South Asian Journal of Research in Microbiology

Volume 15, Issue 1, Page 9-19, 2023; Article no.SAJRM.96775
ISSN: 2582-1989

Emergence of *Raoultella ornithinolytica* Producing Beta Lactamase Enzyme in Different Clinical Specimens in Erbil City

Fattma A. Ali [a*], Ahmed Akil Khudhair Al-Daood [a], Gazang Shakir Ibrahim [a], Chra Ahmed Abdulla [a], Halas Muhammmed Assad [a] and Dlaram Wali Muhammed [a]

[a] College of Health Sciences, Hawler Medical University, Iraq.

Author's contribution

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/SAJRM/2023/v15i1277

Open Peer Review History:
This journal follows the Advanced Open Peer Review policy. Identity of the Reviewers, Editor(s) and additional Reviewers, peer review comments, different versions of the manuscript, comments of the editors, etc are available here: https://www.sdiarticle5.com/review-history/96775

Original Research Article

Received: 28/10/2022
Accepted: 30/12/2022
Published: 15/01/2023

ABSTRACT

Background: *Raoultella ornithinolytica* should never be regarded as merely a saprophytic bacterium that occasionally contaminates bronchial lavage or other deep respiratory samples or surgical sites is an underreported, emerging hospital-acquired infection and is particularly related with invasive operations.*R. ornithinolytica* isolates have significant antimicrobial resistance rates, and physicians should be aware of this so that, before accurate microbiological data are received, an immediate broad-spectrum antibiotic treatment can be instituted..
Objectives: The goal of our study was to retrospectively examine *R. ornithinolytica*, which was isolated from a variety of clinical samples in Erbil, and to examine its epidemiology, antibiotic sensitivity patterns, and ability to produce the enzyme extend spectrum beta lactamase.

**Corresponding author: Email: Fattma.ali@hmu.edu.krd, fattmaabeer@yahoo.com;*

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: Only ten *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 These findings offer a reliable measure of the prevalence of Raoultella ornithinolytica in our region and provide a baseline for future studies that will enable the monitoring of trends over time. If current resistance trends continue, high societal and economic costs can be anticipated; better management of infections caused by resistanceR.ornithinolytica is becoming essential.

Keywords: Raoultella ornithinolytica; antibiotics resistances; ESBL.

1. INTRODUCTION

*"Raoultella ornithinolytica*is Gram-negative, encapsulated, oxidase-negative, catalase-positive, nonmotile, facultatively anaerobic rods formerly known as Klebsiella make up the Enterobacteriaceae family..It is named after the French bacteriologist Didier Raoult" [1]. "*Raoultella spp*. based on their molecular features, have recently been split from the genus Klebsiella" [2]. "This genus was discovered in samples of human tissue as well as water, soil, plants, and occasionally animal mucosa. Type species is *Raoultella ornithinolytica* comb. nov.*Raoultella planticola* comb.nov.and *Raoultella terrigena* comb. Nov [3] *R. ornithinolytica* resides in hospitals and is known to inhabit aquatic habitats" [4]. The results of the 16S rRNA sequence studies, in addition to previously published biochemical and DNA-DNA hybridization data, demonstrated that the genus Klebsiella is heterogeneous and made up of species that form three clusters that also contain members of other genera. These findings are in favor of splitting the genus Klebsiella into two genera. For species in cluster II, the genus name Raoultella is suggested which contained *Klebsiella ornithinolytica, Klebsiella planticola,* and *Klebsiella terrigena,* organisms characterized by growth at 10ºC and utilization of L-sorbose as carbon source [1]. On the basis of new genetic techniques, the bacterium, formerly identified as *Klebsiella ornithinolytica*, was reclassified as Raoultella [1]. Sakazaki et al. provided the first description of this bacterium in 1989 [5]. Raoultella species have never been isolated from clinical specimens and are believed to only exist in aquatic, botanical, and soil habitats [6]. Monnet et al. reported the first isolation of *R.planticola* from neonates in neonatal wards [7]. "There have been few reports of *R. ornithinolytica*, an aquatic gram-negative commensal of the Enterobacteriaceae family, surviving in human saliva. *R. ornithinolytica has developed into a human pathogen in a number of illnesses that are contracted in the community or hospitals*" [8]. "The analysis of 16SrDNA and rpoB sequences produced data that support the varied taxonomic structure of the genus Klebsiella. The same reports that this bacteria has been linked to human diseases such peritonitis and enteric fever-like syndrome have

