

ORIGINAL ARTICLE

Antibiogram and Frequency of BLA-NDM-1 Gene in E.Coli Isolated from Carbapenem Resistant Cases of UTI Patients

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ABSTRACT

Background: Urinary tract infections (UTI) are very common in all the population around the world. Women are the usual victims of the super bug *Escherichia coli* (*E.coli*) and usually suffer from UTI once in a lifetime. The multi-drug resistant (MDR) *E.coli* is very intelligent creature and turned resistant to the antibiotics through transposons, mutations and plasmids. Most of the *E.coli* harbors the gene *bla*-NDM-1 due to which they have become highly resistant to Carbapenem antibiotics.

Objectives: To find the *bla*-NDM-1 gene in Carbapenem resistant *E.coli* isolated from UTI patients. To determine the frequency of Carbapenem resistant *E.coli* in patients attending tertiary care hospital of Peshawar.

Methodology: This study was conducted in Khyber Teaching Hospital Peshawar from January 2019 to June 2019. we collected 87 urinary *E.coli* isolates which were resistant to Carbapenem by disc diffusion method on Mueller Hinton Agar. Polymerase chain reaction (PCR) was done to unyield the presence of *bla*-NDM-1 gene in these 87 cases.

Results: According to this study *E.coli* was the most common causative agent for UTI and females suffered more than male patients. The antibiotics Tigecycline and Colistin showed 100% sensitivity against *E.coli*. Some of the antibiotics like Ampicillin; Meropnem etc were 100% resistant to *E.coli*. The gene *bla*-NDM-1 turned out to be positive in 26.43% of the cases.

Conclusion: This study concluded that all the *E.coli* isolated from UTI patients having *bla*-NDM-1 gene exhibit resistance to Carbapenem. These isolates are very difficult to treat and limited therapeutic options are available.

Keywords: Multi- drug resistant *Escherichia coli*, Carbapenemases, Antibiogram, Plasmids, Polymerase chain reaction, Transposons and Mutations.

INTRODUCTION

Urinary tract infections (UTI) are very common in our society and caused by a lot of microorganisms like *Escherichia.coli* (*E.coli*), *Proteus mirabilis*, *Staphylococcus saprophyticus* and *Kliebsiella. Pneumoniae*. *E.coli* is responsible for 80-90% cases of UTI globally. One thousand different antigenic varieties of *E.coli* occur globally. At least 150 million people suffer from UTI per year, among these 50-60% are females at risk of developing UTI once in a life time (1)

The aim of treating UTI is elimination of pathogens, relief of symptoms, decreasing risk of recurrences and preventing damage to genitourinary tract. Urinary *E.coli* strains are becoming alarmingly resistant to most of available antibiotics in Peshawar and worldwide (2).

The treatment in our society is started without doing urine culture and antimicrobial sensitivity testing, usually when the patients comes to the doctor, they are already on self-medication antibiotics. The misuse and abuse of over the counter available antimicrobials has led to emergence of multi drug resistant (MDR) strains of *E.coli* in our third world countries and worldwide (3).

Antibiotics should only be prescribed by the physicians and efforts should be done by health care workers to educate people about prevention of UTI (4).

There is rapid spread of antimicrobial drug resistance genes by the Carbapenemases *bla* (beta lactam antibiotics, New Delhi metallo beta lactamases) in Pakistan, India,

United Kingdom, Srilanka, China, Turkey and spreading globally by international travel. These genes are called *bla*-NDM-1, are carried on plasmids and there is serious concern for its dissemination in Peshawar due to lack of surveillance programs, infection control committees and diagnostic facilities (5).

The prevalence of *E.coli* patients in Khyber Pakhtunkhwa (KPK) is 24.2% and steadily increasing day by day because of *E.coli* becoming resistant to nearly all the available therapeutic strategies.

In this study we have investigated *bla*-NDM-1 gene in Carbapenem resistant *E.coli* isolated from UTI patients, generated an antibiogram against MDR urinary isolates of *E.coli* and determined the frequency of Carbapenem resistant *E.coli* in patients attending tertiary care hospital of Peshawar (6).

