Gram-Positive Bacteremia in Febrile Children Under Two Years of Age in Babylon Province

A Thesis Submitted

By

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تجرثم الدم المتسبب عن البكتريا الموجبة لصبغة غرام عند الاطفال الحميين دون السنتين في محافظة بابل

رسالة تقدمت بها

شيماء عبيد حسون

بكالوريوس علوم في علوم الحياة

إلى مجلس كلية الطب-جامعة بابل كجزء من متطلبات نيل درجة الماجستير في الإحياء المجهرية

ذو القعدة/ ١٤٢٧ كانون الأول / ٢٠٠٦

بِسْمِ اللَّهِ الرَّحْمَنِ الرَّحِيمِ

﴿سَنُرِيهِمْ آيَاتِنَا فِي الْآفَاقِ وَفِي أَنْفُسِهِمْ
حَتَّى يَتَبَيَّنَ لَهُمْ أَنَّهُ الْحَقُّ أَوَلَمْ يَكْفِ بِرَبِّكَ
أَنَّهُ عَلَى كُلِّ شَيْءٍ شَهِيدٌ﴾

صدق الله العلي العظيم

(سورة فصلت: الآية ٥٣)

الخلاصة

استهدفت هذه الدراسة التحري عن تجرثم الدم المتسبب عن البكتريا الموجبة لصبغة غرام عند الرضع الحميين تبعا لعدد من عوامل الخطورة والمتمثلة بعامل العمر وعامل الجنس ودرجة الحرارة ومدة بقاء الحمى و الإقامة والتغايرات الفصلية وكذلك المسببات البكتيرية المشتركة في إحداث التجرثم.

حيث جمعت ١٦٠ عينة دم من أطفال تتراوح دون السنتين ممن يعانون من الحمى تم إدخالهم إلى ردهة الطوارئ في مستشفى بابل للولادة والأطفال خلال فترة عشرة أشهر (من تشرين الأول ٢٠٠٤ إلى أب ٢٠٠٥).

بعد إجراء فحص زرع الدم للعينات المأخوذة وجد ان٣١عينة(١٩.٤٪) من أصل ١٦٠ عينة دم قد أعطت نتيجة موجبة منها ٢١(٦٨٪) متسببة عن البكتريا الموجبة لصبغة غرام و١٠(٣٢ ٪) متسببة عن البكتريا السالبة لصبغة غرام.

تتمثل المسببات البكتيرية الموجبة لصبغة غرام بالأنواع التالية:

Staphylococcus .aureus (S.aureus) ١٠ عزلات وبنسبة (٣٣٪) وهي العزلة السائدة تليها Coagulase negative staphylococci(CoNS) ٥وبنسبة (١٦٪) و *Streptococcus pyogenes* ٤وبنسبة (١٣٪) و *Listeria monocytogenes* و.*Micrococcus* spp عزلة واحدة وبنسبة (٣٪) لكل منهما.

من بين عزلات البكتريا العنقودية Staphylococci وجدت ٣ عزلات من البكتريا العنقودية الذهبية *S.aureus* مقاومة للمثسيلين وبنسبة (٣٠٪) في حين وجدت عزلة واحدة فقط من البكتريا العنقودية السالبة لفحص التخثر CoNS وبنسبة (٢٠٪).

كذلك اجري فحص حساسية العزلات البكتيرية للمضادات الحياتية بطريقتين (طريقة الانتشار بالأقراص وطريقة التركيز المثبط الأدنى). حيث أظهرت النتائج إن العزلات المقاومة للمثسيلين كانت أكثر مقاومة للمضادات الحياتية من العزلات الحساسة له.

كما أبدت العزلات البكتيرية التالية (*S.aureus* و CoNS و*Micrococcus* spp) نسبة مقاومة بلغت (١٠٠٪) لكل من البنسلين والاموكسلين والامبسلين فيما أبدت نسب مقاومة متفاوتة بالنسبة للسيفالكسين والسيفوتاكسيم والجنتامايسين والكوتريموكسازول والاميكاسين.

أما بالنسبة ل*St.pyogenes* و*L.monocytogenes* فقد أظهرت هذه العزلات نسبة مقاومة معدومة (٠٪) لكل من البنسلين والاموكسلين والامبسلين فضلا عن الجنتامايسين والكوتريموكسازول والاميكاسين بالنسبة ل*L.monocytogenes* .

فيما يتعلق بعوامل الوبائية وعوامل الخطورة أظهرت الدراسة النتائج التالية:

- إن الرضع بعمر اقل من شهر كانوا أكثر عرضة للإصابة بتجرثم الدم بنسبة (٣٢.٥٪).
- الذكور كانوا أكثر عرضة للإصابة من الإناث حيث بلغت نسبة الإصابة لدى الذكور (٥٢٪) مقارنة ب(٤٨٪) للإناث مع عدم وجود فرق معنوي.
- ازدادت معدلات تجرثم الدم بزيادة معدلات درجات الحرارة.
- كانت فترة استمرار بقاء الحمى من يوم إلى يومين الأكثر ارتباطا بحدوث حالات تجرثم الدم.
- كانت أكثر حالات تجرثم الدم حدوثا في الفصل الحار مقارنة بالفصل البارد حيث بلغت نسبة الإصابة في شهري نيسان وأيار (١٩.٣ ٪و٢٥,٨٪) على التوالي.
- كان الرضع الذين يعيشون في المناطق الريفية أكثر عرضة للإصابة بنسبة (٦٧.٧٪) من الرضع الذين يعيشون في المدينة بنسبة (٣٢.٢٪) مع وجود فرق معنوي.
- أظهرت الدراسة إن لدى الرضع الذين يتناولون الرضاعة الطبيعية حالات تجرثم دم اقل وبنسبة (٢١٪) مقارنة بالرضع الذين يتناولون الرضاعة الصناعية بنسبة (٢٥٪) مع وجود فرق معنوي.

Summary

The study aimed to investigate Gram-positive bacteremia in febrile children according to some risk factors represented by age, sex, temperature degree, duration of the fever, residence and seasonal variation and to detect the type of causative bacteria.

One hundred sixty blood samples were collected from children below ٢ years who were suffering from fever and admitted to Emergency Department of Babylon Maternity and Children Hospital in Hilla during a period of ten months (from November ٢٠٠٤ to August ٢٠٠٥).

After culturing the blood samples the results indicated that ٣١(١٩.٤%) blood samples revealed positive cultures consisting of ٢١(٦٨٪) Gram-positive bacterial isolates and ١٠(٣٢٪) Gram-negative isolates. Gram-positive isolates were represented by:

- ***Staphylococcus aureus (S.aureus)*** accounted for ١٠(٣٣٪) and it was the predominant isolate followed by **Coagulase negative Staphylococci (CoNS)** which accounted for ٥(١٦٪), ***Streptococcus pyogenes*** ٤(١٣٪), ***Listeria monocytogenes*** ١(٣٪) and ***Micrococcus*** spp. ١(٣٪).

Among the staphylococcal isolates, ٣(٣٠٪) isolates were Methicillin resistant ***S.aureus*** (MRSA), while ١(٢٠%) isolate was Methicillin resistant **CoNS** (MR**CoNS**).

Antibiotic susceptibility test was also studied by two methods (Disk diffusion method and minimal inhibitory concentration [MIC] method). The

results revealed that Methicillin resistance isolates were more resistant to antibiotics compared with Methicillin sensitive isolates.

S.aureus, **CoNS** and ***Micrococcus*** spp. exhibited the same resistance rate (١٠٠٪) to Penicillin, Amoxicillin and Ampicillin, whereas they were sensitive to Cephalexin, Cefotaxime, Gentamicin, Co-trimoxazole and Amikacin in variant rates.

Streptococcus pyogenes and ***L.monocytogenes*** showed fully sensitive to Penicillin, Amoxicillin and Ampicillin. ***L.monocytogenes*** revealed the same rate of resistance (٠٪) towards Gentamicin, Co-trimoxazole and Amikacin while ***St.pyogenes*** revealed resistance rate of (٢٥٪) to Gentamicin and Amikacin.

Regarding the epidemiological and risk factors for bacteremia in children, it was found that:

- Infants under one month of age were more susceptible to bacteremia than other ages in a rate of (٣٢.٥٪).
- Males were found to be more susceptible than females, the rate of infections was ٥٢٪ and ٤٨٪ respectively with no significant correlation.
- The bacteremic state increased with the increment in body temperature.
- Persisting fever for ١-٢ days in children was more associated with bacteremic state.
- Bacteremia occurred more in hot season compared with cold season and the rate of infection was (١٩.٣٪ and ٢٥.٨%) in April and May.

- The rate of infection in children living in rural area was found to be (٦٧.٧%) while it was(٣٢.٢٪) in urban children with a significant correlation.
- This study revealed that children who were breast-fed have bacteremia in a percentage of (٢١٪) which was less than that in children who were fed artificially (٢٥٪) with a significant correlation.

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We certify that this work was prepared under our supervision at the University of Babylon, college of medicine as partial requirements for the degree of master of science in Microbiology….

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Dedication

To the Daughter of the Holy Prophet and the Wife of Imam Ali

Fatima Al-Zahra

Leader of the women of paradise

And to my mother

Abbreviation	Meaning
°C	Degrees Celsius
ABC	Absolute band count
CA	Community acquired
CDC	Centers of diseases control and prevention
FUO	Fever of unknown origin

FWS	Fever without source
GABHS	Group A beta hemolytic streptococci
hrs	hours
ICU	Intensive care unit
IU	International unit
KFSH	King Faisal Specialist Hospital
KKUH	King Khalid University Hospital
mecA	Methicillin resistance gene
MIC	Minimal inhibitory concentration
ml	Milliliter
mm	Millimeter
MRCoNS	Methicillin resistance Coagulase negative Staphylococci
MRSA	Methicillin resistance ***Staphylococcus aureus***
MSCoNS	Methicillin sensitive Coagulase negative Staphylococci
MSSA	Methicillin sensitive ***Staphylococcus aureus***
NCCLs	National committee for clinical laboratory standards
NICU	Neonatal intensive care unite
NNIC	National nosocomial infection surveillance system
OB	Occult bacteremia
SAB	***Staphylococcus aureus*** bacteremia
SBI	Serious bacterial infection
spp.	Species
U	unit
WBC count	Weight blood cell count
WHO	World health organization
YCH	Yangon children's hospital
µg	Microgram

Abbreviation

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Shaimaa

١-١ :Introduction:

Bacteremia is the presence of viable bacteria in the circulating blood (Spraycar, ١٩٩٥). Bacteria may enter the blood stream giving rise to bacteremia from an existing focus of infection, from a site with the commensally flora or by direct inoculation of contaminated material into the vascular system. These organisms are often cleared from the blood within minutes, so that the bacteremia is silent and transient but if the immune system is overwhelmed or evaded, organisms persist in the blood and bacteremic symptoms would arise (Eykyn, ١٩٩٨). Bacteremia should be distinguished from septicemia in which signs and symptoms of severe diseases are present (Campbell and Mclutosh, ١٩٩٨).

Childhood bacteremia is defined as the presence of bacteria in the bloodstream of a febrile child who was previously healthy; the child does not clinically appear to be ill and has no apparent focus of infection (occult bacteremia) (Lorin, ١٩٩٣; Swindell and Chetham ١٩٩٣).

Patients with occult bacteremia do not have clinical evidence other than fever of a systemic response for infection (Harper and Fleisher, ١٩٩٣).

Bacteremia may also occur in children with focal infections or in children who have sepsis (ie, clinical evidence other than fever of a systemic response to infection). The common causative organisms being isolated from bacteremic patients are Gram-positive organisms represented by ***Staphylococcus aureus ,Streptococcus pneumoniae ,Streptococcus pyogenes ,Coagulase Negative Staphylococci, Enterococcus*** **spp.** ***and Listeria monocytogenes*** while Gram negative organisms which are commonly isolated are ***Escherichia coli, Klebsiella spp., proteus spp., pseudomonas aeroginosa, Enterobacter*** **spp.,** ***Citrobacter*** **spp.,** ***Salmonella*** **spp.,** ***Shigella*** **spp.,** ***Campylobacter*** **spp.,** ***Haemophilus*** **spp.,** ***Neisseria meningitides*** and ***Neisseria gonorrhoeae,*** in addition to anaerobic bacteria (Eykyn, ١٩٩٨)**.**

Bacteremia may be transient, continuous, or intermittent. These are briefly outlined below:

- ***Transient bacteremia****:* incidental bacteremia may occur spontaneously or with such minor events as brushing teeth or chewing food. Transient bacteremia may develop following dental work or other iatrogenic manipulations, but it is generally clinically benign and self-resolved in children who do not have an underlying illness or a turbulent cardiac blood flow.
- ***Continuous bacteremia****:* in septic shock, bacterial endocarditis and other endovascular infection, organisms are released into the bloodstream at fairly constant rate; bacteria are continuously present in the bloodstream.
- ***Intermittent bacteremia***: bacteria can be found intermittently in the bloodstream as in the case of transient seeding of the blood from a sequestered focus of infection, such as an abscess, bacteria are released into blood approximately ٤٥ minutes before a febrile episode (Forbes *et.al.,* ١٩٩٨).

Bloodstream infections continue to be a major cause of morbidity and mortality despite advances in antimicrobial therapy and supportive care. It has been estimated that between ٢٠٠,٠٠٠ to ٤٠٠,٠٠٠ episodes of bloodstream infections occur annually in the United States of America (USA) (Armstrong *et.al.,* ١٩٩٩). In Iraq some studies were carried out about bacteremia and septicemia (Al-Salamy, ١٩٩٩, Al-Charrakh *et.al.,* ٢٠٠٠ and Al-Waznee, ٢٠٠١).

The crude mortality rate among neonates admitted to the main referral hospital in Al-Anbar was ٢٨٪ (Al-Zwaini, ٢٠٠٢), while it was ٣٥٪ among

patients with nosocomial bloodstream infections in Saudi Arabia and most deaths are due directly to infections (Qutub and Akhter, ٢٠٠١).

Fever in infants younger than ١ year old, especially those younger than ٣ months, can signal a serious infection (Torpy *et.al.*, ٢٠٠٤).

Prompt antibiotic therapy usually succeeds in clearing bacteria from the bloodstream. Recurrence may indicate an undiscovered site of infection. Untreated bacteria in the blood may spread, causing infection of other organs such as heart, brain, kidney, spleen and liver.

Because bacteremia in children has a different implications and a different patterns than that in adults (Baker *et.al.*, ١٩٩٣ and Grandsen *et.al.*, ١٩٩٤) and children bacteremia is not quietly enough studied in Babylon province, consequently this project was suggested in an attempt to project a light on this subject. It is aimed at:

١. Estimating the incidence of Gram-positive bacteremia in infants of Babylon Province.
٢. Detecting the rate of Methicillin resistant ***Staphylococcus aureus*** (MRSA) causing bacteremia.
٣. Detecting the relation of bacteremia with the degree of fever.
٤. Determinating the role of some risk factors such as age, sex, residence, duration of fever and seasonal variation on bacteremic infections.
٥. Detecting susceptibility of the isolated causative bacteria to some traditional antimicrobial agents.

١.٢. Literature review:

Neonatal septicemia is a major cause of morbidity and mortality in developing countries (Al-Zwaini, ٢٠٠٢).

Neonatal septicemia remains the major clinical problem in neonatology, with high morbidity and mortality rates. In India, the current neonatal mortality rate is ٤٣.٣٪ per ١٠٠٠ live births (Ramji, ٢٠٠١). The incidence of neonatal septicemia for babies born at the main referral hospital in Al-Anbar was ٩.٢ per ١٠٠٠ live births (Al-Zwaini, ٢٠٠٢).

The majority of neonatal deaths occurs in developing countries and it was estimated to be as high as ٤ millions per year. Most of these deaths occurs because of infections and the commonest types of infections are bacteremia, meningitis and respiratory infection. The case fatality rates is as high as ٤٥٪ and the main pathogenic bacteremia responsible are ***Escherichia coli(E.coli), Klebsiella spp.***, ***Staphylococcus aureus(S.aureus)*** and ***Streptococcus pyogenes*** (David *et.al*., ٢٠٠٤).

Infants in the present series with streptococcal infections may die early, whereas those with staphylococcal or mixed infection most often die later (Eisenfeld *et.al.,* ١٩٨٣).

١.٢.١. Risk factors:

There are many factors that may affect the bacteremic incidence such as sex, age, temperature, seasonal variation and duration of fever, which will be discussed briefly below:

١.٢.١.١. Sex:

No known sex-based difference exists in the prevalence or course of bacteremia (Bass *et.al.,* ١٩٩٣). Shams Al-Deen, ٢٠٠١ in her study on the incidence of bacteremia in children found that it was more prevalent among male patients than female (٦٤.٩٪ versus ٣٥.١٪).

١.٢.١.٢.Age:

The risk of serious bacterial infection, bacteremia, and sepsis is higher in neonates than in infants or children and it is greatest from birth throughout the first month of life. It was found that ***Listeria monocytogenes*** and Group B streptococci were the most common pathogens causing serious bacterial infection (SBI) in this age group (Brook, ٢٠٠٣).

Bacteremia in the neonate younger than ٢٨ days more often produces focal infections than bacteremia in older infants and children. Consequently, the neonatal age group has the highest morbidity and mortality rates (Harper, ١٩٩٥).

In Babylon (Al-Waznee, ٢٠٠١) it was found that ٣٣٪ of septicemic infection occurs in infants of less than ١ year.

Kuppermann *et.al.* ١٩٩٨ considered the age as a predictor of bacteremia and he stated that the incidence of bacteremia proportionally increased with the process of age. He found that bacteremia rates were ١.٢٪ ,٢.٤٪ , ٢.٩٪ and ٤٪ in the children with age groups of ٣-٦ months,٦-١٢months , ١٢-١٨months and ١٨-٢٤ months respectively.

In Saudi Arabia the rate of bacteremia in infants aged (١-٢٤ months) was (٦١٪) at King Khalid University Hospital (Babay *et.al.,* ٢٠٠٥).

Children aged ٦ months to ٢ years were found to be at an increased risk (Swindell and Chetham, ١٩٩٣; Lee and Harper, ١٩٩٨ and Kuppermann, ١٩٩٩).

Sepsis may develop as a complication of localized community acquired infections or may follow colonization and local mucosal invasion by virulent pathogens. Children aged ٣-٣٦ months were reported to be at high risk for occult bacteremia, which occasionally progresses to sepsis (Powell and Stormorken, ٢٠٠٣).

Febrile children younger than ٣ years without a clear source of infection have a small but important risk of sepsis (Brook, ٢٠٠٣).

Studies of occult bacteremia mostly focused on children younger than ٣ years. Some studies showed that age does not affect the risk of developing occult bacteremia (Bass, *et.al*., ١٩٩٣), while other analyses have found that variations in age-based risk are dependent on the infecting organism (Baker and Bell, ١٩٩٩).

١.٢.١.٣.Body temperature:

Most studies define fever as a temperature of at least ٣٨°C in infants younger than ٣ months and a temperature of at least ٣٩°C in children aged ٣-٣٦ months. Hypothermia may be the presenting sign of bacterial infection in young infants. One guideline defined hypothermia as a temperature less than ٣٦°C (Baraff *et.al.,* ١٩٩٣).

Febrile state in which a major pathogen circulates in the blood for hours to days in temporary balance with the body's immune defenses (Baron and Fink, ١٩٨٠).

The risk of bacteremia increases as body temperature rises, but the low overall prevalence of bacteremia limits the usefulness of temperature degree as a clinical tool for risk stratification although an infant or a young child with a temperature higher than ٤٠.٩°C is more than three times more likely to harbor bacteremia than an infant or a young child with a temperature of ٣٩°C, most well-appearing children will not have bacteremia (Luszczak, ٢٠٠١).

A temperature is the clinical indicators of risk for bacteremia and serious bacterial infection among febrile children. Harper and Fleisher (١٩٩٣) noted that a higher temperature was associated with a higher risk of bacteremia. Moreover (Kuppermann *et.al.,* ١٩٩٨) found that in ٣٩-٣٩.٤°C the rate of bacteremia was <٢٪, in ٣٩.٤-٤٠°C was ٢.٣٪, in٤٠-٤٠.٥°C was ٣.٤٪ and >٤٠.٥°C was٤.٥٪) while (Lee and Harper, ١٩٩٨) found the rate of bacteremia at the same ranges of temperature above mentioned were <١٪, ١.١٪, ١.٧٪ and ٢.٦٪ respectively.

Children aged ٢ to ٢٤ months with febrile seizures were found to be at similar risk for occult bacteremia as those with fever alone (Shah *et.al*., ٢٠٠٣).

The degree of temperature elevation has been shown to be associated with the risk of occult bacteremia, meningitis, and serious bacterial infection (SBI). It has been reported that the risk increases as the temperature rises from ٣٩°C to ٤١°C (Kuppermann *et.al.,* ١٩٩٨). This risk stratification has been challenged in recent clinical studies, suggesting that those with temperatures between ٣٨.٥-٤١.١°C all have the same risk of SBI (Baraff, ٢٠٠٠).

Studies in the bacteremia literature were designed to determine the relationship between temperature and risk of occult bacteremia (Baraff *et.al.,* ١٩٩٣).

The upper extreme of the febrile temperature alone is inadequate to distinguish occult bacteremia; however, the risk of bacteremia has consistently been found to increase with increment in temperature (Lee and Harper, ١٩٩٨ and Strait *et.al.,* ١٩٩٩). Studies have shown a variation in risk at given temperatures based on age; this has led to the fever cutoffs listed below:

Table (١-١) Relationship of Age and Fever with Bacterial Infection*:

Age	Temperature(°C)	Rate of Bacterial Infection %
Neonates <١ mo	٣٨-٣٨.٩	٥
	٣٩-٣٩.٩	٧.٥
	>٤٠	١٨
Infants aged ١-٢ mo	٣٨-٣٨.٩	٣
	٣٩-٣٩.٩	٥
	>٤٠	٢٦

*(Bonadio, ١٩٩٣)

One hundred and seventy-five infants of less than ٨ weeks of age, presenting to the pediatric emergency room of the Bronx Municipal Hospital Center in USA with rectal temperature greater than or equal to ٣٨°C, Culture-positive bacterial infections occurred in ٦.٣٪ (n = ١١) (Crain and Shelov, ١٩٨٢).

It was found in Oak Lawn on ١٩٨٧ that in children from three to ٢٤ months of age with rectal temperatures of greater than or equal to ٤٠.٠°C, the prevalence of bacteremia was ٧.٣٪ (Yamamoto *et.al.*, ١٩٨٧).

١.٢.١.٤. Seasonal variation:

A seasonal variation exists among febrile children presented for evaluation. The peak is from late autumn to early spring in children of all ages which is likely because of respiratory and gastrointestinal viral infections. Another peak occurs during the summer in infants younger than ٣ months and is likely because of enteroviral infections and thermoregulation during hot weather (McCarthy, ١٩٩٨). However, other studies do not specifically address seasonal variation associated with bacteremia (Bang *et.al.,* ٢٠٠٥).

