Med. J. Babylon, 7(4) : 42-47. 2009.

Isolation and characterization of *Raoultella ornithinolytica* from Clinical Specimens in Hilla city, Iraq

Samir M. Al-Hulu **Alaa H. Al-Charrakh**
Mohammed A.K. Al-Saadi
Babylon University, College of Medicine, Department of Microbiology

Abstract:

A total of 720 clinical samples were collected from three main hospitals in Hilla city/ Babylon province, Iraq. Samples were screened for presence of *Raoultella* spp., as well as studying their expression of virulence factors. A total of 144 bacterial isolates were recovered and identified as *Klebsiella*-like organisms. Out of these, 11 isolates were identified as *Raoultella ornithinolytica*, which represent 7.6% of all *Klebsiella*-like organisms found. Many virulence factors expressed by *R. ornithinolytica* were studied *in vitro*. All isolates produced capsule and expressed CFA/I, and CFA/III.
Nine isolates (81.8%) were able to produce siderophores. Four isolates (36.6%) were able to produce bacteriocin. All *R. ornithinolytica* isolates were unable to produce extracellular protease, hemolysin, and histamine. All isolates of *R. ornithinolytica* were resistant to penicillin, ampicillin, gentamicin, chloramphenicol, rifampin, cephalothin, cephotaxime, streptomycin, amoxicillin.,but they showed high sensitivity to nitrofurantoin and ciprofloxacin, and all them were completely sensitive to meropenem. *R. ornithinolytica* expressed a high degree of sensitivity to the effect of human serum when they grew in human serum at 37 ^{o}C for 3 hrs. The present study represented the first record of occurrence of *R. ornithinolytica* in human clinical samples in Iraq.

الخلاصة: تضمنت هذه الدراسة جمع 720 عينة سريرية من ثلاث مستشفيات رئيسة في مدينة الحلة/ محافظة بابل، العراق، والكشف عن وجود بكتريا الروالتيلا وكذلك دراسة قابلية هذه البكتريا على انتاج عوامل الضراوة المختلفة. تم الحصول على 144 عزلة بكتيرية وشخصت على انها عزلات مشابهة للكليبسللا، ومن هذه الاخيرة تم تشخيص 11 عزلة عائدة للنوع *Raoultella ornithinolytica*, والتي كانت بنسبة 7.6% من العزلات المشابهة للكليبسللا المعزولة. أظهرت النتائج ان جميع هذه العزلات كانت لها القابلية على انتاج الكبسولة وكذلك عوامل الضراوة (النوع الاول والثاني) وان 81,8% منها كانت منتجة للسايدروفور وان اربع عزلات منها فقط كانت منتجة للبكتريوسين.
أظهرت النتائج ايضا" ان عزلات *R. ornithinolytica* كانت غير قادرة على انتاج انزيمات البروتييز والهيمولايسين وكذلك الهستامين. أظهرت جميع هذه العزلا ت مقاومة عالية لمضادات البنسلين،الامبسيلين،الاموكسلين،الجنتامايسين، الكلورامفينكول، الريفامبين، السيفالوثين، والستربتومايسين غير انها اظهرت حساسية عالية تجاه عقاري النايتروفيورانتون والسبروفلوكساسين وان جميعها كانت حساسة 100% لعقار الميروبنيم. كما أظهرت النتائج ايضا ان عزلات*R. ornithinolytica* كانت حساسة وبدرجة عالية لتاثير المصل البشري عند تنميتها فيه لمدة ثلاث ساعات بدرجة 37° مئوية. ان هذه الدراسة تعد أول تسجيل لوجود بكتريا *R. ornithinolytica* في العينات السريرية البشرية في مدينة الحلة/العراق.

Introduction:

The genus *Raoultella* (formerly *Klebsiella*) is a member of the family Enterobactericeae. The data from the sequence analyses of the 16S rRNA along with previously reported biochemical and DNA–DNA hybridization data confirmed that the genus *Klebsiella* is heterogeneous and composed of species which form three clusters that also included members

of other genera. These data support the division of the genus *Klebsiella* into two genera. The name *Raoultella* is proposed as a genus name for species of cluster II which contained *Klebsiella ornithinolytica*, *Klebsiella planticola*, and *Klebsiella terrigena*, organisms characterized by growth at 10ºC and utilization of L-sorbose as carbon source (1)

Originally, *Raoultella* spp. though to occur solely in aquatic, botanic, and soil environments and has been never been isolated from clinical specimens (2,3). The first isolation of *R.planticola* from neonates in neonatal wards reported by (4).Monnet and his colleagues (5) mentioned that *R.planticola* may found in human clinical specimens and represent 8% of clinical isolates, while, *R. terrigena* is rarely found, which represent as 0.4% among clinical *Klebsiella* strains (6).

