ORIGINAL ARTICLE

MOLECULAR CHARACTERIZATION OF MBL IN UROPATHOGENIC *E. COLI* ISOLATED FROM PATIENTS OF TERTIARY CARE HOSPITAL

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Background: Antibiotic resistance is on an increasing trend, particularly in gram-negative bacteria. The production of metallo β-lactamase (MBL) puts the health sector at great risk as it further limits the treatment option for MDR strain. The current study aims to determine the prevalence, antibacterial sensitivity pattern, and molecular characterization of MBL in Uropathogenic *E. coli* from clinical samples of hospitalized patients in Khyber Pakhtunkhwa. **Methods:** From tertiary care hospitals in Peshawar, 250 Urine samples were collected from indoor hospitalized patients. Gold standard microbiological methods were used to identify UPEC from these clinical samples.For that,urine samples was inoculated onto Cysteine Lactose Electrolyte. Deficient (CLED) agar plate, and MacConkey Agar.Positive growth of *E. coli* identified through Gram staining, colony morphology, Biochemical Tests and *E.coli* 16srRNA gene amplification .Antibiotic sensitivity was determined by the disc diffusion method on Muller Hinton agar. For the detection of MBL production double disc synergy, and a combination disc test of the antibiotics were used. Furthermore, multiplex PCR was used for the molecular characterization of the MBL (bla_{IMP}, bla_{VIM}, and $bla_{NDM)}$ genes. **Results:** Of the 250 samples, only 110 samples were confirmed as Uropathogenic *E. coli* based on colonial morphology, biochemical testing, and molecular level by targeting the 16SrDNA. Female was found more susceptible to UTI compared to male. High prevalence was found in the age group 45–65 years. UPEC was found highly resistance to Ciprofloxacin (90%), followed by Cefotaxime and Ceftriaxone (86%), Ceftazidime and Augmentin (81%), Tazobactam (61%). while the lowest resistance was reported against Meropenum (20%) Imipenem (18%) and Amikacin (37%). PCR-based confirmed prevalence of MBL encoding genes was $bla_{NDM\ (}$42%), bla_{VIM} (32%), and bla_{IMP} (26%). **Conclusion:** The study proposed a higher prevalence of urinary tract infections (UTIs) in females aged group 54–65 years compared to males. An analysis of antibiotic sensitivity revealed Imipenem and Meropenem to be the most effective antimicrobial agents, while Ciprofloxacin, Cefotaxime and Amoxicillin were found to be the less effective. UPEC were found highly resistance to Ciprofloxacin 91%, and ceftazidime 86%, while comparatively less resistance to meropenem, and imipenem,20% and 18% respectively. Genotype BlaNDM of the MBL is highly prevalent (42%) among UPEC.Furthermore, the presence of MBL genes was detected in over 19% of UPEC, and in different combinations.The upraise of the MBLs resistance in uropathogenic *E. coli* is an alarming sign for clinicians to decide on treatment options for complicated UTIs.

Keywords: Urinary tract infection; *Escherichia coli*; Extended spectrum Beta lactamase (ESBL); Metallo beta lactamase (MBL)

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INTRODUCTION

Urinary tract infection (UTI) is a common public health problem particularly in developing countries due to the lack of health education and hygiene practices. It is the second leading cause of morbidity after respiratory tract infection worldwide. Predisposing factors for UTI include prolonged hospital stay, catheterization, female gender, diabetes, children, and very old age. UTI is mostly caused by uropathogenic *Escherichia coli* (UPEC).[1] The UPEC is responsible for more than 95% of community-acquired, and about 80 % of hospital-acquired UTIs while it also causes 3.6–12.6% of complicated UTIs that often lead to urosepsis.[2]

For a long time, the empirical therapy of UTI due to *E. coli* was relayed on the β-lactam antibiotics. However, its widespread uses in agriculture, veterinary and human medicine resulted in the emergence of drug-resistant bacterial strains.[3] These drugs resistance bacteria particularly the ones that produce extended-spectrum β-lactamase (ESBL), and Metallo- β-Lactamase (MBL) also known as carbapenemase

pose a great risk to public health all over the world.[4] Therefore, antibiotic resistance surveillance on a regular basis is necessary to report the newly emerging and dissemination of drug resistance mechanisms and to refine the treatment guideline for empirical antimicrobial therapy.[8] According to the Amber classification, beta-lactamases are classified into (A, B, C, and D). MBL falls in class B, which further subdivides into B1, B2, and B3. Most commonly reported MBLs like IMP, VIM, SIM, GIM, and NDM belong to the B1 class.[5,6]