١.٢.١.٥. Duration of fever:

The duration of fever has been noted to be shorter in patients whose blood cultures eventually become positive for known bacterial pathogens (mean ١٨ hrs.) than in those patients with blood cultures negative for known bacterial

pathogens (mean ٢٥ hrs.) (Bass *et.al.,* ١٩٩٣). Overall, duration of fever was inadequate to identify occult bacteremia clinically (Strait *et.al.,* ١٩٩٩).

١.٢.٢.Pathogenesis:

Under exceptional conditions, some microbes frequently enter the blood stream via some sites. Usually, host defenses to clear these invaders occur without adverse effects on the individual , but when host defenses become compromised or the organism's virulence is enhanced ,illness may result. Host defenses may be impaired by the patient's primary disease, but blood stream invasion today arises commonly in association with instruments used for the patient's care (Campbell and Mclutosh ,١٩٩٨). Even when no underlying disorder is present, consequences of bacteremia may be severe (Amit *et.al.,* ١٩٩٤). Bacteremic infection is more frequently due to organisms that are usually among the endogenous flora of the patient or to that which have replaced the usual microbial flora at one or more body sites especially after hospitalization (McGowan and Shulman, ١٩٩٨).

١.٢.٣. Clinical manifestation:

Recognition of the septic child seems to be difficult. The pediatrician's fundamental dilemma is to differentiate the child with a potentially life–threatening infection from the many children with self–limited or readily

treated infections that are not life–threatening. An awareness of the presence of predisposing conditions to infection in individual children is probably the most helpful guide. However, not all seriously ill children have identified defects in their host defenses, particularly in infancy. Most children with sepsis have obvious and significantly elevated temperatures. However in the very young and those with advanced diseases temperatures actually may be registered in the hypothermic range. Hyperthermia (temperature greater than ٤١°C) implies bacteremia (Crocetti and Barone, ٢٠٠٤)

The primary signs and symptoms of sepsis and its complications include fever, shaking chills, hyperventilation, tachycardia, cutaneous lesions (e.g., petechiae, echymoses, diffuse erythema), and change in mental status such as confusion, agitation, anxiety, lethargy, obtundation or coma. Secondary manifestations include hypotension, cyanosis, symmetric peripheral gangrene (purpura fulminans), oliguria or anuria and jaundice (Powell and Stormorken, ٢٠٠٣).

The following circumstances may increase the chance of developing bacteremia as being stated by (Wessels, ٢٠٠٥):

- Immune suppression, either like in HIV infection or drug therapy
- Antibiotic therapy which changes the balance of bacterial types in the body
- Prolonged or severe illness
- Malnutrition
- Diseases or drug therapy that cause ulcers in the intestines, e.g. chemotherapy for cancer.

١.٢.٤.Etiologies:

The causative agents of bacteremia are variable from time to time, from site to site and from area to another area.

In USA historically with respect to the predominant organisms, it has been reported that in the ١٩٣٠s and ١٩٤٠s, group A streptococci and other Gram-positive bacteria were responsible for most cases of neonatal sepsis.

In the ١٩٥٠s following the introduction of antibiotics, Gram-negative organisms, particularly ***E.coli***, ***Klebsiella***, ***Enterobacter, P.aeruginosa,*** and Enterococci, became the predominant organisms. ***S. aureus*** caused outbreaks of neonatal infection, including sepsis, during the ١٩٥٠s and early ١٩٦٠s (Starr, ١٩٨٥).

In the late ١٩٦٠s and early ١٩٧٠s, group B β–hemolytic Streptococci emerged as an important cause of sepsis. In the early ١٩٨٠s the frequency of sepsis caused by **Coagulase-negative staphylococci** (**CoNS**), particularly ***S. epidermidis*** has increased in several hospitals (Munson *et.al.*, ١٩٨٢ and Baumgart *et.al.*, ١٩٨٣), whereas in other hospitals viridans group of streptococci has emerged as an important cause of sepsis (Broughton *et.al.*, ١٩٨١ and Spiegelblatt *et.al.*, ١٩٨٥).

During ١٩٧٠ to ١٩٧٩ the Gram-positive bacteremia was found to be more frequent than Gram-negative bacteremia which accounted for ٥٢.٥٪ versus ٤١.٨٪ respectively, the Gram-positive organisms represented by (***S.aureus*** ١٠.٨٪, Coagulase-negative Staphylococci **(CoNS)** ٠.١٪, ***Streptococcus pyogenes*** ٢٪ and ***Listeria monocytogenes*** ٠.٧٪) while in ١٩٨٠-١٩٨٩ and ١٩٩٠-١٩٩٥ it was found that Gram-positive was less than Gram-negative which were (٤٥.٤٪ versus ٤٥.٧٪) and (٣٩.٢٪ versus ٤٥.١٪) respectively, and the most common

Gram-positive organisms were ***S.aureus*** ٨.٨, ٧.٦٪, **CoNS** ٠.٨, ١٪, ***Streptococcus pyogenes*** ٢, ٣.٢٪and ***Listeria monocytogenes*** ١, ٠.٣٪ (Eykyn, ١٩٩٨).

Before ١٩٨٠, most late onset or nosocomial infections in neonatal nurseries in industrialized countries were caused by ***S.aureus*** and Gram-negative bacilli (Stoll *et.al.*, ١٩٩٦ and Karlowicz *et.al.*, ٢٠٠٠). For the last ٢٠ years, however, CoNS have predominated and have been responsible for at least half of all late onset infections (Isaacs, ٢٠٠٣).

In Germany between ١٩٨٥-١٩٩٥, ١٠٣٧ bacteremic episodes were recorded in a pediatric tertiary care center and analyzed retrospectively. Berner and his colleagues (١٩٩٨) found high rate(٦٨٪) of bacteremia in neonates resulted by Gram-positive bacteria while Gram-negative bacteria accounted for (٢٩٪).

Bacteria isolated from blood cultures at King Faisal Specialist Hospital (KFSH) in Saudi Arabia between ١٩٨٥ and ١٩٩٩ showed that a higher number of culture-positive patients were detected in ١٩٩٩ (٥٥٪) as compared with ١٩٨٥ (٤٢٪). ***Staphylococcus epidermidis*** was the most predominant organism in ١٩٩٩ (٣٧٪) and also in ١٩٨٥ (٢١٪). Bacteremia caused by ***S.aureus*** decreased from ١٠٪ in ١٩٨٥ to ١٪ in ١٩٩٩. In Saudi Arabia ٨٤,٤٠٠ patients showed an overall rate of bacteremia of ١٥.٧ per ١٠٠٠ admissions. ***S.aureus*** was the most frequently isolated organism (١٣٪) followed by Coagulase negative Staphylococci (١٦٪), ***S.pneumoniae*** ١١.٥٪, Enterococci (٩٪) and beta-hemolytic Streptococci (٩٪). Of the Gram-negative bacteria, the most frequently isolated were ***Salmonella*** **spp.** (٢٠٪), ***E.coli*** (١١٪), ***Klebsiella*** **spp.** (١١٪), ***Pseudomonas*** **spp.** (٨٪) and ***Enterobacter*** **spp.** (٤.٥٪) (Qutub and Akhter, ٢٠٠١).

In Myanmar, at one year study (August ١٩٩٨-July ١٩٩٩) Shwe *et.al.* found that the rate of bacteremia in febrile children as carried out in the Medical Unit (III), Yangon Children's Hospital (YCH), was (٥٤.٢٪) and the commonest organism isolated from children aged ١ month to ١٢ years was ***Salmonella typhi*** (٤٣.١٪) ,***E.coli*** (١٢.٣٪), ***S.aureus*** (٧.٧٪), ***P.aeruginosa*** (٧.٧٪); ***Streptococcus*** **spp.**; ***Shigella*** **spp.**; ***Klebsiella*** **spp.** and ***Acinetobacter*** **spp.** (Shwe *et.al.*, ٢٠٠٢).

Chapman and his colleagues identified ٦٢ infants with sustained infection, from ١٩٩٠ through ٢٠٠١ caused by **CoNS** in ٣٠ and by other organisms in ٣٢ represented by ١٠ Gram-negative, ٢٢ Gram-positive, ***S.aureus*** being the most predominant Gram-positive pathogens which accounted for ٧٢.٧٪ (Chapman and Faix ٢٠٠٣).

In Philadelphia (٢٠٠٢) Bacteremia occurred in ٨ (٢.١٪) of ٣٧٩ children aged ٢ to ٢٤ months with febrile seizures. None of the children with bacteremia had received previous antibiotics. The causative organisms were ***Streptococcus pneumoniae*** in ٧ cases and group A ***Streptococcus*** in ١ case (Shah *et.al.*, ٢٠٠٢).

In Edinburgh on (٢٠٠٢) at Royal Hospital for Sick Children the bacteremic illness rate was (٢.٥٪)in febrile children with temperature >٣٨.٥°C (Osman *et.al.*, ٢٠٠٢).

In Kenya through ١٩٩٨_٢٠٠٢ Prevalence and Outcome of Community-Acquired Bacteremia among Hospitalized Children was (٤٤.٥٪). The causative agents were Gram-positive bacteria represented by ***S.aureus*** ٦.٨٪ and group A streptococci ٤.٢٪. In the Catchment Area of Kilifi District Hospital the rate of bacteremia caused by ***S.aureus*** in infants <٢٨ day was ٠.٤٤ per ١٠٠٠ live births

/year while for those with < ١year age and <٢year age they were ٨٩ and ٥٧ per ١٠٠,٠٠٠/year respectively whereas bacteremia caused by group A streptococci in infants <٢٨ day was ٠.٥٥ per ١٠٠٠ live births /year and for < ١year and <٢year it was ٩٦ and ٦٣ per ١٠٠,٠٠٠/year respectively (Berkley *et.al.,* ٢٠٠٥).

In India(٢٠٠٤) Agnihotri and his colleagues, stated that Gram-negative bacilli predominated (٥٨.٥٪) over Gram-positive cocci (٤١.٥٪), ***S.aureus*** was found to be the most common isolate (٣٥٪) among Gram-positive organisms causing neonatal septicemia .The incidence of Gram-positive and Gram-negative organisms changed little over the ٥ year span, the rate of bacteremia among neonates over that period (July ١٩٩٨-june٢٠٠٣) at the Government Medical College Hospital was ٦٤.٤٪ (Agnihotri *et.al*., ٢٠٠٤).

In Atlanta it was found that ***S.aureus*** and ***E.coli*** were the most common organisms detected in bacteremic children (Diekema *et.al*., ٢٠٠٤).

Recently, it was found at King Khalid University Hospital (KKUH), Saudi Arabia, that (٧٨.٦٪) of the isolates obtained from bacteremic cases were Gram-positive bacteria that included the following types : ***S.epidermidis*** (٥٥.٤٪), ***S.aureus*** (٩.٥٪) of which ١٤٪ were Methicillin resistant types(MRSA), ***Streptococcus pneumoniae*** ٤.٥٪, ٤٠٪ of which were resistant to penicillin and ***Enterococcus faecalis*** ٤٪ whereas Gram-negative bacteria were ٤٤ (٢٠٪) that included ***E.coli*** and ***Klebsiella pneumoniae*** ٣.٦٪ each among bacteremic children aged ١ day to ١٥ years (Babay *et.al*., ٢٠٠٥).

In Najaf in ١٩٩٩ the study detected that neonatal septicemia in the neonatal intensive care unit (NICU) and children unit in Hospital of Maternity and Pediatrics in Najaf was (١٨٪)in neonates (٠-٢٨ days of age). The results showed that Gram-negative organisms constituted the majority of the isolates

٦١.٤٪ from which ***Klebsiella pneumoniae*** accounted for ٣١.٣% while Gram-positive cocci constituted ٣٣.٩٪ and the most Gram-positive pathogen was ***S.aureus*** which accounted for ٨٣.٧% . Anaerobic bacteria were detected in ٣ cases, all of them were ***Clostridium perfringens*** ٢.٧٪ (Al-Salamy, ١٩٩٩).

Later in ٢٠٠٠, the blood specimens were collected from prematures, infants and children (aged from ١ day to ٣ years) who admitted to the Hospital of Maternity and Pediatrics in Najaf during the period from October to December ١٩٩٦. The rate of Gram-positive bacteremia was ١٥.٧٪ consisted of ***S.aureus*** ١٠.٨٪ which was the predominant Gram-positive organisms, and the rate of Gram-negative bacteremia was ٨٤.٢٪, ***Klebsiella*** spp. were the most (Al-Charrakh *et.al.*, ٢٠٠٠). pathogens which accounted for ٦٤.٨٪

And in (٢٠٠١) the rate of bacteremic infants aged (١-٣ mo) incidence was ٢٣٪ of the patients which admitted to the Hospital of Maternity and Pediatrics in Najaf (Shams Al-Deen, ٢٠٠١).

In Babylon, Al-Waznee (٢٠٠١) found that ***S.aureus*** accounted for (٢٦٪)and ***Klebsiella pneumoniae*** accounted for ٥٥٪ in which the predominant organisms in blood stream of infants less than ١year. The incidence of bacteremia in newborn was ٣٦.١٪.

In Ramadi, Al-Zwaini (٢٠٠٢) carried out a study on ١١٨ neonates admitted to the main referral hospital in Al-Anbar with positive blood cultures. The incidence of neonatal bacteremia for babies born at this hospital was ٩.٢ per ١٠٠٠ live births, and mortality rate was ٢٨٪ ,***S.aureus*** accounted for ٣٩٪, ***Klebsiella pneumoniae*** (٣٠٪) and ***E.coli*** (٢١٪) .

Consequently bacteremia may be caused by both Gram-positive and/or Gram-negative organisms which we will discuss briefly below:

١.٢.٥. Gram-positive bacteremia:

A dramatic increase has been noted in the cases of bacteremia caused by Gram-positive cocci both in United States and all around the world (Pittet and Wenzel, ١٩٩٥). Infections with Gram-positive bacilli are now becoming more frequent as well. Among major organisms accounting for bacteremia are:

١.٢.٥.١.Staphylococcal bacteremia :

Bacteremia in children is a big medical problem and ***Staphylococcus*** spp. seems to be the common causative organism in such problem. Bacteremia caused by staphylococci may be more complicated since most strains of staphylococci are highly resistant to antibiotics particularly Methicillin which is referred to as Methicillin resistant ***S.aureus*** (MRSA) and Methicillin resistant **Coagulase-negative staphylococci** (MRCoNS).

A. *Staphylococcus aureus* bacteremia (SAB):

Staphylococcus aureus is a major cause of infection in infants and children. It is particularly common in the pediatrics population as a cause of skin and soft tissue infection, ranging from impetigo, furuncles, and wound infections to septic arthritis and osteomyelitis. Staphylococci are also frequently associated with such life-threatening infection as septicemia, endocarditis, and toxic shock syndrome (Edwards and Baker, ١٩٩٨). ***S.aureus*** bacteremia can occur both in normal (Hieber *et.al.,* ١٩٧٧; Sheargren, ١٩٨٤; Hodes and Brazilai,

١٩٩٠)and immunocompromised hosts (Ladisch and Pizzo, ١٩٧٨). As ubiquitous organisms, staphylococci gain access to the blood stream after colonization of indwelling plastic catheters or other foreign bodies, through breaks in the skin , or from frank infection of wounds. Once the organisms reach the vascular endothelium, the organisms can seed other sites , causing local complications (Edwards and Baker, ١٩٩٨).

S.aureus is increasingly recognized as a common and serious cause of bacteremia. Valles and colleagues reported that ***S.aureus*** was the third most frequent cause of community-acquired bacteremia in a European multicenter study (Valles *et.al*., ٢٠٠٣). In ١٩٤١, Skinner and Keefer stated that ٨٢٪ of ١٢٢ patients treated at Boston City Hospital for ***S.aureus*** bacteremia died of their infection (Miles *et.al*., ٢٠٠٥).

From ١٩٩٠ to ١٩٩٥, according to the National Nosocomial Infection Surveillance system of the Centers for Disease Control and Prevention (CDC), ١٦٪ of hospital-acquired cases of bacteremia in the United States were due to ***S.aureus,*** a proportion second only to that involving Coagulase-negative staphylococci (Burts *et.al*., ٢٠٠٥).

Bloodstream infections due to ***S.aureus*** have been described in children with no apparent focus of infection on examination. One Nigerian study found that ١٥.٨٪ of ٦٠ children diagnosed with septicemia an admission without any identifiable focus had ***S.aureus*** isolated from blood cultures (Odhiambo *et.al*., ١٩٩١). Similarly, another Indian study looking at ١٠٠ febrile children aged ١ month to ٣ years with no focus of infection found that ***S.aureus*** was the most common bacterial isolate from blood cultures (Singhi *et.al*., ١٩٩٢). ***S.aureus*** was

also one of the most frequently isolated organisms in children at high risk of infections, such as sickle cell disease and protein-calorie malnutrition (Issack *et.al.*, ١٩٩٢ and Okuonghae *et.al.*, ١٩٩٣).

At Kilifi District Hospital in Kenya, ***S.aureus*** accounted for around ٥٪ of all organisms isolated from blood cultures in children during the six-year period, and bacteremia resulted by ***S.aureus*** was found to be closely associated with a significant mortality (Ladhani *et.al.*,٢٠٠٤).

Rates of ***S.aureus*** infection have increased during the past two decades. Bacteremia due to ***S.aureus*** has been reported to be associated with mortality rates of ١٥٪-٦٠٪ (Cosgrove *et.al.,* ٢٠٠٣). Neonates had a ١٤-fold increase in mortality, which may be related to not receiving a specific antistaphylococcal antibiotic on admission (Ladhani *et.al.*, ٢٠٠٤).

Children mostly at risk of death associated with bacteremia due to ***S.aureus*** were least likely to have clinical features traditionally associated with this infection (Ladhani *et.al.*, ٢٠٠٤).

Approximately ٢٠٪ of community-acquired and nosocomial bacteremia in the United States are caused by ***S.aureus*** (Wagenvoort *et.al.*, ١٩٩٧).

Incidence of ***S.aureus*** community-acquired (CA) bacteremia in Connecticut was ١٧/١٠٠, ٠٠٠ persons (Morine and Hadler, ٢٠٠١).

Classification of SAB: (Mukonyora *et.al.,* ١٩٨٥)

- Community versus Hospital-acquired

- Primary versus Secondary
- Complicated versus Uncomplicated

Community-acquired: when blood cultures taken within ٤ days of admission to hospital were positive.

Hospital-acquired: if positive cultures were taken more than ٤ days after admission.

Primary infection: This was thought to be the initial staphylococcal focus leading to bacteremia.

Secondary infection: as that following bacteremia.

SAB acquired in hospitals continues to be a frequent and serious complication to hospitalization, and no previous case-control studies dealing with risk factors of this severe disease are available (Jensen *et.al.*, ١٩٩٩).

Finally serious infections caused by ***S.aureus,*** particularly those associated with bloodstream infection, are rapidly increasing in frequency, likely as a result of the proliferation of invasive procedures, the increasing severity of illness of hospitalized patients, suboptimal adherence for infection control practices, selection of resistant strains by antibiotic use, high rates of injecting drug use, and diabetes mellitus. In many cases, being aware of the clinical manifestations and treatment of ***S.aureus*** can help to decrease the incidence of these infections and promote better care for patients who have been-or could be-exposed to the pathogen (Weems, ٢٠٠١).

B . <u>Coagulase-negative staphylococci</u> :

Staphylococci are ubiquitous microorganisms that are pathogenic for humans and animals and widely distributed in the environment. ***Staphylococcus epidermidis*** is a Coagulase-negative strain found universally on the skin and frequently in the nasopharynx. Coagulase-negative staphylococci(CoNS) are the predominant aerobic organisms in the normal bacterial flora of the skin (Meskin, ١٩٩٨).

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 ,١٩٩٤), 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, ٢٠٠٠ and Haimi, *et.al.,* ٢٠٠٢). CoNS are an increasingly a common clinical problem and are currently the most frequent cause of bacteremia in hospitalized patients, particularly in the neonate or child with an indwelling intravascular device (Edwards and Baker, ١٩٩٨). Among the factors that enhance its pathogenicity are the following :(Fulginiti, ١٩٨٤)

١. The administration of immunosuppressive therapy
٢. The use of broad spectrum antibiotics.
٣. The presence of neutropenia.
٤. The need of indwelling catheters and drains.

Data from the National Nosocomial Infections Surveillance System from January ١٩٩٠ to May ١٩٩٩ (NNIS,١٩٩٩) indicated that Coagulase-negative staphylococci are isolated from bloodstream infections in

patients in intensive care units more often than ***S.aureus*** (٣٧.٣٪ versus ١٢.٦٪)respectively .

The incidence of CoNS sepsis was ٣.٤٦ episodes per ١٠٠٠ live births (Isaacs, ٢٠٠٣).

C. Methicillin Resistant *Staphylococcus aureus* infections (MRSA infections) :

MRSA stands for Methicillin-resistant ***Staphylococcus aureus***. MRSA is a type of ***Staphylococcus*** bacterium that has developed resistance to the antibiotic Methicillin and other penicillins. 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, ١٩٩٩) .

Since first described in ١٩٦١ (Jevons ,١٩٦١) , 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.,* ١٩٩٥).

Infections with antibiotic-resistant organisms are thought to result in higher morbidity and mortality rates than in similar infections with antibiotic-susceptible strains (Cosgrove *et.al.,* ٢٠٠٣). Therefore MRSA bacteremia is associated with significantly higher mortality rate than is MSSA bacteremia (Cosgrove *et.al.,* ٢٠٠٣), and it is associated with a

real increase in risk of death, further justifying ongoing MRSA surveillance and control in healthcare facilities(Whitby *et.al.,* ٢٠٠١).

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 (Morine and Hadler, ٢٠٠١).

Community-acquired, Methicillin-resistant ***S.aureus*** (CA-MRSA) is an established pathogen in several areas of the United States (Aguilar *et.al.,* ٢٠٠٣), and they are considered as an emerging problem (Cosgrove *et.al.,* ٢٠٠٣).

MRSA is a variant of ***S.aureus*** that is resistant to all β-lactam antibiotics (Elshafie and Bernardo, ٢٠٠١).

The continuing increase in the incidence of infections caused by MRSA complicates the approach to treat ***S.aureus*** bacteremia. The National Nosocomial Infections Surveillance System of the Centers for Disease Control and Prevention estimated that the prevalence of Methicillin resistance among ***S.aureus*** strains causing nosocomial infections in patients in the intensive care unit (ICU) reached ٥٧٪ in ٢٠٠٢, an absolute increase of ١٣٪ over the ٤٤٪ prevalence in the previous ٥-year period(National Nosocomial Infections Surveillance System, ٢٠٠٣). Among the more than ٨٢,٠٠٠ blood culture isolates reported in hospitalized patients in the United States in ٢٠٠٢, ***S.aureus*** was the second most common pathogen, and ٤٩٪ of ***S.aureus*** isolates were Methicillin resistant (Karlowsky *et.al.,* ٢٠٠٤).

