ISSN: 0974-2115
www.jchps.com Journal of Chemical and Pharmaceutical Sciences

Characterization of Multidrug Resistant Carbapenemases-Producing *Escherichia coli* and *Klebsiella pneumoniae* Isolates from Urinary Tract Infection

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ABSTRACT

ß-lactamase*s* that hydrolyze ß-lactam antibiotics including carbapenems are called carbapenemases, which are either chromosomally or plasmid-encoded. The most prevalent enzymes in *Enterobacteriaceae* are KPC, VIM, IMP, NDM-1 and OXA-48. The present study was focused on isolation and characterization of *Escherichia coli* and *Klebsiella pneumoniae* from urinary tract infection and the detection of carbapenmase (KPC, MBL and OXA- 48) in the tested isolates. A total of 87 urine sample were obtained and cultured. Thirty four isolates of Gram negative bacilli including 20 *E. coli* and 14 *K.pneumoniae* isolates were recovered and tested for carbapenemases production. Phenotypic detection confirmed by Rosco discs while, genotypic detection was based on PCR using specific primers for *bla-IMP* and *bla*-OXA-48. The *bla* OXA-48 genes was detected in 5(25%), of the 20*E. coli* isolates, 20 *E. coli* isolates did not contain bla_{IMP} gene. While 5(35.7%) and 3 (21.4%) of the *K.pneumoniae* isolates were positive for *bla***-***IMP* genes and *bla*OXA-48 genes respectively.

Conclusion: The detection of carbapenemase genes in clinical isolates of *K. pneumoniae* and *E. coli* isolates with UTI should provide impetus to clinicians in consideration of appropriate antimicrobial treatment of UTI caused by *E. coli* and *K. pneumoniae*.

KEY WORDS: carbapenems, Multidrug Resistant, UTI, *Enterobacteriacea*.

1. INTRODUCTION

Urinary tract infection (UTI) is an important worldwide public health problem. Among the most important causes of UTIs are the Gram negative bacteria that belong to *Enterobacteriacea* family specially *Escherichia coli*, which account for as much as 80% of community acquired UTIs. The continuous emergence of resistance to antimicrobial agents among the prevalent pathogens is the most dangerous threat for the treatment of infectious disease. The production of ß-lactamases is the major mechanism of bacterial resistance to ß-lactam antibiotics which considered to be the most widely used class of antibiotics (Thomson, 2010). Carbapenemases belong to the molecular class A, B, and D β-lactamases. Class A and D enzymes have a serine-based hydrolytic mechanism for cleaving the β-lactam ring in antibiotics. Metallo β-lactamases (MBLs), on the other hand, are class B carbapenamases containing zinc at its active site (Ambler, 1980). Mechanisms of resistance to carbapenems include production of β-lactamases, efflux pumps, and mutations that alter the expression and/or function of porins and PBPs. Combinations of these mechanisms can cause high levels of resistance to carbapenems in bacteria such as *K. pneumoniae, P. aeruginosa*, and *A. baumannii* (Canton, 2012). The aim of the present study was to check for the prevalence of carbapenamases production in *K. pneumonia* and *E.coli* isolates from UTI patients.

2. MATERIALS AND METHODS

Isolation of Bacteria: Urine samples from 87 patients with UTI were collected from Hilla Teaching hospital, Childhood and gynecology hospital during April 2015- October 2015. The cultured samples yielded thirty four isolates of Gram negative bacilli including 20 *E.coli* isolates and 14 *Klebsiellapneumonia* isolates. The isolation and identification of isolates were performed by standard bacteriological test (Forbes, 2007) and API-20 E assay. These isolates were tested for carbapenemases production.

Phenotypic Detection of Carbapenemases:

Detection of carbapenemases (KPC, MBL and OXA- 48): Tested isolates were sub-cultured in brain heart infusion broth and a suspension of 0.5 McFarland density was used to seed- inoculate Mueller Hinton agar plates (Himedia, India). After the incubated plates were left to dry for a short time, Rosco discs were applied and the plates were incubated at 35°C for 18-24 hours in ambient air incubator. After 24 hours of incubation, Zones of growth inhibition around Rosco discs zone sizes were recorded and differences in zone sizes were interpreted according to the manufacturer's instructions (ROSCO Diagnostica, Denmark) as shown in table 1.

