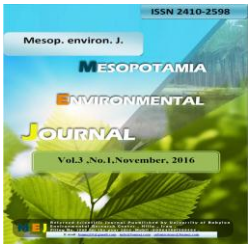
Mesopo. Environ. j., Special Issue C :104-110, 2017
ISSN 2410-2598
proceeding of 1st National conference of science and Art
University of Babylon

Mesopotemia Environmental journal
journal homepage:www.bumej.com



CTX-M producing carbapenem- resistant *Klebsiella pneumoniae* from children with upper respiratory tract infections in Hilla city

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To cite this article:

Abbas,F.M. CTX-M producing carbapenem- resistant *Klebsiella pneumoniae* from children with upper respiratory tract infections in Hilla city.. *Mesop. environ. j*., 2017, Special Issue C.;104-110 .

Abstract

This study was attempted to find out the prevalence of $bla_{\text{CTX-M}}$ gene among carbapenem resistant *Klebsiella pneumoniae* from upper respiratory tract infections . In total ,100 throat samples were collected from children attending Babylon Teaching Hospital for Maternity and Pediatric at a period from November, 2015 to the end of February, 2016 .Fourteenth (14%) isolates were specified as *K.pneumoniae*, these were screened phenotypically for extended spectrum β-lactamase (ESBL) production by disk combination method. Nine (64.3%) isolates were found to be screen-positive. ESBL-producing isolates were submitted to antimicrobial susceptibility testing using Kirby-Bauer disk diffusion method. The highest rates of resistance were observed for penicillin antibiotics (ampicillin and cloxacillin) with (88.88%) and (77.77%) resistance rate, respectively. The lowest rate for carbapenem antibiotics (imipenem, 11.11% and meropenem, 22.22%). Carbapenem resistant isolates were checked by Polymerase Chain Reaction (PCR) method for the presence of $bla_{\text{CTX-M}}$, 2 (100%) isolates gave positive result.

Keywords : *Klebsiella pneumoniae*, ESBL, Carbapenem resistance, CTX-M beta lactamase ,PCR.

Introduction

Antimicrobial resistance is a serious problem in many bacterial pathogens in all developed and developing countries [1, 2]. Extended spectrum β-lactamases (ESBLs) are plasmid -mediated enzymes, efficiently hydrolyze oxyimino-cephalosporins, conferring resistance to extended spectrum cephalosporins and monobactams. They are distributed among Gram-negative bacteria especially

Enterobacteriaceae family. Their prevalence has been increased in later years with great variation among different geographical areas [3;4]. Most ESBL-producing isolates also express plasmid-encoded multidrug resistance. As a result therapeutic options for infection caused by these isolates are limited [5].

The CTX-M enzymes are a group of class A extended spectrum beta- lactamase, have been detected in the second half of 1980s in Europe and derived from the non- bla_{ESBL} of SHV-1 and TEM-1 .Recently, they emerged as the most predominant types within hospitals and communities worldwide [6, 7, 8].The designation CTX is an abbreviation for cefotaximase and indicate that these enzymes have a hydrolytic activity against cefotaxime but not ceftazidime .In recent years, several reports refer to CTX-M mutants exhibiting a significant hydrolytic activity against ceftazidime [9, 10, 11].

This study aimed to determine the prevalence of *K.pneumoniae* isolated from throat samples, determine susceptibility patterns of bacterial isolates, detect bla_{ESBL} gene of CTX-M type by phenotypic and genotypic (PCR) method.

Materials and Methods

Bacterial isolates

A total of 100 throat swabs were collected from children attending Babylon Teaching Hospital for Maternity and Pediatric, Babylon province at a period from November, 2015 to the end of February, 2016. These included: 56 females and 44 males, aged between 5-11 years . All samples were cultured on different selective and differential media such as Blood agar, MacConkey agar (Himedia , India) and Eosin methylen blue agar (Biolife, Italy). The species identification was performed following the standard methods described by Holt *et al*.[12],Collee *et al*.[13] and MacFaddin [14].

