



PHENOTYPIC DETECTION OF ESBL AND MBL IN CLINICAL ISOLATES OF *ENTEROBACTER CLOACAE* AT AL-IMAM AL-HUSSEIN HOSPITAL IN THI-QAR PROVINCE IN IRAQ

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Abstract

The aim of this study was to ascertain the incidence and clinical significance of Metallo β -lactamases (MBL) and Extended spectrum β -lactamases (ESBLs) among *Enterobacter cloacae* isolated from patients at Al-Imam Al-Hussein Hospital. We prospectively collected data on patients with *Enterobacter* infection during a 6-month period from the 1st of January to the end of June 2014.. All of the strains were investigated for antibiotic susceptibility, the presence and expression of (MBL) and ESBLs. 120 *Enterobacter* species isolated from various clinical samples were included in the study Of 15 infections (7 involving The wound, 4 urine and 3 burn female were caused by *Enterobacter cloacae* strains. The strains showed high levels of resistance, especially to CEP³⁰ (100%), TCC^{75/10} (100%), CN¹⁰ (93.33%) and CTR³⁰ (80.33%).

Out of 120 *Enterobacter cloacae* isolates, 8(53.33%) were MBL producers. None of the isolates showed the ESBL. None of the isolates showed the coexistence of ESBL and MBL in the same isolate.

Keywords: ESBL; MBL; *Enterobacter cloacae*.

Introduction

Species of the genus *Enterobacter* have been reported as an important source of intrahospital infections, especially those showing resistance to β - lactame by the production of enzymes like (ESBL) such as TEM, SHV, CTX, VEB, and carbapenemases such as VIM and KPC⁽¹⁾.

Enterobacter species are an important cause of nosocomial infection, but only a few cases of infection due to (MBL)producing *E. cloacae* have been reported in the English literature worldwide^(2,3).

ESBL producing isolates, in addition to being resistant to β -lactame antibiotics, often exhibit resistance to other classes of drugs such as aminoglycosides, cotrimoxazole, tetracycline and fluoroquinolones⁽⁴⁾.

ESBLs are often located on plasmids that are transferable from strain to strain and between bacterial species⁽⁵⁾.

The emergence of (MBLs) in the Enterobacteriaceae is a matter of major concern for clinicians worldwide^(6, 7).

Resistance to carbapenem is predominantly mediated by metallo-betalactamases, a class B type of betalactamases that recognize bivalent metal ions⁽⁸⁾.

Early detection of MBL and ESBL producing organisms is crucial to establish appropriate antimicrobial therapy and to prevent their interhospital and extrahospital dissemination⁽⁹⁾.

Materials and Methods

The study had been conducted at Al-Imam Al-Hussein hospital at Thi-qar province, which is one of the southern province in Iraq for the period from 1st of January to the end of July 2014.

One hundred and twenty swabs were collected from the skin of patients , urine from different sites related to the devices and utensils used in the hospitals.

Swabs were incubated on cultural media; Blood agar, MacConkey agar and Nutrient agar, being prepared according to the manufacturing companies, and incubated at 37°C for (24-48) hours.

Purification of bacterial growth colonies yield pure isolates of bacteria and subsequently their cultural, morphological, microscopical and biochemical characteristics had been studied according to the correlated references^(10,11). For final identification of isolates had been used kits API 20E kit (BioMeriux).

Antimicrobial Sensitivity Tests

Susceptibility for the studied isolates were investigated according to⁽¹²⁾. by using Muller - Hinton agar and the following antibiotics discs: Cephalothin, Ciprofloxacin, Gentamicin, Ceftriaxone, Imipenem, Tetracycline and Ticarcillin-Clavulanic acid. then results were recorded according to⁽¹³⁾.

Detection of ESBL

Method was used Disc approximation to investigate enzymes β -lactmase broad-spectrum, according to what is stated in⁽¹⁴⁾.