Table.1. Rosco KPC/ metallo- beta- lactmase and OXA- 48 confirm kit interpretation as recommended by manufacturer based on increase in zone of inhibition compared to meropenem 10µg diffusion disc.

MRPDP	MRPCX	MRPBO	TEMO	Result
≤ 3	≥ 5	≥ 4		AMPC
≥ 5	≤ 3	≤ 4		MBL
≤ 3	≤ 3	≥ 4		KPC
≤ 3	≤ 3	≥ 4	Ø	OXA-48
≤ 3	≤ 3	≤ 3		ESBL

MRPDP: meropenem+dipidinic acid; **MRPCX**: meropenem+cloxacillin;
MRPBO: meropenem+phenylboronic; **TEMO:** temocillin.

Detection of *bla*Genes by Polymerase Chain Reaction:

DNA Extraction: Genomic DNA of bacteria isolates were isolated according to Gentrapuregene Bact. /kit (Qiagen/USA). Concentrations of DNA were measured by using Nano Drop-spectrophotometer.

Primer Preparation: The primers were synthesized at Accu Oligo / Bioneer /Korea. These primers were provided in a lyophilized form, which were re-dissolved with TE buffer (pH 8) or sterile distilled water to a final concentration of 100 picomoles, and stored at -20°C.

Primer Sequence: The primer sequence for *bla* genes is showed in table 2.

Table.2.The sequence and product size of forward and reverse primers used for amplification *bla* genes

Primer Name	5' – Sequence - 3'	Product size	Reference
bla-OXA-48(F)	GCGTGGTTAAGGATGAACAC	438 bp	Poirelet, 2011
bla-OXA-48(R)	CATCAAGTTCAACCCAACCG		
bla-IMP(F)	GAAGGCGTTTATGTTCATAC	587 bp	
bla-IMP(R)	GTACGTTTCAAGAGTGATGC		Pitoutet, 2005

PCR Conditions: The PCR reaction was performed in a total volum 30 µl containg 10 p mole/µl of each primer, 2x Taq PCR pre-mix (Sol Gent 2x Taq PCR Pre Mix, Sol Gent Co., Ltd.), and 200ng genomic DNA. The reaction mixture was amplified in a GTC thermal cycler (Cleaver Scientific, UK) .To detect *bla*genes (*bla*-OXA-48 and *bla-IMP*) a PCR program was followed as summarized in table 3.

Table.3.PCR conditions

Monoplex gene	Temperature (^{0}C)/ Time					# of cycle
	Initial denaturation	Cycling condition			Final extension	
		denaturation	annealing	extension		
bla-OXA-48	95/ 5 min	95/45 sec	60/45 sec	72/1 min	72/ 8 min	35
bla-IMP	95/ 5 min	95/45 sec	60/45 sec	72/1 min	72/ 8 min	35

Gel Electrophoresis: The amplified PCR products were checked for the expected size on 2% (w/v) agarose gel and visualized after staining with ethidium bromide under ultraviolet exposure. A DNA molecular weight marker (Gene aid/ Korea) was used to estimate the weight of the fragments (Sambrook, 2001).

3. RESULTS AND DISCUSSION

Thirty four isolates collected from midstream urine samples of 87 uncomplicated UTIs patients were identified by using cultural, morphological, and biochemical tests. The isolates included 20 *E.coli* and 14 *Klebsiella pneumoniae*. These isolates were tested for the production of carbapenemase using Rosco discs Fig.1.

