Emergence of *Raoultellaornithinolytica*producing beta lactamase enzyme in different clinical specimens in Erbil city

Abstract

Background:*Raoultellaornithinolytica*is an underreported, emerging hospital-acquired infection and is particularly associated with invasive procedures, should never be considered simply a saprophytic bacterium that occasionally contaminates bronchial lavage or other deep respiratory samples or surgical sites. Physicians should be aware of the high rates of antimicrobial resistance of *R. ornithinolytica* isolates so that immediate broad-spectrum antibiotic treatment can be established before accurate microbiological results are obtained.

Objectives: Our study aimed to carry out a retrospective study on *R.ornithinolytica*isolated from various clinical samples in Erbil city and analyze its epidemiology , antibiotics susceptibility patterns and and producing of Extend spectrum beta lactamase enzyme

. **Materials and methods:** A total of 2350 samples were collected from three different sources(Urine ,High vaginal swab and wound), collected from female and male from patient attending Rizgary hospital and Central lab in Erbil city during the July 2017 to Februry 2018 . Only 10 cases had been identified as *R.ornithinolytica*and all isolates isolated and identified by using microscopical, morphological , biochemical tests and Vitek 2 compact system . Also antibiotics susceptibility testing was performed by using Vitec 2 compact system according to the standard protocol against 13 antibiotics which are (Ampicillin /Sulbactam , Piperacillin/ Tazobactam, Cefazolin, Ceftazidim , Ceftriaxon , Cefepime, Levofloxacin , Trimethoprim/ Sulfamethoxazol, Gentamycin, Tobramycin, Ertapenem, Imipenem, Ciprofloxacin).

Results: Onlyten *R.ornithinolytica*isolates isolated from 2350 distribution according to their source of isolation High vaginal swab 6(0.23%) appeared to be the most dominant specimen than other specimens followed by urine sample 3(0.13%) then wound 1(0.04%).The highest percentage of *R.ornithinolytica. i*solated from female .Most isolates from high vaginal swab sample 6(0.26%) followed by urine 3 (0.13%) then wound 1(0.04), when performing of antibiotic susceptibility the highest resistances rate were to Cefazolin and Trimethoprim / Sulfamethoxazol 10 (100%) followed by Ampicillin / Sulbactam , Piperacillin / Tazobactam, Ceftriaxon , Gentamycin , Tobramycin and Ciprofloxacin 7(70%) for each, 6(60%)isolates ESBL producer, all isolated *R.ornithinolytica* were resistance to more than 8 antibiotics and two isolates completely resistance to all thirteen antibiotics.

Conclusion: The overall prevalence of*R.ornithinolytica*isolates was low in Erbil city and we can be observed and isolated from varies clinical samples and causes serious infections and susceptibility to some antibiotics are low and also most isolates multiresistance and ESBL producers and if prevailing resistance trends continue, high societal and economic costs can be expected , better management of infections caused by resistance *R.ornithinolytica*is becoming essential., these finding offer a reliable measure of prevalence of*Raoultellaornithinolytica* in our

region and provides a baseline for future studies which will enable the monitoring of trends overtime .

Keyward: *Raoultellaornithinolytica,*antibiotics resistances , ESBL.