Recent studies showed that *Raoultella* isolated from clinical specimens has many virulence factors such as capsule, colonization factors antigens (CFA/I and CFA/III), production of siderophore, histamine and bacteriocin (7,8,9). It was also found that *R. planticola* strains were resistant to azteornam, nitrofurantoin, penicillins, and gentamicin but suscebtiple to piperacillin, imipenem, and ciprofloxacin (10, 4).

The aims of this study are to investigate the occurrence of *Raoultella* spp. isolates in human clinical sample in Hilla city, study of some virulence factors contributing in their pathogenesity, and study the susceptibility of these isolates to some antibiotics.

Materials and Methods:

Samples collection:

A total of 720 clinical samples were collected in the present study during the period from November 2006 to March 2007. Clinical samples were collected from patients admitted to the main three hospitals in Hilla-city/Babylon province, (Teaching Hospital of Hilla, Mergan Hospital, Maternity and Pediatric Hospital). Types and numbers of clinical samples collected during the study were as follows: Urine, 503; Stool, 174; Blood,4; Wound10, Burn, 21; and Vagina, 3.

Identification of bacterial isolates:

Bacterial isolates were identified using the traditional morphological and biochemical tests (11), in addition to the tests recommended by (1,12, 13).

Detection of virulence factors of *Raoultella ornithinolytica:*

A number of virulence factors of *Raoultella ornithinolytica* isolates were detected, which included capsule production, Hemolysin production, Extracellular protease, Siderophore production, and Colonization factor antigens (CFA/I &CFA/III).

Siderophore production and Colonization factor antigens were detected as described by (14).

Extracellular protease was detected using M9 media supplemented with 2% agar and supported by 1% gelatin .The plates were cultivated by picking and patching and incubated for 24-28 hrs at 37^{o}C. After that, 3 ml of 5% trichloro acetic acid was added. The presence of clear zone around each colony indicates the positive result (15). *R. ornithinolytica* isolates were also examined for their ability to produce bacteriocin using cup assay method (16) against a sensitive (indicator) *E. coli* isolate (obtained from department of Microbiology/ College of Medicine/Babylon University).

Serum bactericidal assay:

Bacterial isolates of *E. coli*, *Enterbacter* spp., (obtained from department of Microbiology/College of Medicine/Babylon University) and *R. ornithinolytica* were grown in nutrient broth for 4-6 hrs at 37 ^{o}C, harvested, and adjusted to a density of 2 x 10^{6} bacterial cells/ml with physiological saline. Bacterial suspension 25 µl was mixed with 75µl of pooled normal human serum in sterilized test tubes. Viable counts of bacteria were determined according to (11) after incubation for 1, 2, and 3hrs. Each isolate was tested at least three

times. Isolates were considered serum resistant or serum sensitive if the grading was the same in all experiments. Mixture of bacterial suspension and normal saline was used as control (17).

Antibiotic disk susceptibility test:

The antimicrobial resistance patterns of strains to antimicrobial agents was determined using disk diffusion method as recommended by national committee of clinical laboratory standards (18) and interpreted according to (19). The following antimicrobial agents were obtained (from Oxoid, U.K) as standard reference disks as known potency for laboratory use. Penicillin (P) 10 IU; Ampicillin (AMP) 10 µg; Amoxycillin (AMX) 25 µg; Cephalothin (KF) 30 µg; Cephotaxime (CTX) 30 µg; Meropeneme (MEM) 10µg; Gentamicin (GN) 10µg; Streptomycin (S) 10 µg; Rifampin (RD) 30 µg; Tetracycline (TE) 30 µg; Nitrofurantoin (F) 300 µg; Nalidixic acid (NA) 30 µg; Chloramphenicol (C) 30 µg; Ciprofloxacin (CIP) 5µg. All tests were performed on plates of Muller-Hinton agar.

Results and Discussion:

Isolation and identification of bacterial isolates:

A total of 144 clinical isolates were identified as *Klebsiella*-like organisms, out of these, 11 isolates *Raoultella ornithinolytica* were identified which represent (7.6%) of all *Klebsiella*-like organisms. According to our knowledge, through the available data, we think that this study represented the first record of the occurrence of the *R. ornithinolytica* in human clinical specimens in Iraq.

This result agreed with other studies which found that the percentage of *Raoultella* recovered from clinical specimens were ranged between 8-9% (4,20,21) Podschun and his colleagues (20) reported that *R .planticola* isolated from neonates in intensive care unit represented 8.7% of 131 *Klebsiella* isolates in Germany. In another research, they reported that the isolation of *R. planticola* from neonates in neonatal ward represented (9%) of all Klebsiella spp found in Germany (4).