Put a disc containing a mixture of Amoxicillin/ Clavulanic acid (30 μ g /disc) In the center of the petri dish inoculated. Then arranged the disc antibiotics Pipracillin, Cefotaxime,Ceftriaxone after 3 cm From the center of the

Amoxicillin/ Clavulanic acid The observation of the occurrence of zones of inhibition between the disk and the central one or more of the discs mentioned evidence of any positive outcome for the production of an enzyme isolation.

Detection of MBL

Metallo- β -lactamases – combined disc test (MBL-CD) was done using imipenem and combined imipenem/EDTA (10/ 1000 μ g, Becton Dickinson / Sigma) discs and placed on the agar plates. After overnight incubation at 35 C°, inhibition zones of the imipenem with and without EDTA were compared. The test is considered positive if the difference ≥ 6 mm increasing in the zone diameter of imipenem/EDTA .^(15,16)

Results and Discussion

Susceptibility tests for some antibiotics showed different results depending on type of antibiotics used. the resistance was highly against CEP³⁰ (100%), TCC^{75/10} (100%) and CTR³⁰ (80.33%). As showed in the table (1).

The appearance of resistance for β - lactamase antibiotics could be related to many causes ; production of β lactamase enzymes and its effect which lead to the breakdown of the β - lactame cycle in penicillins and cephalosporines changing it into inactive compounds⁽¹⁷⁾, or may be because of the changes being occurred in the porins of the cellular membrane and ultimately its effect on the cell permeability⁽¹²⁾. some Gram -ve bacteria are resistant for β -lactame antibiotic because it has an Efflux pump system which lead to pump the antibiotics from intracellular to extracellular space⁽¹⁸⁾.

The gradual increase in the resistant of Enterobacteriaceae against β -lactame antibiotics (1st and 2nd generation of penicillins and cephalosporines) reduce the efficacy of these antibiotics in eradicating diseases of bacterial etiology completely since these resistance will lead to continuous change in the epidemiology of these disease⁽¹⁹⁾, while the effect of extended spectrum β - lactamase (ESBLs) became more evident against the 3rd generation of penicillins and cephalosporines⁽²⁰⁾.

The high sensitivity of the studied isolates for Imipenem belong to Carbapenems group and one of the recently used antibiotic, could be due to its limited use in Iraq. Although resistant was also recorded among 40% of these isolates, and the cause could be inferred to the development in the mechanism of bacterial resistance such as its production for Carbapenemases enzymes related to β - lactamases enzymes type D and B⁽²¹⁾.

One of the three mechanisms that may explain the resistance of some bacteria against aminoglycosides antibiotics ; production of converted enzymes which inhibit the activity of antibiotics , changing the target of antibiotics , or through the change of the permeability for the cell barrier^(12,22).

Table 1: Results of antibiotic susceptibility tests of isolated strains of *Enterobacter cloacae*

Types of antibiotics	Frequency (%) of:		
	Resistant	Intermediate	Sensitive
CEP ³⁰	15 (100%)	0	0
CIP ⁵	2 (13.33%)	4 (26.66%)	9 (60%)
CN ¹⁰	14 (93.33%)	1(6.66%)	0
CTR ³⁰	12 (80%)	0	3(20%)
IMP ¹⁰	6 (40%)	1(6.66%)	7(46.66)
TE ³⁰	8 (53.33%)	3 (20%)	4 (26.66%)
TCC ^{75/10}	15 (100%)	0	0

Where the percentage of enzymes (MBL) produced by 15 isolates were (53.33%) as showed in the table 2 and distributed to the isolation number 2,5,6,8,9,11,12 and 14 as showed in the table 3 of the bacteria produced this enzyme as showed in (Picture no. 1) describes the potential bacteria *Enterobacter cloacae* to produce an enzyme MBL through the difference inhibition zones of the imipenem with and without EDTA were compared ≥ 6 mm increasing in the zone diameter of imipenem/EDTA as showed in the table3.