In addition, MRSA is no longer a pathogen found primarily in ICUs. Methicillin resistance was present in ٥٢٪ of ***S.aureus*** isolates from ICU patients and ٤٩٪ of ***S.aureus*** isolates from non-ICU patients. In other words, the assumption that MRSA is a problem confined to severely ill patients in the ICU is no longer valid (Chang *et.al.,* ٢٠٠٣).

In a ١:١ matched case-control study of ***S.aureus*** bacteremia, those infected with MRSA became bacteremic later in their hospital stay, more often had a polymicrobial bacteremia and were appropriately treated later. Although mortality attributable to the MRSA bacteremia was greater, this difference did not achieve significance (Austin *et.al.,* ٢٠٠٣).

Community-acquired infections(MRSA) appear to be increased (Lu *et.al.,* ٢٠٠٥) in both adults and children in various regions and countries, including Australia (Maguire *et.al.,* ١٩٩٨), the United Kingdom (Stacey *et.al.,* ١٩٩٨), New Zealand (Rings *et.al.,* ١٩٩٨), Taiwan (Ito *et.al.,* ٢٠٠١), Saudi Arabia (Madani *et.al.,* ٢٠٠١), North America(Jones *et.al.,* ٢٠٠٢), Finland (Salmenlinna *et.al.,* ٢٠٠٢), and Iraq (Al-Sahllawi, ٢٠٠٢).

Incidence rates of MRSA were estimated world wide by the European Antimicrobial Resistance Surveillance System (EARSS), (٢٠٠٤). These estimations were as follows : Austria (١٥٪), Bulgaria (٣١٪), Croatia (٣٧٪), Czech Republic (٧٪), Denmark (<١٪), Estonia (٥٪), Finland (١٪), Hungary (١٤٪), Ireland (٤٢٪), Israel (٤٣٪), Malta (٤٣٪), Poland (١٩٪), Romania (٤٦٪), Slovenia (١٣٪), Spain (٢٥٪) and Sweden (<١٪) (EARSS, ٢٠٠٤).

Compared with MSSA, MRSA is associated with worse outcomes, including longer hospital and ICU stays, longer durations of mechanical ventilation, and higher mortality rates, in addition, the cost of treating patients with bacteremia due to MRSA is higher (Friedman *et.al.,* ٢٠٠٢). Inadequate antibiotic therapy is associated with significant mortality, and perhaps explains why outcomes in MRSA infection are so poor (Melzer *et.al.,* ٢٠٠٣). Emerging data suggest that therapy with vancomycin is less than optimal; treatment failure and mortality rates are substantial whether the cause of bacteremia is MRSA or MSSA. Because MRSA is far more common in healthcare-related or nosocomial bacteremia than in community-acquired bacteremia, empiric antibiotic therapy for patients with healthcare-related bacteremia should include coverage for MRSA (Tacconelli *et.al.,* ٢٠٠٤).

D. Methicillin Resistant CoNS infections (MR CoNS infections):

S.epidermidis is an opportunistic pathogen that can cause serious problems in compromised hosts, especially those who have intravascular or intraventricular prosthetic devices. The frequency of Methicillin Resistant ***S.epidermidis*** has made the problem of therapy formidable. The general pediatrician would do well to seek the advice of a pediatric infectious disease specialist for diagnosis and management of these infections (Meskin**,** ١٩٩٨). In a survey of blood stream infections in United State, Latin America and Canada it was found that Oxacillin-resistance among CoNS accounted for (٧١.٥٪ U.S., ٦٨.٤٪ Latin America, and ٦٥.٦٪ Canada) (Pfaller *et.al*., ١٩٩٩).

١.٢.٥.٢.Streptococcal bacteremia

A. *Streptococcus pneumonia:*

This organism remains important in community-acquired infections, especially in association with pneumonia. Mortality has changed significantly in the past few decades (Afessa *et.al*., ١٩٩٥). Bacteremic pneumococcal strains resistant to penicillin have been associated with occurrence and complicated treatment (Moreno *et.al.,* ١٩٩٥). These strains are often multidrug resistant. This brings renewed interest in pneumococcal vaccine, which can reduce the risk of pneumococcal bacteremia in high risk patients (Farr *et.al.*, ١٩٩٥) .

B. Group A and group B streptococci :

Endemic cases of sepsis caused by group A streptococci are seen in both adults and children (Moses *et.al.*, ١٩٩٥). Toxic shock syndrome may be part of the presentation of adults with bacteremia caused by group A streptococci (Forni *et.al.,* ١٩٩٥). Group B streptococcal sepsis continues to occur with some frequency, especially in older persons and in those with serious underlying conditions(Jackson *et.al*., ١٩٩٥).

C. *Enterococcus* :

Enterococcal sepsis can have severe consequences. Overall mortality is higher than ٣٠٪, with significantly higher mortality in burned patients and other immunocompromised patients (Noskin *et.al*., ١٩٩٥). The appearance of resistance to vancomycin, added to resistance to aminoglycoside and β-lactam drugs, has made therapy for Enterococcal bacteremia much more difficult (Edmond *et.al.*, ١٩٩٥). For some of these strains no antimicrobial treatment is currently available. Prior use of broad spectrum cephalosporins has been implicated as a risk factor

for nosocomial bacteremia with ***Enterococcus faecalis*** (Pallares *et.al.,* ١٩٩٣).

١.٢.٦.Gram-negative bacteremia :

١.٢.٦.١. Gram-negative bacilli:-

Bacteremia caused by Gram-negative bacilli remains common world wide (Uzeun *et.al.,* ١٩٩٢). In large measure this is due to increasing occurrence of antimicrobial resistance. A French study found that ٦٩٪ of intensive care unit(ICU)-associated bacteremias were due to Gram-negative bacilli, compared with ٥٦٪ outside the ICU (Buisson *et.al.,* ١٩٩٥). Among major organisms causing bacteremia in this group are:

A. *Enterobacteriaceae* :

The common members among this group including ***E.coli***, ***Klebsiella*** spp., ***Enterobacter*** spp., ***Proteus mirabilis***, ***Salmonella*** spp. and ***Serratia marcescens***, continue to be a major cause of Gram-negative aerobic bacillary bacteremia(Champerland *et.al.,* ١٩٩٢). Blood stream invasion caused by Gram-negative bacilli has increased, perhaps because the organisms translocate more efficiently from the gastrointestinal tract than do other bacteria (Steffen *et.al.,* ١٩٨٨). Although antimicrobial resistance has been a problem in some areas, it has been less so in others(McGowan, ١٩٩٤). ***E.coli*** is usually the most common blood culture isolate in community-acquired infections(Grandson, ١٩٩١). ***Klebsiella pneumoniae*** continues to be important in both nosocomial

and community-acquired bacteremias(Lee *et.al.,* ١٩٩٤). ***Enterobacter*** spp. have accounted for sizable proportion (between ٣٪ and ١٠٪) of nosocomial bacteremic infections since the ١٩٧٠s in both the United States and Europe (Chow *et.al.,* ١٩٩١ and Anderson *et.al.,* ١٩٩٤). ***Salmonella*** bacteremia was found to be prominent in developing nations, in children and in patients with AIDS (Ramose *et.al.,* ١٩٩٤). ***Shigella*** bacteremia is rare in adults, put patients with AIDS may be at increased risk(Kaplan *et.al.,* ١٩٩٥). The major contexts in which bacteremic ***Enterobacteriaceae*** have been noted are hospital outbreaks associated with intestinal colonization, urinary catheterization, and resistance to many different antimicrobials (McGowan and Shulman, ١٩٩٨).

B. *Pseudomonas* spp. :

For decades, ***Pseudomonas aeruginosa*** has been an appreciable source of both community-acquired and nosocomial bacteremias. High cases fatality rates have characterized cases associated with this organism(Mizushima *et.al.,* ١٩٩٤). Factors especially important in fatal outcomes of ***P. aeruginosa*** infection are shock, granulocyte count less than ٥٠٠/mm^{٣}, inappropriate antimicrobial therapy, development of secondary foci of infection , and presence of AIDS(Fergie *et.al.,* ١٩٩٤). Community-acquired cases of bacteremia caused by ***Pseudomonas*** species other than ***P.aeruginosa*** have been rare, but nosocomial infections with these organisms are seen with increased frequency (Aoun *et.al.,* ١٩٩٢). Nosocomial infections with these organisms tend to appear in association with contamination of a commercial product such

as respiratory therapy equipment, disinfectants, blood gas analysis or blood products(Henderson *et.al.,* ١٩٨٨).

١.٢.٦.٢.Gram-negative coccobacilli :-

Occurrence of sepsis caused by ***Haemophilus influenzae*** in children has decreased in association with the introduction of an effective vaccine(Saarinen *et.al.,* ١٩٩٥). ***Haemophilus*** bacteremia in adults occurs more frequently in the elderly and is usually due to nontypable strains of ***Haemophilus influenzae***(Najm *et.al.,* ١٩٩٥).

١.٢.٧.Mechanism of bacteremia:

The presumed mechanism begins with bacterial colonization of the respiratory passages, moist lining of the urinary tract, lower digestive tract or other internal surfaces. Bacteria may egress into the bloodstream of some children because of host- and organism-specific factors. Once viable bacteria have gained access to the bloodstream, they may be cleared spontaneously, they may establish a focal infection, or the infection may progress to septicemia. The possible sequelae of septicemia include shock, disseminated intravascular coagulation, multiple organ failure, and death (Harper and Fleisher ١٩٩٣ and Bass *et.al.,* ١٩٩٣).

١.٢.٨.Diagnosis:

The risk of bacteremia has been evaluated by multiple diagnostic tests and clinical scales (McCarthy *et.al.,* ١٩٨٠ and Jaffe and Fleisher ١٩٩١). The

diagnostic tests include white blood cell (WBC) count, Erythrocyte Sedimentation Rate (ESR), C reactive protein, morphological changes in peripheral neutrophils, microscopic examination of Buffy coat and quantitative blood cultures (Brook, ٢٠٠٣).

Infants with bacterial disease are often difficult to identify on the basis of clinical presentation alone (Baker *et.al.,* ١٩٩٠). Several children who had serious bacterial illness were identified only in the clinical assessment while other children with serious bacterial illness appeared normal on clinical assessment but had abnormal laboratory results (Baker *et.al.,* ١٩٩٣).

Jaffe and Fleisher concluded that increments in temperature above ٣٩.٠°C provided additional diagnostic specificity for bacteremia only at the expense of unacceptable decreases in sensitivity. Total WBC provided better information (Jaffe and Fleisher, ١٩٩١). Bonsu and Harper concluded that using a white blood cell count as the basis for the decision to obtain a blood culture probably will cause significant errors. Because of the potentially disastrous results of misdiagnosis, blood cultures should be obtained from all infants who are being evaluated for sepsis (Bonsu and Harper, ٢٠٠٣).

Lieu *et.al.* (١٩٩١) and Harper and Fleisher (١٩٩٣) stated that the sensitivity in identifying bacteremia in a febrile child with a leukocyte count above١٠,٠٠٠/microliter was ٩٢ %. While the blood culture has been estimated to have a sensitivity of ٧٠ % in identifying bacteremia.

Al-Majali found that Absolute Neutrophil Count (ANC) is a good predictive test for determining bacterial infection in febrile young children without focus (Al-Majali, ٢٠٠٤), and it was more sensitive and specific than a WBC or absolute band count (ABC) for occult bacteremia detection (Kuppermann, ١٩٩٩). But because ٦ to ١٠٪ of children with bacteremia may develop serious

bacterial infection(SBI), it has been recommended that a blood culture also be considered especially if the child is ill appearing or the degree of illness is uncertain (Health Policy and Clinical Effectiveness Program, ٢٠٠٠).

The blood culture was considered the rapid and reliable detector of bacteremia so it stills the gold standard for diagnosis of bacteremia (Eykyn, ١٩٩٨). Kurlat *et.al.* (١٩٨٩) pointed out that a ٢-days processing period is sufficient to detect positive blood cultures in the asymptomatic term infant, a ٤-day processing period will detect virtually all clinically important infections, and clinical yield from continuing blood culture processing beyond ٤ days does not justify the time and cost involved, since the number of bacteria in the blood in bacteremia may be small. However the volume of blood per culture is a major determinant of the bacterial yield ,regardless of the blood culture system used (Eykyn, ١٩٩٨).

١.٢.٩.Treatment:

The major goal of antibiotic therapy is the elimination of bacteria from the blood as quickly as possible, thus removing the source of shock ,disseminated intravascular coagulation and other pathophysiologic consequences of sepsis (Starr, ١٩٨٥).

Carroll and his colleagues found that children with clinical and laboratory features of occult bacteremia who were treated with antibiotics at the first visit had a better outcome and fewer septic complications than those who did not receive antibiotic initially (Carroll *et.al.,* ١٩٨٣). So antimicrobial agents are important not only for clearing the blood stream but also for preventing secondary infection from developing as a result of blood stream invasion

especially in ***Staphylococcus aureus*** bacteremia (McGowan and Shulman, ١٩٩٨).

Treatment of suspected bacteremia should be initiated promptly once diagnostic studies have been obtained. Initial therapy is dictated by the infant's age and physical location at onset of signs (community or hospital) and by focus of infections (Edwards and Baker, ١٩٩٨).

Antimicrobial agents are chosen by most likely pathogens and their expected susceptibility patterns (Edwards and Baker, ١٩٩٨).

Some asymptomatic newborns are started on antibiotics because of maternal risk factors, such as prolonged rupture membranes, fever, or chorioamnionitis. Others receive antibiotics for minor symptoms that resolve quickly. Patients in these groups are often treated until it is clear that blood cultures are not positive(Kurlat *et.al.,* ١٩٨٩).

One alternative management protocol calls for the outpatient treatment with parenteral antibiotics of all febrile infants judged to be at low risk for serious bacterial illness (Baskin *et.al.,* ١٩٩٢). This approach, however, results in unnecessary antibiotic treatment for many infants, and it may contribute to the development of antibiotic-resistant pathogens (Wald and Dashefsky, ١٩٩١). So, Baker and his colleagues, caution those who choose to treat febrile infants in this way that they must first evaluate the infants carefully and completely and that subsequent evaluation procedures must be strictly carried out (Baker *et.al.,* ١٩٩٣).

It has been suggested that sepsis should be treated with third-generation cephalosporin or extended Gram-negative spectrum penicillin plus an

aminoglycoside (Powell and Stormorken, ٢٠٠٣). The addition of vancomycin is indicated when staphylococcal septicemia is suspected (Starr, ١٩٨٥).

Another method to prevent the incidence of bacteremia is vaccination ,since Shah and his colleagues concluded that widespread use of the pneumococcal conjugate vaccine may further decrease the incidence of bacteremia (Shah *et.al.,* ٢٠٠٢).

٢. ١. ***Materials:***

٢. ١. ١.***Samples***

One hundred and sixty blood samples have been collected from infants below ٢٤ months who were referred to Babylon Maternity and Children Hospital in Hilla suffering from fever (axillary > ٣٨.٣)through a period of ten months from November /٢٠٠٤ to August /٢٠٠٥. One ml venous blood samples were collected in screw cupped tubes containing supportive media (١٠ml Brain Heart Infusion broth without anticoagulant material).

٢. ١. ٢.***Apparatuses:***

Apparatuses used in this study are listed in (Table ٢-١) below:

Table (٢-١) Applied apparatuses used in this study:

Apparatuses	**Source**

Thermometer	(Japan)
Electric oven	Memmert (Germany)
Incubator	Memmert (Germany)
Autoclave	Ormon
Refrigerator	Concord (Italy)
Distiller	Kottermann
Compound Microscope	Olympus (Japan)
Water path	Memmert (Germany)
Sensitive balance	Mettler (USA)
Centerfuge	Hitachi (Japan)
Benson berner	(Germany)

٢.١.٣.Culture Media :

The following culture media were used properly in appropriate experiment:

٢.١.٣.١.Brain Heart Infusion broth *(Biolife)*

It has been used to enhance the bacterial growth when it was necessary.

٢.١.٣.٢.Nutrient agar medium *(Oxiod-U.K.)*

It is a general medium and was used for general experiments to cultivate the bacterial strains.

٢.١.٣.٣.MacConkey agar medium *(Mast-U.K.)*

It was prepared according to manufacturing company, it has been used to isolate Gram-negative bacilli and to differentiate lactose fermenter from non lactose fermenter (Baron *et.al*., ١٩٩٤).

٢.١.٣.٤.Blood agar medium *(Mast-U.K.)*

Blood agar base was prepared according to the manufacturing company, it was autoclaved at ١٢١°C \ for ١٥ minutes, and cooled to ٥٠ °C, and ٥% of human blood was added. This medium was used to cultivate bacterial strains and to detect their ability for blood haemolysis (Baron *et.al.,* ١٩٩٤).

٢.١.٣.٥.Mannitol salt agar *(Biolife)*

This medium was used to isolate and diagnose ***S.aureus*** (Collee *et.al.,* ١٩٩٦)

٢.١.٣.٦.Carbohydrates Fermentation Medium

The medium was used for detecting the ability of microorganisms to ferment Carbohydrates. The medium was prepared according to the method being recommended by (MacFaddin, ٢٠٠٠) as follows:

a) **Medium base:** it was prepared according to (MacFaddin, ٢٠٠٠) by dissolving ١٠ gm of bacto-peptone with ١ gm of bacto-beef extract with ٥ gm of NaCl and ٠.٠١٨ gm of phenol red in ١٠٠٠ml of distilled water and pH was adjusted to ٧.٤. These contents were distributed in test tubes (٥ ml in each). Durham's tube was added to each test tube to detect the gas production.

b) **Sugar solution:** eight sugars were used which were: D-glucose, Sucrose, Mannitol, Lactose, Xylose, Fructose, Arabinose and Salicin. ٠.١ ml of ١٪ concentration from each sugar was sterilized by chloroform and added to each medium base tube**.**

٢.١.٣.٧.(Methyl red- Vogas proskaure) medium *(Oxiod-U.K.)*

This medium was used to detect the ability of the bacteria to complete or partial analysis of glucose.

٢.١.٣.٨.Motility medium

This medium was prepared according to MacFaddin(٢٠٠٠)by adding ٤gm of agar-agar to ١٠٠ ml of nutrient broth in ١٠٠٠ ml of distilled water then sterilized by autoclave at ١٢١^{o}C \ for ١٥ minutes then it was distributed in tubes; it was used to detect bacterial motility (MacFaddin, ٢٠٠٠).

٢.١.٣.٩.Gelatin agar medium

This medium was prepared by adding (٤.٤ gm) of gelatin (Oxiod-U.K.) to nutrient agar medium (Collee *et.al.,* ١٩٩٦) It was used to detect the ability of the bacteria to hydrolyze gelatin.

٢.١.٣.١٠.Esculin media

This media was made from preparation of ٠.٤gm Ferric ammonium citrate and ٤gm Esculin added to nutrient agar and completed the volume to ١٠٠٠ml distilled water, then poured into tubes and all were sterilized by autoclave at ١٢١^{o}C\for ١٥ minutes, then the tubes were slanted (MacFaddin, ٢٠٠٠) .

٢.١.٣.١١.Muller-Hinton agar

This medium was used to test the sensitivity of bacterial isolates to antibiotics.

٢.١.٣.١٢.Nutrient broth *(Biolife)*

This medium was used to grow and preserve the bacteria.

٢.١.٤.Reagents and Solutions ;

٢.١.٤.١.Methyl red reagent

This reagent was prepared by dissolving ٠.١gm of methyl red reagent in ٣٠٠ ml of ٩٥٪ ethyl alcohol, filling the volume up to ٥٠٠ ml with distilled water, and using this indicator for detection of complete glucose hydrolysis (MacFaddin, ٢٠٠٠).

٢.١.٤.٢.Catalase reagent

This reagent was prepared by adding ٣ml hydrogen peroxide ($H_٢O_٢$) to ٩٧ml of distilled water where its concentration becomes ٣%, the solution was used to study bacterial ability to produce Catalase (MacFaddin, ٢٠٠٠).

٢.١.٤.٣.Oxidase reagent

This reagent was prepared by dissolving ١ gm (Tetra-Methyl-Phenylnene-Diamine-Dihydrochloride) in ١٠٠ ml distilled water and stored in dark bottle (Baron *et.al.,* ١٩٩٤).

٢.١.٤.٤.Coagulase test reagent

This reagent was prepared by addition of ٠.٥ ml of fresh human plasma diluted by ١:٥ to ٠.٥ ml to bacterial suspension (Staphylococci) (MacFaddin, ٢٠٠٠). The test was achieved by two methods; slide and tube method.

٢.١.٤.٥.Voges – Proskauer reagent

This reagent contained:

١. (α- naphthol); indicator; which was prepared by dissolving ٥ gm of α-naphthol in ١٠٠ ml of absolute ethylic alcohol.
٢. (KOH solution, potassium hydroxide solution); prepared by dissolving ٤٠ gm of KOH in ١٠٠ ml of distilled water; it was used for partial glucose hydrolysis (MacFaddin, ٢٠٠٠).

٢.١.٤.٦.Frazier's reagent

This reagent was prepared according to the method being recommended by(Collee *et.al.,* ١٩٩٦). It was prepared by dissolving ٥ gm $HgCl_٢$ in ٢٠ ml of concentrated HCL (٩٨٪), with the addition of ١٠٠ ml distilled water. It was used to detect the ability of bacteria to analyze gelatin (Collee *et.al.,* ١٩٩٦).

٢.١.٤.٧.Normal saline solution

Normal saline solution was prepared by dissolving ٩ gm of NaCl in ١٠٠٠ ml distilled water, and distributing it in tubes and autoclaving at ١٢١^{o}C \ for ١٥ minutes. It was used as diluent's solution.

٢.١.٤.٨.Gram stain solutions

The solutions were prepared according to the required microbiological methods (Baron *et.al.,* ١٩٩٤). The solution included four reagents; crystal violate, iodine, alcohol and Safranine.

٢.١.٤.٩.McFarland standard solution:

McFarland standard solution was used in antimicrobial susceptibility test, tube No. (٠.٥) was used which prepared by adding ٠.٠٥ ml of $BaCl_2.2H_2O$ con. (١.١٧٥٪) to ٩.٩٥ ml of H_2So_4 con. (١٪). The tube No.(٠.٥) of McFarland standard was used to compare the bacterial cell in suspension which gives a cell density $١.٥x١٠^{٨}$ cell/ml. (Baron *et.al.,* ١٩٩٤).