surfaced. This species has been linked to histamine seafood poisoning" [9]. These environmental organisms appear to have pathogenicity similar to *Klebsiella pneumoniae* [10], although seldom infecting humans. Bloodstream infections have sporadically been reported; the first invasive Raoultella spp. infection in a human was originally recorded in 1984. Ten cases of *R. ornithinolytica* infection have been recorded, connecting this pathogen to bacteremia, sepsis, soft tissue infections, and other illnesses over the past ten years. R. ornithinolytica is an important but antifungal cause of human infection. [11]. "Hospital acquired urinary tract infections are frequently brought on by the ability of bacteria to colonize the inner surfaces of indwelling urinary catheters. Hence, it is believed that these pathways may be crucial in the pathogenesis of infection. Understanding the connection between R. ornithinolytica human infections and invasive operations like the insertion of venous catheters, intra-vascular prosthesis, or orthopedic devices would be made easier with the help of other virulence factors, such the capacity to build biofilms". [12]. "The percentage of R. ornithinolytica isolates with decreased antibiotic susceptibility was comparatively high. The mechanism of beta-lactam resistance exhibited by R. ornithinolytica isolates depends on a chromosomal bla gene" [13]. "AmpC -lactamases are-lactamases that hydrolyze penicillins, cephalosporins, and cephamycins (cefoxitin), and are not inhibited by clavulanic acid. (β-lactamase inhibitor-β-lactam combinations)" [14].

2. METHODS

2.1 Samples Collection

From six distinct sources, a total of (2350) samples were obtained (urine, wound swab, high vaginal swab, feces, throat swab,csf). Each bacterial isolate was put through a battery of confirmation assays after being collected. The findings revealed that only ten isolates were identified as R. ornithinolytica. Patients who visited the Central Lab and Rizgari Hospital in Erbil City throughout the time period had their clinical samples taken (July 2017 to February 2018) from patients between the ages of 10 and79, both male and female. Blood culture and MacConkey agar plates were cultured aerobically at 37°C for (24–48 hours) in order to isolate bacteria from the samples. Blood culture and MacConkey agar plates were cultured aerobically at 37°C for (24–48 hours) in order to isolate bacteria from the samples. Using morphological and biochemical testing, pure colonies of isolated microorganisms ,Vitek 2 technology was used to identify species and generate antibiograms for infections [15].

2.2 Antimicrobial Susceptibility Test by Vitek 2 System

The Vitek 2 system was used to test all isolates for their resistance to 13 different antibiotics. Because it can accurately identify the "fingerprint" of bacterial resistance mechanisms and phenotypes, the AES is a key part of the Vitek 2 system. 64 micro wells are present on the Vitek 2 card. Antimicrobials or identifying substrates are present in each well. For the identification of organisms and assessing their susceptibility to antibiotics, Vitek 2 provides a complete menu. Because the Vitek 2 test card is sealed, the likelihood of spills, aerosols, and individual contamination is reduced. Compared to microtiter technologies, disposable waste is decreased by more than 80% [15].

2.3 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). An optical reader was used to objectively measure growth in each well When compared to wells containing cephalosporin alone, the proportional decrease in growth in the wells having cephalosporin + clavulanate was thought to be an indication of ESBL development. The results of all phenotypic ESBL interpretations were reported as positive. When the AES suggested phenotypic interpretations other than ESBLs, strains were described as ESBL-negative [15].

3. RESULTS

3.1 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.

3.2 Relation between *R.ornithinolytica* and Gender (Female, Male)

Out of 2350 samples, 10 samples were isolated for *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.