And the reason for his resistance to the developments in the mechanisms of bacterial resistance, such as production of enzymes that belong to Carbapenemases enzymes β - lactamases class D and B as well as the presence of blaOXA-23 gene genes that encode for resistance to this antibiotic^(23,24).

Acquired Metallo- β -lactamases (MBL) in pseudomonas spp. have recently emerged as one of the most worrisome resistance mechanism because of their capacity to hydrolyze all β -lactame antibiotics including penicillins, cephalosporins and carbapenems, with the exception of aztreonam. Nosocomial infection producing MBL (Metallo- β -lactamase) positive isolates of *Enterobacter clocae* is important to identify because it poses not only therapeutic problem, but also a serious concern for infection control management⁽²⁵⁾.

Table 2: Total number of MBL producer *Enterobacter cloacae* in present study are as follow:-

Total MBL isolates of <i>Enterobacter cloacae</i>	120
MBL producer	08 (53.33%)
MBL non-producer	112 (93.33%)

Table 3: Total number of MBL producer *Enterobacter cloacae* in present study are as follow:-

No.	IMP	IMP + EDTA	MBL
1.	16	19	
2.	0	15	+
3.	0	0	
4.	0	0	
5.	18	25	+
6.	18	27	+
7.	0	0	
8.	16	22	+
9.	33	45	+
10.	0	0	
11.	18	24	+
12.	15	26	+
13.	20	25	
14.	0	17	+
15.	16	21	

None of the isolates showed the ESBL because of the lack of mutations in the genes for the enzymes encoded Beta lactamase type TEM and SHV, leading to a change in the order of the amino acids of the enzyme active site. Examples include enzyme TEM-12 and TEM-2, which replaces the two amino acid Serine instead on-site Arginine 164 and Lysine instead of glutamate Active 104 and lead these changes to the analysis of antibiotics is of great importance, such as ceftazidime and ceftizoxime as show in figure 2 ⁽²⁶⁾.