٢. ٢. *Methods:*

٢. ٢. ١.*Questionnaire :-*

The following data were recorded directly from the parents of infants :

**** Age.***

**** Sex.***

**** Residence, Rural or Urban.***

**** Type of feeding.***

**** Previous admission to hospital***

**** Clinical features, like: Fever, Vomiting, diarrhea….etc.***

****Duration of fever***

Antibiotic treatment, the patient must not have taken any antibiotics for the last ٣days at least to avoid the negative false results.

٢. ٢. ٢.Collection of samples:-

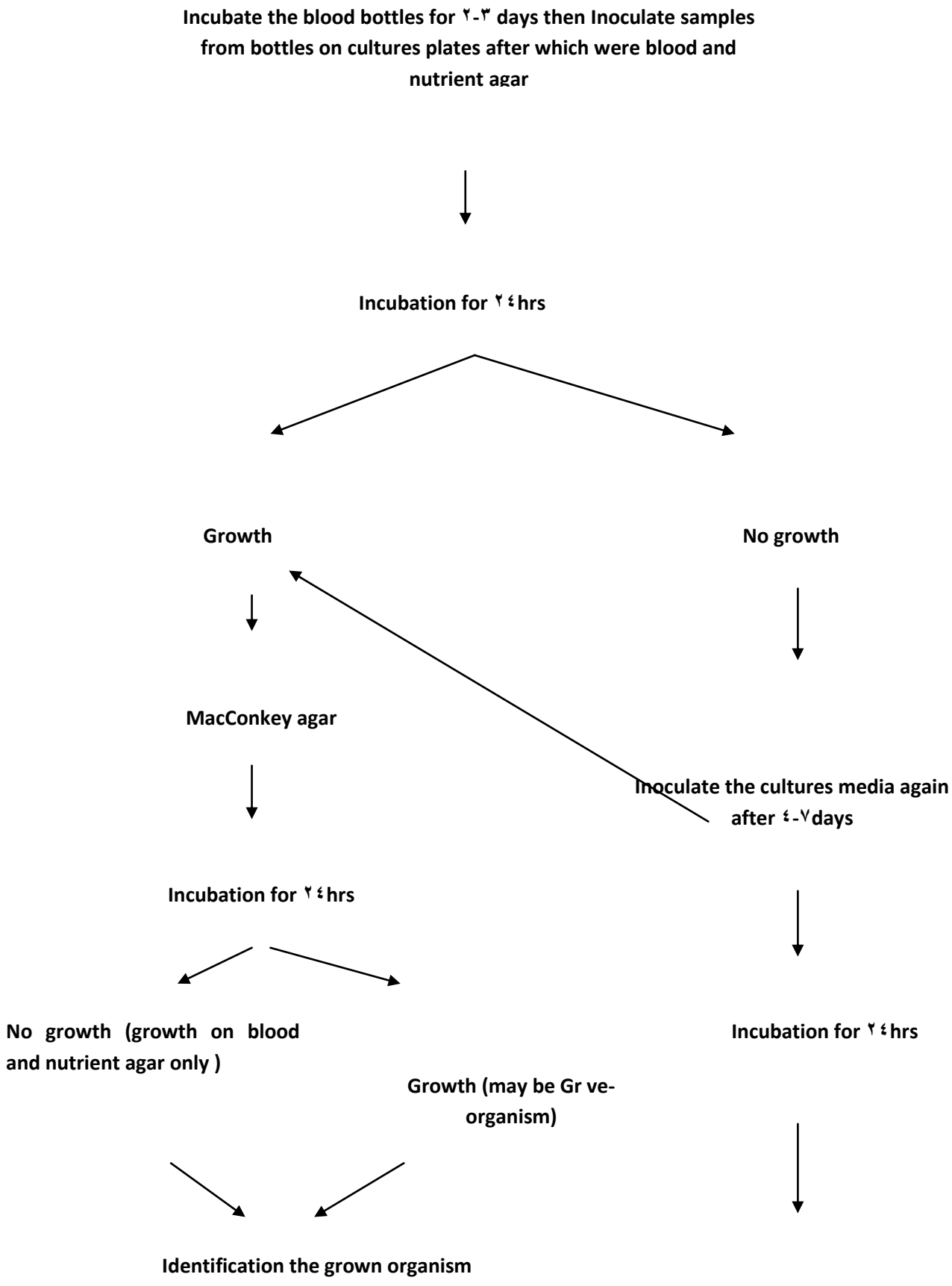
Blood sample was drawn from infant according to (Fischbach, ٢٠٠٠) as follows :

١. Sterilization of the proposed venipuncture site with povidone-iodine followed by ٧٠٪ alcohol and allowed skin to dry.
٢. The top of culture bottle was disinfected by povidone-iodine and air dried.
٣. Drawing approximately ٠.٥ to ١ ml of venous blood by venipuncture.
٤. Discarding the needle from the syringe and replacing it with a second sterile needle before injecting the blood sample into culture bottle which contained Brain-Heart Infusion broth.
٥. The blood sample was inoculated into culture bottle and transferred immediately to the laboratory to incubate it at ٣٧ ^{o}C for ٢-٧ days.

٢. ٢. ٣.Laboratory tests:-

After the incubation period, the signs of growth appeared in the blood culture e.g.; gas production, turbidity, heamolysis and flocculation.

٠.١ ml was taken from the blood culture then these tests were proceeded as indicated in Figure (٢-١) below:


Incubate the blood bottles for ٢-٣ days then Inoculate samples from bottles on cultures plates after which were blood and nutrient agar
Incubation for ٢٤ hrs
Growth
No growth
MacConkey agar
Inoculate the cultures media again after ٤-٧ days
Incubation for ٢٤ hrs
No growth (growth on blood and nutrient agar only)
Growth (may be Gr ve-organism)
Incubation for ٢٤ hrs
Identification the grown organism

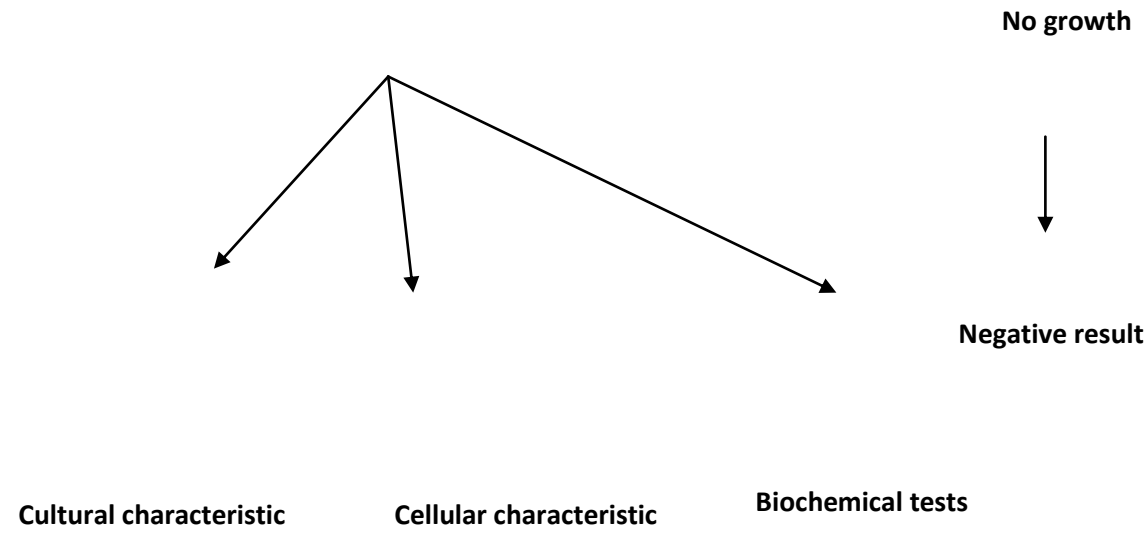


Figure (٢-١): The diagnostic steps of blood culture.

٢. ٢. ٣. ١.Cultural characteristics:

The following colonial characteristics were considered for the identification of bacterial isolates: shape and colour of colonies, the ability of heamolysis, forms of colonies, transulaencancy and pigments production.

٢. ٢. ٣. ٢.Cellular characteristics:

The characteristics were investigated by doing Gram stain for tested organism through which shape of bacterial cell, the reaction with gram stain and the arrangement of the bacterial cells with each other can be studied.

٢. ٢. ٣. ٣.Biochemical tests

٢. ٢. ٣. ٣. ١. Oxidase test

This test was done by rubbing the tested bacterial colony on the filter paper moistened by several drops of Oxidase reagent; when the colony was coloured with violet colour the result was recorded as positive (Baron *et.al.,* ١٩٩٤).

٢. ٢. ٣. ٣. ٢ Catalase test

This test was done by transferring a small quantity of the bacteria colony from a ٢٤hrs. culture medium into a clean slide and adding one drop of (٣٪ H_2O_2). The result was positive when the gas bubbles appear (MacFaddin, ٢٠٠٠).

٢. ٢. ٣. ٣. ٣. Coagulase test

١. ***Slide Coagulase test (clumping factor)***

A pure colony of staphylococci was emulsified in a drop of sterile distilled water to form milky suspension on a microscope slide with a minimum spreading, and a drop of fresh human plasma was added to the suspension with mixing, then the positive result appeared as a coarse clumping which is visible by the naked eye within ١٠ sec. (MacFaddin, ٢٠٠٠).

٢. ***Tube Coagulase test***

The test was used to detect the free Coagulase by adding ٠.٥ ml of diluted human plasma to ٠.٥ ml of centrifuged supernatant at ١٨-٢٤ hrs. pure broth culture of ***Staphylococcus*** spp. in tube

then incubated the tube at ٣٧°C for up ٤ hrs. and observed it every ٣٠ min for clotting by slant the tube, if no clot was visible after ٤hrs,the tube was left at room temperature for over night to confirm the result (MacFaddin, ٢٠٠٠).

٢.٢.٣.٣.٤. Growth on Mannitol salt agar

This medium was streaked by tested bacteria and incubated at ٣٧°Cfor ٢٤hrs. If the medium changes in to yellow colour, the test is positive and the tested organism is ***S.aureus*** (Merck, ١٩٨٥)

٢.٢.٣.٣.٥. Esculin test

The slant tubes containing Esculin medium was streaked by testing bacteria, incubated at ٣٧°Cfor ٢٤hrs. If the organism was grown in an Esculin slant tube changing the colour to dark brown, the result was positive (Baron *et.al.,* ١٩٩٤) .

٢.٢.٣.٣.٦. Methyl red test

The tubes of the (MR-VP broth) were inoculated with the selected bacterial colonies and incubated at ٣٧°C for ٢٤ hrs., then (٥ drops) of methyl red reagent were added to it. The appearance and observation of red colour means a positive result and a complete analysis of glucose (MacFaddin, ٢٠٠٠).

٢.٢.٣.٣.٧. Voges – Proskauer test

The tubes of (MR-VP broth) were seeded with the specific bacterial culture and were incubated at ٣٧°C for ٤٨ hrs., then we read the result by adding (٠.٦ ml of α- naphthol reagent) and (٠.٢ ml

of ٤٠٪ KOH solution); appearance of red colour after ١٥ min. means positive result due to partial analysis of glucose, which produce acetone or (Acetyl methyl-carbinol) (MacFaddin, ٢٠٠٠).

٢.٢.٣.٣.٨. Motility test

The tubes that contained the medium specific for motility stabbed with the specific bacterial culture which was incubated at ٣٧°C for ٢٤hrs. the distribution of growth outer of stabbing region means positive result (MacFaddin, ٢٠٠٠).

٢.٢.٣.٣.٩. Gelatin hydrolysis test

The gelatin medium plates were seeded with the specific bacterial inoculums by streaking method, incubated at ٢٢°C for ٢-٣ days, the plates were treated with Frazier's reagent for ٥-١٠ min., the positive result was the translucence around the colonies (MacFaddin, ٢٠٠٠).

٢.٢.٣.٣.١٠. Hemolysin production

The blood agar plates were inoculated by tested bacteria, incubated at ٣٧°C for ٢٤-٤٨ hrs. and the appearance of clear zone around the bacterial colony referred to (β-hemolytic) or green zone referred to (α-hemolytic)(Baron *et.al.,* ١٩٩٤).

٢.٢.٣.٣.١١. Bacitracin sensitivity test

Bacitracin disc (٠.٠٤U) was placed with sterile forceps to adhere to blood agar inoculated of B-hemolytic streptococci by streaking, incubated at ٣٧°C for ١٨-٢٤hrs.. The positive result is inhibition of

bacterial growth around the disc which indicated ***Streptococcus pyogenes*** (Collee *et.al*., ١٩٩٦).

٢.٢.٣.٣.١٢. Antibiotic susceptibility test.

This test was carried out by two methods:

١. ***Disc Diffusion Method:***

Antibiotic disc	*Symbol*	*Potency*	*Manufacture*	*Zone diameter nearest whole mm*

This was performed on Mueller–Hinton agar with the following antibiotic discs {Table(٢-٢)} according to Kirby-Bauer disc diffusion method (Bauer *et.al*., ١٩٦٦). Sensitivity was read after incubation for ٢٤ hrs. at ٣٥ °C. Isolates were regarded as sensitive or resistant according to NCCLS criteria (NCCLS, ٢٠٠٠).

Table(٢-٢) Antibiotics discs with standard zone diameter(NCCLS, ٢٠٠٠):

				Sensitive	*Medium*	*Resistant*
Penicillin G	**P**	**١٠ IU**	***Bioanalysis***	>٢٩	____	<٢٨
Ampicillin	**AMP**	**١٠*μg***	***Bioanalysis***	>٢٩	____	<٢٨
Amoxicillin	**AMO**	**٢٥*μg***	***Bioanalysis***	>٢٠	____	<١٩
Co-trimoxazole	**SXT**	**٥*μg***	***Bioanalysis***	>١٦	١١-١٥	<١٠
Cefotaxime	**CTX**	**٣٠*μg***	***Bioanalysis***	>٢٣	١٥-٢٢	<١٤
Cephalexin	**CL**	**٣٠*μg***	***Bioanalysis***	>١٨	١٥-١٧	<١٤
Gentamicin	**CN**	**١٠*μg***	***Bioanalysis***	>١٥	١٣-١٤	<١٢
Amikacin	**AK**	**٣٠*μg***	***Bioanalysis***	>١٧	١٥-١٦	<١٤
Oxacillin	**OX**	**١*μg***	***Al-Razii***	>١٣	١١-١٢	<١٠

٢. *Determination of minimum inhibitory concentration(MIC):*

We followed the method of (Baron *et.al.*, ١٩٩٦), list in {Table (٢-٣)}:

Table(٢-٣) Antibiotics used in MIC test with standard MIC ranges(NCCLS, ٢٠٠٠):

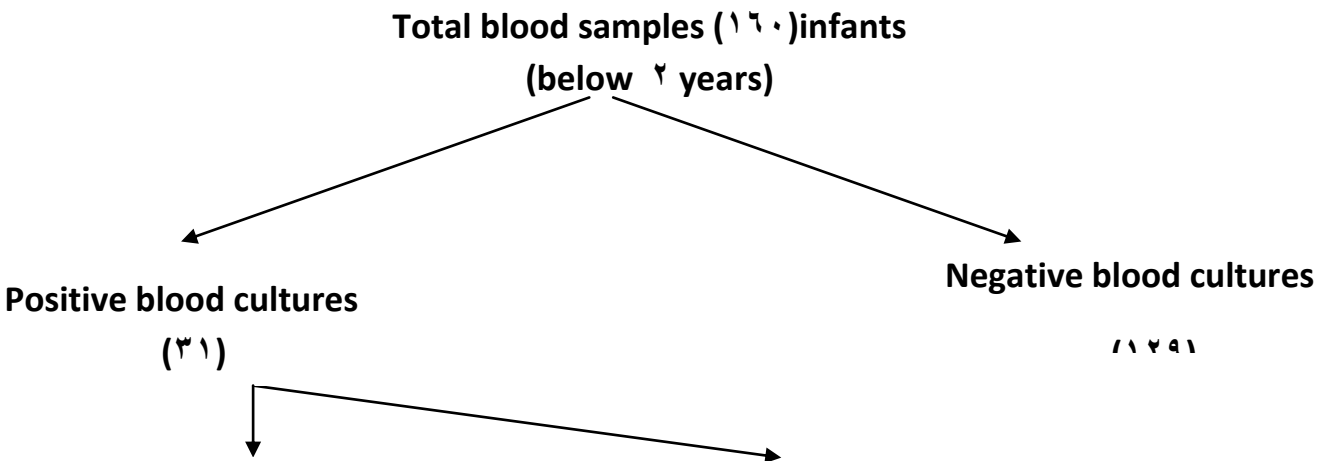
Antibiotic	*Manufacture*	*Suggested ranges for MIC determinations(mg/L)*		*MIC(μg/ml)*		
		Staphylococcus	*Streptococcus*	*Susceptible*	*Intermediate*	*Resistant*

Crystal Penicillin	**Ajanta/ India**	٠.٠٣-١٢٨	ـــــ	<٠.١٢	ـــــ	>٠.٢٥
Ampicillin	**Simrone/ Palghar**	٠.٠٣-١٢٨	٠.٠٠٨-٨	<٠.٢٥	ـــــ	>٠.٥
Amoxicillin	**Simrone/ Palghar**	٠.٠٠٨-١٦	ـــــ	<٤/٢	ـــــ	>٨/٤
Cefotaxime	**Ajanta/ India**	٠.٥-١٢٨	٠.٠٠٤-١٢٨	<٨	١٦-٣٢	>٦٤
Cephalexin	**Ajanta/ India**	٠.٥-١٢٨	٠.٠٠٤-١٢٨	<٨	١٦	>٣٢
Gentamicin	**Troge/ India**	٠.٠٠٨-١٢٨	١-١٢٨	<٤	٨	>١٦
Amikacin	**Troge/ India**	٠.٠٠٨-١٢٨	١-١٢٨	<١٦	٣٢	>٦٤

٢.٢.٣.٣.١٣. Preservation of identified bacterial isolates

Bacterial isolates preserved in nutrient broth which contains ٥٪ glycerol and kept in freeze at -٢٠^{o}C for need (Cruickshank, ١٩٧٢).

٢.٣.Study menu:



٢.٤.*Statistical analysis* :

Data were analyzed by using the $\chi^{٢}$ test and **Z** test, ***p***=٠.٠٥.

٣: Results :

The results shown in Figure (٣-١) indicate that ١٩.٤% (٣١/١٦٠) of the total children being investigated in this study were bacteremic, since they revealed positive results for blood culture, while ٨٠.٦٪ (١٢٩/١٦٠) had not.

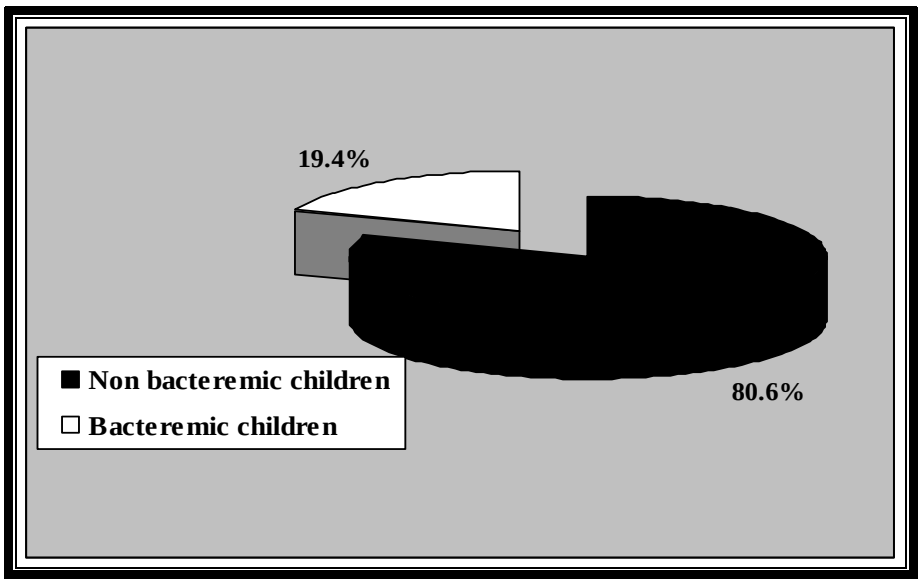


Figure (٣-١): Rate of bacteremia among febrile children.

Figure (٣-٢) shows the ratio of Gram-positive bacteria to Gram-negative, since Gram-positive accounted for ٦٨٪ (٢١/٣١) versus ٣٢٪ (١٠/٣١) for Gram-negative.

Biochemical test	***S.aureus***	**CoNS**	***Micrococcus* spp.**	***St.pyogenes***	***L.monocytogenes***

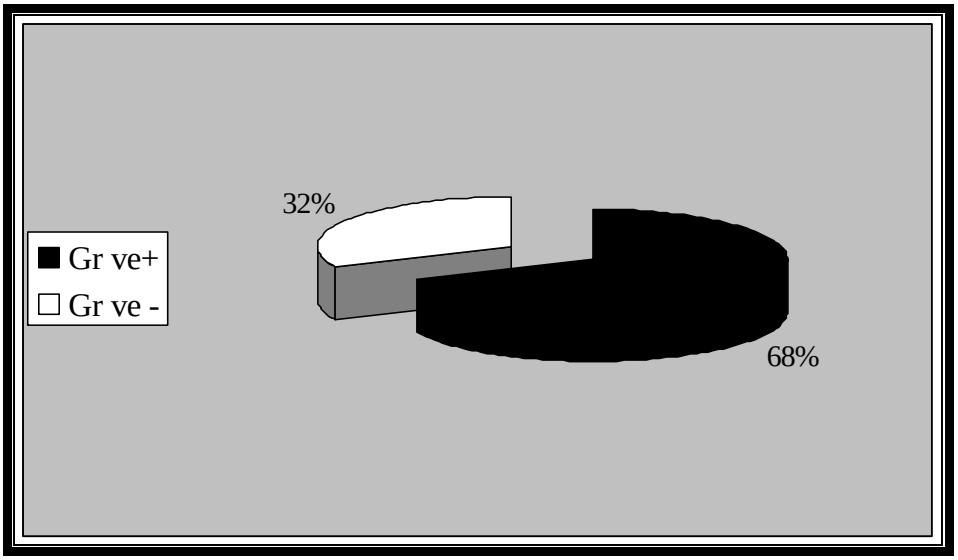


Figure (٣-٢): Rate of Gram-positive to Gram-negative isolates.

Table (٣-١) shows the results of biochemical tests being used for identification and isolation of Gram-positive bacteria. The results were compared with the referential results reported by Baron *et.al.,* (١٩٩٤); Collee *et.al.,* (١٩٩٦) and McFadden (٢٠٠٠).