METHODOLOGY

It was an experimental study. The samples were taken from Khyber Teaching Hospital, Peshawar from January 2019 to June 2019. The samples were analyzed at Centre of Microbiology and biotechnology (COBAM) Peshawar. A total of 87 samples were taken by Non-Probability Convenient Sample Technique.

All the urine samples of *E. coli* isolates, which were resistant to Carbapenem were taken. The urinary samples were taken from male and female patients between the age of 18 to 60 years. Gram negative rods other than *E. coli*

and sensitive to Carbapenem antibiotic urinary samples were excluded from this study.

Antibiogram of urinary isolates of *E. coli* of urinary isolates was obtained by disc diffusion method. The extraction of DNA from Carbapenem resistant strains of urinary isolates of *E. coli* was done by using ThermoScientific Gene Jet Genomic DNA Purification kit. A 48 hours broth cultures were used from pure colonies of *E. coli* for DNA extraction.

The Carbapenem resistant gene blaNDM-1 was amplified by using conventional PCR machine LABNET INTERNATIONAL USA using specific forward primer F'GGCAGTCGCTTCCAACGGT and reverse primer R'GTAGTGCTCAGTGTCGGCAT were used.

Initial denaturation was done at 94C for 10 mins then again denaturation was done at 94C for 30 sec Annealing was done at 57C for 30 sec. Followed by extension for 45

seconds at 72C. Final extension was done at 72C for 7 mins. Cooling was done at 4C for 15 mins.

RESULTS

In this study we included 87 urinary isolates of *E. coli* resistant to Carbapenems. The urinary samples of patients in the age range of 18-60 years were included in the study. There were 32 (36.78%) male patient urine samples and 55(63.21%) female patient urinary samples. All the urinary *E. coli* isolates were tested against 18 antibiotics by disc diffusion method on Mueller Hinton agar, the urinary samples showed different behavior of resistance and sensitivity as seen in the antibiogram in Table 1. On PCR, out of 87 urinary samples of *E. coli* resistant to Carbapenems, bla-NDM-1 gene was positive in 23 (26.43%) cases as shown in Table 2.

Table 1: antibiogram of *e. coli* isolated from patients of uti (n= 87)

Antibiotics	Abbreviations	Sensitive (n)	%	Intermediate (n)	%	Resistant (n)	%
Amoxicillin-clvulanate	Amc	0	0	0	0	87	100
Ampicillin	Amp	0	0	0	0	87	100
Cefoparazone	Scf	59	67.8	6	6.8	22	25.28
Piperacillin-tazobactam	Tzp	54	62.06	7	8.04	26	29.88
Cefipime	Fep	0	0	0	0	87	100
Cefotaxime	Ctx	0	0	0	0	87	100
Ceftazidime	Caz	0	0	0	0	87	100
Aztreonam	Atm	0	0	0	0	87	100
Meropnem	Mem	0	0	0	0	87	100
Amikacin	Ak	73	83.90	5	5.74	9	10.34
Doxycycline	Do	32	36.78	5	5.74	50	57.47
Ciprofloxacin	Cip	40	45.97	8	9.19	39	44.82
Levofloxacin	Lev	42	48.27	8	9.19	37	42.52
Trimethoprim-sulfamethoxazole	Sxt	21	24.13	7	8.04	59	67.81
Chloramphenicol	C	36	41.37	8	9.19	43	49.42
Fosfomycin	Fos	46	52.87	5	5.74	36	41.37
Colistin	Co	87	100	0	0	0	0
Tigecycline	Tgc	87	100	0	0	0	0

Table 2: distribution of carbapenem resistant gene of *E. coli*

Carbapenem resistant gene	Frequency (n)	Percentage %
Bla- ndm-1	23	26.43

DISCUSSION

Urinary tract infections are very common all over the world and were reported for the first time in Egypt in 1550 BC. Out of 150 million people who suffer from this disease per year, 12.6 % are females while 3 % are males but this number is constantly on the rise (7). According to this study out of 87 urinary samples 63.21% were females and 36.78% were male patients.