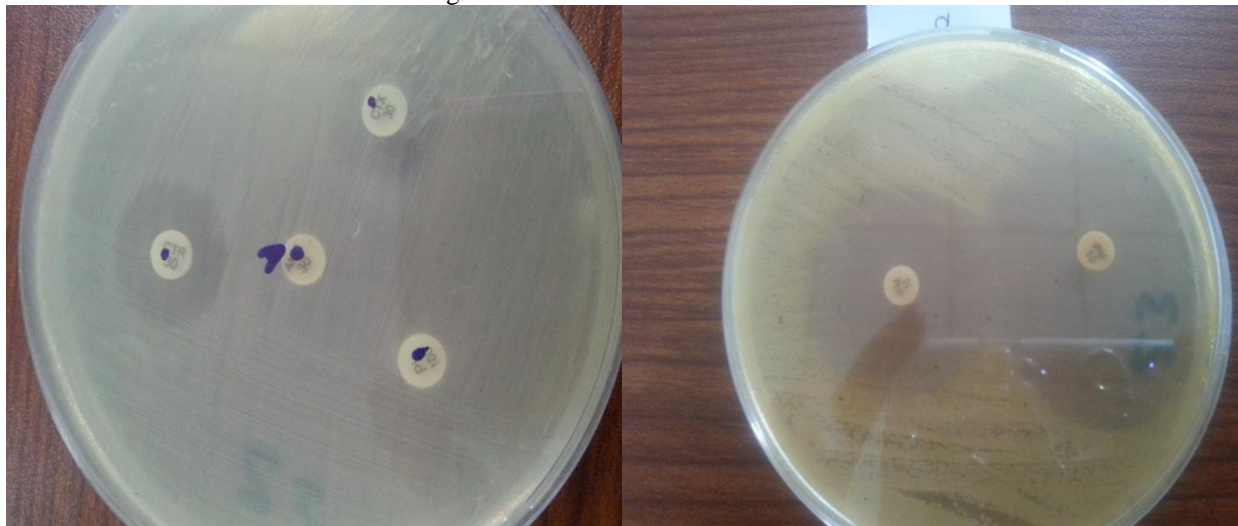


Figure.2 ESBL negative

Figure.1 MBL positive

References

- Sader HS, Castanheira M, Mendes RE, Toleman M, Walsh TR, Jones RN. 2005. Dissemination and diversity of metallo- β -lactamases in Latin America: report from the SENTRY Antimicrobial Surveillance Program. *Int J Antimicrob Agents*. 25:57-61.
- Jeong, S. H., K. Lee, Y. Chong, J. H. Yum, S. H. Lee, H. J. Choi, J. M. Kim, K. H. Park, B. H. Han, S. W. Lee, and T. S. Jeong. 2003. Characterization of a new integron containing VIM-2, a metallo- β -lactamase gene cassette, in clinical isolates of *Enterobacter cloacae*. *J. Antimicrob. Chemother*. 51:397– 400.
- Luzzaro, F., J. D. Docquier, C. Colinon, A. Endimiani, G. Lombardi, G. Amicosante, G. M. Rossolini, and A. Toniolo. 2004. Emergence in *Klebsiella pneumoniae* and *Enterobacter cloacae* clinical isolates of the VIM-4 metallo- β -lactamase encoded by a conjugative plasmid. *Antimicrob. Agents Chemother*. 48:648-650.
- Dinesh, S. Chandel, Judith A. Johnson, Rama Chaudhry et al. 2011. Extended spectrum beta-lactamase-producing Gram-negative bacteria causing neonatal sepsis in India in rural and urban settings. *J. Med. Microbiol*.60: 500 507.
- Mark E. Rupp and Paul D. Fey. 2003. Extended spectrum beta-lactamase (ESBL)-Producing Enterobacteriaceae. *Drugs*. 63(4): 353-365.
- Galani, I., M. Souli, Z. Chryssouli, K. Orlandou, and H. Giamarellou. 2005. Characterization of a new integron containing blaVIM-1 and aac(6₋)-IIc in *Enterobacter cloacae* clinical isolate from Greece. *J. Antimicrob. Chemother* 55:634–638.
- Van Nierop, W. H., A. G. Duse, R. G. Stewart, Y. R. Bilgeri, and H. J. Koornhof. 1998. Molecular epidemiology of an outbreak of *Enterobacter cloacae* in the neonatal intensive care unit of a provincial hospital in Gauteng, South Africa. *J. Clin. Microbiol*. 36:3085–3087.
- Anil Rajput, Bhavin Prajapati, Bimal Chauhan, Atit Shah, Toral Trivedi, and Mina Kadam. 2012. Prevalence of Metallo-beta-lactamases (MBL) producing *Pseudomonas aeruginosa* in a Tertiary care Hospital. *Indian. J. Basic.Appl. Med. Res*. 1(4): 304-308.
- Nirav P. Pandya, Sweta B. Prajapati, Sanjay J. Mehta, Kunjan M. Kikani and Pratima J. Joshi. 2011. Evaluation of various methods for detection of metallo- β -lactamase (MBL) production in gram negative bacilli. *Int. J. Biol. Med.Res*. 2(3): 775-777.
- Retty, A. F., Danil, F. S., & Aice, S. W. (2007). *Bailey and Scott's of Diagnostic microbiology*, 12th ed. Press, Houston, Texas.
- Alexander, S. K., & Strete, D. (2001). *Microbiology a photographic Atlas for Laboratory*, 1st ed. Inc. San Francisco.
- Nester, E. W., Anderson, D. G., Roberts Jr., E., Pearsall, N. N., & Nester, M. T. (2004). *Microbiology, A human perspective*, 4th ed. McGraw Hill, pp.499–502.