Table (٣-١) Biochemical tests used for identification of Gram-positive bacterial isolates:

Hemolysis	**β**	**-**	**-**	**β**	**β**
Catalase	**+**	**+**	**+**	**-**	**+**
Oxidase	**-**	**-**	**+**	**-**	**-**
Coagulase					
Clumping factor(slide test)	**+**	**-**	**-**	**N**	**N**
Tube test	**+**	**-**	**-**	**N**	**N**
Sugars fermentation					
Glucose	**F**	**-**	**F**	**F**	**F**
Mannitol	**F**	**-**	**-**	**-**	**-**
Fructose	**F**	**F**	**-**	**F**	**-**
Xylose	**-**	**-**	**-**	**F**	**-**
Lactose	**F**	**-**	**-**	**F**	**-**
Sucrose	**F**	**F**	**-**	**-**	**-**
Salicin	**-**	**-**	**-**	**-**	**F**
Arabinose	**-**	**-**	**-**	**F**	**-**
Vogas-proskaure	**N**	**N**	**N**	**N**	**+**
Methyl red	**N**	**N**	**N**	**N**	**+**
Mannitol fermentation	**+**	**-**	**-**	**N**	**N**
Esculin hydrolysis	**N**	**N**	**N**	**-**	**+**
Motility	**N**	**N**	**N**	**-**	**+**
Gelatin liquefaction	**+**	**-**	**+**	**-**	**-**
Bacitracin	**N**	**N**	**N**	**Sensitive**	**N**

β = beta hemolysin

F = sugar fermentation

+ = positive result . N = not done

Figure (٣-٣) below shows the types and percentage of bacterial isolates being detected in blood samples which consist of Gram-positive bacteria represented by ***S.aureus*** ١٠(٣٣٪), **CoNS** ٥(١٦٪), ***St.pyogenes*** ٤(١٣٪), ***L.monocytogenes*** ١(٣٪) and ***Micrococcus*** spp.١(٣٪), while Gram-negative bacterial isolates ١٠(٣٢٪) but not diagnosis to their genus level.

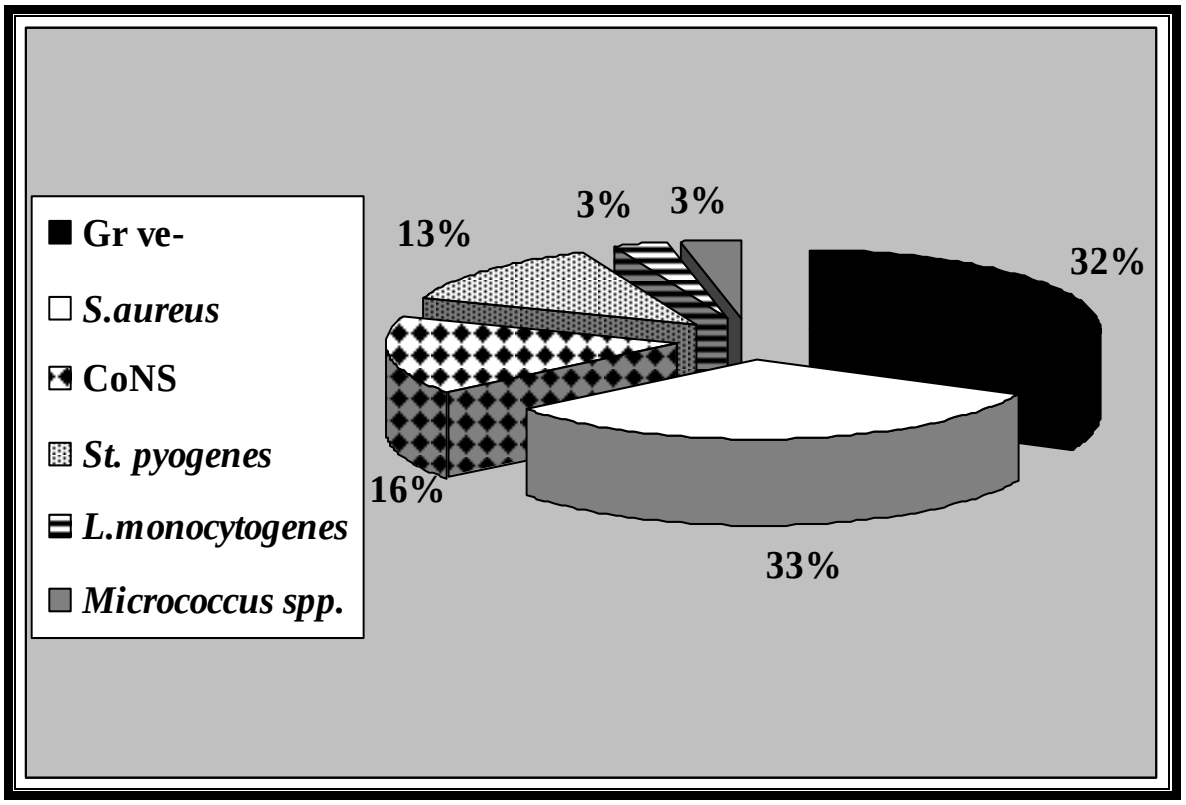


Figure (٣-٣): Types and percentage of bacterial isolates from blood samples of bacteremic children.

The distribution of bacteremic infection according to age is represented in Figure (٣-٤). The results showed that bacteremia were more in infants <١ month than in other age groups.

The study showed non significant relation of Total (Gram-positive and Gram-negative bacteremia) and Gram-positive bacteremia with age factor (***P***>٠.٠٥).

$\chi^{٢}_{calculator}$ (Total) = ٤.٨

$\chi^{٢}_{calculator}$ (Gram-positive) =٥.٥

$\chi^{٢}_{table}$ = ٩.٤

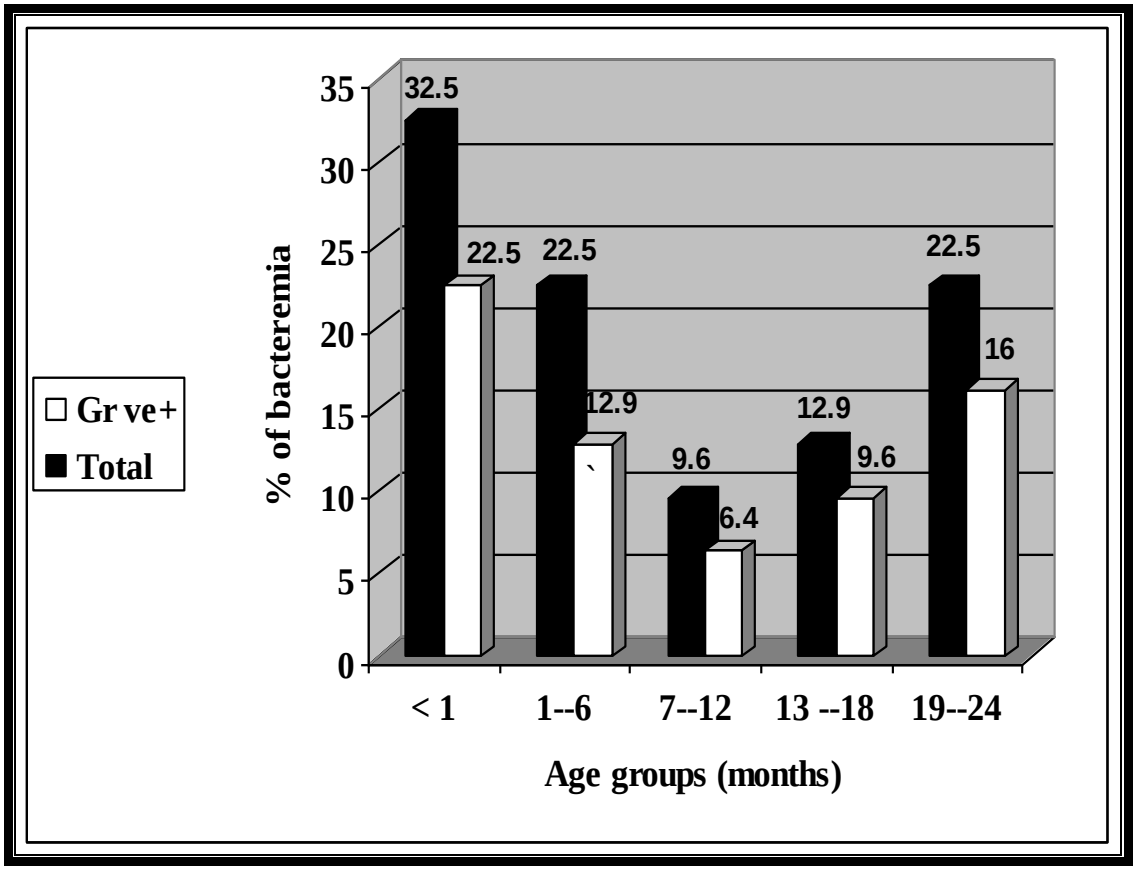


Figure (٣-٤): Distribution of bacteremic infections according to age (months).

The relation with the sex as a risk factor is shown in Figure (٣-٥), the study showed non significant difference between Gram-positive bacteremia and sex factor (***P***>٠.٠٥).

$Z_{calculator}$ =١.٠٦

Z = ١.٩٦

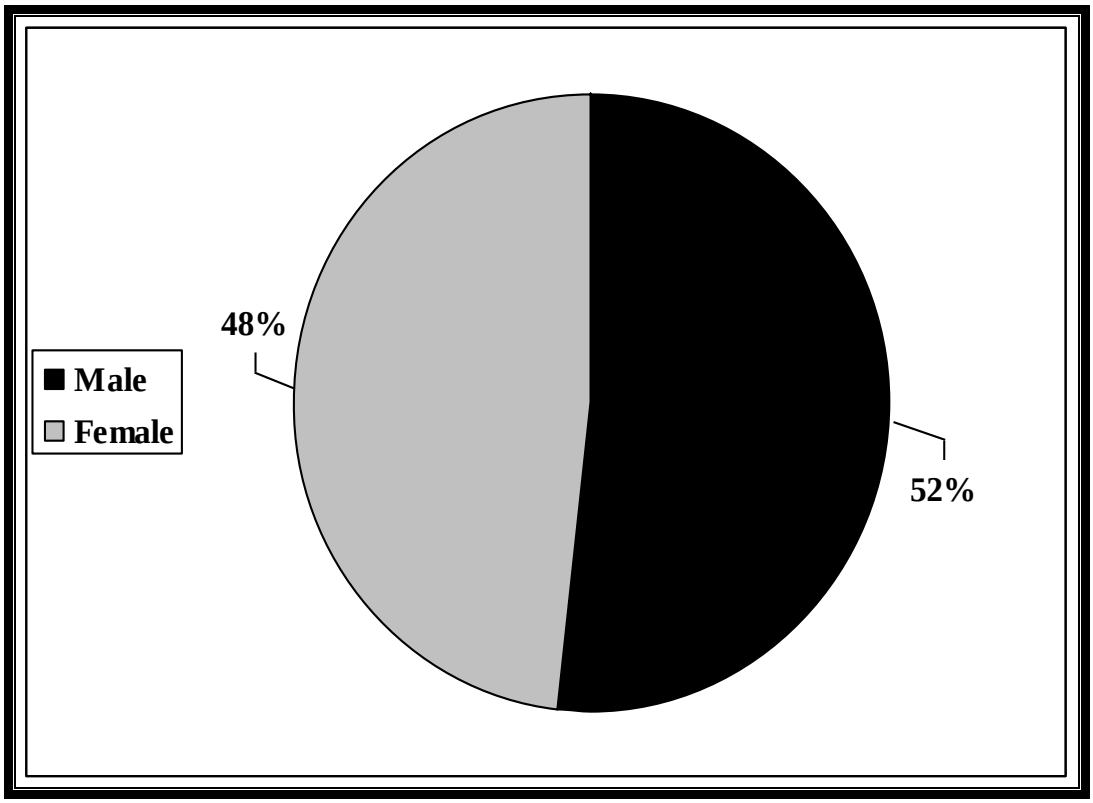


Figure (٣-٥): Distribution of Gram-positive bacteremic infections according to sex.

The study shows non significant difference of Total (Gram-positive and Gram-negative bacteremia) and Gram-positive bacteremia with temperature degree (***P***>٠.٠٥) as shown in Figure (٣-٦).

$\chi^{٢}_{calculator}$ (Total) = ١.٤

$\chi^{٢}_{calculator}$ (Gram-positive) =٤.٥

$\chi^{٢}_{table}$ = ٩.٤

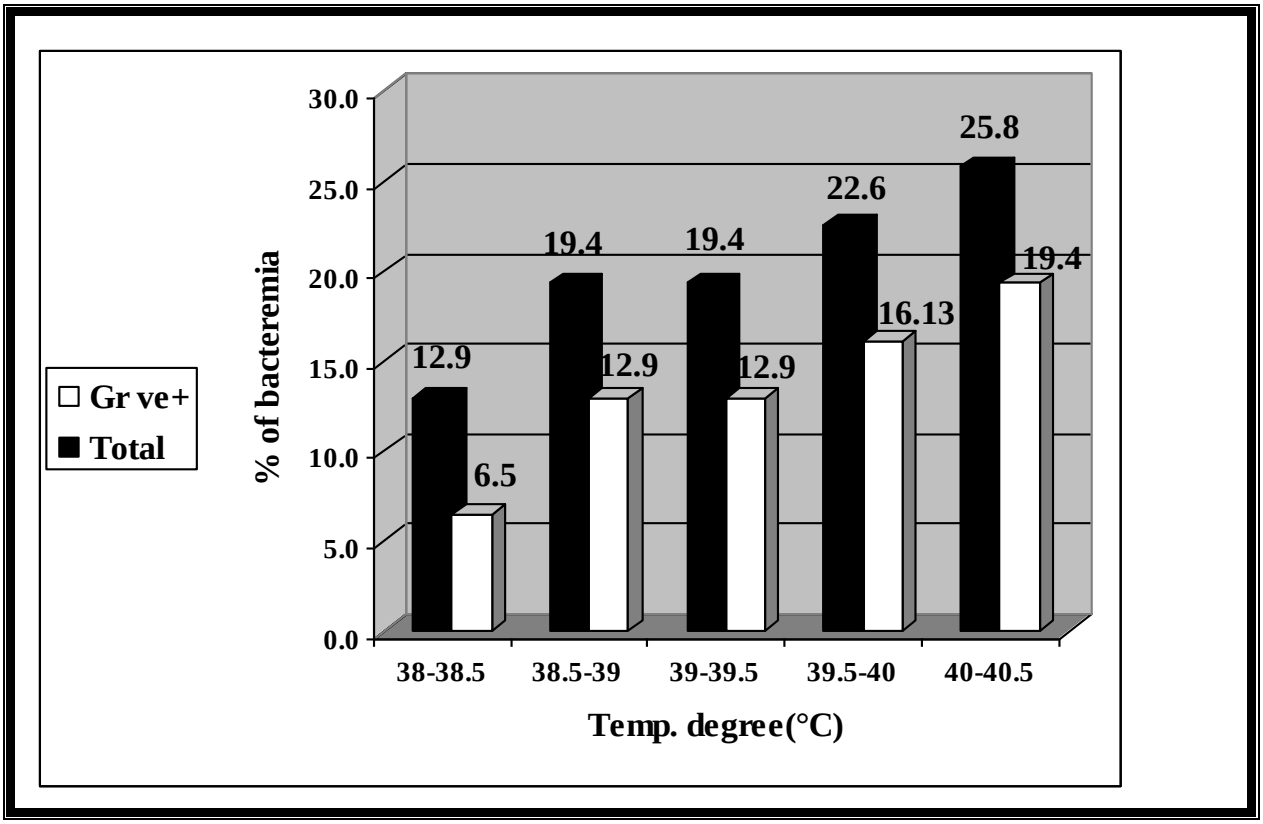


Figure (٣-٦): Distribution of bacteremic infections according to temperature degree (°C).

The result shows that persisting fever for ١-٢ days in children was more associated with bacteremia.

The study shows a significant correlation of Total (Gram-positive and Gram-negative bacteremia) and Gram-positive bacteremia with duration of fever (***P***<٠.٠٥) as shown in figure (٣-٧).

$\chi^{2}_{calculator}$ (Total) = ٣٦.٧

$\chi^{2}_{calculator}$ (Gram-positive) =١٩.٥

χ^{2}_{table} = ٩.٤

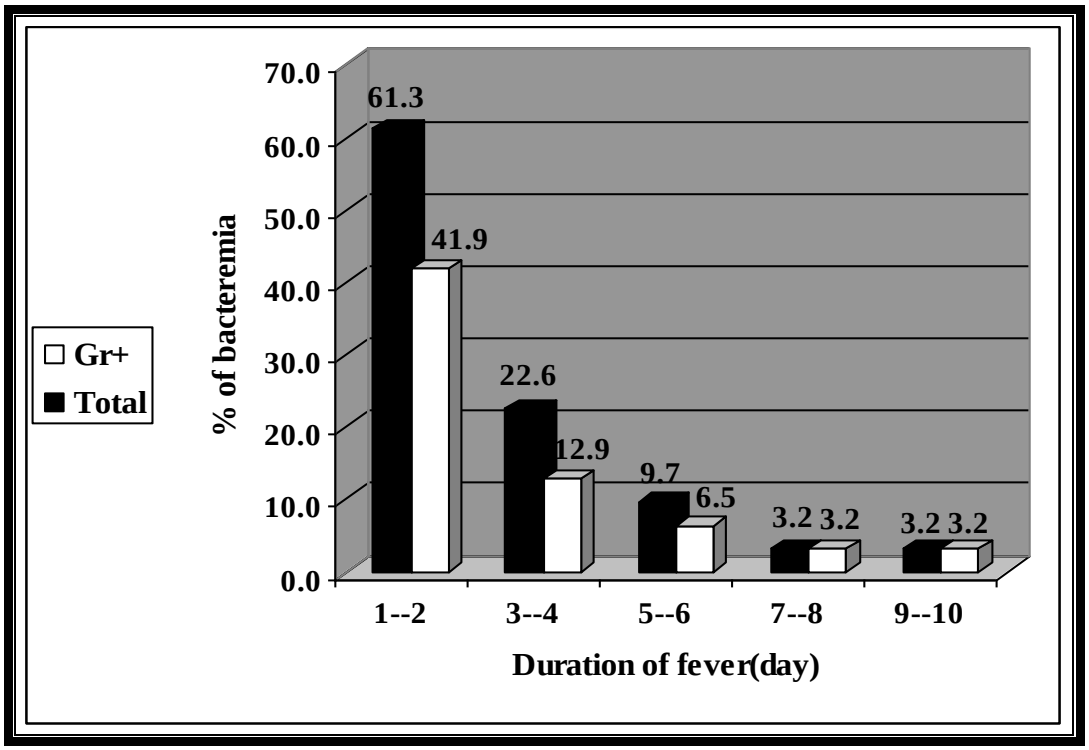


Figure (٣-٧): Distribution of bacteremic infections according to duration of fever (day).

The distribution of bacteremic infections according to the residence is presented in Figure (٣-٨). The study revealed a significant difference of Total (Gram-positive and Gram-negative bacteremia) and Gram-positive bacteremia with the residence factor (***P***<٠.٠٥).

$Z_{calculator}$ (Total) =٢.٣

$Z_{calculator}$ (Gram-positive) = ٣.١

Z = ١.٩٦

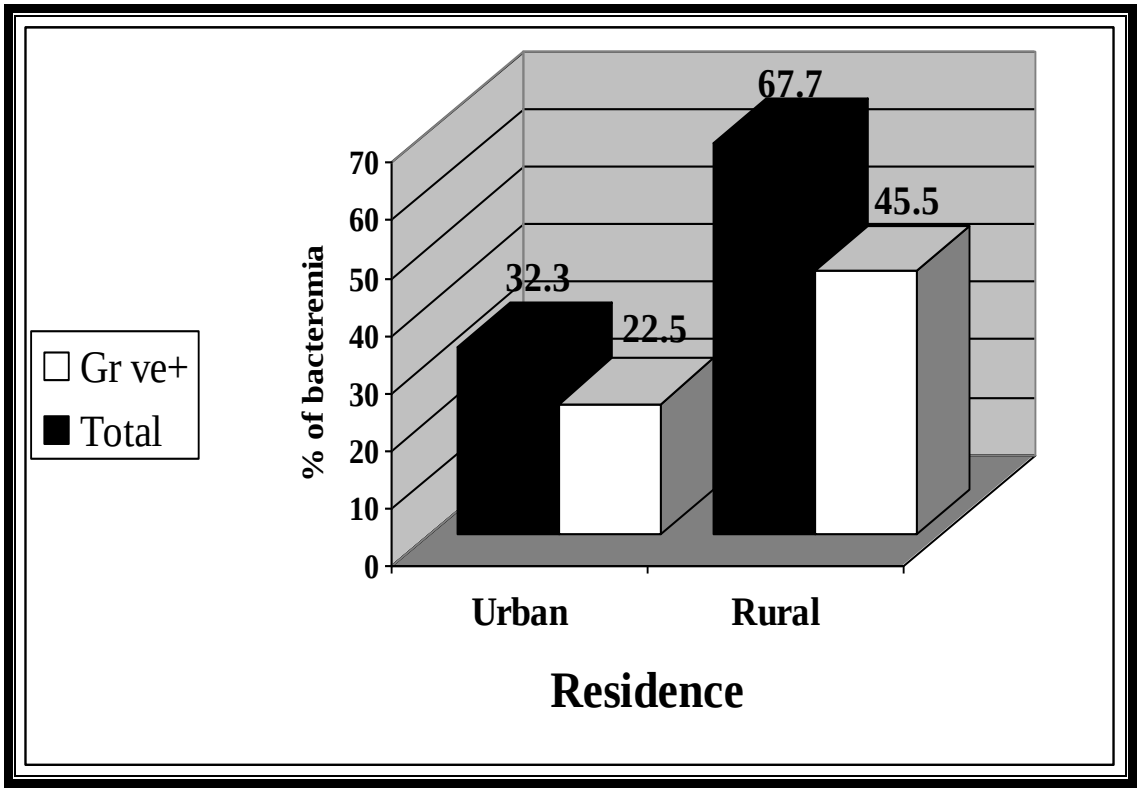


Figure (٣-٨): Distribution of bacteremic infections according to the residency.

Figure (٣-٧) illustrates the rate of bacteremia in children through ١٠ months period from November ٢٠٠٤ to August ٢٠٠٥. the result shows that bacteremia was occurred more in April and May than other months.

The statistical analysis showed a significant difference of Total (Gram-positive and Gram-negative bacteremia) and Gram-positive bacteremia with seasonal variation (***P***<٠.٠٥).