Initially, it was proposed that the MBLs encoded genes are found on chromosomes, but now plasmid-mediated MBLs have also been reported around the globe.[7] The MBLs enzyme renders the activity of beta-lactam antibiotics by hydrolyzing the amide bond that links with the beta-lactam ring. Thus, they not only degrade the carbapenem group of antibiotics but also the rest of the beta-lactam antibiotics excluding the monobactam antibiotics (9). Furthermore, the worldwide spread of MBL is due to horizontal gene transfer and the lack of proper MBL inhibitors. Thus MBLs act as a potential weapon of bacteria against the antibiotics that enable the bacterial community to survive in the presence of antibiotics.[10]

Keeping the importance of the drug resistance issues, the current study aimed to determine the prevalence of the MBLs encoding genes in uropathogenic *E. coli* and to find the association of the MBL genotypes with commonly prescribed antibiotics for UTI.

MATERIAL AND METHOD

One hundred and ten **(**110) positive *E. coli*. Calculated by sample size calculator with prevalence of MBL producing *E.coli* as 7.0% based on reference study(8)with assumption of 95% confidence interval and margin of error as 5%.

It was a cross-sectional study carried out at Hayatabad Medical Complex, Peshawar and processed in microbiology laboratory of IPDM, Khyber Medical University, Peshawar in a duration of six months. In the study the Non-probability convenient sampling techniques were used.Inclusion criteria: Patients having UTI due to Uropathogenic E. coli. Patients willing to participate in the study.

Exclusion criteria:Gram Negative bacteria other than E-coli. Patients under the treatment of antibiotics. Patients not willing to participate.

Ethical clearance was taken from ethical committee of KMU, Peshawar and the concerned hospital and consent was taken from hospitalpatient/attendant.

A total of 250 urine midstream samples were collected from hospitalized patients in a leak-proof sterile urine container at Hayatabad Medical Complex (HMC) Peshawar. The samples were transported to the microbiology lab of the Khyber Medical University (KMU) Peshawar for further analysis.

All the samples were processed for the identification of uropathogenic *E. coli* following a standard technique that is colonial morphology, gram staining, motility, and biochemical tests.[11] Antibiotic susceptibility tests (AST) were performed using the Kirby-Bauer disc diffusion method (12). The antibiotic discs used in the study were Meropenem, (MEM-10ug), Imipenem (IMP-10ug), Pipra/Tazobactum (TZO-100/10 ug), Ciprofloxacin (CIP-30ug), Ceftriaxone (CRO-10ug), Cefotaxime (CXT-30ug), Ceftazidime (CAZ-30ug), Ampicillin (AMP-10ug), and Amikacin AK -30ug) provided by thermos-Scientific™ Oxoid™ UK. The result of the AST was interpreted according to the recommendation of the Clinical and Laboratory Standard Institute 2022 (CLSI-2022) (13) using *E. coli* ATCC25922 as a standard.

Double disc diffusion synergy was used for the identification of MBL production following the CLSI-2022 guideline. Carbapenem (Meropenem, and Imipenem) was selected for this. The zone of inhibition was measured by comparing the zone of meropenem alone with that of Meropenem + EDTA.

For detection of MBLs genotype in *E. coli*, the plasmid was extracted via thermos scientific plasmid DNA extraction kit, following the manufacturing guideline. The confirmation of plasmid was done on 1.5% agarose gel and was stored at -20 ^{0}C for further use. Polymerized chain reaction (PCR) was performed to determine the Metallo beta lactamases enzyme genes using specific reaction conditions, and primers. The primers used for MBL genes were blaVIM, blaIMP, and blaNDM.[14] The details of the primer sequence and PCR reaction condition are listed in the below table. The MBLs genes were confirmed on the basis of their product size by running it on 1.5% agarose gel with 100 pb ladder as control.

RESULTS

Out of 250 samples, 110 samples were confirmed for UPEC with the help of colonial morphology, gram staining, biochemical testing, and molecularly PCR based by targeting the 16SrRNA of UPEC. The prevalence of UTI was found higher in females

(62%) compared to their male counterparts (38%), with the most susceptible age groups being 55–64 years which was 32.7%, (36) followed by the age group 45–54 years at 23.6% (25), while the lowest incidence of UTI was reported in the age group 15–24 years is 5.5% (6).

The antibiotics susceptibility profile of UPEC was checked against the nine antibiotics that are commonly prescribed for the treatment of UTI both in hospital setup and community. UPEC shows high resistance to Ciprofloxacin 90% followed by 86% to cefotaxime and ceftriaxone, 81% to co. amoxiclav, 81% to ceftazidime, 61% to tazobactam, and 37.2% to amikacin, while lowest although comparatively high resistance to meropenem 20% and Impenum 18%. Out of 110 UPEC samples, 21(19.09%) were phenotypically confirmed as MBL producers. A total of 21 uropathogenic *E. coli* were screened for the presence of plasmid-mediated metalo- beta-lactamases genes ($bla_{IMP,}$ $bla_{VIM,}$ and $bla_{NDM).}$ The genome size of the Bla_{IMP} (260bp), bla_{VIM} (350bp), and bla_{NDM} (740bp). The Amplified product of the gene bla_{IMP} is compared with the 100bp DNA ladder shown in the below figure.