3.3 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 Fig. 1.

3.4 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 Fig. 2,on the other hand isolates were ESBL producers and multi resistance.

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%

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%

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
	33.36%	39.82%	26.80%	100%

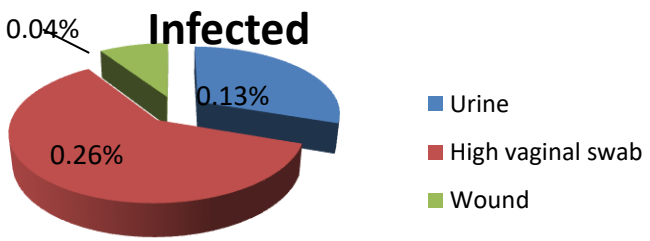

Fig. 1.The incidence of *R. ornithinolytica* in different clinical specimens

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

Antibiotics	No. Sensitive	Percentage%	No. Resistant	Percentage%
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%

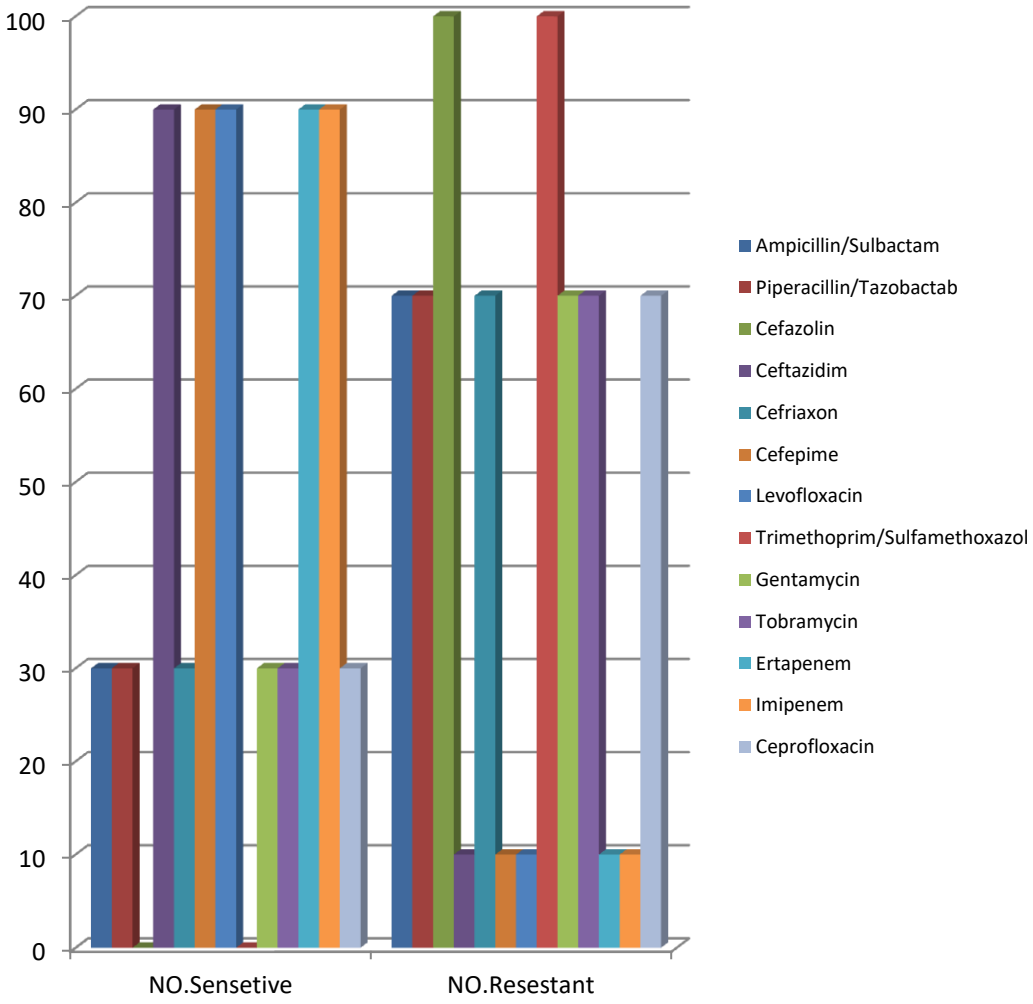

Fig. 2.The number and percentage of antibiotic resistance among *R. ornithinolytica*