$\chi^{٢}_{\text{calculator}}$ (Total) = ٢٠.٣

$\chi^{٢}_{\text{calculator}}$ (Gram-positive) =١٧.٦

$\chi^{٢}_{\text{table}}$ = ١٦.٩

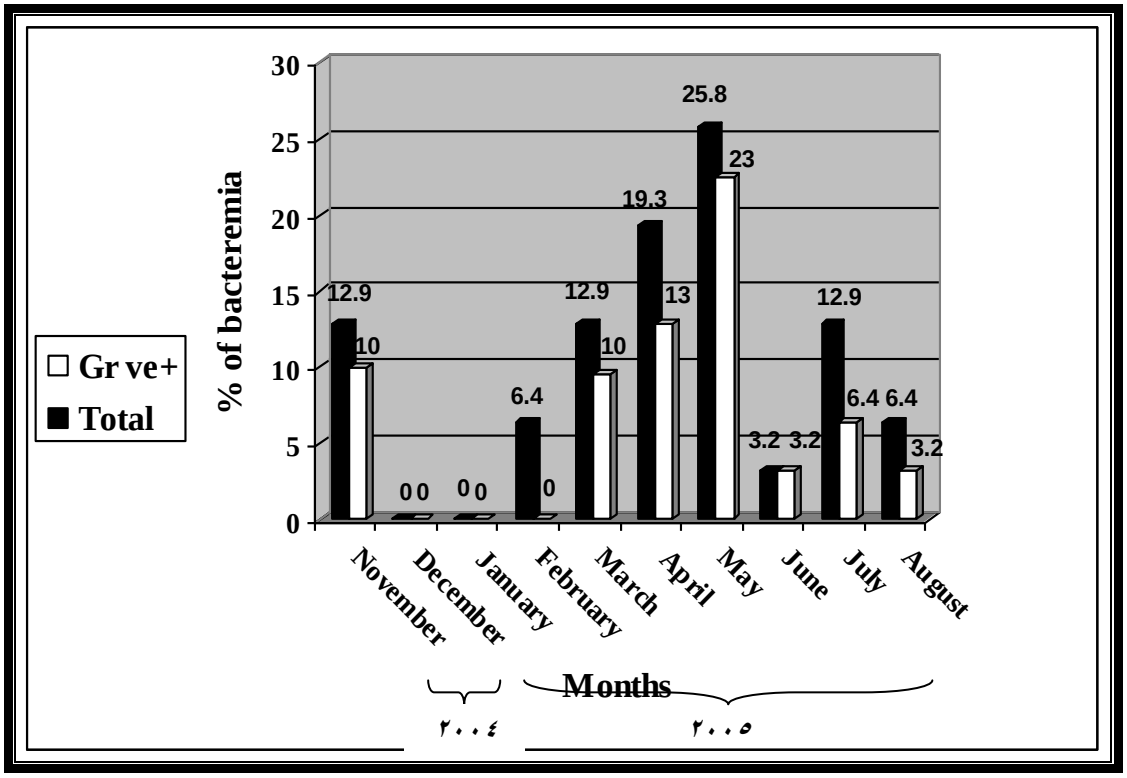


Figure (٣-٩): Seasonal variation of bacteremia in febrile children.

The detection of bacteremic rate in relation to feeding pattern is shown in Table (٣-٢). The children on artificial feeding were more susceptible for bacteremia than those on breast feeding with a significant correlation with bacteremia (***P***<٠.٠٥).

$Z_{calculator}$ = ١.٩٧ Z = ١.٩٦

Table (٣-٢) Distribution of bacteremic infections according to feeding pattern:

Feeding pattern	No. of febrile children	No. of Bacteremic children(%)
Breast feeding	٨٥	١٨ (٢١)
Artificial feeding	٤٠	١٠ (٢٥)
Total	١٢٥	٢٨ (٢٢.٤)

The clinical features associated with Gram-positive bacteremic infection are shown in Table (٣-٣). The study revealed no significant correlation of Gram-positive bacteremic with The clinical features (***P***>٠.٠٥).

$\chi^{2}_{calculator}$ (Gram-positive) =٣.٤ χ^{2}_{table} = ١١.٠٧

Table (٣-٣) The clinical features in Gram-positive bacteremic children:

Clinical features	No. (%)
Fever with no other complain	١٠ (٤٧.٦)
Fever + fit	٣ (١٤.٣)
Fever +cough	٣ (١٤.٣)
Fever +tachypnea	٢ (٩.٥)
Fever +jaundice	٢ (٩.٥)
Fever +frequent bowel motion	١ (٤.٩)
Total	٢١(١٠٠)

The results shown in Figure (٣-١٠) reveal the distribution of Gram-positive isolates according to infantile age. ***S.aureus*** infected all age groups, **CoNS** are more frequent in infants under the age of one month, ***St.pyogenes*** occurs in children older than one year, while ***L.monocytogenes*** and ***Micrococcus*** spp. occur only in children under one month of age.

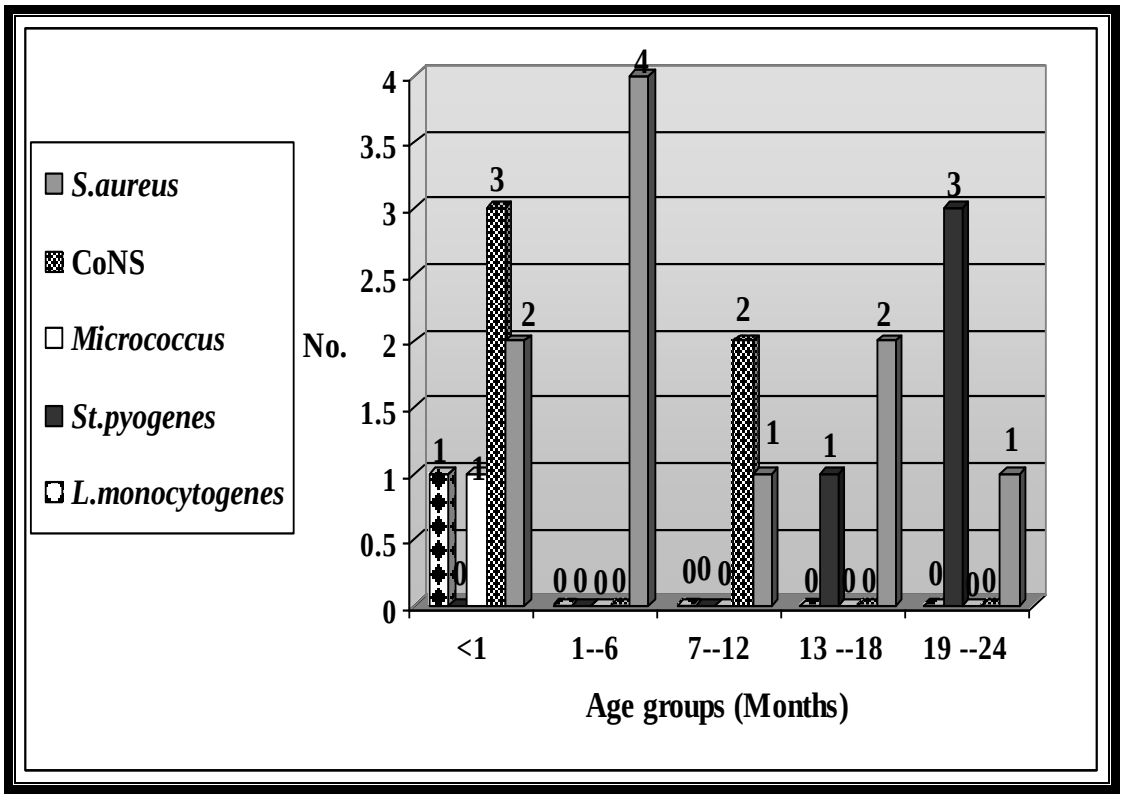


Figure (٣-١٠): Distribution of Gram-positive bacterial isolates according to age groups.

Figure (٣-١١) shows the percentage of Methicillin resistant ***S.aureus*** (MRSA) from all ***S.aureus*** isolates (١٠ isolates).

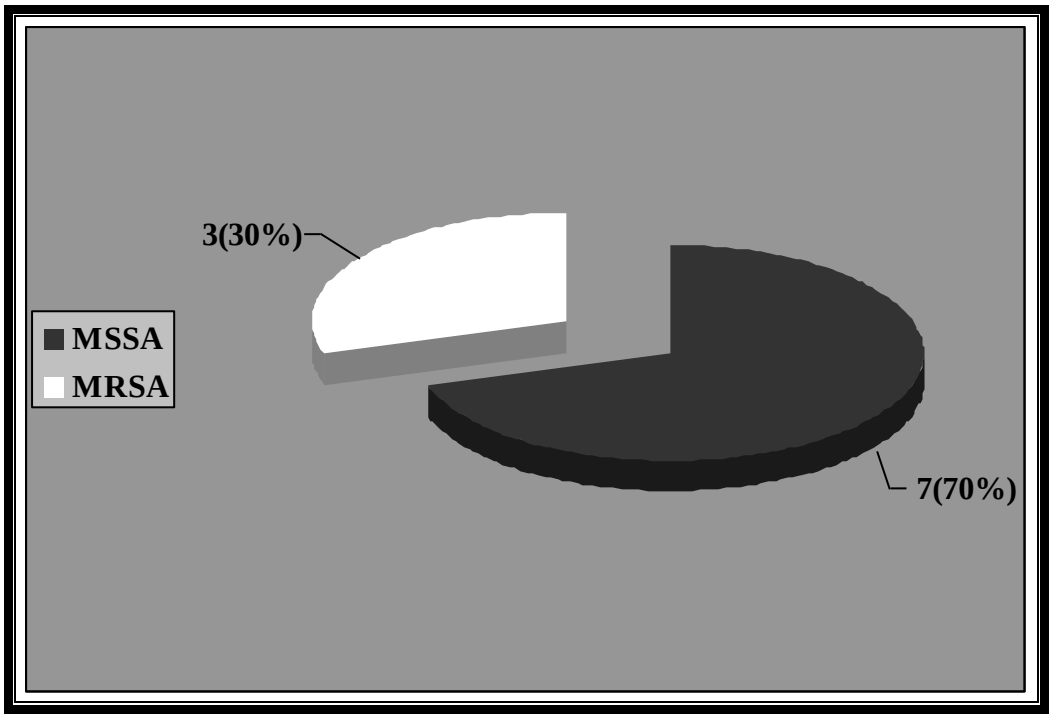


Figure (٣-١١): Rate of MRSA and MSSA.

The results of susceptibility test of ***S.aureus*** (MRSA and MSSA) toward some of traditional antibiotics by using Disc diffusion method and MIC method are shown in Figures (٣-١٢A) and (٣-١٢B) respectively.

In both methods, MRSA isolates were more resistant to Cefotaxime, Cephalexin and Gentamicin antibiotics in comparison with MSSA.

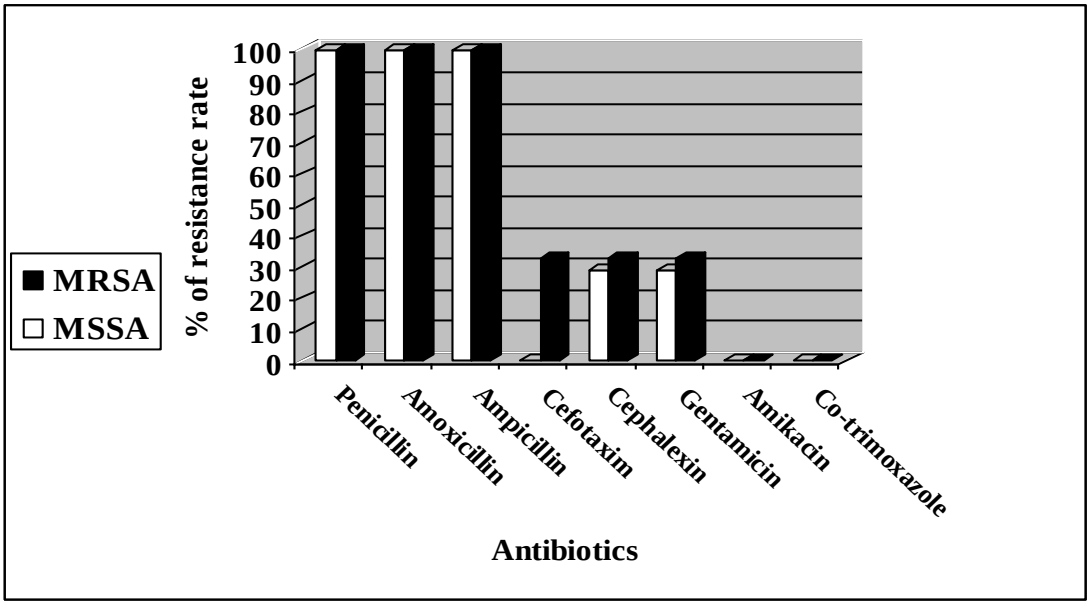


Figure (٣-١٢A): Resistance rate of ***S.aureus*** strains (MRSA and MSSA) to different antibiotics (Disc diffusion method).

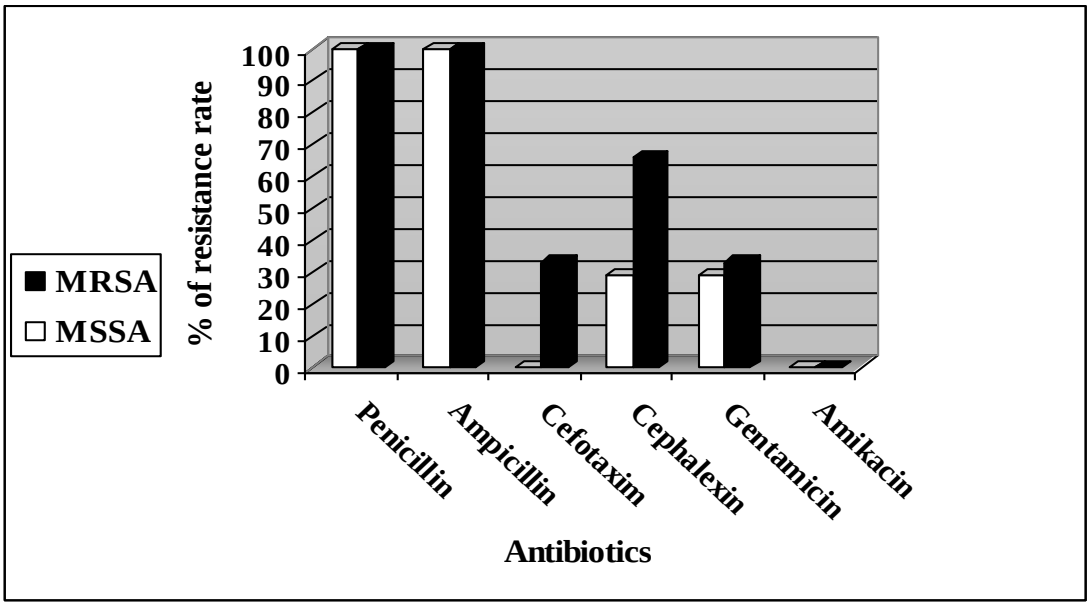


Figure (٣-١٢B): Resistance rate of ***S.aureus*** strains (MRSA and MSSA) to different antibiotics (MIC method).

Figure (٣-١٣) shows the percentage of Methicillin resistant CoNS (MRCoNS) from all CoNS isolates (٥ isolates).

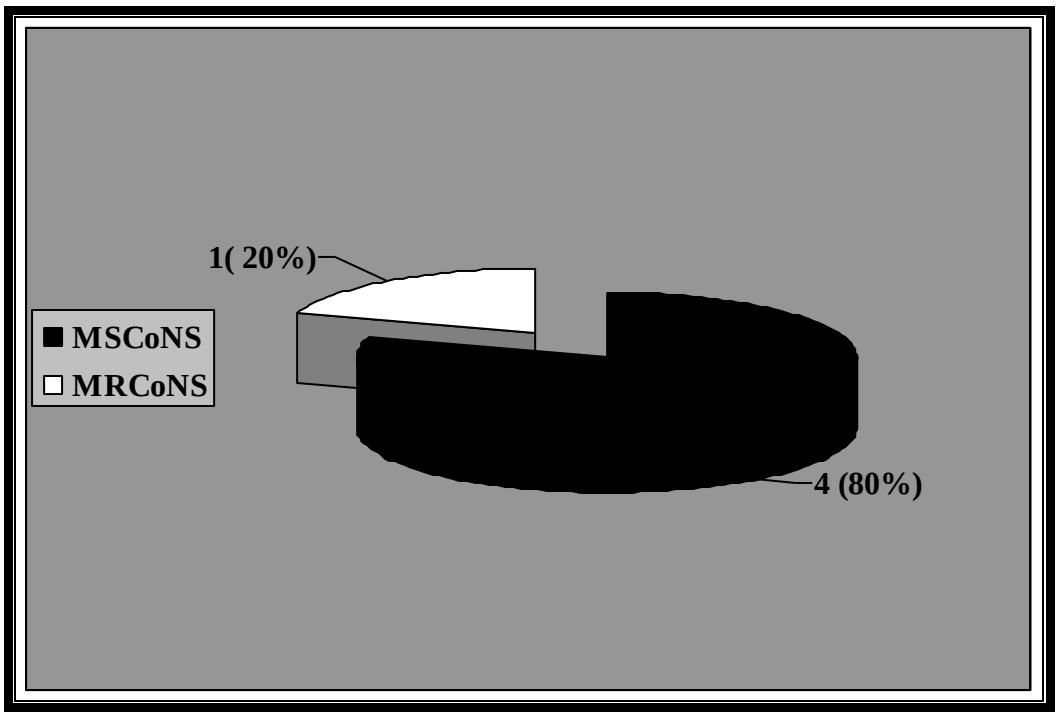


Figure (٣-١٣): Rate of MRCoNS and MSCoNS.

The results of susceptibility test of **CoNS** (MRCoNS and MSCoNS) toward some of traditional antibiotics by using Disc diffusion method and MIC method are shown in Figures (٣-١٤A) and (٣-١٤B) respectively.

In both methods, MRCoNS isolates were more resistant to Cefotaxime, and Gentamicin antibiotics in comparison with MSCoNS.

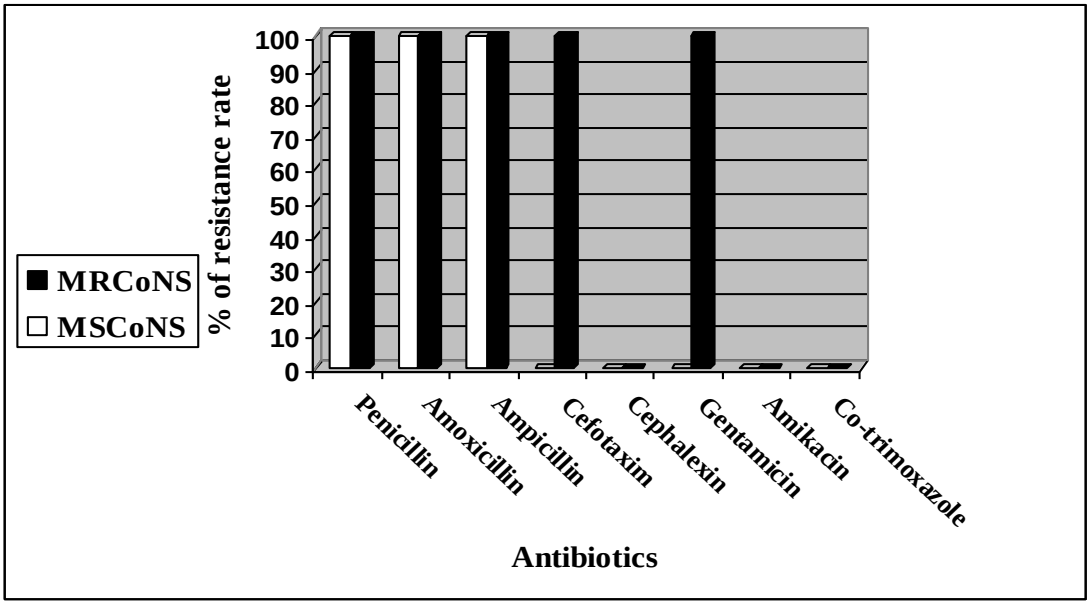


Figure (٣-١٤A) Resistance rate of **CoNS** strains (MRCoNS and MSCoNS) to different antibiotics (Disc diffusion method).

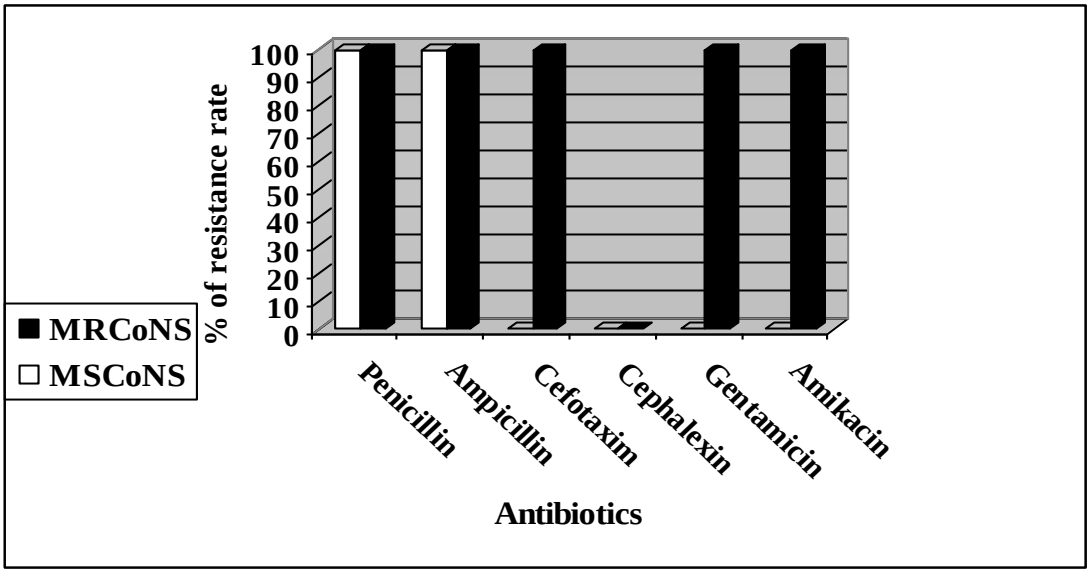


Figure (٣-١٤B) Resistance rate of **CoNS** strains (MRCoNS and MSCoNS) to different antibiotics (MIC method).

Figures (٣-١٥A and B), show the results of susceptibility test of ***Micrococcus*** spp., represented for some antibiotics by using Disc diffusion method (A) and MIC method(B). Its revealed that ***Micrococcus*** spp. was full resist to Penicillin, Ampicillin and Amoxicillin and sensitive to other antibiotics.

