Detection of *bla*OXA-23 Gene Dissemination among Various Bacterial Pathogens Isolated from Urinary Tract Infection (UTI) in Al-Najaf Al-Ashraf

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Abstract

This study aimed to explore the dissemination of bla_{OXA-23} gene among bacterial causative agents of urinary tract infections. A total of 54 samples of urine from patients with Urinary Tract Infection (UTI) during the period from November, 2021 to March, 2022, out of 36 samples were positive growth. Gram-negative bacteria were 27 (75%) (Which include 11(31%) of isolates were identified as *E. coli*, 7(19%) of isolates identified as *K. pneumoniae*, 6(17%) *Acinitobacter bumanni*, 2(8%*) proteus merabilis,* and 1(3%) *Pseudomonas aeruginosa*.). While 9 (25%) were gram-positive isolates 6 (17%) were *S. aureus* and 3(5%) were *Enterococcus faecalis*. Carbapenems susceptibility was performed by disk diffusion method, where Meropenem antibiotic showed that out of 36 isolates 19 (55%) were resistant (6 (31.5%) *A. bumanni*, 3 (15.8%) *S. aureus*, 3 (15.8%) *E. feacalis*, 2 (10.5%) *E. coli*, 2 (10.5%) *K .pneumoniae*, 2 (10.5%) *P. mirabilis* and 1 (5.4%) *P. aeruginosa*), as for Imipenem antibiotic showed that out of 36 isolates 16 (45%) were resistant (6 (37.5) *A. bumanni*, 3 (18.7%) *K. pneumonia*, 3 (18.7%) *S. aureus*, 2 (12.5%) *E. coli*, 1 (6.3%) *P. merablis* and 1 (6.3%) *P. aeruginosa*.). Total DNA extract samples were used in bla_{OXA-23} gene detection, results showed that total *Acinetobacter baumannii* isolates(6) were positive for bla_{OXA-23} gene, then in *K. pneumoniae* out of 7 isolates 4 were positive for bla_{OXA-23} gene, out of 11 *E. coli* isolates 2 were positive for bla_{OXA-23} gene, in *Proteus mirabilis* and *pseudomonas auroginosa* isolates 2 and 1 respectively all were positive for bla_{OXA-23} gene, while in all isolates of *S. aureus* and *Enterococcus faecalis* bla_{OXA-23} gene was not detected. We conclude that various types of gram negative UTI causative agents harbored bla_{OXA-23}, meanwhile its absence in isolated gram positive isolates.

1. Introduction

UTI is a common disorder, accounting for 1%–3% of consultations in general medical practice. The prevalence of UTI in women is about 3% at the age of 20, increasing by about 1% in each subsequent decade. In males, UTI is uncommon, except in the first year of life and in men over 60, when it may complicate bladder outflow obstruction [1]. As reported by the National Ambulatory Medical Care Survey, UTI alone is responsible for nearly seven million patient visits in outpatient department (OPD) as well as up to one million visits in hospital emergency department, resulting in about 100,000 hospitalizations. Nearly 50–60% of all women suffer from an episode of UTI at least once in their lifetime [2]. Carbapenems are considered to be reliable and effective antibiotic agents against most pathogenic bacteria because of their broad antibacterial spectrum and are used in the treatment of serious nosocomial infections caused by cephalosporin-resistant bacteria [3].The bla_{OXA-23} gene belongs to the class D carbapenemase gene cluster that was considered as the first group of OXA-type B-lactamases conferring carbapenem resistance, capable of hydrolyzing broad-spectrum cephalosporins and carbapenems [4]. The bla_{OXA-23} gene can be encoded on the chromosome as well as within plasmids and has been associated with mobile genetic elements. The group was considered as the first group of OXA-type B-lactamases bla_{OXA-23} conferring carbapenem resistance and has been reported worldwide. It has gained clinical importance due to its wide dissemination worldwide, especially in strains of *Acinetobacter baumannii*, and was considered as the most common and predominant resistance gene within this bacterium [5]. Meanwhile, presence of the bla_{OXA-23} gene in *Escherichia coli* is unique and was reported once from Singapore [4].