Also the results were approximately compatible with previous results (21) since *R. planticola* represented (6%) of all clinical *Klebsiella* isolates recovered in Hilla-city. However, in another studies *R. planticola* represented only (1%) of clinical isolates in Georgia and Brazil, respectively (22,13). *R.planticola* isolates were not detected in this study, and this result was in accordance with that result obtained by Watanakunakron and Jura (23) who showed that *R. planticola* isolates were not appeared in any case in the area of study in United States.

Results in present study also found that most isolates 8 (72.7%) of the 11 *R. ornithinolytica* were isolated from 174 rectal swab and stool samples and this result agreed with the finding obtained by (24) who found that the principle reservoirs of transmission of *Klebseilla* in hospital setting are the gastrointestinal tract of patient and hand of hospital personal. Moreover, this result in the present study agreed with (4) who showed that a high rate (68%) of *Klebsiella* was isolated from feaces. The result also agreed with the finding obtained by (25) who found that intestinal colonization considered as the main reservoir for *R. planticola*.

The results also indicated that only 3 (27.3%) isolates of *R. ornithinolytica* were detected in 503 urine samples, and no isolates were detected in blood, vagina, ear, and wound samples. The absence of *R. ornithinolytica* in these clinical samples may attribute to the few number of samples taken in this study.

Detection of virulence factors:

Results in the present study showed that all *R. ornithinolytica* isolates were able to produce capsule but they were unable to produce hemolysin on blood agar, and 9 (81.8%) of the isolates were able to produce siderophore (**Table 1**).These results are similar to that

reported by (7), who found that *Klebsiella* spp.isolates have the ability to provide iron and produce siderophore without hemolysin production. According to the result of the present study, there is an association may be present between aerobactin synthesis and the virulence factor of *R. orinthinolytica* isolates.

The results also revealed that all isolates of *R. ornithinolytica* were able to express the colonization factor antigens (CFA/I, and CFA/III) (**Table 1**). The production of CFA/I by *R. ornithinolytica* isolates expressed by hemagglutination of human red blood cells (group A) in presence of mannose and this result agreed with findings obtained by, (26) and (27) who found that CFA/I cause mannose-resistant hemagglutination which agglutinate human group A erythrocytes, and agreed with finding obtained by (28), who found that *Klebsiella pneumoniae* that have CFA/I classified under " mannose specific" bacterial lectins.

The expression of colonization factors antigens (CFA/I, and CFA/III) by *R. ornithinolytica* isolates may play an important role in pathogenesis and virulence of these bacteria which enhance their ability to colonize the intestinal and urogenital tracts. This result agreed with findings obtained by other studies which showed that *Klebsiella, Raoultella* expressed type (I, III) pili which mediate bacterial binding to mucous or epithelial cell of urogenital, respiratory and intestinal tract (7).

The results in Table 1 also showed that all isolates of *R. ornithinolytica* were unable to produce protease and these results fitted with fact that *Klebsiella* were unable to produce extracellular protease (gelatinase). The extracellular enzymes of bacteria like protease, lipases, and nuclease are not shown to have a direct role in invasion or pathogenesis, but these enzymes presumably may associate in bacterial nutrition or metabolism (29).

Detection of Bacteriocin production:

The ability of *R. ornithinolytica* isolates to produce bacteriocin was also tested. Results showed that 4 (36.4%) of *R. ornithinolytica* isolates were able to produce bacteriocin (**Table 1**). The production of bacteriocin by *Klebsiella* and *Raoultella planticola* strains was recorded by several authors (30, 31).

In this study, all bacteriocin-producing isolates were isolated from urine and stool samples and this finding assumed the bacteriocin may be a virulence factor of *Raoultella* pathogencity. Morever, It was found that the cytolysin of *Enterococus faecalis* posses both hemolysin and bacteriocin which may increase the persistence of these bacteria in blood stream and their resistance to serum, indicating that the bacteriocin is essential for virulence and pathogencity of *Enterococcus* in septicemia (32). On other hand, Viddotto and his co-workers (33) found that the bacteriocin activity of *E. coli* isolates is not essential for virulence and pathogencity of producing isolates but it aids them in their competition.