Introduction

*Raoultellaornithinolytica*is belonging to family Enterobacteriaceae, it consist of gram–negative, encapsulated, oxidase negative, catalase positive ,nonmotile, facultatively anaerobic rods formerly designated *Klebsiella* .It is named after the French bacteriologist Didier Raoult.[1].*Raoultella spp*. Have recently been separated from the genus *Klebsiella*based on their molecular characteristics [2].This genus was recovered from water , soil , plants , and occasionally mammal mucosa , that including human specimens . Type species is *Raoultellaornithinolytica* comb. nov.*Raoultellaplanticola*comb.nov.and*Raoultellaterrigena* comb. nov.[3]*R.ornithinolytica* is known to inhabit aquatic environments and can be found in hospital environments[4].The data from the sequence analyses of the 16SrRNA 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]. Formerly known as *Klebsiella ornithinolytica*, the bacterium was reclassified as *Raoultella* based on new genetic approaches[1] . This bacterium was first described in 1989 by Sakazaki*et al*.[5].*Raoultella spp*. though to occur solely in aquatic, botanic, and soil environments and has been never been isolated from clinical specimens [6]. The first isolation of *R.planticola* from neonates in neonatal wards reported by Monnet *et al*[7].*R.ornithinolytica* is a Gram-negative aquatic–commensal of the *Enterobacteriaceae* family that has occasionally been reported to survive in human saliva.*R. ornithinolytica* has become a human pathogen in many community-acquired and/or hospital-acquired infections[8] .The results of the analyses of 16S rDNA and rpoB sequences support the heterogeneous taxonomic structure of the genus *Klebsiella*. *R.ornithinolytica* was until recently often confused with genus *Klebsiella* this species has been related for causing histamine fish poisoning ,same reports appeared , that this bacteria has been related to human infections such as peritonitis and enteric fever like syndrome [9]These environmental organisms, infrequently causing human infections, appear to have pathogenicity similar to that of *Klebsiella pneumoniae* [10] . The first human *Raoultella spp*. invasive infection was described in 1984, and

bloodstream infections have been reported rarely [11] .Over the past decade, *R.ornithinolytica* has emerged as antifungal, but important causal agent of human infection,ten cases of *R.ornithinolytica* infection have been reported, linking this pathogen to bacteremia and sepsisand soft tissue and other infections[12] . The ability to colonize the inner surfaces of indwelling urinary catheters is a major cause of hospital-acquired urinary tract infections. It is thus thought that these mechanisms could play an important role in the pathogenesis of infection. Other virulence factors, such as the ability to form biofilms, will help us to better understand the relationship between *R. ornithinolytica* human infections and invasive procedures such as the implantation of venous catheters, intra-vascular prostheses, or orthopedic devices [13] .The proportion of *R. ornithinolytica* isolates with reduced susceptibility to antibiotics was relatively high . The presence of a chromosomal bla gene is key tothe mechanism of beta-lactam resistance displayed by *R.ornithinolytica*isolates[14] . AmpC β –lactamases are β –lactamase enzymes that hydrolyze narrow , broad , and extended spectrum cephalosporins and cephamycins (cefoxitin), penicillins and not inhibited by clavulanic acid(β-lactamase inhibitor-β-lactam combinations) [15].

METHODS

Samples collection

A total of (2350) samples were collected from six different sources (urine, wound swab, high vaginal swab, feces, throat swab,csf). After collection all bacterial isolates were subjected to a series of confirming tests. Results showed that only (10) isolates were indicated as *R.ornithinolytica*. Clinical samples were collected from patients attending Rizgari hospital and Central Lab in Erbil city during the period (July 2017 to February 2018) from male and female patients with the age of 10-79 years .For isolation of microorganisms, the specimen was directly inoculated on culture media; Blood culture and macConkey agar plates were incubated aerobically at 37°C for (24-48) hours. Pure colonies of isolated microorganisms were identified using morphological, biochemical tests, Species identification and antibiograms for pathogens were performed using Vitek 2 system [16].

Antimicrobial susceptibility test by Vitek 2 system

All isolates of were screened for their susceptibility to 13 used antibiotics by using Vitek 2 system **w**ith its ability to provide accurate "fingerprint" recognition of bacterial

resistance mechanisms and phenotypes, the AES is a critical component of Vitek 2 technology .The Vitek 2 card contains 64 micro wells. Each well contains identification substrates or antimicrobial. Vitek 2 offers a comprehensive menu for the identification and antibiotic susceptibility testing of organisms. The Vitek 2 test card is sealed, which minimizes aerosols, spills, and personal contamination. Disposable waste is reduced by more than 80% over microtiter methods [16].

Phenotypic screening for ESBL

Each isolate was tested using the VITEK 2 system with the antimicrobial susceptibility test extend AST-EXN8 card. This system was designed to perform both screening and confirmatory tests for phenotypic detection of ESBL on the same plate.The test comprises a panel of six wells containing ceftazidime 0.5 mg⁄ L, cefotaxime 0.5 mg⁄ L and cefepime 1.0 mg⁄ L, the rest of three wells were filled with same three antibiotics in combination with clavulanic acid (4, 4 and 10 mg⁄ L, respectively). Growth in each well was quantitatively assessed by means of an optical reader. The proportional reduction in growth in wells containing Cephalosporin plus clavulanate compared with those containing the cephalosporin alone was considered to be indicative of ESBL production. All phenotypic interpretations of ESBLs were reported as a positive ESBL screening result. Strains were reported as ESBL-negative whenever phenotypic interpretations other than ESBLs were proposed by the AES [16].