2. Material and Methods

2.1 Isolation and Identification

A total of 54 samples were collected from non-duplicate patients with urinary tract infection. All were admitted to clinical laboratories, in Al-Najaf province/Iraq during the period from November, 2021 to March, 2022. Samples were manipulated as in (Collee, *et al*., 1996). Positive cultures then identified by the automated VITEK-2 compact system using ID-GN and GP cards.

2.2 Antibiotic Susceptibility Test

To test the susceptibility of carbapenems two agents

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(imepenem 10µg and meropenem 10µg) were used where the discs were provided by Bioanalyse Company (Turkey). The test was performed by the disk diffusion method [6].

2.3 Genomic DNA Extraction (Total DNA)

DNA extraction by the boiling method, bacterial suspensions were incubated at 99°C for 15 min in water bath, and immediately cooled on ice for 15 min then left to reach room temperature and centrifuged at 8000 rpm for five minutes, subsequently the supernatant contain DNA was collected in 1.5ml eppendorf tube (Yamagishi, *et al*., 2016).

2.4 Oligonucleotides and Polymerase Chain Reaction

The oligonucleotide primers provided by (Macrogen, Korea) were re-suspended according to the manufacturer's instructions as stock suspension. A working primer tube was prepared by diluting with TE buffer molecular grade. The final picomoles depended on the procedure of each primer. Template DNA and primers were thawed before use, PCR mixture contained the following constituents; 5 µl DNA template, Primer (F+R) 2µl for each, 12.5 µl Master mix, and completing the volume with Molecular grade water to yield 25 µl as final volume. The PCR cycling program parameters conditions were; initial denaturation at 94 $C^0$for 3 minutes with 35 cycles of denaturation 95C^0/25sec, annealing 52 C^0/45 seconds and extension 72 C^0/50 seconds followed by final extension step with 72 C^0 for 5 minutes [7].

3. Results and Discussion

3.1 Isolation and Identification

The data revealed that the percentage of total growth of bacterial isolates from non-repetitive 54 patients were 36 (66.6%) gave positive bacterial growth, while 18 (33.4%) sample were with no growth. Out of 36 isolates, gram-negative bacteria were 27 (75%) and gram-positive bacteria were 9 (25%).

According to the results of the characteristics of colony morphology, microscopic examination and VITEK-2 system, it was revealed that out of total 36 clinical samples collected during the study period, out of 27 gram-negative isolates 11(31%) of isolates were identified as *E.coli*, 7(19%) of isolates identified as *K. pneumoniae*, 6(17%) *Acinitobacter baumannii*, 2(8%*) proteus merabilis,* and 1(3%) *Pseudomonas aeruginosa*, while out of 9 gram-positive isolates 6 (17%) were *S. aureus* and 3(5%) were *Enterococcus faecalis*. This result agreed with (Flores-Mireles, *et al*., 2016), were *E. coli* the first causative agent (65%) followed by *K. pneumoniae* (8%) while *S. aureus*, *E. faecalis* (3%) and (11%) respectively.

In current study the antimicrobial susceptibility of 36 isolates to some carbapenems antibiotics were evaluated. The results revealed different resistance phenotypes for 2 disk antibiotics belong to carbapenems class (Imipenem and Meropenem) (Figure-1). The antimicrobial testing of UTI isolates for Meropenem antibiotic showed that out of 36 isolates 19 (55%) were resistant (6 (31.5%) *A. bumanni*, 3 (15.8%) *S. aureus*, 3 (15.8%) *E. feacalis*, 2 (10.5%) *E. coli*, 2 (10.5%) *K .pneumoniae*, 2 (10.5%) *P. merablis* and 1 (5.4%) *P. aeruginosa*), as for Imipenem antibiotic showed that out of 36 isolates 16 (45%) were resistant (6 (37.5) *A. bumanni*, 3 (18.7%) *K. pneumonia*, 3 (18.7%) *S. aureus* , 2 (12.5%) *E.coli*, 1 (6.3%) *P. merablis* and 1 (6.3%) *P. aeruginosa*.).