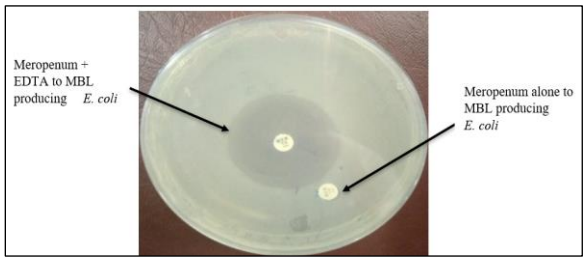

Figure-1: Molecular detection of MBL producing genes in uropathogenic *E. coli*

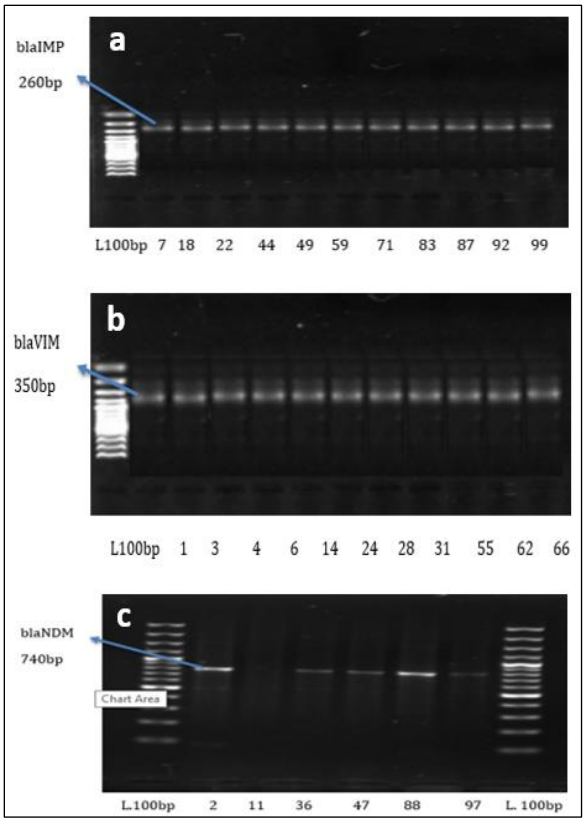

Figure-2: (a), shows the PCR amplified product (260bp) of IMP gene against 100bp leader, (**b)**, VIM gene products (350bp) with 100bp leader, and (**c)**, are of NDM gene products (740pb) with 100bp leader run on 1.5% agarose gel. Among the 110 UPEC isolates, 19% were phenotypically confirmed as MBL producers which harbored $bla_{NDM,}$ gene(42%), blavim gene(32%) harbored bla_{IMP} (26%).

Table-1: Details of primer size, annealing temperature, and target genes.

Target Gene	Primers	Primer Sequence (5-3P)	Size	Annealing Temp	Reference
NDM	NDM	F: GGTTTGCGATCTGGTTTTC R: CGGAAATGGCTCATCACGATC	780bp	53C	(7,14)
VIM	VIM	F: CGAATGCGCACCAG R: TGGTGTTTGGTCGCAAT	350bp	52C	(7,14)
IMP	IMP	F: GTTTAACAAAACAACCACC R: GGAATAGAATGGCTTAACTCT	260bp	52C	(7,14)

Table-2: Frequency and distribution of UTI among males and females with respect to different age groups:

Parameters	Frequency of UTI	Frequency of UTI	Male (%)	Female (%)
Gender		38%	62%	
Age wise prevalence	15-24 years		0 %	5.5 %
	25-34 years		0 %	9%
	35-44 years		4.5%	10%
	45-54 years		9%	14.5%
	55- 64years		10%	22.7%
	65 years and above		14.5 %	0%

Table-3: Antibiotic susceptibility profile of Uropathogenic *Escherichia coli*.