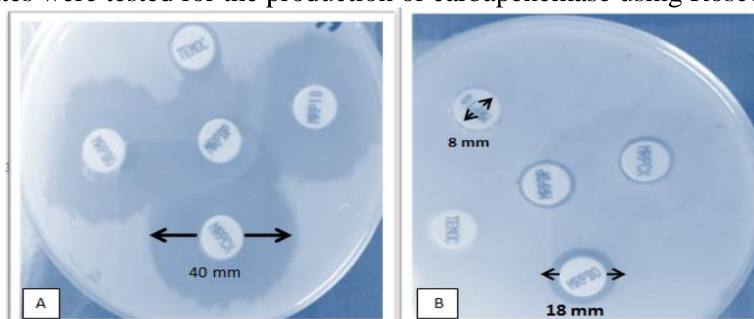

Figure.1.A represent positive results while B represent negative results

In PCR experiments using specific primers for *bla* IMP and *bla*-OXA-48,the *bla* OXA-48 genes was detected in 5(25%) of the *E. coli* isolates Figure 2. While 3 (21.4%) and 5 (35.7%) of the *K. pneumonia* isolates were positive for *bla* OXA-48 and*bla-IMP* genes respectively Figure 3 and Figure 4.

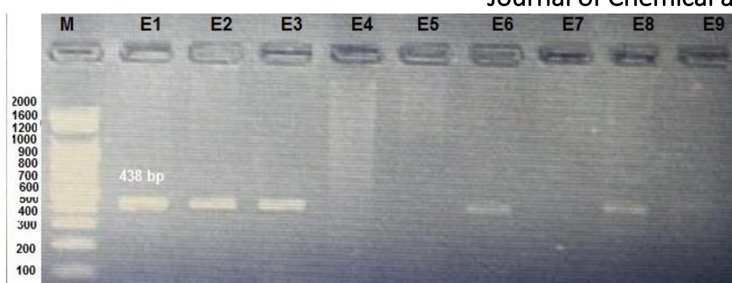

Figure.2. Electrophoresis of *E. coli* for detection of *bla*OXA-48 gene (438 bp) using 1% agarose for 1 hour at 70 volt.

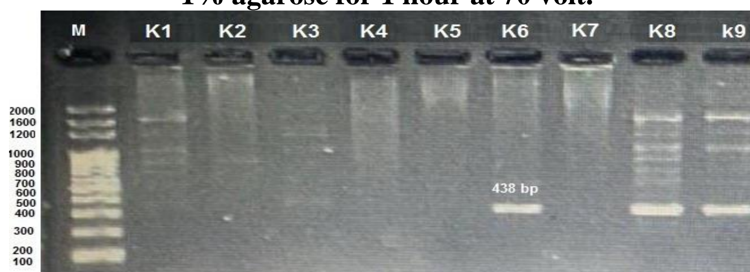

Figure.3. Electrophoresis of *Klebsiella pneumonia* for detection of *bla* OXA-48 gene (438 bp) using 1% agarose 1 hour at 70 volt

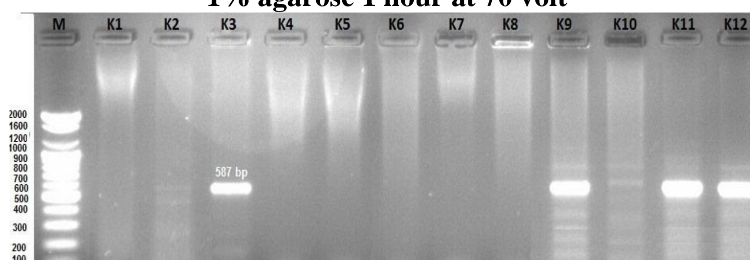

Figure.4. Electrophoresis of*Klebsiella pneumonia* for detection of*bla-IMP* gene(587 bp) using 1% agarose1 hour at 70 volt

In the present study, 34 isolates from patients with UTI, including 20 *E. coli* and 14 *K. pneumonae* were screened for carbapenemase production by Rosco discs and PCR assay. The results revealed that out of the thirty four clinical isolets, 20 *E. coli* isolates did not contain bla_{IMP} gene, neither did 2 *K. pneumonia* isolates have bla_{IMP} gene. However, $bla_{OXA\text{-}48\ like}$ genes, were detected in 5 *E. coli* isolates and 3 *K. pneumonia* isolates. A single *K. pneumonia* isolate (E9) was positive for both of bla_{IMP}, $bla_{OXA\text{-}48\ like}$ and screen tests as shown in table 4.