Antibiotic resistance of *Raoultella ornithinolytica*:

All isolates of *Raoultella ornithinolytica* (100%) were found to be resistant to penicillin,ampicillin , gentamycin, chloromphincol, rifampin, cephalothin, cefotaxime, lincomycin, streptomycin, amoxicillin, calthromycin, azithromycin, and (90.9%) resistant to tetracycline, (63.6%) were resistant to naldixic acid (**Figure 1**). This result agreed with other studies (10,34), which found that all strains of *Klebsiella* spp are resistant to ampicillin, pencillin and amoxicillin. The Results also showed that all isolates were resistant to cefotaxime and cephalothin (**Figure 1**).The result in the present study is compatible with other researchers who found that chromosomally encoded resistance to first and second generation cephalosporins in strains of *Klebsiella* has been emerged in many hospitals (10). Result also showed that all *Raoultella ornithinolytica* were resistant to gentamycin and streptomycin (**Figure 1**), and this result agreed with findings obtained by several studies which showed that strains of *Klebsiella* have a highest level of resistant to gentamycin and other aminoglycoside antibiotics (35). The results also showed that (90.9%)

of *Raoultella ornithinolytica* isolates were resistant to tetracycline and (63.6%) of them were resistant to naldixic acid.

Depending on the results of antibiotics sensitivity observed in this study, *Raoultella ornithinolytica* may be regarded as multi-drug resistant by expressing resistance to more than 14 antibiotics and this result may be compatible with findings obtained by (36,21) who showed that *Klebsiella, Raoultella planticola* isolates expressed multi-drug resistance to several antibiotics.

Serum resistance of *Raoultella ornithinolytica*:

The effect of human serum on the growth of *E. coli*, *Enterobacter* spp. and *R. ornithinolytica* was detected. Figure (**2-a**) shows the effect of human serum on the growth of *E. coli*, *Enterobacter* spp and *R. ornithinolytica* after one hr. of incubation on nutrient agar. *R. ornithinolytica* was the most sensitive to the effect of serum, when compared to the other bacterial types, on the same manner, *E. coli* was less effected by serum. On the other hand the result in figure (**2-b**) showed the high effect of serum on the growth of all bacterial isolates after 2 hr, of incubation. After 3 hr, of incubation *R. ornithinolytica*, was the most sensitive bacterial isolate when compared to the other isolates (**Figure 2-c**).

The results in figure (2 a, b, c) Statistically by using χ^2 test showed that there was a strong relationship between the bacterial types and serum resistance ($P< 0.01$) . Results also showed that the serum resistance profile of *E. coli* and *Enterobacter* spp. after first hour of incubation tends to be similar to that of after third hour of incubation. After two hours of incubation , both these bacterial isolates were sensitive to the serum by comparing to the relative rate of growth in control, this may be due to the fact that serum components may need to time to exhibit their effect on bacterial growth which was clearly observed after 2 hrs., but after 3 hrs. bacterial isolates may develop a mechanism of induced resistance that makes bacterial isolates able to exhibit a degree of serum-tolerance. *R. ornithinolytica* exhibited a high degree of serum sensitivity when compared with the growth rate in control. The serum-sensitive profile of *R.. ornithinolytica* may explain that this bacteria did not isolate from samples, in this study. This sensitivity may limit the pathogenic properties of R. *ornithinolytica* in normal subjects (immunocomptence), at the same time the pathogenic capacity increases in immunocompromized patient as well as in age extremes.

Most strain of gram-negative bacteria are sensitive to the bactericidal effect of human serum, whereas, pathogenic strains often exhibit serum resistance properties (37). The main role of the serum bactericidal systems is thought to prevent microorganisms to invading and persisting in the blood. Even differences in the degree of bacterial serum susceptibility may determine whether a strain is able to infect as well as the length of time it takes the organisms to establish the infection (38).

References:

1- Drancourt M.; Bollet, C.; Corta, A. and Rousselier, P. (2001). Phylogentic analyses of *Klebsiella* species delineate *Klebsiella* and *Raoultella* gen. nov., with descripition of *Raoultella ornithinolytica* com. nov., *Raoultella terrigena* com. nov. and *Raoultella planticola* comb. nov. Internet. J. System. Evolution Microbiol. **51**: 925-932.

2- Bagley, S. T.; Seidler, R. J.; Albot, H. W.J.; and J. E. Morrow. (1978). Isolation of *Klebsiella* from living wood. Appl. Environ. Microbiol. **36**: 178-185.

3- Sakazaki, R. ; Tamura, K.; Kosako, Y.; and Yoshizaki, E. (1989). *Klebsiella ornithinolytica* spp *Nov*., formerly known as ornithino-positive *Klebsiella oxyotoca*. Curr. Microbiol. **18**: 201-206 (Abstract).

