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Research Article

ESBL PREVALANCE IN URINE SPECIMENSOF CANCER PATIENTS AT A TERTIARY CARE CANCER CENTRE IN KERALA

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ABSTRACT

Objective: The objective of this study documents the prevalence of extended-spectrum beta-lactamase (ESBL)-producing multiple drug resistance uropathogens reported prospectively for a period of 3 months from February 2020 to April 2020 and its susceptibility to the commonly recommended antibiotics for urinary tract infections (UTI).

Methods: Identification and characterization of the uro-pathogens from 200 samples were done by routine smear microscopy, culturing, Kirby–Bauer disc-diffusion assay, and double disc synergy test.

Results: Thirty percent of samples were culture-positive pointing *Escherichia coli, Klebsiella pneumoniae, Pseudomonas Aeruginosa,* and *Serratia marcescens* (51.9%, 36.5%, 9.61%, and 1.92%, respectively). Almost all ESBL producers were found to be multi-drug resistant that includes *E. coli* (56.6%), *K. pneumoniae* (36.74%) and *P. aeruginosa* (6.66%) warranting prompt need of surveillance for effective clinical management.

Conclusion: The current study pointed the emergence and incidence of ESBL producing Gram-negative bacilli that are multi-drug resistant and causing UTI among cancer patients.

Keywords: Extended-spectrum β-lactamase, Gram-negative bacilli, Urinary tract infection.

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INTRODUCTION

Cancer patients are inexplicably at ten times higher risk of developing infections than non-cancer patients. Urinary tract infections (UTI) are the second most commonly diagnosed infectious illness in cancer patients [1]. Untreated UTI can result in serious complications such as kidney damage, renal scarring, and renal failure.

UTI is commonly caused by Gram-negative bacteria (GNB) such as *Escherichia coli*, *Proteus* spp., *Klebsiella* spp., *Enterobacter* spp., *Citrobacter* spp. and also Gram-positive bacteria, *Staphylococcus saprophyticus*, *Enterococcus* spp. besides Coagulase-negative bacteria [2]. GNB-producing Extended-Spectrum Beta-Lactamase (ESBL) has emerged as a significant challenge to undertake with present antibiotics. The bacteria which are responsible for causing UTIs have more aggressive virulence factors that enhance their host cell attachment, colonization as well as virulence [1].

Drug-resistant microbes have been an escalating problem worldwide since the introduction of the first antibiotic in the 1940s. Although the antimicrobial susceptibility pattern among bacteria varies from country to country, UTI is treated often by broad-spectrum antibiotics, and treatment is started empirically without performing culture and sensitivity [3]. This inappropriate and non-judicious use of antibiotics has resulted in the emergence of multi-resistant strains of bacterial pathogens worldwide. According to a survey conducted by the European Survey of Antibiotic Consumption, multidrug-resistant (MDR) bacterial strains were accountable for a mortality rate of nearly about 25,000 Europeans/year usually due to complications during UTIs. Hence, it is necessary to circumvent the non-judicious use of antibiotics that lead to the emergence of antimicrobial resistance and the most appropriate antibiotics should have opted for first-choice empiric treatment of UTI [4].

Plasmid-mediated beta-lactamase-producing Gram-negative bacilli (GNB) were discovered inGreecein the 1960s. In 1983, plasmid-mediated beta-lactamases capable of hydrolyzing the third-generation cephalosporins, known as the extended-spectrum drugs were discovered. These enzymes are referred to as ESBLs and they confer resistance to most beta-lactam antibiotics, including the third-generation cephalosporins and monobactam antibiotics sparing the cephamycins [5]. Infections with these ESBL-producing organisms have been associated with poor outcomes. Currently, carbapenems constitute the best treatment option for infections caused by resistant organisms causing invasive site infections. A concern, however, is the difficulty of reliably identifying ESBL-producing organisms in many clinical laboratories, making it likely that their prevalence is underestimated and knowledge among clinicians still lacking. Phenotypic detection of ESBLs is based on the resistance they confer to oxyimino-beta-lactam substrates and the ability of a beta-lactamase inhibitor, usually clavulanate, to block this resistance [6]. Other acquired enzymes, notably AmpC-type beta-lactamases that are by plasmid as well as chromosomal genes, can provide oxyimino-beta- lactam resistance but are resistant to inhibition by clavulanate and confer resistance to cephamycins, which ESBLs donot.