Results

Distribution of *R.ornithinolytica*according to location of Erbil city

Prevalence of *R.ornithinolytica*was highest in Central Lab (0.25%) ,While the lowest prevalence of *R.ornithinolytica* found in Rizgari Lab (0.27%) as show in table (1).

Table (1): Distribution of *R.ornithinolytica*according to location of Erbil city

Hospitals	NO.of (+ve)samples	NO.of(-ve)samples	Total
Rzgari lab	**4**	**1428**	**1432**
	0.17%	**60.70%**	**60.93%**
Central lab	**6**	**912**	**918**

	0.25%	**38.81%**	**39.06%**
Total	**10**	**2340**	**2350**
	0.42%	**99.57%**	**100%**

Relation between *R.ornithinolytica*and gender (female , male)

Out of 2350 samples ,10 samples were isolatedfor*R.ornithinolytica*,the highest percentage of *R.ornithinolytica*in female 9 (0.38%) was higher than those in male patients 1 (0.043% as show in table (2).

Table(2): Relation between *R.ornithinolytica* and gender(female,male)

Patient	**(+ve) samples**	**(-ve)samples**	**Total**
Male	**1**	**1069**	**1070**
	0.043%	**46.63%**	**45.53%**
Female	**9**	**1271**	**1280**
	0.38%	**54.09%**	**54.47%**
Total	**10**	**2340**	**2350**
	0.43%	**99.57%**	**100%**

The incidence of *R.ornithinolytica* in different clinical specimens.

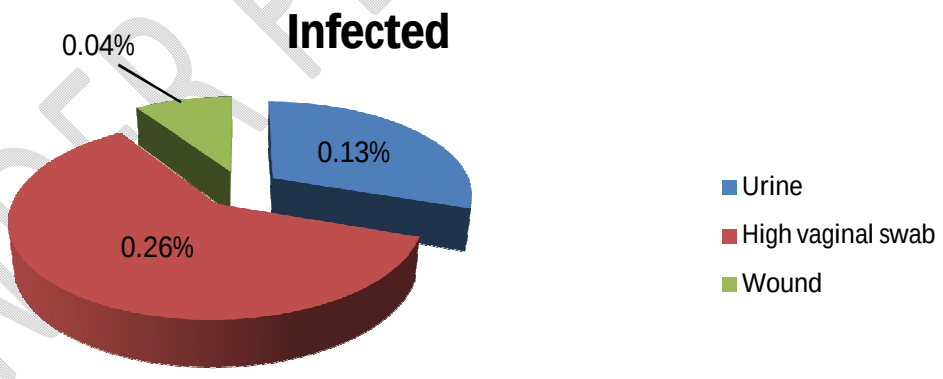
Out of 2350 samples 10 sample isolates distribution according to their source of isolation More than half of these infections were contributed by female genital tract 6(0.26%)and in the second position comes UTI, 3(0.13%)while only one (0.04%)wound infections has been detected . as in table (3) and in figure(1).

Table(3)The incidence of *R.ornithinolytica*in different clinical specimens

Patient	**Number and percentage of *R.ornithinolytica***

	Urine	High vaginal swab	Wound	Total
Infected	**3**	**6**	**1**	**10**
	0.13%	**0.26%**	**0.04%**	**0.43%**
Uninfected	**781**	**930**	**629**	**2340**
	33.23%	**39.57%**	**26.76%**	**99.56%**
Total	**784**	**936**	**630**	**2350**

Antibiotics	NO.Sensitive	Percentage %	NO.Resistant	Percentage %
	33.36%	**39.82%**	**26.80%**	**100%**



Figure(1):The incidence of *R.ornithinolytica* in different clinical specimens.