3.5 Antibiotic Susceptibility Patterns for ESBL Producer *R. ornithinolytica* Isolates

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

4. DISCUSSION AND CONCLUSION

4.1 Frequency of Isolated *Raoultella ornithinolytica*

The greatest recorded case of *R. ornithinolytica* infection in humans occurred during a 12-year period at four university hospital centers in France. The greatest recorded case of R. *ornithinolytica* infection in humans occurred during a 12-year period at four university hospital centers in France. 86 instances of *R. ornithinolytica* (formerly *Klebsiella ornithinolytica*) were found in the literature [16], with half of these cases being published in 2015 [17]. Out of 2350 samples that were tested in 2017 in Erbil city by Rizgary and Central laboratories, 10(3.93%) (positive for *R. ornithinolytica*, 4(0.71%)) isolates were obtained from Rizgary Hospital, while 6(0.25) isolates were obtained from Central laboratory. In other words, the Vitek 2 compact system, along with microscopical, morphological, and biochemical analyses, allowed us to identify *R. ornithinolytica* in(0.43%) of the samples we collected in 2017., our study yielded lower findings than those noted by Hansen from Thi-Qar [18]. Twenty Raoultella spp. isolates were detected in Iraq from 229 positive specimens, whereas *R. ornithinolytica* reported 16 isolates, or 6.98% of the total Raoultella recovered from clinical specimens [19]. Additionally discovered that the majority of isolates [8] (72.7%) of the [20] *R. ornithinolytica* were isolated from 174 rectal swab and stool samples and Kuhn et al. [21] who discovered that the primary reservoirs of *Klebseilla* transmission in hospitals are the gastrointestinal tract of patients and hands of hospital personnel and outcomes). reported by Podschun *et al* [12] 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; the organism was isolated from blood. Research has shown that a high rate (68%) of Klebsiella was isolated from faces [15] The second patient was a 97-year-old woman who had a huge renal cyst that was obstructing her colic. The cyst's fluid culture was positive for *R. ornithinolytica* [22]. An child with visceral heterotaxy and *R. ornithinolytica* bacteremia was the third case to be documented. Just this particular example displayed noticeable skin flushing, which may have been caused by a histamine reaction [23]. Research has demonstrated that the incidence of these organisms in clinical settings might vary geographically and that between 0.2% and 19.0% of isolates initially identified by 16S rRNA analysis as Klebsiella spp. were *Raoultella spp* [10]. Studies and research on infections caused by *R. ornithinolytica* in humans are urgently needed. Sadly, given how challenging it is to distinguish the bacterium using phenotypic techniques, this is an underappreciated problem. Health risks associated with this bacteria should not be disregarded. To distinguish the Raoultella species, a variety of phenotypic biochemical identification tests were available. A number of biochemical reactions were used to identify *R. ornithinolyti*ca, and the primary positive reactions were seen for urea, ornithine and lysine decarboxylase, citrate, glucose, and sucrose. It typically grows at a temperature of 10 °C with L-sorbose being used as a carbon source [18]. All of the bacterial isolates were identified by a battery of laboratory tests to ensure that the results were accurate and that the isolates belonged to *R. ornithinolytica*. A number of biochemical reactions were used to identify *R. ornithinolytica*, and the primary positive reactions were seen for urea, ornithine and lysine decarboxylase, citrate, glucose, and sucrose. It typically grows at a temperature of 10 °C with L-sorbose being used as a carbon source [18]. All of the bacterial isolates were identified by a battery of laboratory tests to ensure that the results were accurate and that the isolates belonged to R. ornithinolytica. Gram negative bacteria that are red to pink in color, road-shaped, occurring singly, in pairs, in short chains, and irregularly spaced apart make up this bacterial cell from the smear preparation. R. ornithinolytica tested positive for catalase as well, it turned out. Other biochemical tests were conducted, such as H2S and Voges-Proskauer, which were negative for Raoultella, and the organism appeared to be oxidase negative, which means that the bacteria is not producing cytochrome c oxidase enzyme (colorless color). The immediate formation of oxygen bubbles is evidence that the organism is catalase positive. can be recognized on a Gram stain as a rare human pathogen that is a Gram-negative