4- Podschun, R.; Acktun, H.; Okpara, J.; Linperkamp O.; Ullman, U. and Borneff, M. Lipp (1998).Isolation of *Klebsiella planticola* from Newborns in Neonatal word . Clinc. Microbiol Notes., **36** (8) : 2331-2332.

5- Monnet, D., J. Freney, Y. Brun, J. M. Boeufgras, and J. Fleurette. (1991). Difficulties in identifying *Klebsiella* strains of clinical origin. Zentralbl. Bakteriol. Parasitenkd. Infektionskr. Hyg. Abt. 1 Orig. 274:456–464.

6- Podschun, R., and U. Ullmann. (1992). Isolation of *Klebsiella terrigena* from clinical specimens. Eur. J. Clin. Microbiol. Infect. Dis. **11:**349–352.

7- Podschun, R.; Sievers, D.; Fischer, A.; Ullmann, U.(1993). Serotypes, hemma-gglutinins, siderophore synthesis, and serum resistance of *Klebsiella* isolates causing human urinary tract infections. J. infect. Dis. **168**: 1415-1421.

8- Podschun, R.; and Ullmann, U. (1998). *Klebsiella* species. as nosocomial pathogens, epidemiology, taxonomy, typing methods, and pathogencity factors. Clin. Microbiol. Rev. **11**(4). 589-603.

9- Kanki, M.; Yoda, I.; Tsukamoto, T.; Slibata, T. (2007). *Klebsiella pneumoniae* produce no histamine: *Raoultella planticola* and *Raoultella ornithinolytica* strains are histamine producers. Appl. Environ. Microbiol.**68**: 3462-3466.

10-Liu, Y.; Mee, B.J.; and Mulgare, L. M. (1997). Identification of clinical isolates of indole-postive *Klebsiella* species, including *Klebsiella planticola* and genetic and molecular analysis of their β-lactamase. J. Clin. Microbiol. **35**:2365-2369.

11-McFaddin, J. F. (2000). Biochemical tests for identification of medical bacteria. Lippincott Williams and Wilkins. Philadelphia. USA.

12-Hansen, D. S.; Aucken, H. M.; Abiola, T.; and Podschun, R. (2004). Recommended test panel for differentiation of *Klebsiella* species on the basis of a trilateral interlabratory evaluation of 18 biochemical tests. J. Clin. Microbiol. **42**, (8): 3665-3669.

13-Alves, M. S.; Dias, R. C. D. S.; DeCastro A. C. D.; Riely, L. W.; and Moreira, B. M. (2006). Identification of clinical isolates of indole-positive and indole negative *Klebsiella* spp. J. Clin. Microbiol. **44**: 3640-3646.

14-Sambrook, J.; and Rusell, D.W. (2001). Molecular cloning. A laboratory manual. 3rd. Cold Spring. Harber Laboratory Press, N. Y.

15-Piret, J.; Millet, J.; and Demain, A. (1983). Production of intracellular protease during sporulation of *Bacillus brevis*, Eur. J. Appl. Microbiol. Biotechnol. **17**: 227-230.

16-Al-Qassab, A. O.; and Al-Khafaji, Z. M. (1992). Effect of different conditions on inhibition activity of enteric lactobacilli against diarrhea-causing enteric bacteria. J. Agri. Sci., **3** (1): 18-26.

17-Sahly, M.; Aucken, H.; Benedi, V. J.; Forestier, C.; Fussing , V. Hansen, D. S., Ofek, I. Podschun, R, Sirot; D.; Tomas, J. M.; Sandrang; D.; Ullmann, U. (2004) . Increased serum resistance in *Klebseilla pneumoniae* strains producing extended-spectrum β-lactamases. Antimicrob. Agents Chemother. **48**: 3477- 3482.

18-National Committee for Clinical Laboratory Standards (NCCLS) (2003a). Performance standards for disk susceptibility tests, 8th ed. Approved standard. M 100-S13. National Committee for Clinical Laboratory Standards. Wayne, pa.

19-National Committee for Clinical Laboratory Standards (NCCLS) (2003b). Performance standards for antimicrobial susceptibility testing: Approved standard. M2-A8. National Committee for Clinical Laboratory Standards. Wayne, Pa.

20-Podschun, R., and U. Ullmann. 1994. Incidence of *Klebsiella planticola* among clinical Klebsiella isolates. Med. Microbiol. Lett. 3:90–95.

21-Al-Charrackh, A. H. (2007). Isolation of *Raoultella planticola* from clinical specimens in Hilla city . Proceedings of 3rd Conference of Microbiology and Biotechnology, Basrah, Iraq.