The number and percentage of antibiotic resistance among *R.ornithinolytica*

All *R.ornithinolytica*isolated were tested for antimicrobial susceptibility testing,*R. ornithinolytica* isolates showed highest sensitive (90%) to cefepime &imipenem,Ertapenem and Ceftazdium and levofloxacin, as shown in table (4) and in figure (2),on the other hand isolates were ESBL producers and multi resistance.

Table (4) The number and percentage of antibiotic resistance among *R.ornithinolytica*

Ampicillin/Sulbactam	**3**	**30%**	**7**	**70%**
Piperacillin/Tazobactam	**3**	**30%**	**7**	**70%**
Cefazolin	**0**	**0%**	**10**	**100%**
Ceftazidim	**9**	**90%**	**1**	**10%**
Ceftriaxon	**3**	**30%**	**7**	**70%**
Cefepime	**9**	**90%**	**1**	**10%**
Levofloxacin	**9**	**90%**	**1**	**10%**
Trimethoprim/Sulfamethoxazol	**0**	**0%**	**10**	**100%**
Gentamycin	**3**	**30%**	**7**	**70%**
Tobramycin	**3**	**30%**	**7**	**70%**
Ertapenem	**9**	**90%**	**1**	**10%**
Imipenem	**9**	**90%**	**1**	**10%**
Ciprofloxacin	**3**	**30%**	**7**	**70%**

