Journal of Natural Sciences Research www.iiste.org
ISSN 2224-3186 (Paper) ISSN 2225-0921 (Online)
Vol.6, No.8, 2016

Emergence of Raoultella ornithinolytica producing AmpC -Beta lactamases in the different clinical specimens

Intidhaar N.Abid
Department of pathological analysis ,College of Science , Thi-Qar University , Thi- Qar , Iraq

ABSTRACT

The incidence of AmpC β –lactamases producing member of Enterobacteriaceae is increasing in recent years. The aim of this study was to evaluate AmpC β –lactamases production by clinical isolates of *Raoultella ornithinolytica* by phenotypic detection (AmpC disc test (ADT) and modified three dimensional test(MTDT)) . Twenty isolates(8.73%) of *Raoultella* sp. were identified among 229 (70.89%) different bacteria (gram negative and gram positive) that isolated from different clinical specimens (urine 8(9.8%), burns 5(12.19%), wound5(6.3%) and stool 2(8.6%)) .Three species of *Raoultella* were isolated in this study that included *Raoultella ornithinolytica (16(6.98%)), Raoultella terrigena* (3 (1.31%)) and *Raoultella planticola*(1(0.43%)). Thirteen (81%) and10(62.5%) of *Raoultella ornithinolytica* were resist to cefoxitin and amoxicillin-clavulanate respectively,10 out of 13 cefoxitin resistant and all isolates that resistant to amoxicillin-clavulanate were produced AmpC β –lactamases by two phenotypic tests .The AmpC β – lactamases producers were distributed to 3(100%) from burns ,6(75%)from urine and 1(33.3%) from wounds .The study showed AmpC β – lactamases producers were also high resist to other antibiotics that included tetracycline (90%) and ciprofloxacin(80%),and all isolates (100%) were sensitive to imipenem .

KEYWORDS: *Raoultella ornithinolytica*, AmpC β –lactamases**,** clinical specimens,

INTRODUCTION

The genus *Raoultella* is belonging to family Enterobacteriaceae ,it consist of gram – negative , capsulated , oxidase negative, 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 *Raoultella ornithinolytica* comb. nov. *Raoultella planticola* comb. nov.and *Raoultella terrigena* comb. nov. (1,3) . *Raoultella ornithinolytica* was until recently often confused with genus Klebsiella – this species has been related for causing histamine fish poisoning . Some reports appeared , that this bacteria has been related to human infections such as peritonitis and enteric fever – like syndrome (1,4,5) A surface water isolate of *Raoultella* sp. having ability to multidrug and multimetal resistance ,these drugs like ampicillin , amoxicillin / clavulanic acid (6) *Raoultella* spp.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 (7) 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(7,9).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) (10).Because the clinical significance of the AmpC β –lactamases , and the studies of the AmpC β –lactamases present in *Rauoltella onithinolytica* has not been performed ,the purpose of this study was to determining for the presence of AmpC β –lactamases in clinical isolates of *Rauoltella onithinolytica* .

MATERIALS AND METHODS

Specimens collections

323 different clinical specimens were collected from some hospitals (Al- Hussein and Maternity & Children) in Al- Nasiriyah city , Thi –Qar province , Iraq. These specimens included urine (132) ,burns (58) ,blood (15) ,wound(95) ,and stool (23) the specimens were cultured on MacCokey' s agar and blood agar (Himedia , India) , isolates identification was performed by routine laboratory methods including API 20 E system(BioMerieux , France) .

Antibiotic susceptibility test

Antibiotic susceptibility testing was performed by the Kirby Bauer method on Mueller Hinton agar (BD& BBL ,USA) according to CLSI protocols(11) The antibiotics were a cefoxitin** (CX)(30 µg)),moxicillin-clavulanate* ((AMC)(20 µg /10 µg)), chloramphenicol* ((C) (30 µg)),cephalothin * ((CF)(30 µg)) , imipenem * ((IPM)(10 µg)), ceftazidime * ((CAZ)(30 µg)) , ciprofloxacin** ((CIP)(5 µg)), ceftriaxone ** ((CRO)(30 µg)) , tetracycline** ((TE)(30 µg)), cefotaxime** ((CTX)(30 µg)) , gentamycin ** ((CN)(10 µg)), cephalexin**

((CL)(30 µg)),tobramycin** (TB)(10 µg), amikacin** ((AK)(30 µg)) , cefepime ** ((CFP)(30 µg)), amipicillin* ((AM)(10 µg)) , co–trimethaxazole ** ((CO)(1.25-23.75) µg), nalidixic acid** ((NA)(30 µg)),
*: BD& BBL ,USA
**: Bioanalyse , Turkey