The Clinical Laboratory Standards Institute (CLSI) recommends screening the isolates of *E. coli, Klebsiella pneumoniae, Klebsiellaoxytoca, and Proteus mirabilis* by disc diffusion (DD) and dilution antimicrobial susceptibility tests. The phenotypic confirmatory tests for ESBL production include Cephalosporin/clavulanate combination discs and the broth/agar dilution method demonstrating a synergistic activity between a cephalosporin and a beta-lactamase inhibitor. Other methods of ESBL detection are the double-DD tests [the agar supplemented with clavulanate] the disk replacement method, and the three-dimensional test. Commercially available methods for ESBL detection include the E-test for ESBL.

This study is aimed at documenting the prevalence of ESBL producing multiple drug resistance uropathogens and their susceptibility to the antibiotics generally used for the treatment of UTI.

METHODS

The study was conducted among Cancer patients in the Microbiology division of a tertiary cancer Centre. It is a hospital-based cross-sectional study that was carried out among 200 urine specimens from medical, surgical, and allied super-specialty units. The factors like sex, age, date of admission, diagnosis, etc. were also recorded.

This study was carried out for 3 months from February 2020 to April 2020. All Urine samples received in the microbiology lab during the study period were analyzed.

Samplecollection

Patients were instructed to self-collect the urine specimens by following the standard methods with aseptic precautions.A clean catch mid-stream urine sample that was collected aseptically in a wide mouth leak-proof specimen container was transported to the laboratory for culture.

Urine specimens were inoculated on Blood Agar, MacConkey agar, and Cysteine lactose deficient medium agar using a calibrated loop. A quantitative culture method was used to inoculate on the culture plates. The inoculated culture plates were incubated at 37°C for 18–24 h. After reading and interpreting the culture results, the clinically significant isolates were identifiedas per the Manual of Clinical Microbiology [4].

Determination of ESBL production using double disc synergy test (DDST)

DDST was used to verify the ability of an organism to produce an ESBL enzyme on Muller – Hinton agar (MHA) using third-generation cephalosporin (Ceftazidime and Cefotaxime). At least three to five well-isolated colonies of the same morphological type were selected from an agar plate culture. The top of each colony was touched with a loop, and the growth was transferred into a tube containing 4–5 mL of a suitable broth medium such as peptone water. The culture was incubated at 37°C. The turbidity of the actively growing broth culture was adjusted with sterile saline or broth to obtain turbidity optically comparable to that of the 0.5 McFarland's standard by visually comparing the inoculum tube and the 0.5 McFarland'sstandard. Optimally within 15 min, a sterile cotton swab was dipped into the adjusted suspension. The swab was rotated several times and pressed firmly on the inside wall of the tube above the fluid level to remove excess inoculums. It was then swabbed onto the MHA plate and this procedure was repeated by swabbing two more times, rotating the plate approximately 60°C each time to ensure an even distribution of inoculums. Complete drying of the MHA plates was ensured.

The discs which contained amoxicillin-clavulanate were placed in the center of the culture swabbed MHA plate. The cefotaxime and ceftazidime discs were placed 15 mm and 20 mm apart respectively, center to center to that of the amoxicillin-clavulanate disc. The plates were inverted and placed in an incubator set to 35°C within 15 min after the disc was applied.

After 16–18 h of incubation, each plate was examined. A clear-cut enhancement or synergy of inhibition between the cephalosporin disc and Beta-lactamase inhibitor disc were interpreted as positive for ESBL production. All strains positive for ESBL production were stored at –20°C for further analysis. Bacteria considered significant ESBL producers were tested for antibiotic susceptibility using the Kirby Bauer DD method according to CLSI guidelines.

Antibiotic susceptibilitytesting

Bacterial isolates considered significant as ESBL producers were tested for antibiotic Susceptibility using the Kirby Bauer DD method according to CLSI guidelines. A panel of antibiotic discs (HIMEDIA) Ampicillin, Piperacillintazobactam, Levofloxacin, Ciprofloxacin, Norfloxacin, Nitrofurantoin, Cefuroxime, Cefotaxime, Ceftazidime, Imipenem, Meropenem, and Amikacin was used for the susceptibility test. The result was interpreted as whether the organism was sensitive or moderately sensitive or resistant to the antimicrobial agents by using CLSI guidelines. *E. coli* ATCC no: 25922 were used as quality control strains for antibiotic susceptibilitytests.The method for inoculation of bacterial isolates on MHA plates was similar as practiced in DDST.