Antibiotics name	Sensitivity	Intermediate sensitive	Resistance
Amikacin	63 (57.2%)	6 (5.45%)	41 (37.2%)
Imepenum	88 (80%)	2 (1.8%)	20 (18.1%)
Meropenem	86 (78.1%)	2 (1.8%)	22 (20%)
Ceftazidime	17 (15.5%)	3 (2.7%)	90 (81.8%)
Ceftriaxone	15 (13.6%)	0 (00%)	95 (86.3%)
Cefotaxime	15 (13.6%)	0 (00%)	95 (86.3%)
Amoxicillin and clavulanic	18 (16.3%)	3 (2.7%)	89 (80.9%)
Piperacillin/tazo	40 (36.3%)	2 (1.8%)	68 (61.8%)
Ciprofloxacin	9 (8.1%)	2 (1.8%)	99 (90%)

DISCUSSION

Urinary tract infection is a global health problem that is most prevalent in females and children. The emergence of drug resistance particularly the acquisition of MBL among the pathogen that causes UTIs puts the human population at great risk mainly in the developing world where health facility is already in overwhelming situation.[15] Uropathogenic *E. coli* is the predominant organism that causes UTI, in all age groups male, and female, both in the community population and in hospital setup. Surveillance study on antimicrobial among uropathogenic organism is scars in developing countries.[16] In this study high prevalence of UTI was found in females (62%) compared to males (38%), particularly at the reproductive stage of life with the predominant organism being *E. coli* as 44%. This finding is in agreement with a study conducted in Iran 43.5, Nigeria 44.1%, and India 44%, but lower than a study in Poland where UPEC was 58% in HIV-positive patients (17–19, 23). In the current study, UPEC shows high resistance to Ciprofloxacin 90% followed by 86% to cefotaxime, ceftriaxone, and 81% to co. amoxiclav, 81% to ceftazidime. In close proximity results to this study were reported from India, and Turkey where resistance to Ciprofloxacin was above 90%, while to cephalosporin 2nd, 3rd, and 4th generation was between 75% to 88%. Although a slightly lower rate of resistance was reported among UPEC to cefotaxime 77%, ceftriaxone, 78%, and ceftazidime 72% from other parts of Pakistan.[20–21]

Resistance to imipenem and meropenem was among the lowest 18% and 20% respectively. Higher resistance was reported to Imipenem 34.5% in India.[23] While the lowest 8% resistance to each antibiotic has been reported from Mexico.[24] In the current study, MBL production was confirmed phenotypically among 21 (19.09%) of the clinical isolate in contrast to our study a high result was reported from Saudi Arabia 62.5% phenotypically while Mexico, had the lowest resistant rate of 8%, and 10% although Iran, and India having similar resistance pattern 26% and 34%.

Metalo-beta-lactamase synthesis is an important mechanism for the resistance to carbapenem group of antibiotics, several genes are responsible for the synthesis of such enzyme. We included three genotypes of this enzyme in our study that is $bla_{NDM,}$ $bla_{VIM,}$ and $bla_{IMP.}$ The prevalence of different MBL genotypes in this study is bla_{NDM} 42%, $bla_{VIM,}$ 32%, and bla_{IMP} 26%. Different MBL genotype prevalence has been reported from different countries even different centers. But a similar pattern of MBL genotype prevalence has been reported from India where Bla_{NDM} at 63% followed by Bla_{VIM} at 18.6% and less than 10% $Bla_{IMP.}$[23] In China higher prevalence was found of Bla_{NDM} 83% and Bla_{IMP} 17% while Bla_{VIM} was not included in the study.[25]

CONCLUSION

Based on the data, there is a higher prevalence of urinary tract infections (UTIs) in females aged group 54-65 years compared to males. An analysis of antibiotic sensitivity revealed Imipenem and Meropenem to be the most effective antimicrobial agents, while Ciprofloxacin, Cefotaxime and Amoxicillin were found to be the less effective. UPEC were found highly resistance to Ciprofloxacin 91%, and ceftazidime 86%, while comparatively less resistance to meropenem, and imipenem,20% and 18% respectively. Genotype BlaNDM of the MBL is highly prevalent (42%) among UPEC. Furthermore, the presence of MBL genes was detected in over 19% of UPEC, and in different combinations. Additionally, some isolates indicated the phenotypic and genotypic occurrence of carbapenem resistance genes. This situation is a cause for concern, highlighting the need for antibiotic prescriptions to be based solely on clinical diagnosis via urine culture. The current situation is worse, required surveillance study across the country. Antibiotics prescription must be made based on culture sensitivity to avoid spread of

Recommendations:

Animal husbandry practices should be closely monitored for antibiotic usage, and routes of transmission should be identified to prevent transmission between animals and humans. Over-the-

counter availability of antibiotics is a major concern in the generation of drug resistance among pathogens. Furthermore, environmental dissemination of antibiotics through various routes could lead to the emergence of XDR and MDR pathogens.

AUTHORS' CONTRIBUTION

SA, AAK: Conceptualization of the study design, literature search. MA, NT: Data collection, data analysis, interpretation. SA, MA, IU: Write-up, proof reading.

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