AmpC β –lactamases screening testing

R. ornithinolytica isolates were screened for AmpC β - lactamases by standard disc diffusion breakpoint for cefoxitin or moxicillin-clavulanate . Isolates resistant to cefoxitin or moxicillin-clavulanate were suspected of being AmpC producers (12)**.**All isolates of both groups were tested for AmpC β –lactamases production by following phenotypic tests:

AmpC Disc Test (ADT):

This test was done as described by Black *et al.*(13) .It was used Tris – EDTA to permeabilize the bacterial cell and release β –lactamase into the medium. AmpC discs were prepared by applying 20µl of a 1:1 mixture of sterile saline and 100 X Tris EDTA to sterile filter paper discs . the discs were allowed to dry and stored at 2-8 °C .Alawn of cefoxitin susceptible *E .coli* ATCC 25922 was inoculated a Mueller Hinton agar(BD& BBL ,USA) plate .Before use , the AmpC discs were rehydrated with 20µl of sterile saline and 8-10 colonies of test bacterium was applied to a disk , Acefoxitin disc (30 µg) placed on the inoculated Mueller Hinton agar . the inoculated AmpC disc was placed almost touching the cefoxitin disc with the inoculated disc face in contact with the agar surface . The plates were then incubated overnight at 37°C .plates were examined for an indentation or a flattening of the zone of inhibition , indicating positive result ,or the absence of a distortion , indicating of negative result.

Modified Three Dimensional Test(MTDT)

Confirmation of AmpC enzyme production was detected by modified three dimensional test as described by Manchanda & singh(14) . 10-15 mg fresh overnight growth from Mueller Hinton agar was taken in a mirocentrifuge tube (200 µl) .50 µl peptone water was added and centrifuged at 800 g for 15 min.,crude enzyme extract was prepared by repeated freeze thawing for five to seven times . Lawn culture of *E .coli* ATCC 25922 was done on Mueller Hinton agar plates and cefoxitin (30 µg) disc was placed on the surface of plate . A 3 cm linear slit was cut using sterile scalpel blade 3 mm away from the cefoxitin disc . At the other end of the slit a well was cut .30 µl of crude enzyme extract was put in the well . the plates were then incubated overnight at 37°C . Isolates showing distortion of zone of inhibition was taken as positive for AmpC production , and isolates with no distortion of zone of inhibition was taken as AmpC non production.

RESULTS

Twenty isolates of *Raoultella* sp. Were identified from 229 positive specimens for bacterial culture (gram positive and gram negative) *Raoultella* sp. Registered 8.73% among all isolates . species of *Raoultella* sp. That detected in this study were *Raoultella ornithinolytica in the first order which registered 16 isolates in percentages 6.98%*, followed by *Raoultella terrigena (3)* isolates in percentages 1.31% and 1(0.43%) isolates for *Raoultella planticola* (Table 1).

TABLE 1.Numbers & percentages of *Raoultella ornithinolytica* isolates from different clinical specimens

Type of Specimens	**No. of specimens**	**No. & (%) of *positive spciemens***	**No. & (%) of *Raoultella* sp.**	**No. & (%) of *Raoultella ornithinolytica***	**No. & (%) of *Raoultella terrigena***	**No. & (%) of *Raoultella planticola***
Urine	132	81(61)	8(9.8)	8(9.8)	-	-
Burns	58	41(70.6)	5(12.19)	3(7.31)	1(2.43)	1(2.43)
Blood	15	5 (33.3)	-	-	-	-
Wounds	95	79(83.15)	5(6.3)	3(3.79)	2(2.53)	-
Stool	23	23(100)	2(8.6)	2(8.69)	-	-
Total	323	229(70.89)	20(8.73)	16(6.98)	3(1.31)	1(0.43)

The results in table 1 showed , the higher emergency for *Raoultella ornithinolytica* was 8 isolates in urine specimens .

Results of table 2 appeared 13(81%) and 10 (62.5%) isolates of *Raoultella ornithinolytica* were resistant to cefoxitin moxicillin-clavulanate respectively .

TABLE 2. Screening tests of *Raoultella ornithinolytica* isolates to production AmpC β - lactamases

Antibiotics	No. &(%)of resistant isolates	No. &(%)of intermediate isolates	No. &(%)of sensitive isolates
Cefoxitin	13 (81)	3(19)	-
Amoxicillin-clavulanate	10(62.5)	-	6(37.5)

All amoxicillin-clavulanate resistant isolates and 10 of 13 cefoxitin resistant isolates of *Raoultella ornithinolytica* were found to be positive to production AmpC β – lactamases by AmpC disc test (ADT) and modified three dimensional test (MTDT)
(Table 3) .