Screening of extended spectrum β-lactamase production (Recommended by CLSI,2010)

The screening test for ESBLs production was performed using a phenotypic confirmatory test, clavulanic acid disk combination method as previously described [15].

Antimicrobial susceptibility test

All ESBL-positive isolates were subjected to Kirby –Bauer disk diffusion assay to determine their susceptibility profiles against a panel of 12 antibiotics from 6 different classes on Mueller- Hinton agar plates (Oxiod, England) [16]. The following antibiotic disks were tested : ampicillin (10µg), cloxacillin (10 µg) , amoxicillin- clavulanic acid (10 µg) , cefotaxime (30 µg), ceftazidime (30 µg), ceftriaxone (30 µg), cefoxitin (30µg), imipenem (10µg), meropenem (10µg), erythromycin (15µg), levofloxacin (5µg) and norfloxacin (10 µg). After 18 hrs of incubation at 37 C^{o},the zones of inhibition were measured and compared with the Clinical and Laboratory Standards Institute (CLSI) guidelines [17]. *Escherchia coli* ATCC 25922 (College of Medicine ,University of Kufa) was used as quality control.

Molecular detection of *bla*$_{CTX-M}$ gene

DNA preparation

DNA of bacterial isolates was prepared following the protocol described by Pospiech and Neuman [18] with some modifications and used directly for PCR as DNA template.

PCR amplification

Bla$_{CTX-M}$ gene was identified by Polymerase Chain Reaction method using the following sets of primers (Bioneer, Korea) CTX-M/F (F: CGCTTTGCGATGTGCAG) and CTX-M/R (R: ACCGCG ATCGTTGGT) (550 bp), in a 25 µl reaction volume using 12.5 µl Go Taq Green Master Mix 2X (Promega, USA), 5 µl DNA template, 2.5 µl of 10 pmol/ µl of specific up stream primers and, 2.5 µl of 10 pmol/ µl of specific down stream primers and 2.5 µl nuclease-free water. The PCR cycling parameters comprised: an initial denaturation at 94 C˚ for 30 sec, followed by 35 cycles of denaturation at 94 C˚ for 30 sec, anneling at 60 C˚ for1 min, extension at 72 C˚ for 1 min and a final extension step of 72 at 10 min [19]. The PCR reaction product was analyzed using 1.5% agarose gel at 70 volts for 2-3 hrs , after staining with ethidium bromide (0.5 mg /ml),the product was visualized under UV-Transilluminator ,then photographed with Gel documentation system. 100 bp DNA Ladder (Bioneer, Korea) was used to assess PCR product size.

Results and Discussion

Out of 100 clinical samples,23 isolates were belonged to *Klebsiella* spp., of which 14 (14%) isolates were identified as *K.pneumoniae*. This result higher than the finding of Ndip *et al*,who detected 11% prevalence rate for *K.pneumoniae* obtained from throat samples of school children in Buea, Cameroon [20]. Other study identified 8% prevalence rate for non-fermenter Gram-negative bacteria including *K. pneumoniae* recovered from throat swabs in China hospitals [21] .

Due to the extensive use of broad spectrum cephalosporins, numerous outbreaks of infection caused by ESBL-producing *K.pneumoniae* have been detected in different geographical areas [22].

In this investigation ESBL production was screened phenotypically by disk combination method, 9 (64.3%) isolates were determined to be screen-positive (Table-1). Vasumathi *et al*., identified 13(35.1%) isolates of *Klebsiella* spp. as ESBL producer by this method recovered from intensive care unit in Chennai, India [23]. However, definitive detection of ESBL-producing isolates was more reliable by molecular techniques [24].

Table (1):Number and percentage of extended spectrum beta lactamase producing *K.pneumoniae* using disk combination method.

No. of *K.pneumoniae* isolates	**No. (%) of ESBL-positive isolates**	**No. (%) of ESBL-negative isolates**
14	9 (64.3%)	5(35.7%)

ESBL-harboring isolates can mediate resistance to beta-lactam antibiotics [25, 26]. In this study, ESBL-producing *K.pneumoniae* isolates revealed high level of resistance to the most antibiotics tested

with highest rate for penicillin antibiotics (ampicillin and cloxacillin) with (88.88%) and (77.77%) resistance rate, respectively. The next most resistant antibiotic was amoxicillin-clavulanic acid (66.66%), (table- 2).

