ayers S., Friedman S., Chen S., McDermott P.F., McDermott S., Wagner D.D. and Meng J.(2001).The isolation of antibiotic resistant Salmonella from retail ground meats. N. Engl. J. Med. ,345:1147–1154.

References

- Whitfield, C. and Valvano M.A. (1993). Biosynthesis and expression of cell surface polysaccharides in Gram-negative bacteria. *Adv. Microbiol. Physiol.* , 35:135–246.
- Williams, P. and Griffiths E.(1992).Bacterial transferrin receptors, structure, function and contribution to virulence. *Med. Microbiol. Immunol.* , 181: 301-322.
- Williams, P.H. (1979). Novel iron uptake system specified by ColV plasmids: an important component in the virulence of invasive strains of *Escherichia coli*. *J. Infect. Immun.* , 26:925-932.
- Wolff, H.(1998).Methods for the detection of male genital tract inflammation. *Andrologia.*, 30(1):35-39.
- Wolff, H.(1995). The biologic significance of white blood cells in semen. *Fertil. Steril.* , 63:1143-1157.
- Wolff , H. , Politch J.A. , Martinez A. , Haimovici F. , Hill J.A. and Anderson D.J.(1990). Leukocytospermia is associated with poor semen quality . *Fertil. Steril.* ,53:528-536.
- Wolner-Hanssen, P. and Mardh P.A.(1984). in vitro tests of the adherence of *Chlamydia trachomatis* to human spermatozoa. *Fertil. Steril.*, 42: 102- 107.
- Wood, G.C., Hanes S.D., Croce M.A., Fabian T.C. and Bougher B.A. (2002).Comparison of ampicillin-sulbactam and imipenem-cilastin for the treatment of *Acinetobacter ventilator-associated pneumonia*. *Clin. Infect. Dis.* , 34: 1425–1430.

References

World Health Organization (WHO) .(1984).Work shop on the standardized investigation of the infertile couple. Moderator P. Rowe; Coordinator M. Darling. In: Proceedings of 11th World Congress on Fertility and Sterility. Eds. Harrison, R. F., et. al. MTP Press Ltd, Lancaster, Boston.; PP: 427-431.

World Health Organization (WHO) (1999): WHO laboratory manual for the examination of human semen and sperm- cervical mucus interaction, 4th edition; Cambridge, Cambridge University Press. UK.

Xu, C., Sun G.F., Zhu Y.F. and Wang Y.F.(1997). The correlation of *Ureaplasma urealyticum* infection with infertility. *Andrologia*, 29:219-226.

Yamaguchi, K., Domon H., Miyazaki S., Tateda K., Ohno A., Ihii K., Matsumoto T. and Furuya N.(1998).In vitro and in vivo antibacterial activities of CS-834, a new oral carbapenems. *J. Antimicrob. Agents Chemother.* , 42:555-563.

Yassin ,H.(1990).Chronic otitis media microbiological and epidemiological study Master thesis, College of Medicin, university of Basrah , Iraq.

York, M. , Gibbs L. , Chehab F. and Brooks G.(1996). Comparison of PCR detection of *mecA* gene with standard susceptibility testing

References

methods to determine methicillin resistance in coagulase-negative staphylococci. J. Clin. Microbiol., 34: 249–253.

Zahac, L., Mady U., Lig Y. and Yu Y.(2003). The states of drug resistance and amp C gene expression in *Enterobacter cloacae*. J. Chine. Med. , 116: 1244-1247.

Zaneveld, L.J.D., and Polakoski K.L.(1977).Collection and Physical examination of the ejaculate. In: Techniques of Human Andrology. Hafez, E.S.E. (eds.), Elsevier, Biochemical Press, North- Holland. , pp. 147-172.

Zavos, P.M. , Correa J.R. and Zarmakoupis- Zavos P.N.(1998). Antisperm antibody treatment mode, levels of antisperm antibodies after incubation with TEST- Yolk buffer and filtration using the sperm prep. II method. Fertil. Steril.,69: 517-521.