Antibiotic activity for 36 isolates For Meropenem revealed 10 (27.7%) and 7 (19.4%) were sensitive and intermediate respectively while for Imipenem revealed 13(36.1%) and 7 (19.4%) were sensitive and intermediate respectively. (Corrêa, *et. al* 2012) revealed that Imipenem and Meropenem resistance rates were 71.4% and 69.7%, respectively. Which agree with current study where incidence of high resistance rates in both Imipenem and Meropenem (16 (45%) and 19 (55%)) respectively.

3.2 Molecular Detection of blaOXA-23 Gene among UTI Isolates

The results of PCR amplification of *bla*$_{OXA-23}$ gene were revealed that out of 36 isolate, there were 15 (41.7%) isolates positive for *bla*$_{OXA-23}$ gene.

Figure (1) PCR result of *bla*$_{OXA-23}$ gene detection showed *Acinitobacter bumanni* 6 isolates all were positive for *bla*$_{OXA-23}$ gene, then in *K. pneumoniae* out of 7 isolates 4 were positive for *bla*$_{OXA-23}$ gene, out of 11 *E.coli* isolates 2 were positive for *bla*$_{OXA-23}$ gene, in *Proteus merablis* and *pseudomonas auroginosa* isolates 2 and 1 respectively all were positive for *bla*$_{OXA-23}$ gene, while in all isolates of *S. aureus* and *Enterococcus faecalis bla*$_{OXA-23}$ gene was not detected, and for The *bla*$_{OXA-23}$ gene was significantly detected in gram negative bacteria accounting for 15 isolate were positive to *bla*$_{OXA-23}$ gene, while gram positive bacteria were negative for *bla*$_{OXA-23}$ gene. A native study by [8] showed that all isolates of *A. baumannii* were positive for *bla*$_{OXA-23}$ gene. The results agree with (Al Atrouni, *et al*., 2016) mentioned that out of 119 *A. baumannii* isolates, 76.5% were resistant to carbapenems. The most common carbapenemase was the OXA-23-type, found in 82(69%) isolates.

While in Hilla province [9] recorded that the presence of *bla*$_{OXA-23}$ gene was 15 (88.2%) of *K. pneumoniae* isolates. This result was consistence with current study where *bla*$_{OXA-23}$ gene occurrence in *K. pneumoniae* isolates was (57.1%)

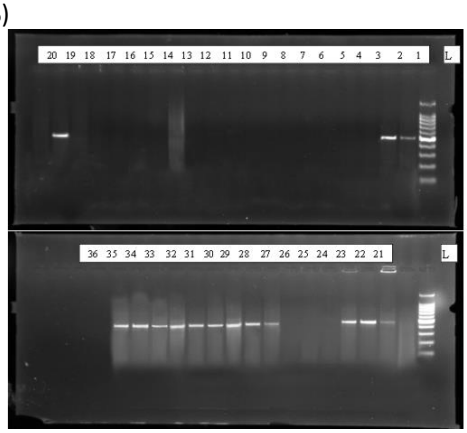

Figure-1: Electrophoresis diagram of Monoplex PCR amplified products for extracted plasmid DNA of 36 **isolats**

from patients with UTI using specific OXA-23 primer show positive products at 501 bp. Line L, molecular size marker,

and products migrated at 75 volt for 90 minutes and stained with ethidium bromide. *E. coli* (1-11), *S. aureus* (12-17), *K. pneumonia* (18-24), *E. faecalis* (25-27), *P. merablis* (28-29), *P. aeruginosa* (30) and *A. baumannii* (31-36). Ladder (1000 bp).

4. Conclusion

From all that we conclude that all bacterial isolates from UTI showed high resistance to carbapenems where *Acinitobacter bumanni* was the highest in bla_{OXA-23} gene prevalence, which represent a high risk of health in hospitals.

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