22-Westbrook, G. L.; Hara, C. M.; Roman, S. B.; Miller, j. (2000). Incidence and identification of *Klebsiella planticola* in clinical isolates with emphasis on Neoborns. J. Clin. Microbiol., **38**:1495-1497.

23-WatanaKunakorn, C. L.; and Jura, J. (1991). *Klebsiella* bacterimia: a review of 196 episodes during a decade (1980-1989). Second. J. Infect. Dis. **23**: 399-405.

24-Kuhn, I.; Ayling-Smith, B.; Tullus, K.; and Burman, L.G. (1993). The use of colonization rate and epidemic index as tools to illustrate the epidemiology of faecal Enterobacteriaceae strains in Swedish neonatal wards. J. Hosp. Infect. **23**: 287-297.

25-Seleden, R.; Lee, S.; Wang, W.L.; Bannett, J. V.; and Eickhoff, T.C. (1971). Nosocomial *Klebsiella* infections: intestinal colonization as a reservoir. Ann. Intern. Med. **74**: 657-664.

26-Evans, D. G.; Evans, D. J. Jr; and Toja, W. (1977). Hemaglutination of human group A erythrocytes by enterotoxogenic *Escherichia coli* of isolated from adults with diarrhea: Correlation with colonization factors. Infect. Immune. **63**:4849-4856 (Abstract).

27-Evans, D. G.; and Evans, D. J. Jr .(1978).New surface- associated heat-labile colonization factor antigen(CFA/II) produced by enterotoxogenic *Escherichia coli* of serogroup O6 and O8 .Infect. Immun. 21 :638-647.

28-Maayan, M C.; Ofek, I.; Meddalia, D.; Aonson, M.(1985). Population shift in mannose-specific fimbriated phase of *Klebsiella pneumoniae* during experimental urinary tract infections in mice. Infect. Immun. **49**:785 -789.

29-Brooks, G. F.; Butel, J. S.; and Morse, S. A. (2004). Jawetz, Melnick and Adelberg's. Medical Microbiology. Twenty-Third ed. McGraw Hill Education (Asia). Singapore.

30-Bauernfeind, A.; Petermuller, C.; and Schneider, R. (1981). Bacteriocins as tools in analysis of nosocomail *Klebsiella pneumenia* infections. J. Clin. Microbiol. **14**:15-19. (Abstract).

31-Al-Charrackh, A. H.**,** Yousif S. Y.**,** and Al-Janabi H. S. (2007) Antimicrobial spectrum of the action of bacteriocins from *klebsiella* isolates**.** Proceedings of 3rd Conference of Microbiology and Biotechnology, Basrah, Iraq.

32-Ike, Y.;Hashimoto, H.; and Clemell, D. B. (1984). Hemolysin of *Streptococcus faecalis* subspecies *zymogenes* contributes to virulence in mice. Infect. Immun. **45**: 528-530.

33-Viddoto, M. C.; Furlaneto, M. C.; and Parugini, M. R. E. (1991). Virulence factors of *Eschericia coli* in urinary isolates. Braz. J. Med. Biol. Res. **24**: 365-373.

34-Stock, I.; and Widemann, B. (2001). Natural antibiotic susceptibility of *Klebsiella pneumoniae, K. oxyotoca, K. planticola, K. ornithinolytica* and *K. terrigena* strains. J. Med. Microbiol. **50**: 396-406.

35-Noriega, E. R.; Leibowitz, R. E.; Richond, A.S.; Rutinstein, E.; Schaefler, S.; Simberkoff, M. S.; and Rahal, J.J. (1975). Nosocomial infections caused by gentamicin-resistant, streptomycin-sensitive *Klebsiella*. J. Infect. Dis. **131** (suppl): S 45-S50.

36-Al-Charrakh, A. H. (2005). Bacteriological and genetic study on extended – spectrum Beta-lactamases and bacteriocins of *Klebsiella* isolated from Hilla city. Ph. D. Thesis. College of Science. Baghdad University.

37-Olling, S. (1977). Sensitivity of gram negative bacilli to the serum bactericidal activity: A marker of the host parasite relationship in acute and persisting infections. Secand. J. Infect. Dis. **10** (supp l.): 1-40.

38-Taylor, P. W. (1983). Bactericidal and bacteriolytic activity of serum against gram-negative bacteria. Microbiol. Rev. **47**: 46-83.