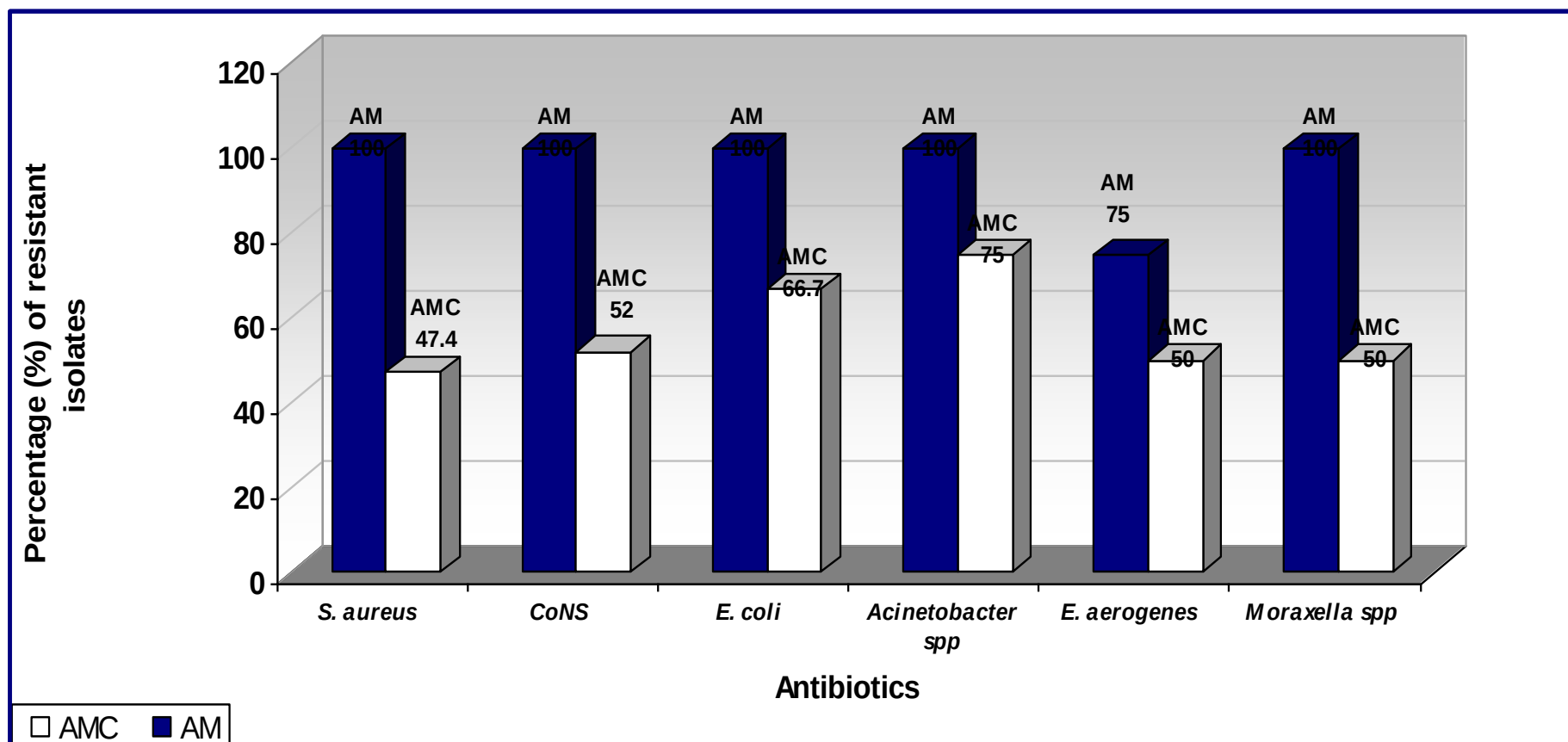


Figure (3-2) Resistance of bacterial isolates to amoxicillin and amoxicillin–clavulanic acid .