TABLE 3. The frequency of AmpC β - lactamases producing *Raoultella ornithinolytica* isolates by phenotypic detection methods

Method	No. &(%)of AmpC β - lactamases producing isolates
AmpC disc test (ADT)	10(62.5)
Modified three dimensional test(MTDT)	10(62.5)

The results in table 4 were revealed the total numbers of isolates that produce AmpC β – lactamases were obtained from different clinical specimens that included 3(100%) ,6(75%) and 1(33.3%) isolates from burns , urine and wounds specimens respectively.

TABLE 4. distribution of AmpC β – lactamases producing *Raoultella ornithinolytica* isolates according to different clinical specimens

Specimens	No. of *Raoultella ornithinolytica* Isolates	No. &(%)of AmpC β – lactamases producing isolates
Urine	8	6(75)
Burns	3	3(100)
Blood	-	-
Wounds	3	1(33.3)
Stool	2	-
Total	16	10(62.5)

Table 5 revealed the antibiotic susceptibility of AmpC β – lactamases producing *Raoultella ornithinolytica* isolates to different antibiotics .The results showed that all (100%)of AmpC β – producers were

resistant to cefotaxime , cephalexin , ampicillin and high rates of resistance(90%) were observed to ceftriaxone , cephalothin and tetracycline. Isolates were appeared resistant to most other antibiotics in ranging from 60%-80% .All isolates were sensitive to imipenem (100%) , and it was found higher levels of sensitivity (60%) to cefepime and ceftazidime antibiotics .

TABLE 5. Antibiotic susceptibility of AmpC β – lactamases producing isolates (n=10)

Antibiotics	No. &(%)of resistant isolates	No. &(%)of sensitive isolates
Cefepime	4(40%)	6(60%)
Amipicillin	10(100%)	0(0%)
Cephalothin	9(90)	1(10)
Imipenem	0(0%)	10(100%)
Ceftazidime	4(40%)	6(60%)
Cefotaxime	10(100%)	0(0%)
Ceftriaxone	9(90%)	1(10%)
Cephalexin	10 (100%)	0(0%)
Tetracycline	9 (90%)	1(10%)
Ciprofloxacin	8(80%)	2(20%)
Gentamycin	6(60%)	4(40%)
Chloramphenicol	7(70%)	3(30%)
Tobramycin	6(60%)	4(40%)
Amikacin	7 (70%)	3(30%)
Co-trimethaxazole	7(70%)	3(30%)
Nalidix acid	6(60%)	4(40%)

DISCUSSION

Raoultella ornithinolytica is found in aquatic environment **,** fish and insects and produces histamine poisoning in fish(15) . In human, some cases are related to these bacteria such as soft tissue infections , urinary tract infections in adults and neonatal infections are reported (16). In this study *Raoultella* sp. Were isolated from different clinical specimens that included urine , burns , wounds and stool, the emergency of these bacteria in these specimens may be related to considered these bacteria are opportunistic pathogens which have ability to cause some infections under different conditions (17) . *Raoultella ornithinolytica* was registered high percentage than other *Raoultella* species that isolated in this study , and was founded in urinary tract infections with high rate ,this may be due to it is part of the normal flora in the lower intestine and can be introduced to urinary tract in many way (18) these results are identical with those obtained by Al-Hulu et al. (2009)who found , 11 isolates of *Raoultella ornithinolytica* were isolated from clinical specimens in Hilla city , Iraq . *Raoultella terrigena* and *Raoultella planticola* were detected in this study ,one isolate of *Raoultella terrigena* was obtained in burn specimen and two of these bacteria were detected in wound specimens .whereas , one isolate of *Raoultella planticola* was isolated from burns specimens ,thus , this study may be considered the first record of presence of *Raoultella terrigena* and *Raoultella planticola* in human clinical specimens in Al –Nasiriyha city , Thi- qar province , Iraq.