Carbapenems belong to the β-lactam group of antibacterial agents that consists of (imipenem, meropenem, doripenem, ertapenem, panipenem and biapenem). Emergence of carbapenemases in *Enterobacteriaceae* and nonfermentative bacteria poses a serious therapeutic problem in hospitals because carbapenems are often the antibiotics of last resort for the treatment of serious infections caused by multidrug-resistant Gram-negative bacteria. These bacteria have the potential to spread rapidly within the hospital environment and also across the continents (Nagaraj 2012). β-lactam antibiotics, particularly the third generation cephalosporins, are the most important drug class used to treat serious community-acquired or nosocomial infections caused by *E. coli* (Pitout, 2012).

It is argued that the main reservoirs of OXA-48-harbouring *K. pneumonia* and *E. coli* are in the countries of North African and Turkey (Nordmann and Poirel, 2014). The successful spread linked to a single self-conjugative of blaOXA-48 is 62 kb IncL/ M plasmid which was so far only identified in Enterobacteriaceae (Poirel, 2012). More recently, a chromosomal location of bla OXA-48 was reported from isolates in the UK, France, Egypt, Lebanon and Switzerland (Beyrouthy, 2014), it was noticed in fowl not only among humans (Al Bayssari, 2015). Philippon (1994), reported the comparison between ESBL producing strains and non ESBL producers reporting showed that ESBL-producers were significantly more resistant to cephalosporins, quinolones, aminosides, trimethoprim/sulfamethoxazole and amoxicillin/clavulanic acid than non-ESBL methods. The genes encoding ESBLs are normally located in transferable plasmids that may also show other resistance determinants, such as those prevent to chloramphenicol, trimethoprim, sulphamides, aminoglycosides, tetracyclines, and quinolones.

Table.4.Screened for Carbapenemase production by Rosco discs and PCR assay

Isolates	*bla*$_{IMP}$		*bla*$_{OXA-48\ like}$		Isolates	*bla*$_{IMP}$		*bla*$_{OXA-48\ like}$	
	Screen test	PCR	Screen test	PCR		Screen test	PCR	Screen test	PCR
E1	Negative	Negative	Positive	Positive	E18	Negative	Negative	Negative	Negative
E2	Negative	Negative	Positive	Positive	E19	Negative	Negative	Negative	Negative
E3	Negative	Negative	Positive	Positive	E20	Negative	Negative	Negative	Negative
E4	Negative	Negative	Negative	Negative	K1	Negative	Negative	Negative	Negative
E5	Negative	Negative	Negative	Negative	K2	Positive	Negative	Negative	Negative
E6	Negative	Negative	Positive	Positive	K3	Positive	Positive	Negative	Negative
E7	Negative	Negative	Negative	Negative	K4	Negative	Negative	Negative	Negative
E8	Negative	Negative	Positive	Positive	K5	Negative	Negative	Negative	Negative
E9	Positive	Negative	Negative	Negative	K6	Negative	Negative	Positive	Positive
E10	Negative	Negative	Negative	Negative	K7	Negative	Negative	Negative	Negative
E11	Negative	Negative	Negative	Negative	K8	Negative	Negative	Positive	Positive
E12	Negative	Negative	Negative	Negative	K9	Positive	Positive	Positive	Positive
E13	Negative	Negative	Negative	Negative	K10	Negative	Positive	Negative	Negative
E14	Negative	Negative	Negative	Negative	K11	Negative	Positive	Negative	Negative
E15	Negative	Negative	Positive	Negative	K12	Negative	Positive	Negative	Negative
E16	Negative	Negative	Negative	Negative	K13	Positive	Negative	Negative	Negative
E17	Negative	Negative	Positive	Negative	K14	Negative	Negative	Negative	Negative

4. CONCLUSION

Carbapenemase genes in clinical isolates of *K. pneumoniae* and *E. coli* from urine of UTI patients should provide impetus to clinicians in consideration of appropriate antimicrobial treatment of UTI caused by *E. coli* and *K. pneumonia*.

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