anaerobic bacillus closely related to Klebsiella. However, due to its rarity, it may be challenging to identify. Although Raoultella can resemble Klebsiella in appearance, Alves et al. [24] stated that Raoultella will typically be oxidase-and Voges-Proskauer-negative and that culture and biochemical assays will typically indicate fermentation of lactose and acid generation on MacConkey agar. Additionally, Raoultella should not produce H2S and will typically not show arginine or phenylalanine Raoultella fermented glucose, used citrate, lysine, malonate, and l-sorbose, were urease-and methyl red-positive, were nonmotile, positive for indole production, histamine assimilation, and growth at 10°C. Additionally, Raoultella shouldn't produce H2S and typically won't show arginine or phenylalanine utilization. There are three phyletic lines in the genus Klebsiella that it shared with other Enterobacteriaceae species, such as 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,*and R.terrigena; and cluster III contains *K. oxytoca,* sproser *et al*[25] ,kwon *et al* [26] R. ornithinolytica cases can be hard to distinguish from K. oxytoca in clinical labs using common phenotypic identification tools like the API 20E test kit and Vitek 2 GN ID card. Only one biochemical test—the ornithine decarboxylase (ODC) test—was available in the API 20E system to distinguish between *R. ornithinolytica and K. oxytoca.* The Vitek 2 system had five biochemical tests that might distinguish ODC-negative R. ornithinolytica isolates, however due to the lack of specificity, this method had to be verified by molecular identification (16S rRNA gene sequencing).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 obtainel" [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.

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

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].

4.2 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 female which 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.

4.3 Antibiotic Susceptibility Testing for *R. ornithinolytica*

Cefepime & imipenem, Ertapenem, Ceftazdium, and levofloxacin exhibited the highest levels of sensitivity(90%) in *R. ornithinolytica* isolates, indicating that these antibiotics remain active against this bacteria and can be used as a therapeutic treatment. Cefazolin and Trimethoprim/Sulfamethoxazol *exhibited the highest levels of resistance(70%) in these isolates.*. In our investigation, the percentages of isolates resistant to ceftriaxone, Gentamycin, and Ciprofloxacin(4%,6%, and1%, respectively) were greater than those reported by (Zhou) [32]. Six incidences of ampicillin resistance have been reported [33]. *R. ornithinolytica* environmental isolates are susceptible to amino-and carboxypenicillin when combined with clavulanic acid. There has been some reported resistance to antibiotics such ciprofloxacin and cotrimoxazole [34] Given that the patient received 2 weeks of SXT therapy prior to the isolation of Ro25687, it is important to note that all isolates were susceptible to trimethoprim-sulfamethoxazole (SXT) except for *R. ornithinolytica* strain Ro25687. This suggests that the patient was not infected by the same strain or that the use of antibiotics contributed to SXT resistance under the selective pressure of SXT usage.. Only North America has seen reports of carbapenemase-resistant R. *ornithinolytica* strains thus far [33]. 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 [35]. 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 While Al-Hulu [35] from Hila, Iraq, claimed that *R. ornithinolytica* has recently developed resistance to a wide variety of antibiotics, the majority of clinical isolates of this organism were shown to be resistant to all classes of antibiotics, including 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.*Raoultella spp* 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]. *Raoultella spp* are often susceptible to most beta-lactam agents, however isolates that produce extended-spectrum beta lactamases and carbapenemases have emerged . Although typical beta-lactam medications may often kill Raoultella spp., some isolates have arisen that manufacture extended-spectrum beta lactamases and carbapenemases [36]. MDR's growth as a global health issue is frequently linked to bacteremia, uti, newborn infections, and the presence of underlying illnesses. Growing bacterial resistance to antimicrobials poses a serious threat to human health as well as an economic issue, ultimately resulting in the survival of the resistant germs and the eradication of the susceptible ones [37]. Alterations to cell membranes are major mechanistic routes linked to resistance in