The ability of bacteria to production of AmpC β – lactamases have become a major therapeutic challenge , these enzymes resistant to penicillins , cephamycins ,, extended spectrum cephalosporins , monobactams and β – lactam inhibitors (19, 10). Isolates resistant to cefoxitin or amoxicillin / clavulanic acid were suspected of being AmpC β – lactamases producers (12),of 13 cefoxitin resistant isolates of *Raoultella ornithinolytica* , 10 isolates were positive for AmpC β – lactamases production by two phenotypic tests((AmpC disc test (ADT) and Modified three dimensional test(MTDT)) , three cefoxitin resistant isolates were negative to production AmpC enzymes by phenotypic tests. The resistance of isolates to cephamycin (cefoxitin) may be due to porin mutations (20). In this study , all isolates that have resistant to AMC (Amoxicillin \ Clavulanic acid) were positive for AmpC β – lactamases production by two phenotypic tests , these results are due to AmpC β – lactamases activity is not affected by the extended spectrum β – lactamases inhibitor clavulanic acid (10), these results were in accordance with results being reported by Reuland *et al*., (2015) who found *Raoultella ornithinolytica* was positive for AmpC β – lactamases in the phenotypic confirmation test(21) . In this study *Raoultella ornithinolytica* was isolated from different clinical specimens(urine , burns , wounds and stool), the burns and urine isolates were found to have high percentages of AmpC β – lac tamases.

Results of this study were appeared that , AmpC β – lactamases producers of *R. ornithinolytica* were resistant to other antibiotics in high rates especially β – lactam antibiotics such as cefotaxime , cephalexin , ampicillin ,ceftriaxone and cephalothin . Many gram negative bacteria produce a chromosomally mediated AmpC β – lactamases which , when hyperproduced , may result resistance to cephamycin , aztreonam , penicillins , narrow – broad and extended spectrum cephalosporins (10). Also , AmpC β – lactamases producing isolates were resistant to other β – lactam antibiotics that included tetracycline , this resistance may be due to AmpC enzymes have been located of plasmid . AmpC plasmids often carry multiple resistances to other antibiotics such as tetracycline , chloramphenicol and sulfonamide (22,23).The results showed that imipenem was the most effective antibiotic against AmpC β – lactamases producing *R. ornithinolytica* , this results may be due to carbapenems are remain very stable to the action of AmpC β – lactamases(24). The results of this study conclude , the emergence of *Raoultella* sp. In different clinical specimens , which included some species such as *Raoultella ornithinolytica*, *Raoultella terrigena* and *Raoultella planticola* , thus this study may be considered as a preliminary record about occurrence of these species especially *Raoultella terrigena* and *Raoultella planticola* in human clinical specimens in Thi- Qar province in Iraq. The results showed high prevalence of AmpC β – lactamases producing *R. ornithinolytica* isolates that detected by phenotypic tests , and the imipenem was the most effective antibiotic against these isolates . This study invite to increased efforts of surveillance for and the study of occurrence and prevalence of other species which belonging to genus *Raoultella* in clinical specimens and study their virulence factors and their resistance against effective antibiotics .This study recommend that reasonable use of the antibiotics especially β – lactam antibiotics may be the key to reduction of AmpC β – lactamases production and other β – lactamases enzymes.

Acknowledgment

My great thank to all staff which help me in Al –Hussein and Bent Al – Huda hospital of Maternity & Children in Al- Nasiriyah city , Thi –Qar province, for providing clinical samples and to staff of lab . in Bent Al – Huda hospital for their help to complete this work

REFERENCES

1. Drancourt, M; Bollet, C; Carta, A; Rousselier, P .(2001). "Phylogenetic analyses of Klebsiella species delineate Klebsiella and Raoultella gen. nov., with description of Raoultella ornithinolytica comb. nov., Raoultella terrigena comb. nov. and Raoultella planticola comb. nov.". International journal of systematic and evolutionary microbiology 51 (Pt 3): 925–32.
2. Park,J.S.;Hong,K.H.;Lee,H.J.;Choi,S.H.;Song;J.H.;Song,K.H.;Kim,H.B.;Park,K,U.;Song,J.and Kim,E.(2011). Evaluation of three phenotypic identification systems for clinical isolates of *Raoultella ornithinolytica* , J . Med. Microbiol. , 60:492-499.
3. Jain, K.; Radsak, K.; Mannheim, W. (1974). "Differentiation of the Oxytocum group from *Klebsiella* by deoxyribonucleic acid hybridization". *Int J Syst Bacteriol* **24** (4): 402–407.
4. Sibanda , m.(2014). Primary peritonitis caused by Raoultella ornithinolytica in a 53 -year- old man. JMM case reports DO |10.| 099/ JMMCR.0.002634.
5. Morais, V.P.;Daporta,M.T.;Bao,A.F.and Campello,M.G.(2009).Enteric fever –like syndrome caused by Raoultella ornithinolytica(*Klebsiella* ornithinolytica).Letters to the editor.J.Clin.Microbiol.,47(3):868-869.
6. Koc,S.;Kabatas,B.and Icqen,B.(2013).Multidrug and heavy metal –resistant Raoultella planticola isolated from surface water . Bull Environ Contam Toxicol.US national library of medicine national institutes of health .,91(2):177-83.