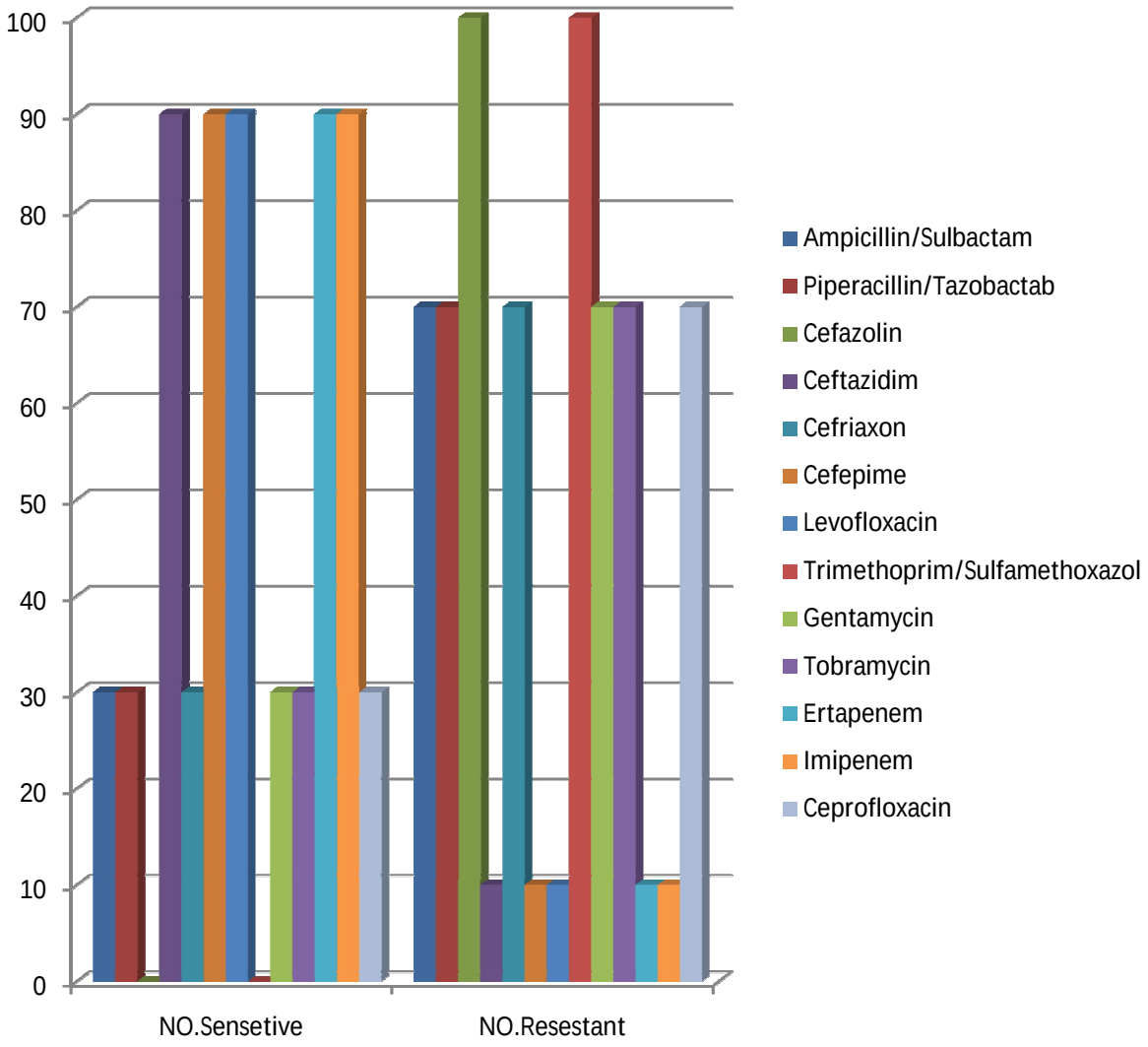


Figure (2)The number and percentage of antibiotic resistance among *R.ornithinolytica*

Antibiotic susceptibility patterns for ESBL producer *R.ornithinolytica*isolates

The bacterial isolates revealed remarkable variation in their resistance and sensitive antibiotics used,but in generalmost isolates of *R.ornithinolytica*were multi drug resistance to more than seven antibiotics and six of them ESBL producers as in table (5).

Table (5): Antibiotic susceptibility patterns for ESBL producer *R.ornithinolytica*isolates

Antibiotics	R1	R2	R4	R5	R7	R10	NO.S	NO.R
Ampicillin/Sulbactam	R	R	R	R	R	R	0	6
Piperacillin/Tazobactam	R	R	R	R	R	R	0	6
Cefazolin	R	R	R	R	R	R	0	6
Ceftazidim	S	R	S	S	S	S	5	1
Ceftriaxone	R	R	R	R	R	R	0	6
Cefepime	S	R	S	S	S	S	5	1
Levofloxacin	S	R	S	S	S	S	5	1
Trimethoprrim/Sulfamethoxazole	R	R	R	R	R	R	0	6
Gentamicin	R	R	R	R	R	R	0	6
Tobramycin	R	R	R	R	R	R	0	6
Ertapenem	S	R	S	S	S	S	5	1
Imipenem	S	R	S	S	S	S	5	1
Ciprofloxacin	R	R	R	R	R	R	0	6