Qiwen Yang Hui Zhang et al reported from China that 63.2% of UTI were due to *E. coli*, 12.2% due to *Klebsiella pneumoniae* (*K. pneumoniae*), 11.5% were microorganisms other than *Enterobacteriaceae*. While in this study all 87 cases of UTI were due to *E. coli* (8).

In this study the incidence of UTI was highest in the age group of people between 31-40 years (35.63%) followed by people in age group of 21-30 years (21.83%). The age group between 16 and 20 years showed 20.69% cases of UTI. 51-60 years old showed (12.64%) and 41-50 years of age showed (10.34%) cases of UTI in Peshawar. Menyfah Q. alanazai et al reported the incidence of UTI in

different age groups of Saudi Arabia (Riyadh). In children between ages of 0-12 years 32.29% cases were positive for UTI. In the age range of 13-64 years 23.34% of people had UTI. In people above 65 years 37.50% cases of UTI were reported (9).

In this study the *E. coli* urinary isolates showed 100% resistance to Carbapenem group of antibiotics which are more or less in accordance with the study conducted in Surabaya by Alimsardjono et al. They reported 97.7% resistance of UTI cases to Meropnem (10).

Sundaramoorthy et al reported 30.30% resistance to Cefoparazone-Sulbactam, compared to our study in which we reported 25.28% resistance to the antibiotics (11).

In our study 29.88% cases were resistant to Piperacillin –Tazobactam, while Sarah Mellisa Norgaard et al reported from USA 4% resistance in UTI patients (12).

We reported 67.8% UTI cases resistant to Trimethoprim-Sulfamethoxazole while Ian A.Critchley reported from America that 32.1% cases were resistant to the drug (13).

Ana Carolina C.Campos et al reported from Brazil that only 2% cases were resistant to Fosfomycin compared to 41.37% cases in this study showed resistant to the antibiotic (14).

In this study no cases were resistant to antibiotic Tigecycline, which seems to be a good treatment option, compared to Manju Raj Purohit et al reported 0.1% resistant to the antibiotic from India (15)

Mohammad Asaduzzaman et al reported from Bangladesh 24.8% resistance to Colistin while in our study there was 0% resistance to the antibiotic (16).

The bla-NDM-1 gene is responsible for most cases of Carbapenem resistant cases of E.coli causing UTI globally. In this study 26.43% cases were positive for the gene compared to the study conducted in Vietnam in which Cuong Q.Hoang et al reported 22% cases harboring bla-NDM-1 gene (17).

CONCLUSION

This study concluded that all the E.coli isolated from UTI patients having bla-NDM-1 gene exhibit resistance to Carbapenem. These isolates are very difficult to treat and limited therapeutic options are available.