Huang *et al*.[27] found that ESBL-producing *K.pneumoniae* isolates exhibited the highest resistance rate 100% against ampicillin antibiotic. In Cameroon, Ndip *et al*.[20] demonstrated 60% resistance rate for ampicillin by *K.pneumoniae* isolates recovered from school children. The reason behind higher resistance may be due to frequent use of these antibiotics in Hilla city.

However, the lowest rates were noticed for carbapenem antibiotics with (11.11 %) and (22.22 %) resistance rate for imipenem and meropenem, respectively (Table-2) . Also, Huang *et al*. [27] identified 1.9% resistance rate toward imipenem among ESBL-producing *K.pneumoniae* isolates.

Table(2):Antibiotics resistance pattern of ESBL -producing *K.pneumoniae* isolates against various antibiotics(n=9).

Antibiotic class	**Agent used**	**No.(%) of resistant ESBL-positive isolates**
Penicillins	ampicillin	8 (88.88%)
	cloxacillin	7 (77.77%)
β –lactams/β- lactamase inhibitor combinations	amoxicillin-clavulanic acid	6(66.66%)
Cephems	cefotaxime	5(55.55%)
	ceftazidime	4(44.44%)
	cefriaxone	5(55.55%)
	cefoxitin	5(55.55%)
Penems	imipenem	1(11.11%)
	meropenem	2(22.22%)
Macrolides	erythromycin	4(44.44%)
Quinolones	levofloxacin	3(33.33%)
	norfloxacin	3(33.33%)

Nowadays, CTX-M enzymes are spread widely in both hospitals and communities [28]. PCR amplification analysis revealed the existence of bla_{CTX-M} gene in all 2(100%) carbapenem -resistant isolates. (Fig. 1). In China, one study reported CTX-M extended spectrum β –lactamase coupled with KPC-2 as the predominant carbapenemase encoding gene among carbapenem-resistant *K.pneumoniae* isolates [29] . While other study achieved by Nasehi *et al*., showed that all 3 *K.pneumoniae* isolates recovered from throat samples were negative for CTX-M β-lactamase in Iran [30] . This may be due to limited number of tested samples.

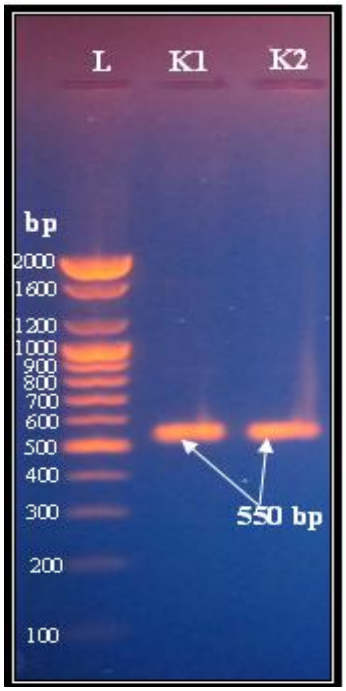

Figure (1): PCR amplification of *bla*$_{CTX-M}$ gene (550 bp) among carbapenem-resistant *Klebsiella pneumoniae* isolates. Lane (L), DNA moleculer size marker (100- bp Ladder). Lane (1,2) of *K. pneumoniae* isolates showing positive result with *bla*$_{CTX-M}$ gene.

Conclusion

The present work emphasizes the existence of ESBL-producing *K.pneumoniae* among children, their presence with carbapenem resistance will be create significant therapeutic problems. Thus, effective control measurements and laboratory detection of ESBL-producing strains by phenotypic or molecular methods will limit the spread of these agents.

Acknowledgement

I would like to express great thank to medical staff in Babylon Teaching Hospital for Maternity and Pediatric for their facilities.

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