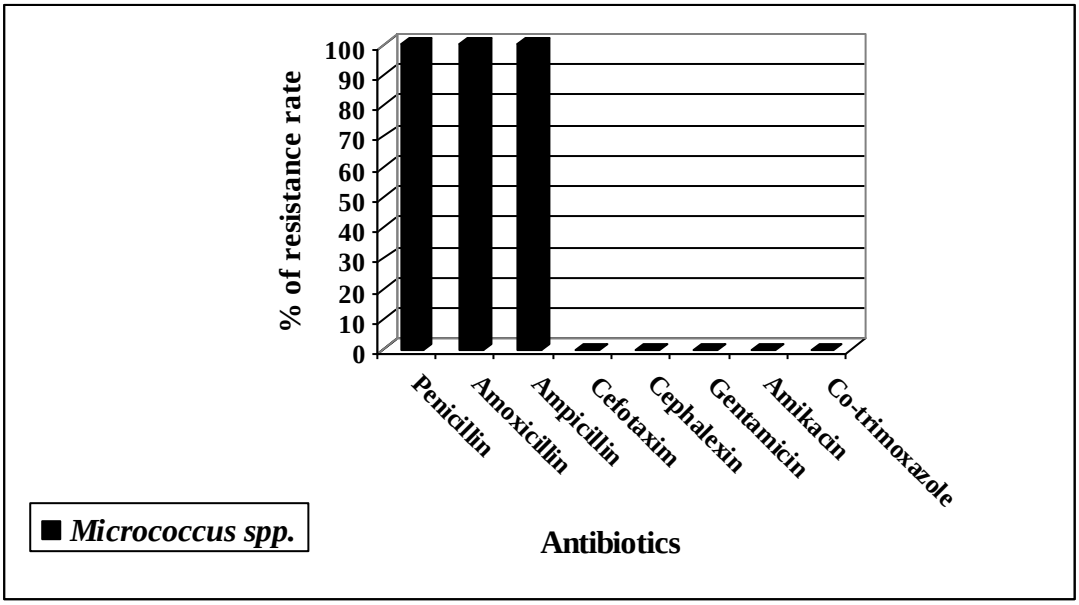


Figure (٣-١٥A) Resistance rate of ***Micrococcus*** spp. to different antibiotics (Disc diffusion method)

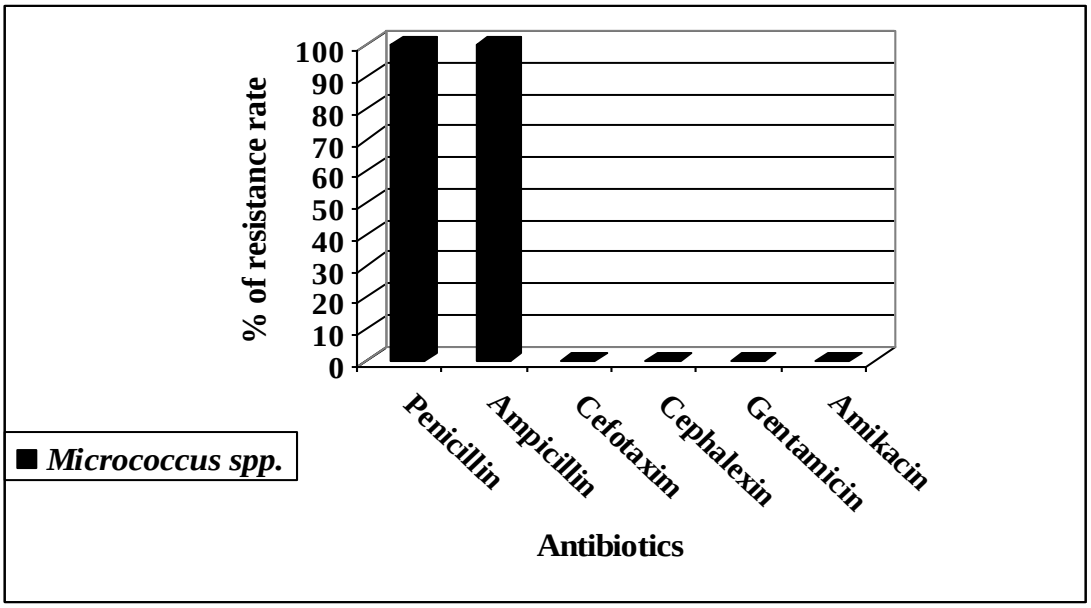


Figure (٣-١٥B) Resistance rate of ***Micrococcus*** spp. to different antibiotics (MIC method).

Figures (٣-١٦A and B), show the results of susceptibility test of ***Streptococcus pyogenes***, represented for some antibiotics by using Disc diffusion method (A)

and MIC method(B). Its revealed that ***Streptococcus pyogenes*** was sensitive to Penicillin, Ampicillin and Amoxicillin and variable resistance to other antibiotics.

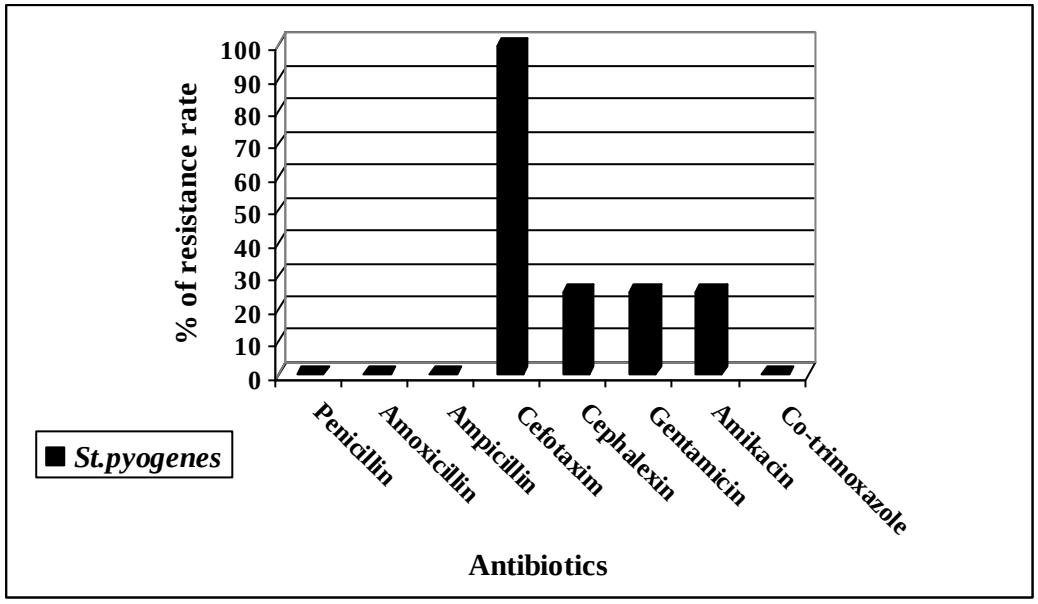


Figure (٣-١٦A) Resistance rate of ***Streptococcus pyogenes*** to different antibiotics (Disc diffusion method).

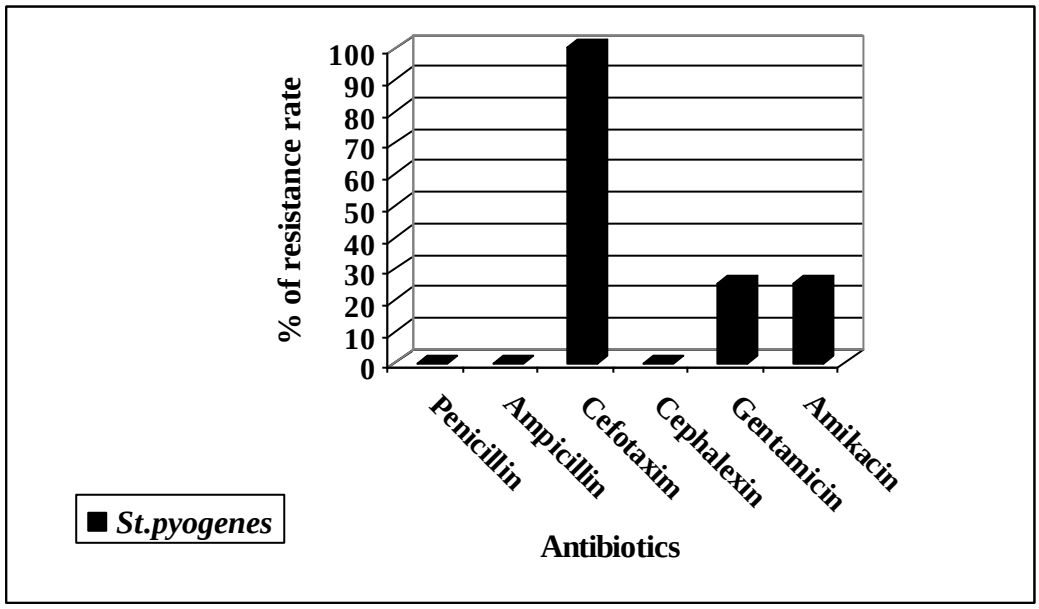


Figure (٣-١٦B) Resistance rate of ***Streptococcus pyogenes*** to different antibiotics (MIC method).

Figures (٣-١٧A and B), show the results of susceptibility test of ***Listeria monocytogenes***, represented for some antibiotics by using Disc diffusion method (A) and MIC method(B). Its revealed that ***Listeria monocytogenes*** was resist to Cephalexin and sensitive to other antibiotics.

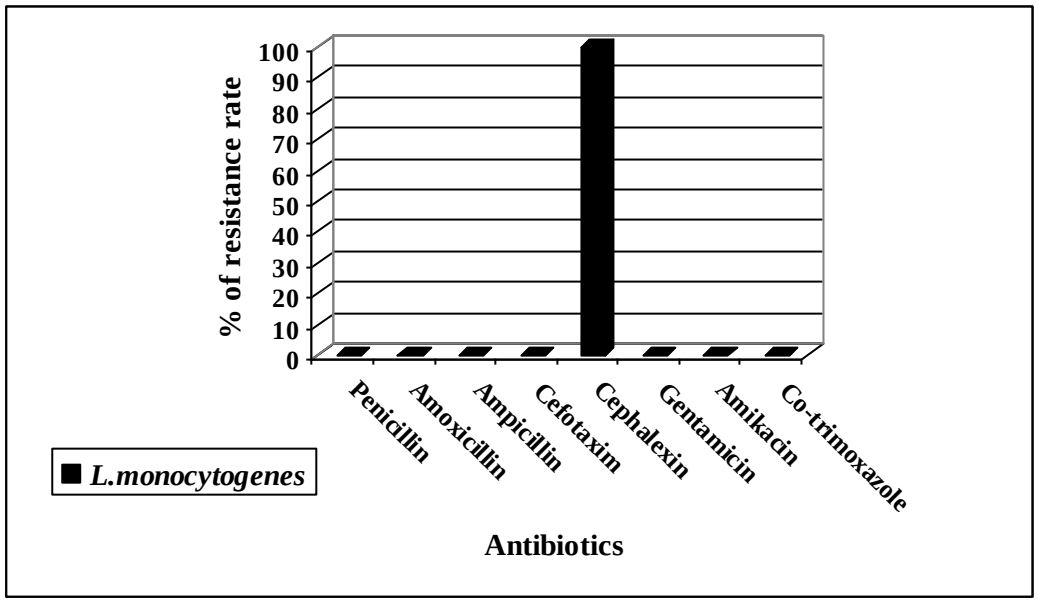


Figure (٣-١٧A) Resistance rate of ***Listeria monocytogenes*** to different antibiotics (Disc diffusion method).

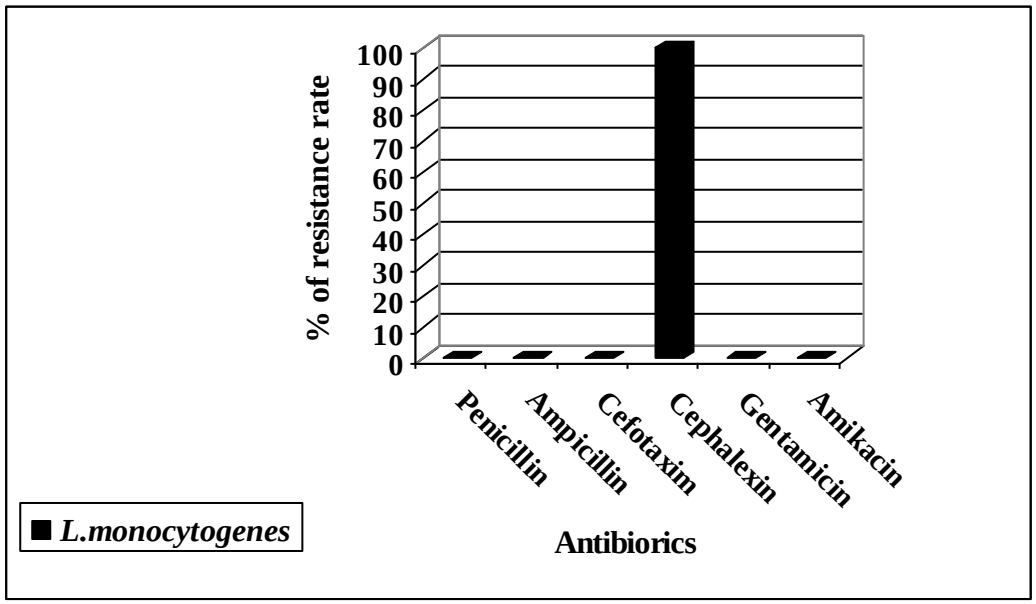


Figure (٣-١٧B) Resistance rate of ***Listeria monocytogenes*** to different antibiotics (MIC method).

٤.١ : Discussion:

According to the clinical features of one hundred sixty febrile children (below ٢٤ months) and after cultural results of their blood samples, the study found that thirty one children (١٩.٤%) have bacteremia (Figure ٣-١); this result agrees with Shams Al-Deen (٢٠٠١) who stated that the rate of bacteremia among infants aged ١-٣ months was (٢٣%) but it was higher than an American study (Liu *et.al.,* ١٩٨٥) which found that the rate of bacteremia in children aged ١-٢٤ months was (٧.٧٪), while it was less than the Saudi study, where the rate was (٦١٪) in the same age groups (Babay *et.al.,* ٢٠٠٥).

Consequently the results of bacteremia in children seem to be variable. This variation may be related to difference in sample size or to the population studied (patients seen in the office versus patients seen in emergency room) (Liu *et.al.,* ١٩٨٥). The cause of fever in the remaining febrile children (٨٠.٦%) may be non bacterial cause, where most of the investigated young children with fever had no focus of infection presented with a self limiting viral illness (Ede and Gilio, ١٩٩٩ and Brook, ٢٠٠٣).

٤.١.١: Etiology:

The increased frequency and changing etiology of bacteremia may be a result of varying patient population (Qutub and Akhter, ٢٠٠١), nosocomial

acquisition of bacteria by the mother and the neonate, the immune response of population groups and geographic and climatic differences and microbiologic techniques in pathogen isolation (Aurangzeb and Abdul Hameed, ٢٠٠٣).

In this study, ٣١ febrile children were bacteremic being caused by both Gram-positive ٢١(٦٨٪) and Gram- negative organisms ١٠(٣٣%) (Figure ٣-٢). This result was in accordance with other studies, where the percentages of Gram-positive and Gram- negative organisms isolated from bacteremic children were ٦٨٪ and ٢٩٪ respectively (Berner *et.al.,* ١٩٩٨). Chapman and Faix (٢٠٠٣) stated that Gram-positive organisms accounted for ٨٤% of the positive culture results whereas Gram-negative organisms accounted for ١٦٪ in bacteremic infants. However the results were in contrast with local studies (Al-Charrakh *et.al.,* ١٩٩٦; Al-Salamy, ١٩٩٩ and Shams Al-Deen, ٢٠٠١) who found that Gram- negative organisms being isolated from bacteremic children more than Gram-positive organisms; variable results are expected in such studies and the variation can be attributed to various risk factors represented by (children's age, gestational age, body weight). Another study pointed out that the etiology may even vary at different times within the same place (Aurangzeb and Abdul Hameed, ٢٠٠٣).

After biochemical tests carried out on Gram-positive isolates (Table ٣-١) the following causative agents of bacteremia were detected in this study:

Staphylococcus aureus was predominant Gram-positive isolates which accounted for (٣٢%) from all isolates (Figure ٣-٣). This result agreed with some other studies, since an Indian study looking at febrile children aged ١ month to ٣ years with no focus of infection found ***S.aureus*** to be the most common bacterial isolate from blood cultures (Singhi *et.al.,* ١٩٩٢). Al-Charrakh *et.al.*

(١٩٩٦) and Shams Al-Deen (٢٠٠١) reported that ***S.aureus*** was predominant Gram-positive organisms in bacteremic children with a percentage of ١٠.٨% and ٢٩.٧٪ respectively. Al-Waznee (٢٠٠١) also found that ***S.aureus*** was predominant and accounted for ٢٦٪ in infants less than ١ year. Consequently, bacteremia due to ***S.aureus*** in children continues to carry a significant mortality, which may be related to not receiving specific antistaphylococcal antibiotics on admission (Ladhani *et.al.,* ٢٠٠٤). The reason that make ***S.aureus*** to be more invasive among Gram-positive organisms and an important pathogen that leads to fatal bacteremia can be ascribed to the virulence factors, since ***S.aureus*** has four proteins having important roles in pathogenicity by allowing bacteria to avoid host defenses and by acting as adhesions, which have been characterized : protein A (immunoglobulin binding protein), fibronectin binding proteins, collagen binding protein and the fibrinogen binding protein (clumping factor) (Foster and McDevitt, ١٩٩٤).

Predominance of ***S.aureus*** may be related to its ability to resist many antibiotics especially Methicillin (MRSA) which facilitated its widespread to environment (Mukoyor *et.al.,* ١٩٨٥).

Coagulase negative staphylococci (**CoNS**) appear to be the major pathogen world wide and associated with significant morbidity and mortality in neonates and infants (Weisman, ٢٠٠٤) and it is considered the most common organisms associated with infantile bacteremia (Babay *et.al.,* ٢٠٠٥). In this study **CoNS** represented the second pathogen among Gram-positive isolates (١٦٪) of all isolates (Figure ٣-٣), which agreed with an Indian study (Jain *et.al.,* ٢٠٠٤) which found that **CoNS** in infants with septicemia was (١٦.٣٪). Sastre *et.al.* (٢٠٠٢) had reported the combined results of ٢٧ acute care hospitals in Spain where ٥٨٪ of all isolates were Gram-positive organisms mainly ***Staphylococcus***

epidermidis which accounted for ٤٢٪, in bacteremic infants, also Babay *et.al.* (٢٠٠٥) found it ٥٥.٤٪ in bacteremic children.

The reason of high rate of CoNS isolates may be related to the use of broad spectrum antibiotics (Fulgintti, ١٩٨٤) and to the role of specific adhesion and slime produced by CoNS, since slim can reduce the immune response and opsonophagocytosis (Kloos and Bannerman, ١٩٩٤).

Streptococcus pyogenes (GABHS) in this study accounted for (١٣٪) from all isolates (Figure ٣-٣). With this respect, there were three studies (Wheeler *et.al.,* ١٩٩١; Schlieveret *et.al.,* ١٩٩٦ and Abuhammour *et.al.,* ٢٠٠٤) which have documented an incidence ranging from ٠.٥-٢% of GABHS bacteremia in children. The rate of bacteremia due to this organism has risen; theories to account for the apparent increase in incidence and severity have involved possible changes in susceptibility of the population as well as changes in the virulence of the organism itself (American Academy of Pediatrics, ١٩٩٨). The majority of the infections have been caused by strains of GABHS expressing M-protein and producing the pyrogenic exotoxins A, B, or both (Wheeler *et.al.,* ١٩٩١).

Micrococcus spp. was found as normal inhabitants of human skin and mucous membranes and was usually regarded as contaminants in clinical isolates (Gahrn-Hansen, ١٩٨٥). Nevertheless, strains identified as ***Micrococcus*** spp. have been involved as causative organisms in invasive diseases such as bacteremia. Since ١٨٨٢ Ogston stated that "***Micrococcus*** spp., when limited in its extent and activity, causes acute suppurative inflammation, and when more extensive and intense in its action on the human system may cause virulent forms of septicemia and pyaemia" (Ogston, ١٨٨٢). Recently it was found that ***Micrococcus*** spp. could cause many life-thereating infections in addition to

bacteremia like endocarditis, central nervous system infection, peritonitis and pneumonia (Eiff *et.al*., ١٩٩٥).

In the present study ***Micrococcus*** spp. was found to account for (٣%) from all isolates (Figure ٣-٣). It can be associated with other infections rather than bacteremia such as pneumonic infection in infants in a frequency of ٨.٧% (Al-Hamawandi, ٢٠٠٥).

Listeria monocytogenes in this study was also found to be implicated in infant's bacteremia. The body defense against ***Listeria monocytogenes*** and other intracellular pathogens is by cell-mediated immunity; therefore it is not surprising that individuals whose cell-mediated immunity is suppressed are more susceptible to the devastating effects of Listeriosis (Joklik *et.al.,* ١٩٩٢).

The immune systems of fetuses and newborns are very immature, deficiencies in immunoglobulin M and complement activity associated with the neonatal state may contribute to the propensity of infants to develop Listeriosis (Bortulussi *et.al.,* ١٩٨٦).

Listeria monocytogenes accounted for (٣%) from all isolates (Figure ٣-٣) and found only in neonates. Ako-Nai *et.al*. (١٩٩٩) found that ***Listeria monocytogenes*** represented (٨.٤%) of septicemic neonates. The results presented in this study showed low prevalence of neonatal ***Listeria*** compared with the study above. This may be attributable to aggressive food regulation and industrial clean-up efforts.

٤.١.٢: Age factor:

Older and newer reports of bacteremia in febrile infants suggested that its incidence varied according to age (Crain and Shelov, ١٩٨٢, Baker *et.al.,* ١٩٩٣ and Baker and Bell, ١٩٩٩).

All patients being investigated in this study were below ٢ years. This age group seems to be more liable for bacteremia because they have a low immunoglobulin G antibody response to encapsulated bacteria (Baker, ١٩٩٩).

The results indicated that bacteremia in infants less than ١ month were more occur than other age groups (٣٢.٥%) (Figure ٣-٤), which goes with (Pantell, ٢٠٠٤) who found that bacteremia occurred with greatest preponderance in infants younger than ١ month. This relatively high overall rate of bacterial disease in this age group is likely to be related to the lower level of immunocompetence in younger infants. Infants in the first few months of life have been shown to have decreased opsonin activity, macrophage function, and neutrophil activity that the immune system is not fully developed (Baker, ١٩٩٩).

٢٢.٥% of patients being tested in this study having bacteremia were between (١-٦) months of age. Some study pointed that infants aged ٣to ٢٤-Month with fever and no major source of infection were at highest risk for occult bacteremia (OB) (Jaskiewicz *et.al.,* ١٩٩٤).