microorganisms, and these changes result in reduced drug uptake [38]. This bacterium, along with other dangerous bacteria, became resistant to several antibiotic groups due to antibiotic abuse, which takes the form of using antibiotics without a prescription or in an inappropriate manner.. Increased bacterial susceptibility to antimicrobials poses a serious threat to human health and an issue for the economy, allowing resistant bacteria to survive and killing susceptible ones. The results of the current study showed that R. ornithinolytica isolates had a high level of resistance, indicating the need for local or national studies to characterize and monitor multiresistane antibiotics and to design tactics that would hasten management and control Also, hospitalized patients and staff could significantly lower the frequency and spread of such instances by using antibiotic combination therapy against multiresistant bacteria and practicing good hygiene. The ecology and physiology of the bacteria can influence antibiotic resistance patterns, and these differences may point to different modes and processes of resistance acquisition.

4.4 Antibiotic Resistance Patterns and ESBL Producing for *R. ornithinolytica Isolates*

The indiscriminate use of antimicrobials over prolonged periods has led to emergency of 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 [36]. 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 resistance The prevalence of ESBLs among clinical isolates differ in different countries and in different hospital and are rapidly changing over time [36]. “A surface water isolate of *Raoultella spp*. having ability to multidrug and multimetal resistance ,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 *Raoultella ornithinolytica* were resist to ampicillin , cephalothin and other groups of beta lactam antibiotics .Some isolates of *Raoultella ornithinolytica* 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].

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Drancourt M, Bollet C, Carta A. Rousselier phylogenetic analyses of *Klebsiella species* delineate *Klebsiella* and *Raoultella* gen. nov; with description of *Raoultella ornithinolytica* comb. nov, *Raoultella terrigena* com. nov. and *Raoultella planticola* com.nov. International Journal of Systematic and Evolutionary Microbiology. 2001.51(pt3):925-32.
2. Park JS, Hong KH ,Lee HJ, Choi SH, Song JH, Song KH, Kim HB, Park KU, Song J, Kim E. Evaluation of three phenotypic identification systems for clinical isolate of *Raoultella ornithinolytica.*, J. Med. Microbiol. 2011;60:492-499.
3. Jain K, Radsak K, W Mannheim. Differentiation of the Oxytocum group from *Klebsiella* by deoxyribonucleic acid hybridization. Int J Syst Bacteriol. 1974; 4(4):402-407.
4. Kanki M, Yoda T, Tsukamoto T, Shibata T.*Klebsiella pneumoniae* produces no histamine: *Raoultella planticola* and *Raoultella ornithinolytica* strains are histamine producers. Appl Environ Microbiol. 2002;68:3462–6.
5. Sakazaki R, Tamura K, Kosako Y, Yoshizaki E. *Klebsiella ornithinolytica* spp

Nov, formerly known as ornithino-positive *Klebsiella oxyotoca*. Curr. Microbiol. 1989; 18:201-206.