AM: amoxillin , AMC: amoxicillin–clavulanic acid

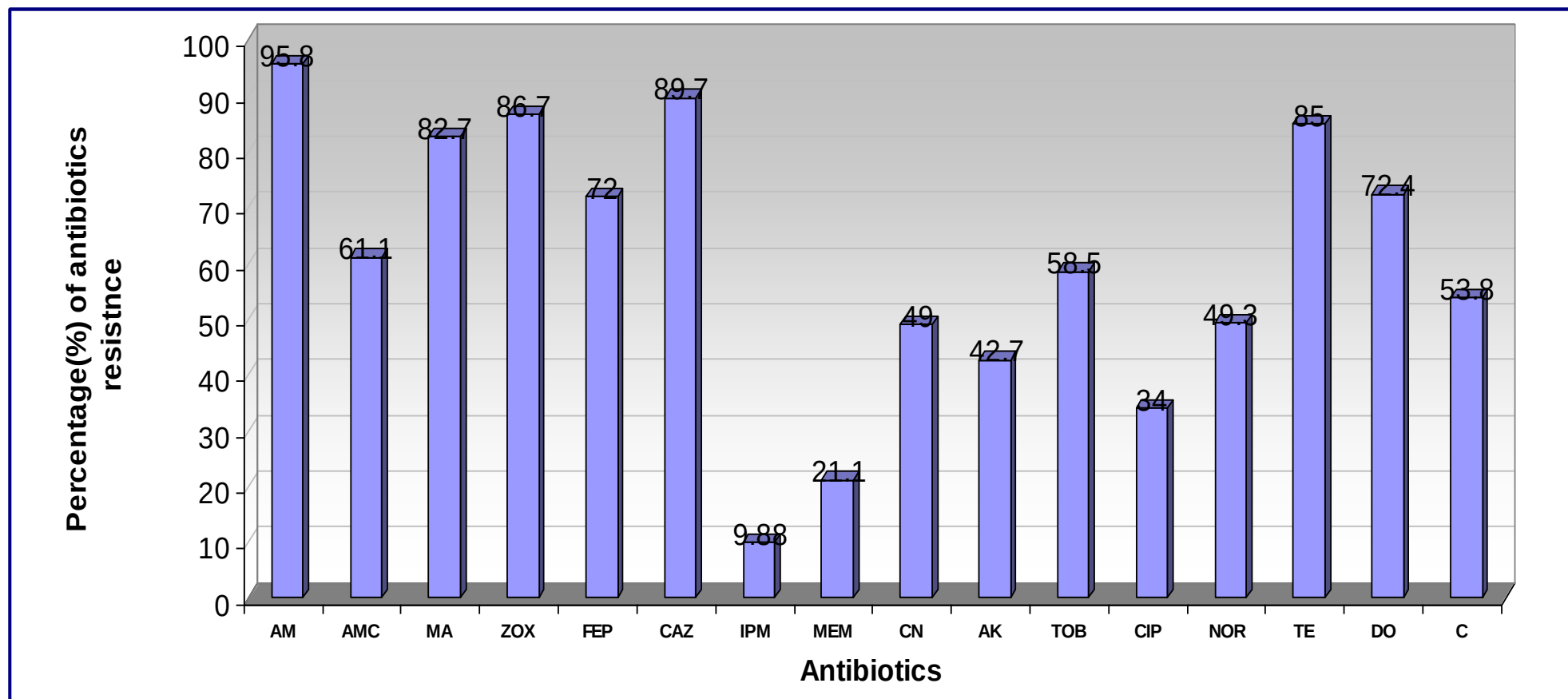


Figure (3-1): Resistance of bacterial isolates to several antibiotics.

AM : amoxicillin , AMC: amoxicillin –clavulanic acid , MA :cefamandole , ZOX: ceftazidime , FEP : cefepime , CAZ : ceftizoxime , IPM: imipenem , MEM: meropenem , CN: gentamycin , AK: amikacin , TOB: tobramycin, TE :tetracycline , DO: doxycycline , CIP: ciprofloxacin , NOR : norfloxacin , C: chloramphenicol