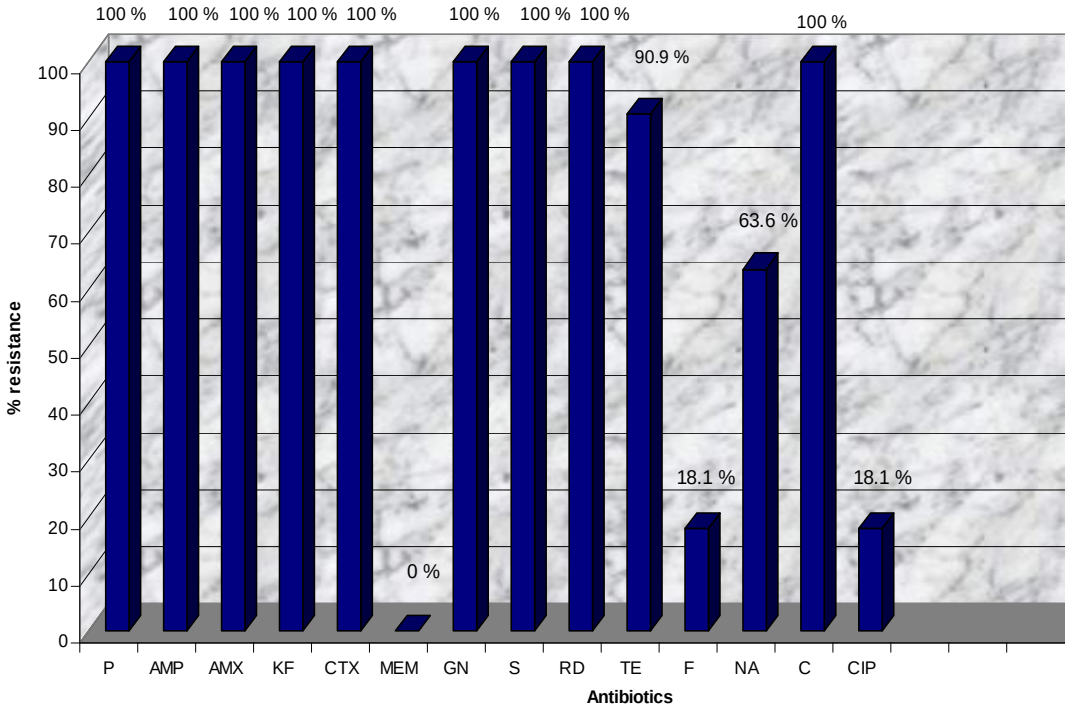
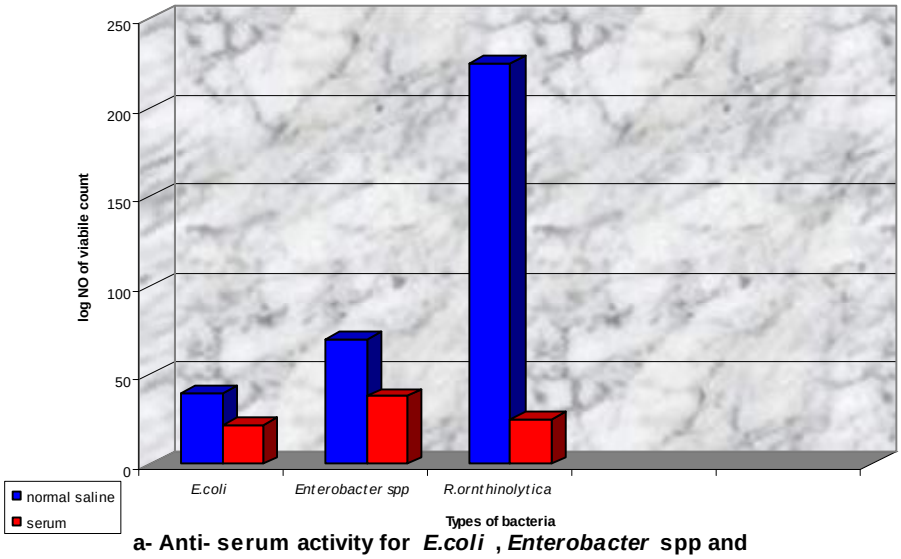