- 13- Clinical and Laboratory Standard Institute (CLSI). (2007). Performance standards for antimicrobial susceptibility testing, 17th informational Supplement, 27(1), Pennsylvania USA.
- 14- Jarlier, V.; Nicolas, M.; Fournier, G.; & Philippon, A. (1988). Extended broad-spectrum β -Lactamases conferring transferable resistance to newer β -lactam agents in Enterobacteriaceae: Hospital prevalence and susceptibility patterns. Rev. Infect. Dis. 10(4): 867-78.
- 15- Berge's, L.; Rodriguez-Villalobos, H.; Deplano, A. and Struelens, M. (2007). Prospective evaluation of imipenem/EDTA combined disc and E-test for detection of metallo- β -lactamase producing *Pseudomonas aeruginosa*. J Antimicrobial Chemother., 50 : 812-813.
- 16- Yong, D.; Lee, K.; Yum, J.; Shin, H.; Rossolini, G. and Chong, Y. (2002). Imipenem-EDTA disk method for differentiation of metallo- β -lactamase-producing clinical isolates of *pseudomonas* spp. and *Acinetobacter* spp. J Clin Microbiol., 40: 3798-3801.
- 17- Pfeillif D.; Janes E. and Weideman B. (2000): Role of Penicillin binding protein, the initiation of the ampc β -Lactamas Expression in *Enterobacter cloacae*, J. Antimicrob. Agent Chemother. 44 : (1) 169 – 72.
- 18- Schweizer H.P. (2003): Efflux as a mechanism of resistance to anti-Microbial in *Pseudomonas aeruginosa* and related bacteria: Unanswered questions. J. Genetic and molecular research 2(1):48 – 62.
- 19- Sahm, F. D., Thornsberrry, C., Mayfield, C. D., Jones, E. M., & Karlowsky, A. J. (2001). Multidrug-resistant urinary tract isolates of *Escherichia coli*, prevalence and patient Demographics in the United State in 2000.
- 20- Woodford N.; Ward E. and Kaufmann M.E. (2006): Molecular Characterization of *Escherichia coli* isolates producing CTX – M – 15 extended – spectrum β –lactamase (ESBL) In Health Protection Agency, U.K.
- 21- Laclero M.; Glupcynski Y. and Tulkens P. (1999): Aminoglycosides Activity and resistance, J. Med. Microb. 43 (4): 727 – 37.
- 22- Levinson W. and Jawetz E. (2004): Medical Microbiology and Immunology examination and board review, 8th ed. Lange Medical books, McGraw – Hill, New York.
- 23- Coelho, J.M.; Turton, J.F.; Kaufmann, M.E.; Glover, J.; Woodford, N.; Warner, M.; Palepon, M.F.; Pike, R.; Pitt, T.K.; Patel, B.C. and Livermore, D.M. (2006). Occurrence of Carbapenem-resistant *Acinetobacter baumannii* clones at multiple hospitals in London and Southeast England. J. Clin. Microbiol. 44 (10): 3623-27.
- 24- Taneja, N.; Maharwal, S. and sharma, M. (2003). Imipenem resistance in nonfermenters causing nosocomial urinary tract infections. Indian. J. Med. Sci. 57(7): 294-99.
- 25- Mehul S Chaudhari¹ Tanuja B Javadekar² Govind Ninama³ Neelam Pandya¹ Jivraj Damor⁴ (2011) A STUDY OF METALLO-BETA-LACTAMASE PRODUCING PSEUDOMONAS AERUGINOSA IN CLINICAL SAMPLES OF S.S.G. HOSPITAL Vol 1 Issue 2 Oct – Dec 2011 : ISSN 2249 4995
- 26- Al-Marjani, Mohammed F. 2010. Antibiotics Resistance of Bacteria to Antibiotics. Ministry of Higher Education and Scientific Research. AL- Mustansiriya University/iraq