6. Bagley ST, Seidler RJ, Albot HWJ, Morrow JE. Isolation of *Klebsiella* from living wood. Appl. Environ. Microbiol. 1978;36:178-185.
7. Monnet DJ, Freney Y, Brun JM, Boeufgras, Fleurette J. Difficulties in identifying *Klebsiella* strains of clinical origin. Zentralbl Bakteriol. Parasitenkd. Infektionskr. Hyg.Abt. 1 Orig. 1991;274: 456–464.
8. Heggendorn FL, Gonçalves LS, Dias EP, Silva A, Junior M, Galvão M, Lutterbach M. Detection of sulphate-reducing bacteria in human saliva ActaOdontol Scand. 2013;71:1458-1463.
9. Morais VP, Daporta MT, Bao AF, Campello MG, Andrés GQ. Enteric fever-like syndrome caused by *Raoultella ornithinolytica (Klebsiella ornithinolytica*) J Clin Microbiol. 2009;47:868–9.
10. Murray PR, Baron EJ, Jorgensen HJ, Landry ML, Pfaller MA. Manual of clinical microbiology, 9th edition. ASM Press, Washington, DC. 2007;1.
11. Deeb LS, Bajaj J, Bhargava S, Alcid D, pitchumoni CS. Acute suppuration of the pancreatic duct in a patient with tropical pancreatitis. Case Rep Gastroenterol. 2008;2(1):2732.
12. Podschun R, Sievers D, Fischer A, Ullmann U. Serotypes, hemma-gglutinins, siderophore synthesis, and serum resistance of *Klebsiella* isolates causing human urinary tract infections. J Infect Dis. 1993;168:1415-1421.
13. Walckenaer E, Poire Ll, Leflon-Guibout V, Nordmann P, Nicolas-Chanoine MH. Genetic and biochemical characterization of the chromosomal class A β-lactamases of *Raoultella* (formerly *Klebsiella*) *planticola* and *Raoultella ornithinolytica.* Antimicrob Agents Chemother. 2004;48: 305-312,
14. Hostacká A, Klokocníková L. Antibiotic susceptibility, serum response and surface properties of *Klebsiella* species Microbios. 2001;104:115-124.
15. Eigner U, Schmid A, Wild U, Bertsch D, Fahr AM. Analysis of the comparative workflow and performance characteristics of the VITEK 2 and Phoenix systems. Journal of Clinical Microbiology. 2005;43: 3829-3834.
16. Zhou G, Guo S, Luo Y, Ye L, Song Y, Sun G, *et al.* NDM-1-producing strains, family *Enterobacteriaceae*, in hospital, Beijing, China Emerg Infect Dis. 2014;20:340-342.
17. Richter SS, Sercia L, Branda JA, Burnham CA, Bythrow M, Ferraro MJ, *etal* .Identification of *Enterobacteriaceae* by matrix-assisted laser desorption/ionization time-of flight mass spectrometry using the VITEK MS system .Eur J Clin Microbiol Infect Dis. 2013;32:1571-1578.
18. Hansen DS, Aucken HM, Abiola T, Podschun R. Recommended test panel for differentiation of Klebsiella species on the basis of a trilateral interlaboratory evaluation of 18 biochemical tests. J Clin Microbiol. 2004;42:3665-3669.
19. Al-Charrackh AH. Isolation of *Raoultellaplanticola* from clinical specimens in Hillacity. Proceedings of 3rd Conference of Microbiology and Biotechnology, Basrah, Iraq. 2007.
20. Alves MS, Riley LW, Moreira BM. A case of severe pancreatitis complicated by *Raoultella planticola* infection. J. Med. Microbiol. 2007;56:696-698.
21. Kuhn I, Ayling-Smith B, Tullus K, Burman G. 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. 1993.23:287-297.
22. Vos B, Laureys M. Giant renal cyst as cause of colic obstruction. Rev Med Brux. 2009;30:107–9.
23. Mau N, Ross LA. *Raoultella ornithinolytica* bacteremia in an infant with visceral heterotaxy. Pediatr Infect Dis J. 2010; 29:1–2.
24. Alves MS, Riley LW, Moreira BM. A case of severe pancreatitis complicated by *Raoultella planticola* infection. J. Med. Microbiol. 2007;56:696-698.
25. Spro$er C, Mendrock U, Swiderski J, Lang E, Stackebrandt E. The phylogenetic position of Serratia, Buttiauxella and some other genera of the family *Enterobacteriaceae*. Int J Syst Bacteriol. 1999;49:1433±1438.
26. Kwon SW, Go SJ, Kang HW, Ryu JC, Jo JK. Phylogenetic analysis of Erwinia species based on 16S Rrna gene sequence. Int J SystBacteriol. 1997;47:1061±1067.
27. Tenover FC. Mechanisms of antimicrobial resistance in bacteria. Am J Infect Control. 2006;119:S3-S10.
28. Seleden R, Lee S, Wang WL, Bannett JV, Eickhoff TC. Nosocomial Klebsiella