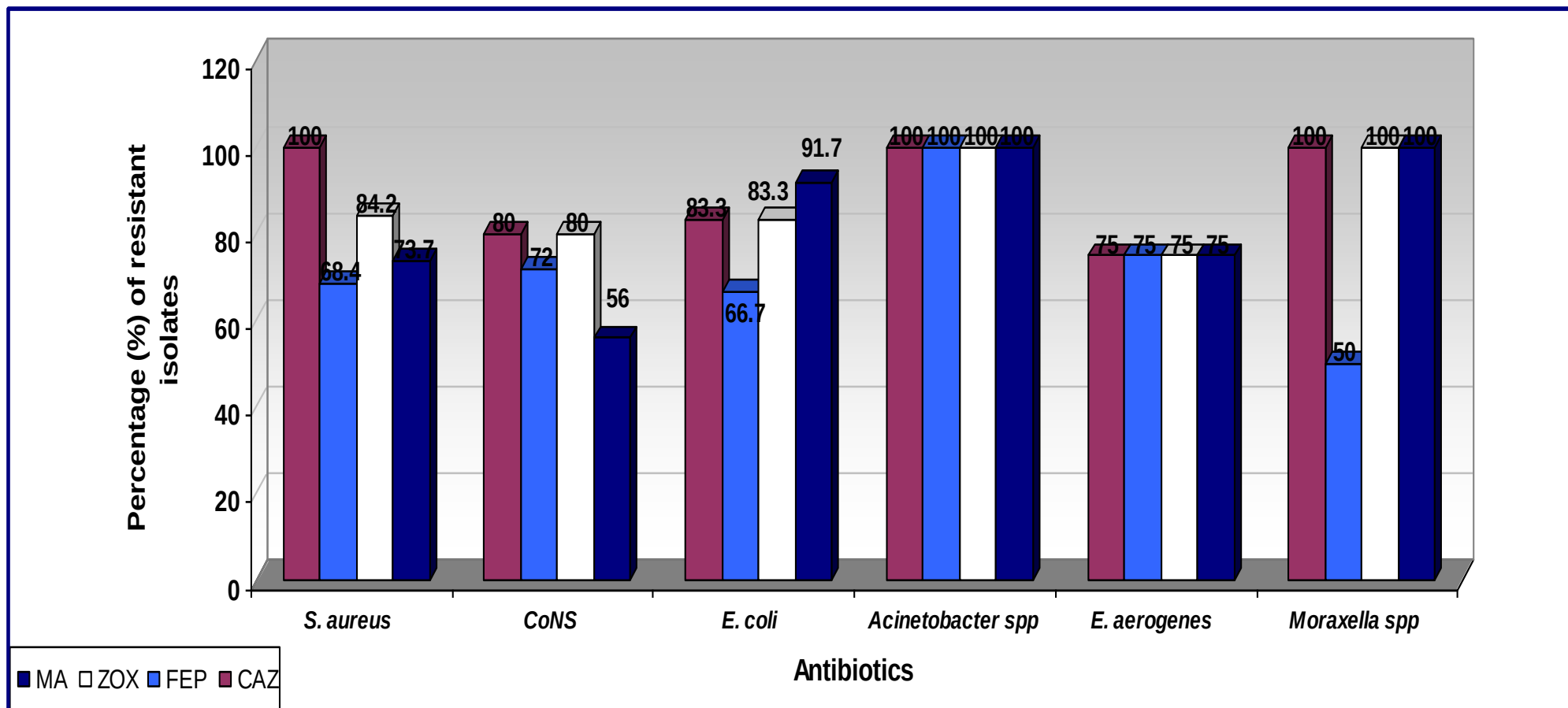


Figure (3-3) Resistance of bacterial isolates (gram positive and gram negative) to cephalosporins.