DISCUSSION

Frequency of isolated *Raoultellaornithinolytica*

The series of human infections caused by *R. ornithinolytica* managed at four university hospital centres in France over the last 12 years is the largest reported to date. A literature search identified 86 reported cases of *R. ornithinolytica* (formerly *Klebsiella ornithinolytica*)[17]and half of these cases were reported in 2015[18].Out of 2350 samples that were subjected to Rizgary and Central laboratory in 2017 in Erbil city, 103(.93%) were positive for *R. ornithinolytica,* 4(0.71%) were isolated from Rizgary Hospital while 6(0.25) isolates were collected from Central laboratory .In other words (0.43%)of the samples collected in 017 were identified as *R. ornithinolytica*by using microscopical, morphological, biochemical tests and Vitek 2 compact system. our study lower than results recorded by(Hansen) from Thi-Qar[19] ,Iraq who found that twenty isolates of *Raoultella spp*. were identified from 229 positive specimens while *R. ornithinolytica* registered 16 isolates in percentages 6.98% of *Raoultella* recovered from clinical specimens [20]. Also found that most isolates 8 (72.7%) of the 11 *R. ornithinolytica* were isolated from 174 rectal swab and stool samples and Kuhn *et al*[21]who founded that the principle reservoirs of transmission of *Klebseilla* in hospital setting are the gastrointestinal tract of patient and hand of hospital Personal and resulte reported by Podschun*et al*[13] .showed that a high rate (68%) of *Klebsiella* was isolated from faces, there are only three case reports of human infection by *R .ornithinolytica*, the first patient was an 82-year-old woman in whom the microorganism caused an enteric fever-like syndrome, and the organism was isolated from blood[16] The second patient was a 97-year-old woman who presented with a giant renal cyst, which caused colic obstruction. The fluid culture isolated from the cyst was positive for *R.ornithinolytica*[22] .The third reported case was of*R.ornithinolytica* bacteremia in an infant with visceral heterotaxy. Only this case showed marked skin flushing, which was possibly related to a histamine reaction[23] .Studies have shown that 0.2 to 19.0% of isolates initially identified as *Klebsiella spp* were *Raoultella spp*. by 16S rRNA analysis and that the prevalence of these organisms in clinical settings can vary geographically [10] There is an imminent need for studies and researches that address infections by *R.ornithinolytica*in human beings. Unfortunately, this is an underestimated issue since the bacterium is difficult to identify from the use of phenotypic methods. This bacterium represents a serious risk to health and should not be neglected. Several phenotypic biochemical identification tests were available to differentiate the Raoultella species. Identification test of *R. ornithinolytica* was performed using a series of biochemical reactions and the basic positive reactions were observed in case of urea, ornithine and lysine decarboxylase, citrate, glucose, and sucrose. It generally grown at 10°C with utilization of L-sorbose as a carbon source[19] .To make certain on accurate results, that the isolates belong to *R. ornithinolytica*all of the bacterial isolates were confirmed on their identification by using a series of laboratory tests. All *R. ornithinolytica*isolated and identified by using microscopical, morphological, biochemical tests and Vitek 2 compact system , the colonies on blood agar medium showing shiny moist cream color appearance and sticky from mild to strong with non hemolysis (in 24h culture) ,but after 48h the culture show hemolysis, and mucoid ,lactose fermenting(pink) bacterial and mucoid colonies in macConkey agar, this bacterial cell from smear preparation are Gram negative bacterium,red to pink in color road shaped,accuring singly,

in pair,in short chain and irregular in arrangement . *R. ornithinolytica*turned out to be positive for catalase test as well. the immediate formation of oxygen bubbles is evidence that the organism is catalase positive,appeared to be oxidase negative which means that the bacteria is not producing cytochrome c oxidase enzyme(colorless color),other biochemical tests performed such as H2S and Voges–Proskauer which were negative for *Raoultella ,* can be identified on a Gram stain as a Gram-negative anaerobic bacillus closely related to Klebsiella, but may be difficult to recognize due to its rarity as a human pathogen. Alves *et al.*[24] noted that *R.ornithinolytica* can appear similar to Klebsiella, but culture and biochemical tests will typically show fermentation of lactose and acid production on MacConkey agar and Raoultella will typically be oxidase- and Voges–Proskauer-negative. Additionally, Raoultella should not produce H2S and will typically not show arginine or phenylalanine utilization,Raoultella fermented glucose, utilized citrate, lysine, malonate and l-sorbose and were urease- and methyl red-positive and were nonmotile, positive for indole production, histamine assimilation and growth at 10°C.The genus *Klebsiella* consists of three phyletic lines which were shared with other members of the Enterobacteriaceae, including Enterobacter aerogenes, Erwinia and Tatumella. Cluster I comprises *K.pneumoniae* subspecies *pneumoniae, rhinoscleromatis* and *ozaenae*, and *K. granulomatis*; cluster II contains *R.ornithinolytica, R. planticola,*andR.terrigena; and cluster III contains *K. oxytoca,* sproser *et al*[25] ,kwon *et al*[26] Cases of *R. ornithinolytica* are difficult to distinguish from *K.oxytoca* in clinical laboratories using routine phenotypic identification methods such as the Vitek 2 GN ID card, and API 20E test kit .The API 20E system had only one biochemical test discriminating between *R. ornithinolytica* and *K. oxytoca;* these needed to be completed by an ornithine decarboxylase (ODC) test.TheVitek 2 system had five biochemical discriminating tests that can identify ODC-negative *R.ornithinolytica* isolates, but this technique had to be confirmed by molecular identification (16S rRNA gene sequencing) due to the lack of specificity.[2].