REFERENCES

- Flores-Mireles AL, Walker JN, Caparon M, Hultgren SJ. Urinary tract infections: epidemiology, mechanisms of infection and treatment options. *Nature reviews microbiology*. 2015 May;13(5):269-84.
- Luna-Pineda VM, Ochoa SA, Cruz-Cordova A, Cazares-Dominguez V, Reyes-Grajeda JP, Flores-Oropeza MA, Arellano-Galindo J, Castro-Hernandez R, Flores-Encarnacion M, Ramirez-Vargas A, Flores-Garcia HJ. Features of urinary Escherichia coli isolated from children with complicated and uncomplicated urinary tract infections in Mexico. *PloS one*. 2018 Oct 4;13(10):e0204934.
- Ny S, Edquist P, Dumpis U, Gröndahl-Yli-Hannuksela K, Hermes J, Kling AM, Klingeberg A, Kozlov R, Källman O, Lis DO, Pomorska-Wesołowska M. Antimicrobial resistance of Escherichia coli isolates from outpatient urinary tract infections in women in six European countries including Russia. *Journal of global antimicrobial resistance*. 2019 Jun 1;17:25-34.
- Lee DS, Lee SJ, Choe HS. Community-acquired urinary tract infection by Escherichia coli in the era of antibiotic resistance. *BioMed research international*. 2018 Sep 26;2018.
- Mahalingam N, Manivannan B, Khamari B, Siddaramappa S, Adak S, Bulagonda EP. Detection of antibiotic resistance determinants and their transmissibility among clinically isolated carbapenem-resistant Escherichia coli from South India. *Medical Principles and Practice*. 2018;27(5):428-35.
- Somily AM, Habib HA, Absar MM, Arshad MZ, Manneh K, Al Subaie SS, Al Hedaithy MA, Sayyed SB, Shakoor Z, Murray TS. ESBL-producing Escherichia coli and Klebsiella pneumoniae at a tertiary care hospital in Saudi Arabia. *The Journal of Infection in Developing Countries*. 2014 Sep 12;8(09):1129-36.
- Jhang JF, Kuo HC. Recent advances in recurrent urinary tract infection from pathogenesis and biomarkers to prevention. *Tzu-Chi Medical Journal*. 2017 Jul;29(3):131.
- Zhang H, Yang Q, Liao K, Ni Y, Yu Y, Hu B, Sun Z, Huang W, Wang Y, Wu A, Feng X. Antimicrobial susceptibilities of aerobic and facultative gram-negative bacilli from intra-abdominal infections in patients from seven regions in China in 2012 and 2013. *Antimicrobial Agents and Chemotherapy*. 2015 Oct 19;60(1):245-51.
- Alanazi MQ, Alqahtani FY, Aleanizy FS. An evaluation of E. coli in urinary tract infection in emergency department at KAMC in Riyadh, Saudi Arabia: retrospective study. *Annals of clinical microbiology and antimicrobials*. 2018 Dec;17(1):1-7.
- Herdianty H, Alimsardjono L, Indiatuti DN. Resistance Patterns of Escherichia coli and Klebsiella pneumoniae Bacteria Against Amikacin, Ceftazidime, Meropenem, Nitrofurantoin Antibiotics in Elderly Patients with UTI in RSUD Dr. Soetomo. *JUXTA: Jurnal Ilmiah Mahasiswa Kedokteran Universitas Airlangga*. 2019 Jan 30;10(1):20-4.
- Vijayanapathy S, Karthikeyan VS, Mallya A, Mythri KM, Viswanatha R, Keshavamurthy R. Antimicrobial resistance patterns in a tertiary care nephro-urology center in South India. *Journal of Integrative Nephrology and Andrology*. 2018 Jul 1;5(3):93.
- Nørgaard SM, Jensen CS, Aalestrup J, Vandenbroucke-Grauls CM, de Boer MG, Pedersen AB. Choice of therapeutic interventions and outcomes for the treatment of infections caused by multidrug-resistant gram-negative pathogens: a systematic review. *Antimicrobial Resistance & Infection Control*. 2019 Dec;8(1):1-3.
- Critchley IA, Cotroneo N, Pucci MJ, Mendes R. The burden of antimicrobial resistance among urinary tract isolates of Escherichia coli in the United States in 2017. *PLoS One*. 2019 Dec 10;14(12):e0220265.
- Campos AC, Andrade NL, Ferdous M, Chlebowicz MA, Santos CC, Correal JC, Lo Ten Foe JR, Rosa AC, Damasco PV, Friedrich AW, Rossen JW. Comprehensive molecular characterization of Escherichia coli isolates from urine samples of hospitalized patients in Rio de Janeiro, Brazil. *Frontiers in Microbiology*. 2018 Feb 16;9:243.
- Purohit MR, Lindahl LF, Diwan V, Marrone G, Lundborg CS. High levels of drug resistance in commensal E. coli in a cohort of children from rural central India. *Scientific reports*. 2019 Apr 30;9(1):1-1.
- Asaduzzaman M, Baral K, Islam MM, Nayem A, Alam J, Juliana FM, Hossain N, Das B, Asma R, Tapati SJ. Susceptibility pattern of second line antibiotic colistin against gram negative bacteria causing urinary tract infection in selected areas Dhaka city, Bangladesh. *Eur J Biomed Pharm Sci*. 2018;5(3):874-9.
- Brink AJ, Coetzee J, Clay CG, Sithole S, Richards GA, Poirel L, Nordmann P. Emergence of New Delhi metallo-beta-lactamase (NDM-1) and Klebsiella pneumoniae carbapenemase (KPC-2) in South Africa. *Journal of clinical microbiology*. 2012 Feb;50(2):525-7.