7. Walckenear,E.;Poirel,L.;Leflon-Guibout,V.;Nordmann,P.and Nicolas-Chanoine,M.(2004).Genetic and biochemical characterization of the chromosomal class A β – lactamases of Raoultella(formerly *Klebsiella*) planticola and Raoultella ornithinolytica .American Society of Microbiology . Antimicrobial Agents and Chemotherapy , 48(1):305-312.
8. Al-Hulu,S.M.;Al-Charrakh,A.H. and Al-Saadi,M.A.K.(2009).Isolation and characterization of Raoultella ornithinolytica from clinical specimens in Hilla city , Iraq.Med.J.Babylon, 7(4): 42-47.
9. Hostacka, A., and Klokocnikova,L. (2001). Antibiotic susceptibility, serum response and surface properties of *Klebsiella* species. Microbios 104:115-124.
10. Jacoby, G. A.(2009). AmpC β-Lactamases. Clin Microbiol Rev.; 22(1): 161–182.
11. Clinical Laboratory Standards Institute .(2003). Performance standards for antimicrobial disk susceptibility tests . 8th ed .Approved standard M2-A8 .Wayne ,Pa .
12. Clinical Laboratory Standards Institute .(2009). Performance standards for antimicrobial susceptibility testing . 19th ed .Informational supplement M100-S19 .Wayne ,Pa .USA.
13. Black, J.A.; Moland, E.S.and Thomson, K.S. (2005).AmpC disk test for detection of plasmid – mediated AmpC β-Lactamases in Enterobacteriaceae lacking chromosomal AmpC β-Lactamases .J.Clin. Microbiol. , 43:3110-3.
14. Manchanda,V.and Singh,N.P.(2003). Occurrence and detection of AmpC beta-lactamases among Gram-negative clinical isolates using a modified three-dimensional test at Guru Tegh Bahadur Hospital, Delhi, India. J.Antimicrob Chemother ., 51(2):415-8.
15. Kanki,M.;Yoda,T.;Tsukamoto,T.and Shibata,T.(2002).*Klebsiella pneumonia* produces no histamine : Raoultella planticola and Raoultella ornithinolytica strains are histamine producers .Appl Environ Microbial ., 68:3462-3466.
16. Chun, J.; Yun, J.W.; Huh ,H.J and Lee, N.Y.(2015). Clinical characteristics of Raoultella ornithinolytica bacteremia infection 43:59-64.
17. Tortora , G.J.; Funke , B. R. and Case , C.L. (2002) . Microbiology An introduction , 8th . ed . PEARSON .Benjamin Cummings . p: 924.
18. Berg, R.D.(1990) . Bacterial translocation from the gastrointestinal tract . Compr Ther.16(10): 8- 15.
19. Thomson,K.S.(2001). Controversies about extended – spectrum and AmpC-beta – lactamases . Emerg. Infect. Dis. 7:333-336.
20. Thomson,K.S.(2010).Extended – spectrum - β-Lactamase, AmpC ,and carbapenemase Issues .J.Clin.Microbiol.American Society for microbiology , p:1019-1025.
21. Reuland,E.A.;Halaby,T.;Hays,J.P.;Jongh,D.M.C.;Snetselaar.H.D.R.;Keulen,M.;Elders,P.J.M.;Savelkoul,P.H .M.;Vandenbroucke-Grauls,C.M.J.E. and Al- Naiemi,N.(2015). Plasmid-mediated AmpC: Prevalence in community –Acquired isolates in Amsterdam , the Netherlands ,and risk factors for carriage , PLOS ONE|DO| :10.1371/ journal.pone.0113033.
22. Horii,T.;Arakawa,Y.;Ohta,M.;Sugiyama,T.;Wacharotayankun,R.;Ito,H.and Kato,N.(1994).Characterization of a plasmid-borne and constitutively expressed bla Mox-1 gene encoding AmpC –type β-Lactamase . Gene 139:93-98.
23. Stapleton,P.D.;Shannon,K.P.and French,G.L(1999). Carbapenem resistance in *Escherichia coli* associated with plasmid – determined CMY-4 β-Lactamase production and loss of an outer membrane protein .Antimicrob.Agents Chemother. 43:1206-1210.
24. Heller,I;Grif,K.and Orth ,D.(2012).Emergence of VIM-1- carbapenemase – producing *Enterobacter cloacae* in Tyrol ,Austria .J. Med. Microbial ., 61: 567-71.