MA: cefamandole, ZOX: Ceftizoxime, FEP: Cefepime, CAZ: ceftazidime

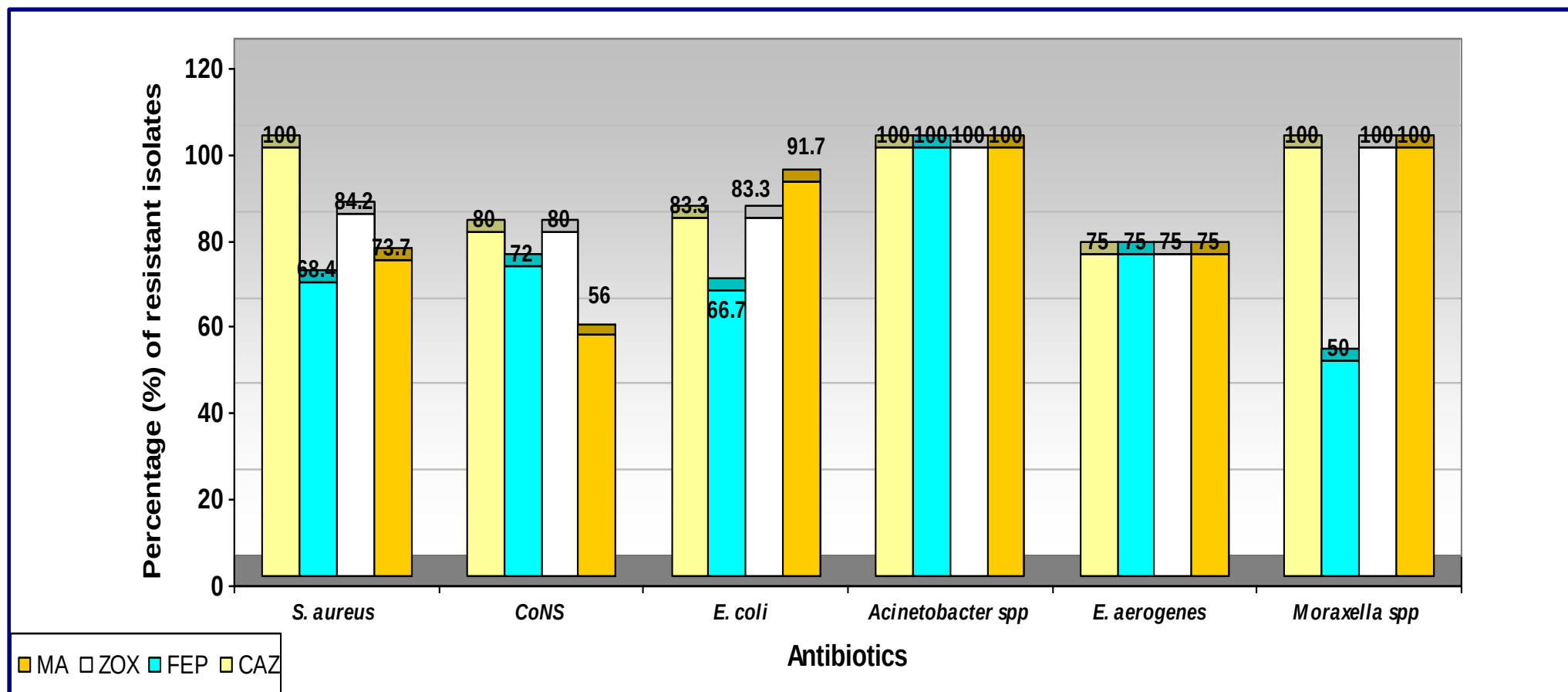


Figure (3-4) Resistance of bacterial isolates (gram positive and gram negative) to cephalosporins .

MA: cefamandole , ZOX: ceftizoxime ,FEP: cefepime ,CAZ: ceftazidime

Table (3-6) virulence factor of gram negative bacterial isolate.

Bacterial isolate	No. of isolate	Hemolysin production n(%)	Capsule Production n(%)	Siderophore Production n(%)	Bacteriocin production n(%)	Lipase production n(%)	Extracellular protease production n(%)	CFA /I** n(%)	CFA/III* n(%)
<i>E.coli</i>	12	5 (41.7) β -hemolysis	2(16.7)	12 (100)	10(83.3)	9 (75)	5 (41.7)	8 (66.7)	12(100)
<i>Enterobacter aerogenes</i>	8	4 (50) β -hemolysis	0 (0.0)	6 (75)	2 (25)	7(87.5)	0 (0.0)	6 (75)	8(100)
<i>Acinetobacter</i> spp	4	0 (0.0)	0 (0)	3 (75)	1 (25)	0 (0.0)	0 (0.0)	2 (50)	4(100)
<i>Moraxella</i> spp	2	0 (0.0)	0(0.0)	2 (100)	0 (0.0)	1 (50)	0 (0.0)	1(50)	2(100)

** CFA I, colonization factor antigen 1

* CFA III, colonization factor antigen 3