The incidence of R. ornithinolytica in different specimens

R. ornithinolytica is an underreported, emerging hospital-acquired infection and is particularly associated with invasive procedures, should never be considered simply a saprophytic bacterium that occasionally contaminates bronchial lavage or other deep respiratory samples or surgical sites. Physicians should be aware of the high rates of antimicrobial resistance of *R. ornithinolytica* isolates so that immediate broad-spectrum antibiotic treatment can be established before accurate microbiological results are obtained [27].In presence study out of 2350 samples only 10 (0.43%) case of infection with *R.ornithinolytica* were detected. More than half of these infections were contributed by female genital tract and in the second position comes UTI,while only one wound infections has been detected .The result agree with the finding obtained by Seleden*et al*[28] ,who found that that only 3 (27.3%) isolates of *R. ornithinolytica* were detected in 503 urine samples, but disagree with this result about no isolates were detected in vagina, ear, and wound samples, Kuhn *et al*[21] recorded that the principle reservoirs of transmission of this bacteria in hospital setting are the gastrointestinal tract of patient and hand of hospital personal. Till now the cases reported of *R.ornithinolytica*has a very low mortality rate and a good prognosis unlike other gram negative bacteria .Although *R. ornithinolytica* a rare hospital infection, it should be kept in mind as a cause, by virtue of the fact that it is a part of the flora in natural environment.The pathogenic potential of *R.ornithinolytica*

isolates in human disease has become increasingly important. Many cases of biliary tract infection, urinary infection,and bacteria caused by *R.ornithinolytica* have been reported[29] .Infections by *R. ornithinolytica* are exceedingly rare in humans and have been reported as bloodstream, urinary tract and soft tissue infections in adults and as fatal neonatal infections. Most adult cases are linked with underlying diseases, especially malignancies [23].

Relation between *R.ornithinolytica*and gender

The prevalence of *R.ornithinolytica*was analyzed according to persons gender among the10 case is positive, almost all infections were detected in femalewhich were 9 case (0.38%),and 1 case is positive in men, in our study the higher rate of *R.ornithinolytica*was found in females compere to male, this is may be due to that we collected the sample more from female than in male . On the other hand Alonso *et al*[30] recorded that 79 cases of female and male infected by *R.ornithinolytica*mean age was 62.2 years , the percentage of infection greater for male patients (63.3%) than female while results reported by Ahmed and Ali[31]showed the presence of R.*ornithinolytica* 1.4% isolated only from women with vaginitis.

Antibiotic susceptibility testing for *R. ornithinolytica*

R. ornithinolytica isolates showed highest sensitive (90%) to cefepime &imipenem,Ertapenem and Ceftazdium and levofloxacin and this finding indicates that this antibiotics remain active against this bacteria and can be used as therapeutic treatment, while highest resistance (70%) to cefazolin and Trimethoprim/Sulfamethoxazol. In our study, the proportions of isolates resistant to ceftriaxone, Gentamycin , and Ciprofloxacin were higher than reported by (Zhou)[32] (4%, 6%, and 1%, respectively). No isolate resistant to carbapenems was observed in this study. Resistance to ampicillin has been reported in six cases[34] .The combination of amino- and carboxypenicillin with clavulanic acid makes *R. ornithinolytica* environmental isolates susceptible to these molecules.[35] .Some resistance to antibiotics such as ciprofloxacin and cotrimoxazole has been observed[33] . It is worth noting that all isolates were susceptible to trimethoprim-sulfamethoxazole (SXT) except *R. ornithinolytica* strain Ro25687, which indicates that the patient was not infected by the same strain or that the use of antibiotics led to SXT resistance under the selective pressure of SXT usage, given that the patient received 2 weeks of SXT therapy before the isolation of Ro25687. To date, carbapenemase-resistant *R. ornithinolytica* strains have been reported only in North America [34]. In the future, screening of *R. ornithinolytica* clinical isolates for reduced susceptibility to antibiotics will improve our understanding of the mechanisms underlying increased antibiotic resistance while Al-Hulu [36] from Hila /Iraq reported that now-a-days, *R. ornithinolytica* acquired resistance against broad range of antimicrobials, most of the clinical isolates of *R. ornithinolytica* were found resistant to all class of antimicrobials such as ampicillin, amoxicillin, cephalothin, cephotaxime, chloramphenicol, penicillin, gentamicin, rifampin, and streptomycin but Nitrofurantoin and meropenam showed highest sensitivity for *R. ornithinolytica* in all the clinical samples in hilla city/ Iraq and study in Erbil recorded by(Ahmed and Ali(number) [31] showed that *R. ornithinolytica*was (100%) resistant to Amikacin, Aztreonam, Clindamycin and Cefoxitin.*Raoultellaspp* are often susceptible to most beta-lactam agents, however isolates that