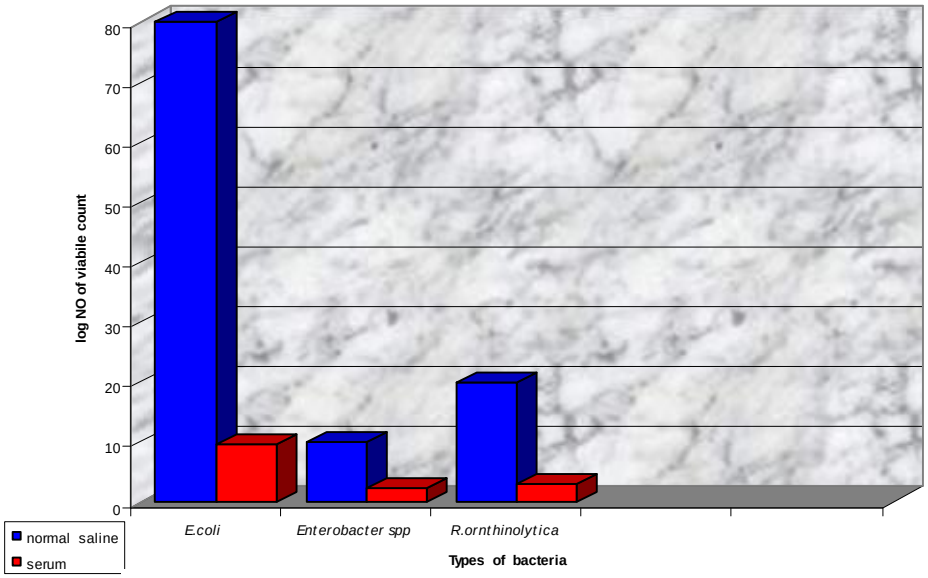
Figure (1): Resistance of 11 *R. ornithinolytica* isolates to 14 antibiotics.

Table (1): Virulence factors of *Raoultella ornithinolytica* isolates

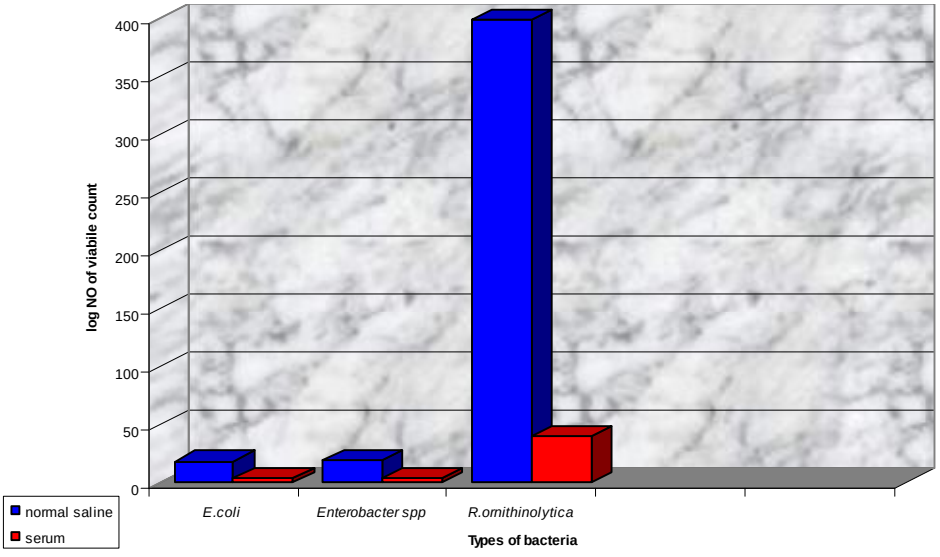
Isolate designations	Clinical sample	Capsule	Siderophore	Hemolysin	Colonization factor antigen		Extracellular protease	Bacteriocin Diameter in mm
					CFA/I	CFA/III		
*R.ornithinolytica*398	urine	+	+	-	+	+	-	+(**34**)
*R.ornithinolytica*487	urine	+	+	-	+	+	-	-
*R.ornithinolytica*104	urine	+	+	-	+	+	-	+(**12**)
*R.ornithinolytica*26	stool	+	+	-	+	+	-	+(**34**)
*R.ornithinolytica*13	stool	+	+	-	+	+	-	-
*R.ornithinolytica*27	stool	+	+	-	+	+	-	-
*R.ornithinolytica*32	stool	+	+	-	+	+	-	+(**12**)
*R.ornithinolytica*70	stool	+	-	-	+	+	-	-
*R.ornithinolytica*99	stool	+	+	-	+	+	-	+
*R.ornithinolytica*163	stool	+	-	-	+	+	-	-
*R.ornithinolytica*45	stool	+	+	-	+	+	-	-



a- Anti- serum activity for *E.coli* , *Enterobacter* spp and *R.ornithinolytica* after1 hr of incubation.



b- Anti-serum activity for *E.coli* , *Enterobacter* spp and *R.ornithinolytica* after 2 hour of incubation.



Figure(3-2-c): Anti -serum activity for *Ecoli*, *Enterobacter* spp and *R.ornithinolytica* after 3 hour of incubation.

Figure (2 a, b, c): Anti - serum activity for *E. coli*, *Enterobacter* spp, and *R.. ornithinolytica* after 1, 2 and 3 hours of incubation.