infections: intestinal colonization as a reservoir. Ann. Intern. Med. 1971;74:657-664.
29. Chun S, Yun JW, Huh HJ, Lee Clinical NY. Characteristics of *Raoultella ornithinolytica* bacteremia Infection. 2015;43:59-64.
30. Alonso MP, Rojas1 LR, Campo1 RD, Cantón R, Morosini. Comparison of different methods for identification of species of the genus Raoultella: report of 11 cases of Raoultella causing bacteraemia and literature review. Clin Microbiol Infect. 2016.22:252–257.
31. Ahmad SS, Ali FA. Detection of ESBL, Ampc and metallo Beta- lactamase mediated resistancein gram- negative bacteria isolatedfrom women with genital tractinfection1. European Scientific Journal. 2014;10(9).
32. Zhou G, Guo S, Luo Y, Ye L, Song Y, Sun G, *et al.* NDM-1-producing strains, family *Enterobacteriaceae*, in hospital, Beijing, China Emerg Infect Dis. 2014;20:340-342.
33. Brooks GF, Butel JS, Morse SA. Jawetz Melnick. Adelberg's.Medical Microbiology.Twenty-Third ed. McGraw Hill Education (Asia). Singapore; 2004.
34. García-Lozano T, Pascual Plá FJ, Aznar O. [*Raoultella ornithinolytica* in urinary tract infections. Clinical and microbiological study of a series of 4 oncologic patients]. Med Clínica. 2013; 141:138-139.
35. Al-Hulu SM, Al-Charrakh AH, Al-Saadi MAK. Isolation and characterization of *Raoultella ornithinolytica* from clinical specimens in Hilla city. Iraq Med J Babylon.2009;7:42-47.
36. Mumtaz S, Ahmad M, Aftab I, Akhtar N, Hameed A. *in vitro* comparative efficacy of Carbapenims β-lactamase β-lactamase inhibitor combinations against multidrug resistant gram negative bacilli. Gomal Journal of Medical Science. 2018;6(1):1-6.
37. Tije TN, Sheth PM, Lastovetska O, Chung C, Patel SN, Melano RG. Molecular characterization of *Klebsiella pneumonia* carbapenemase (KPC) producing *Enterobacteriacae*in Ontario, Canada. 2008-2011. PLoS One. 2014;9:e116421. 10.1371/ Journal Pone. 0116421.
38. Sambrook J, Rusell DW. Molecular cloning. A laboratory manual.3rt cold .Spring.Harber Laboratory Press. N.Y. 2001.
39. Koc S, Kabatas B, Icqen B. Multidrug and heavy metal –resistant *Raoultella planticola* isolated from surface water. Bull Environ Contam Toxicol. US National library of Medicine National Institutes of health. 2013;91(2):177-83.
40. Walckenaer E, Poire Ll, Leflon-Guibout V, Nordmann P, Nicolas-Chanoine MH. Genetic and biochemical characterization of the chromosomal class A β-lactamases of *Raoultella* (formerly *Klebsiella*) *planticola* and *Raoultella ornithinolytica* .Antimicrob Agents Chemother. 2004;48: 305-312.
41. Hostacká A, Klokocníková L. Antibiotic susceptibility, serum response and surface properties of *Klebsiella* species. Microbios. 2001;104:115-124.
42. Hageman JC, Fridkin SK, Mohammed JM, Steward CD, Gaynes RP, Tenover FC. Antimicrobial proficiency testing of national nosocomial infections surveillance system Hospital Laboratories. Infection Control Hospital Epidemiology Journal. 2003;24: 356-361.

Peer-review history:
The peer review history for this paper can be accessed here:
https://www.sdiarticle5.com/review-history/96775