Infants aged ٧-١٢ months have (٩.٦%) rate of bacteremia this agreed with Liu *et.al.* (١٩٨٥) who found (٦.٢٪) rate of bacteremia among infants aged ٧-١٢ months. children aged ١٩-٢٤ months have ٢٦.٧%. Infants of these age groups were reported to have less risk for developing bacteremia because they are immune competent than smaller age groups (Sinkinson and Pichichero, ١٩٩١ and McCarthy, ١٩٩٨).

Moreover Kuppermann *et.al.* (١٩٩٨) and Lee and Harper (١٩٩٨) concluded that all children below ٢٤ months were at higher risk for bacteremia.

In this study there was non significant difference between all age groups and the rate of bacteremia (***p***>٠.٠٥).

٤.١.٣: Sex factor:

The rate of Gram-positive bacteremia in male and female was (٥٢%) and (٤٨%) respectively (Figure ٣-٥), with non significant difference (***p***> ٠.٠٥). This result is agreeable with some other studies Murray *et.al.* (١٩٨١); Bass *et.al.* (١٩٩٣) and Al-Eissa *et.al*. (٢٠٠١) which stated that no sex based difference exists in the prevalence of bacteremia. However Shams Al-Deen (٢٠٠١) found that the bacteremic rate in male and female was (٦٣.٩٪) and (٣٥.١٪) respectively with significant difference. These relative variations in results of some studies can be ascribed to some environmental factors and to the patients conditions themselves (like the age, the immune state).

٤.١.٤: Temperatures degrees:

The study found that bacteremic incidence increased with the increase in temperatures degrees (Figure ٣-٦) but with non significant correlation (***p***> ٠.٠٥). The results were in accordance with many results being reported by other researchers: McCarthy *et.al*. (١٩٧٧); Baron and Fink (١٩٨٠); Fleisher *et.al*. (١٩٩٤); Kuppermann *et.al*. (١٩٩٨); Lee and Harper (١٩٩٨); Strait *et.al*. (١٩٩٩) and Luszczak (٢٠٠١) concluded that the risk of bacteremia increased as temperature rises, that infant or young child with a temperature higher than ٤٠.٩°C is more than three times more likely to harbor bacteremia than an infant or a young child with a temperature of ٣٩°C. By contrast Shams Al-Deen (٢٠٠١) found that in infants at >٤٠°C the rate of bacteremia declined. The high rate of temperature degree which associated with bacteremia was explained by the fact that, the component of the host's response to bacterial invasion is the release of small molecular weight proteins called cytokines. Cytokines are produced and released from polymorphonuclear cells (PMNs) and phagocytes in response to infectious stimuli. Some cytokines can affect the brain's thermoregulatory center and raise the hypothalamic set point. These processes lead to the development of fever (McCarthy, ١٩٩٨).

٤.١.٥: Duration of fever:

In the present study it was found that (٦١.٣٪) of patients who had fever for ١-٢ days were more associated with bacteremia (Figure ٣-٧). This result agreed with Jaffe who pointed out that persistent fever for ٤٨ hours was associated with an increased risk of bacteremia and serious bacterial infection (Jaffe *et.al.,* ١٩٨٧).

Bass *et.al.* (١٩٩٣) has noted that the duration is shorter in patients whose blood cultures is positive for bacterial pathogens (mean ١٨ hrs.) than in patients with negative blood cultures for bacterial pathogens (mean ٢٥ hrs.).

Another study found that the median duration of fever in patients with bacteremia and without bacteremia was the same, one to two days, but the mean class of patients with bacteremia was significantly lower than that of patients without bacteremia (Teach and Fleisher, ١٩٩٧).

Overall, duration of fever was inadequate to identify occult bacteremia clinically (Strait *et.al.,* ١٩٩٩).

The statistical analysis showed a significant correlation between the duration of fever and the rate of bacteremia (***P***<٠.٠٥).

٤.١.٦: Residence factor:

To compare between rural and urban areas regarding frequency of bacteremia among children, the study found that bacteremia was more in those living in rural areas (٦٧.٧%) while it was(٣٢.٢%) in those living in urban areas (Figure ٣-٨), although the statistical analysis showed a significant difference between the rate of infection among two areas(***p***< ٠.٠٥).

This difference may be mainly related to their low socioeconomic status, malnutrition and lack of medical facilities(Yagupsky and Giladi, ١٩٨٧), in addition to possible factors like poor hygiene in the delivery room and in neonatal care units, no colostrum feeding and umbilical or skin infections during the early neonatal period (Bang *et.al.,* ٢٠٠٥).

٤.١.٧: Seasonal variation factor:

Bacteremia over a period of ١٠ months in this study was more noticed in April and May than in other months (Figure ٣-٩). In this study, a significant correlation between the seasonal variation and bacteremia was found (***P***<٠.٠٥).

This result is in accordance with McCarthy (١٩٩٨) who found that the peak of bacteremia occurs during the summer in infants younger than ٣ months and is likely to be due to the circulation of enteroviruses at that time of the year. Therefore the newborn is at greatest risk for bacterial sepsis in high environmental temperature.

It has been reported that bacteremia can occur throughout the year but is more frequent during winter, spring, and early summer months (American Academy of Pediatrics, ١٩٩٨).

But, it has been found in some studies (Bang *et.al.,* ٢٠٠٥) that non significant seasonal variation was observed in the incidence of sepsis.

٤.١.٨: Type of lactation:

The study found that among ١٢٥ suckling children, ١٨ children out of ٨٥ (٢١٪) were breast-fed have bacteremia while ١٠ children out of ٤٠ (٢٥٪) who were artificially-fed have bacteremia (Table ٣-٢). It was found a significant correlation between the feeding pattern and bacteremia (***P***<٠.٠٥).

These results are agreeable with Shams Al-Deen (٢٠٠١) who found that children with artificial feeding have higher risk of bacteremia than those on breast-fed. This indicates that natural breast feeding decreases the incidence and/or severity of a wide range of infectious diseases including bacterial meningitis, bacteremia, diarrhea, respiratory tract infection, necrotizing enterocolitis, otitis media, urinary tract infection, and late-onset sepsis in preterm infants. In addition, postneonatal infant mortality rates in United States were reduced by ٢١٪ in breast-fed infants (Chen and Rogan, ٢٠٠٤).

It is well understood that human milk contains bacterial and viral antibodies, including relatively high concentrations of secretory IgA which prevents microorganisms from adhering to intestinal mucosa. It also contains substances that inhibit growth of many common microorganisms. Antibodies in human milk are thought to provide local gastrointestinal immunity against organisms entering the body via this route. They probably account at least partially for the lower incidence of diarrhea as well as otitis media, pneumonia, bacteremia and meningitis during the first year of life in infants who are breast fed exclusively versus artificial fed for the first ٤ months of life (Heird, ٢٠٠٣).

٤.١.٩: Clinical features:

The general clinical features associated with Gram-positive bacteremia are shown in (Table ٣-٣) with no significant correlation with bacteremia.

The common clinical features of children being investigated through this study were only fever (٤٧.٦%). This result agreeable with Babay *et.al*. (٢٠٠٥) which pointed out that fever was the most common presentation and

predicator of blood stream infection in children aged (١-١٢m). Bonadio found that ١٥٪ of infants with documented fever at presentation had serious bacterial infections. As with older children the magnitude of fever is predictive of serious bacterial infection (Bonadio, ١٩٩٠).

This result related to that Fever represents a normal physiologic response that may result from the introduction of an infectious pathogen into the body and is hypothesized to play a role in fighting and overcoming infections (Kai, ١٩٩٦ and Adam, ١٩٩٦).

The rest clinical features are usually associated with bacteremia (fit, cough, tachypnea and frequent bowel motion). Some features may help to identify the causative organisms. Infections above the diaphragm are more likely to be due to Gram-positive organisms. Infections in the abdomen, including the biliary and urinary tracts are more likely to be due to Gram-negative bacteria. However, no secure methods are available other than those based on laboratory diagnosis for differentiating Gram-positive from Gram-negative causes of bacteremia (McGowan and Shulman, ١٩٩٨).

٤.١.١٠: Distribution type of bacterial isolates according to age groups:

Bacterial pathogens causing infantile bacteremia vary according to the age of the patient. Figure (٣-١٠) shows the main organisms being isolated from bacteremic infants less than one month of age. ***S.aureus*** ٢ isolates; **CoNS** ٣ isolates; ***Micrococcus*** spp. ١ isolate and ***Listeria monocytogenes*** ١ isolate. This distribution is quite agreeable with Brook (٢٠٠٣) who found that the main

causes of bacteremia in children under ١ month were ***S.aureus*** and ***Listeria monocytogenes.***

L. monocytogenes isolated only from this age group which agrees with Boukhari who found that the rate of listerial infection was highest among infants of less than one month (Boukhari *et.al*., ١٩٩٩).

Micrococcus spp. was also reported to be mostly isolated from infants less than one month (Al-Hamawandi, ٢٠٠٥).

CoNS are especially important as a cause of infection in neonates (Kloos and Bannerman, ١٩٩٤) and they are the most frequent blood culture isolate associated with bloodstream infection in infants older than ٣ days (Rubin *et.al.,* ٢٠٠٢). **CoNS** occurred in smaller, more premature infants than ***S.aureus*** (Chapman and Faix, ٢٠٠٣ and Healy *et.al.,* ٢٠٠٤).

The results of this study found that ٣ isolates of **CoNS** were isolated from infants of less than one month. While ***S.aureus*** isolates were isolated from all infantile age groups in a variety of percent; this is in agreement with Ladhani *et.al.* (٢٠٠٤) who stated that ***S.aureus*** could infect all age groups.

In infants under one month, two isolates of ***S.aureus*** were detected. It has been reported that the most common pathogen causing neonatal bacteremia is ***S.aureus*** (Bhutta *et.al.,* ١٩٩١; Dawudu *et.al.,* ١٩٩٧; and Kaushik *et.al*., ١٩٩٨).

Most ***S.aureus*** isolates were isolated from infants aged under ١ year, the result agrees with the results of other studies which found that the peak incidence of ***S.aureus*** bacteremia was observed in children <١ year of age (Philip *et.al*., ٢٠٠١).

Prevail ***Streptococcus pyogenes*** isolates were isolated from infants aged >١٨ months; this result may confirm the results being reported by Park who found that most infectious agents associated with occult bacteremia in young children aged ٣-٣٦ months were ***S.aureus*** and ***Streptococcus pyogenes*** (Park, ٢٠٠٠). Moreover, the incidence of streptococcal bacteremia was much higher in children than in adults (Huang *et.al.,* ٢٠٠١).

٤.١.١١: Antibiotic susceptibility:

Antibiotic susceptibility test was performed to show the effect of some antibiotics on the bacterial isolates by using two methods (Disc diffusion method {Kirby–Bauer technique} Figures (٣-(١٢,١٤,١٥,١٦,١٧)A) and MIC method Figures (٣-(١٢,١٤,١٥,١٦,١٧)B).

Gram-positive bacterial resistance continues to be an important clinical therapeutic problem. Increasing multidrug resistance in Methicillin resistant ***Staphylococcus aureus*** (MRSA), Methicillin resistant Coagulase Negative Staphylococci (MRCoNS) are at the forefront of current treatment concerns (Linden, ١٩٩٨).

S.aureus :

Penicillin was introduced for the treatment of Staphylococcal infections in the early of ١٩٤٠s (Feder, ٢٠٠٠). By the end of that decade, much of the Staphylococcal strains were strongly produced β-lactamase and were resistant to Penicillins (Berber and Dowzenko, ١٩٤٨). In ١٩٦٠s, semisynthetic Penicillins

(Methicillin, Oxacillin) and cephalosporins were developed, which were not inactivated by β-lactamase (Rolinson *et.al.,* ١٩٦٠). In ١٩٦١, MRSA was first described (Jevons, ١٩٦١).

The mechanism of resistance was a change in the Penicillin binding proteins (PBPs) within the cell membrane. This change in the PBPs resulted in Penicillins and cephalosporins binding less avidly to PBP ٢a (Gutmann, ١٩٩٧). Resistance of the PBPs to Penicillins and cephalosporins was chromosomally mediated by the ***mec***-A gene (Boyce, ١٩٩٨).

Methicillin resistant ***Staphylococcus aureus*** (MRSA) detected in this study was ٣٠٪ from all ***S.aureus*** isolates (Figure ٣-١١), which agrees with Denniston and Andrew (٢٠٠٥) who found that (٢٧٪) of infants had MRSA bacteremia and with Al-Sahllawi (٢٠٠٢); and also agrees with Mee-Marquet *et.al.* (٢٠٠٤) who found MRSA to be accounted for ٣٣٪ while it was detected in ١٤٪ only in Saudi study (Babay *et.al.,* ٢٠٠٥)

We found that ***S.aureus*** isolates were highly resistant to many antibiotics by both methods of susceptibility tests {Disc diffusion and MIC methods (٣-١٢A; B)}. MRSA and MSSA exhibited the same rate of resistance to Penicillin and Ampicillin in both methods and Amoxicillin in disc diffusion method. This could be explained by the fact that all Staphylococcal strains produce β- lactamase which inactivate the β- lactam ring compounds (Prince, ١٩٩٨).

Cephalosporins (Cefotaxime and Cephalexin) resistance by the disc diffusion method shows that ***S.aureus*** isolates are more sensitive to these antibiotics. MSSA have a resistance rate up to ٢٩٪ while MRSA isolates exhibited high resistance rate toward these antibiotics (٣٣٪) for each antibiotics.

In MIC method MSSA showed the same rate of resistance in the previous method, while MRSA revealed more resistance (٣٣٪ and ٦٦٪) to Cefotaxime and Cephalexin; these results were in agreement with those results being reported by Starr (١٩٨٥) who found that most strains of ***S.aureus*** were resistant to Penicillin and Ampicillin but susceptible to Cephalosporins. Some studies reported that some extended-spectrum beta-lactamase -producing organisms were not resistant to all cephalosporins (Paterson *et.al.*, ٢٠٠١). The results showed that the third generation Cephalosporins was shown to be effective against ***S.aureus*** which agreed with (Meremikwu *et.al.,* ٢٠٠٥).

In both methods MSSA and MRSA revealed a remarkable resistance rate to Gentamicin.

Amikacin and Co-trimoxazole were more effective against ***S.aureus*** strains, both MSSA and MRSA, since no resistance to Amikacin was seen in both methods and to Co-trimoxazole in disc diffusion method. These results were in accordance with Healy and co-worker (٢٠٠٤) who reported that MRSA isolates were highly sensitive to vancomycin, Gentamicin, Trimethoprim-sulfamethoxazole and Clindamycin. However the results were in contrast with Chi *et.al.* (٢٠٠٤) who found that MRSA strains had a high degree (٦٥.٧٪) of resistance toward Trimethoprim-sulfamethoxazole.

Generally resistance to antibiotics was observed more in the Methicillin-resistant isolates compared with Methicillin sensitive isolates which agreeable with (Kumhar *et.al.*, ٢٠٠٢) and that could be attributed to the structural gene for Penicillin-binding protein which was responsible for the intrinsic resistance of MRSA (Suzuki *et.al.,* ١٩٩٢). The resistant organisms produce PBPs that have low affinity for binding β-lactam antibiotics (Chambers, ٢٠٠١).

CoNS:

CoNS are common isolates from blood cultures, and an increasing proportion is now Methicillin resistant, therefore the National Committee for Clinical Laboratory Standards (NCCLS) recently issued new criteria for zone sizes applicable to Oxacillin disc sensitivity testing for CoNS and the British Society of Antimicrobial Chemotherapy (BSAC) has also issued guidelines (Tan, ٢٠٠٢). In this study **CoNS** isolates exhibited multiple antibiotics resistance, including resistance to Methicillin.

Methicillin resistant CoNS (MRCoNS) were found in (٢٠٪) from all **CoNS** isolates (Figure ٣-١٣), which agrees with Cotton *et.al.* (١٩٨٩) who reported that ***S.epidermidis*** resisted oxacillin up to (٢٠٪) among children with nosocomial sepsis, whereas Jain *et.al.* (٢٠٠٤) found that the rate of MRCoNS was recorded as high as (٦٦ %).

CoNS appear resistant to many antibiotics like ***S.aureus*** Figures (٣-١٤A, B).

MSCoNS and MRCoNS showed the same resistance rates (١٠٠٪) to Penicillin and Ampicillin in Disc diffusion and MIC methods and Amoxicillin in disc diffusion method. A ١ results have been reported by Fluit *et.al.* (٢٠٠٠) and Jain *et.al.* (٢٠٠٤) who found that Penicillin resistance was frequent (٩٤%) and (١٠٠٪). That's related to CoNS which have ***mec***-A gene which encoded for Penicillinase production (Suzuki *et.al.,* ١٩٩٢).

MSCoNS revealed high sensitivity towards Cephalosporins (Cefotaxime and Cephalexin) in both methods with a resistance rate of ٠%. While MRCoNS showed (١٠٠٪) resistance rate toward Cefotaxime but (٠٪) to Cephalexin in both methods.

Amikacin resistance was (٢٥%) in both methods. Similar results were reported by Jain *et.al*. (٢٠٠٤) who found that Amikacin resistance was relatively rare (١٩%). Gentamicin and Co-trimoxazole in the disc diffusion method were more effective on MSCoNS with (٠٪) resistance rate, while MRCoNS showed (١٠٠٪) resistance rate toward Gentamicin and Amikacin in both methods and with (٠٪) toward Co-trimoxazole in disc diffusion method.

Like ***S.aureus***, **CoNS** had resistance to antibiotics and was more seen in the Methicillin-resistant isolates compared with those who were Methicillin sensitive (Jain *et.al.,* ٢٠٠٤). Another non-Penicillin-binding protein-dependent mechanism such as hyperproduction of ß-lactamase, or the presence of other low-affinity Penicillin-binding proteins (Geha *et.al.,* ١٩٩٤), could be responsible for this phenomenon.

The number of multiple-drug-resistant strains, including Methicillin-resistant CoNS, has increased, and the majority of CoNS causing neonatal septicemia are resistant to the traditional antibiotics used to treat newborn infants (Baumgart *et.al.,* ١٩٨٣ and Stoll *et.al.,* ١٩٩٦).

Micrococcus* spp.*:

Similarly, ***Micrococcus*** spp. exhibited a similar antibiotic sensitivity profile to that expressed for ***Staphylococci.*** Figures (٣-١٥A, B).

Micrococcus spp. were resistant to Penicillin and Ampicillin in both methods and to Amoxicillin in the disc diffusion method in a percentage of (١٠٠٪),while sensitive to other antibiotics like Cefotaxime, Cephalexin, Amikacin, Gentamicin and Co-trimoxazole at resistance rate (٠٪). Data about ***Micrococcus*** spp. susceptibilities to antibiotics are relatively rare. Strains recovered from clinical sources were susceptible to most antibiotics (Selladurai

et.al., ١٩٩٣); however, clinical isolates resistant to Penicillin have been reported (Magee *et.al*., ١٩٩٠; Adang *et.al.,* ١٩٩٢ and Eiff *et.al*., ١٩٩٥).This has been confirmed by the results of this study. It was found that strains were resistant to Penicillin. It was also found that Gentamicin was more active than Amikacin (Eiff *et.al*., ١٩٩٥). The results were similar to Al-Hamawandi's results who found that ***Micrococcus*** spp. resisted Ampicillin and Amoxicillin in a frequency of (١٠٠٪), but were fully sensitive to Cefotaxime (Al-Hamawandi, ٢٠٠٥). The mechanism of β-lactam resistance in ***Micrococcus*** spp. is controlled by chromosome as well as plasmid genes (Garner *et.al*., ١٩٨٨).

Streptococcus pyogenes:

St.pyogenes was Penicillin and Ampicillin sensitive in both methods and Amoxicillin and Co-trimoxazole in disc diffusion method at resistance rate (٠٪); the results are shown in Figures (٣-١٦A; B).

Regarding Cephalosporins, the organism exhibited variable resistance rates, that was (١٠٠٪) to Cefotaxime in both methods and (٢٥٪ and ٠٪) to Cephalexin in disc diffusion and MIC methods respectively and the same resistance rate (٢٥٪) was observed to both Amikacin and Gentamicin in both methods.

Penicillin is uniformly active against ***St.pyogenes*** and has remained the antibiotic of choice for the treatment of infections caused by this organism (Etesse *et.al.,* ١٩٩٩). The explanations for this remarkable state of continued susceptibility to Penicillin are that β-lactamase may not be expressed or may be toxic to the organism and/or that low-affinity Penicillin-binding proteins are either not expressed or render organisms nonviable (Horn *et.al.,* ١٩٩٨).

Listeria monocytogenes :

Figures (٣-١٧A, B) show that ***L.monocytogenes*** was more sensitive to Penicillin, Ampicillin, Cefotaxime, Gentamicin and Amikacin in both methods and Amoxicillin and Co-trimoxazole in disc diffusion method with resistance rate of (٠٪) while it was fully resistant to Cephalexin in rate (١٠٠٪) in both methods.

These results were in accordance with many reports which found that ***Listeria*** is susceptible to a number of antibiotics, including Penicillin, Ampicillin, Trimethoprim/sulfamethoxizole and aminoglycosides, but resistant to Cephalosporins (Schuchat *et.al.,* ١٩٩١; Boukhari *et.al.*, ١٩٩٩ and Walsh *et.al.,* ٢٠٠١). Therefore cephalosporins are not recommended (Schuchat *et.al.,* ١٩٩١).

Gilbert *et.al.* ٢٠٠١ stated that the antibiotics being most active against ***Listeria*** are Ampicillin, Gentamicin, and Trimethoprim/sulfamethoxizole. So the empirical use of Ampicillin to cover febrile infants for ***L. monocytogenes*** infections is most justifiable in the first month of life (Brown *et.al.,* ٢٠٠٢).

Generally, resistance to antibiotics may be related to usage of concentration of antibiotics lower than the minimal inhibitory concentrations (MIC) or what is called sub lethal concentration (Nafeesa *et.al.,* ٢٠٠١), or to the widespread use of antibiotics in hospitals which provided a reservoir of antibiotic-resistant microorganisms (Kloos and Bannerman, ١٩٩٤).

٤-٢: Conclusions:

١. Bacteremia in infants aged < ١ month was relatively more than other age groups.
٢. Gram-positive organisms were predominant pathogens in bacteremic infants and ***S.aureus*** was the most common one.
٣. Increment in temperature was associated with the increased probability of bacteremic infection in infants below two years.
٤. Cephalexin and Amikacin were the most effective drugs on Gram-positive bacteria causing bacteremia in infants.
٥. MRSA and MRCoNS were more resistant to many antibiotics than sensitive stains.

٤-٣: Recommendations:

١. Blood culture must be done for any infant with high temperature.
٢. Use new methods of blood culture system which evaluated blood samples in a shorter time.
٣. Febrile infants should receive empirical antibiotics like (Amikacin in combination with Cefotaxime) until the result of blood culture is confirmed to minimize the risk of spreading infection.
٤. There is a need for more studies to cover all microorganisms that cause blood stream infection in children with broad age groups.

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