produce extended-spectrum beta lactamases and carbapenemases have emerged and MDR emergence of *R. ornithinolytica* is a global health problem commonly associated with bacteremia, urinary tract infection, neonatal infections, and exist in underlying existing infection[27]. *Raoultellaspp* are often susceptible to most beta-lactam agents, however isolates that produce extended-spectrum beta lactamases and carbapenemases have emerged [37]. MDR emergence as a global health problem commonly associated with bacteremia, urinary tract infection, neonatal infections, and exist in underlying existing infection . Increasing resistance in microorganism for antimicrobials becomes a major threat to health and economic problem which ultimately leads to allowing survival of the resistant bacteria and death of the susceptible ones. Major mechanistic pathways associated with resistant in microorganism are cell membrane alterations, which lead to decreased uptake of drug [38] The antibiotic abuse in the form of using antibiotics without indication or in an inappropriate way made this bacterium along other pathogenical bacteria resistant against many antibiotics groups . Increasing resistance in microorganism for antimicrobials becomes a major threat to health and economic problem which ultimately leads to allowing survival of the resistant bacteria and death of the susceptible ones.The current study revealed a high percentage of resistance in *R. ornithinolytica*isolates, highlighting the necessity for local or country-based investigations to characterize and monitor multiresistane antibiotic and to develop strategies that will accelerate and management and control. In addition, the application of antibiotic combination therapy against multiresistane and maintenance of proper hygiene by hospitalized patients and staff could effectively reduce the rate and dissemination of such cases. Differences in antibiotic resistance patterns can result from the ecology and physiology of the bacteria and may suggest distinct modes and mechanisms of resistance acquisition

Antibiotic resistance patterns and ESBL Producing for *R.ornithinolytica isolates*

The indiscriminate use of antimicrobials over prolonged periods has led to emergencyof MDR strain. Whenever new and effective antibiotic is introduced bacteria after exposure to this antimicrobial, acquire resistance through different mechanism, commonest being the production of β-lactamase. Production of ESBLs by this organisms have made even the third generation cephalosporins ineffective. To combat this MDR strains new and more effective antibiotics are required [37]In interested finding in this part of the study was a association of R.ornithinolytica with β-lactamase. Therefore in present study vitek 2 system were used for detection β-lactamase production among *R.ornithinolytica* and showed that 6(60%)isolates of *R.ornithinolytica* ESBL producer, in general all isolated *R.ornithinolytica* were resistance to more than 8 antibiotics and one isolates completely resistance to all thirteen antibiotics(multi drug resistanceThe prevalence of ESBLs among clinical isolates differ in different countries and in different hospital and are rapidly changing over time [37]. A surface water isolate of *Raoultella spp*. having ability to multidrug and multimetalresistance ,these drugs like ampicillin , amoxicillin / clavulanic acid [39] .*Raoultellaspp*.have a penicillinase that related β –lactam resistance pattern. Penicillinase of *Raoultella spp*. That related β –lactam resistance suggesting the presence of a chromosomal β –lactam gene [40] .In 2009 Al Hulu *et al*. founded that all isolates of

Raoultellaornithinolytica were resist to ampicillin , cephalothin and other groups of beta lactam antibiotics .Some isolates of *Raoultellaornithinolytica* have resistant to amipicillin and other antibiotics ,this resistance can be associated with the presence of β –lactamases[41]. Many clinical microbiological laboratories still face significant problems ESBL screening and identification as ESBL pathogenesis can present with variations in the vitro pattern of resistance of β-lactamase agent. Proficiency testing study performed by the World Health Organization and Centers for Disease Control have raised concerns about the current ability of many clinical laboratories to detect ESBL-Producing microorganism [42].

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