The antimicrobial disc was dispensed onto the surface of the inoculated agar plates and was pressed down to ensure complete contact with the agar surface distributed evenly so that they were no closer than 24 mm from the center to the center. The plates were inverted and placed in an incubator set to 35°C within 15 min after the disc was applied. After 16–18 h of incubation, each plate was examined. The diameter of the zones of complete inhibition was measured.

RESULTS

A total of 200 urine specimens were collected during the study period out of which 60 (30%) showed culture-positive (Table 1).

Microbial etiology of urinary tract infections causing organisms were studied (Table 2). GNB were found to cause UTI among 86.6% of totalcases.

Among the GNB, *E. coli* was liable for 51.9% of infections followed by *K. pneumoniae* (36.5%), *Pseudomonas aeruginosa* (9.61%), and *Serratia marcescens* (1.92%) (Table 3). The incidence of the ESBL producing GNB causing UTI among cancer patients was studied (Table 4). The ESBL producers can cause infection at any stage (Figs. 1 and 2) However; prevalence was high among the elderly age group. There was 46.6% UTI and 38.4% caused by ESBL producers within the elder population. In both the overall UTI and UTI caused by ESBL producers, women were found to be more susceptible (Figs. 3 and 4).

Among the ESBL producers, 56.6% were *E. coli*, while *Klebsiella pneumonia* and *P. aeruginosa* were 36.74 and 6.66% respectively

Table 1: Culture results of the urine specimens

Total number of specimens (n)	Culture positive specimens (%)	Culture negative specimens (%)
200	60 (30%)	140 (70%)

Table 2: Microbial etiology of UTI: Organism based on groups

Pathogens	Total number of isolates (n=60)	Percentage
Gram-positive cocci	4	6.66
Budding yeast	4	6.66
Gram-negative bacilli	52	86.6

UTI: Urinary tract infections

Table 3: Etiology of gram-negative bacilli causing UTI

Pathogen	Total number of isolates (n=52)	Percentage
Escherichia coli	27	51.9
Klebsiella pneumoniae	19	36.5
Pseudomonas aeruginosa	5	9.61
Serratia marcescens	1	1.92

UTI: Urinary tract infections

Table 4: ESBL producers

Organism	Total (n=52)	Percentage
ESBL producers	30	57.69

ESBL: Extended-spectrum beta-lactamase

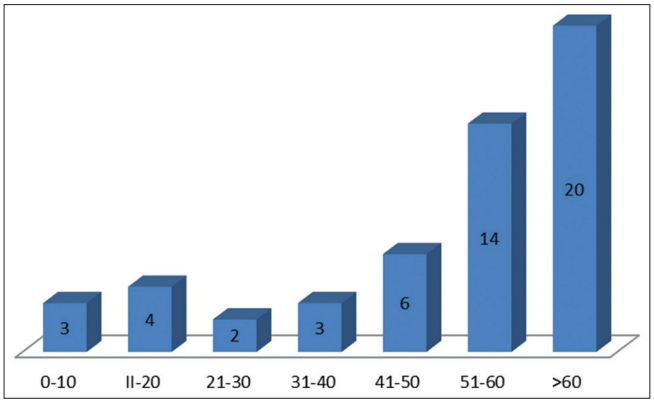

Fig. 1: Age distribution of patients with Gram-negative bacilli causing urinary tract infections

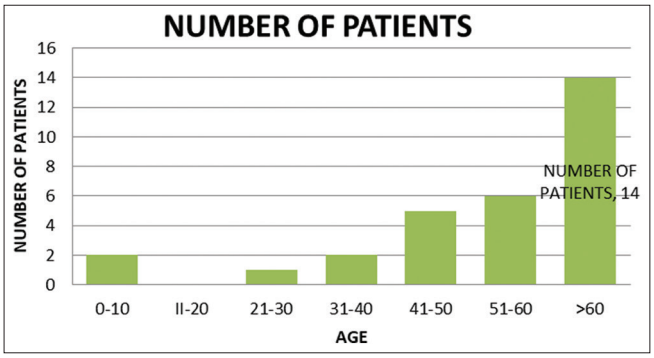

Fig. 2: Age distribution of patients with Extended-Spectrum Beta-Lactamase producers causing Urinary tract infections

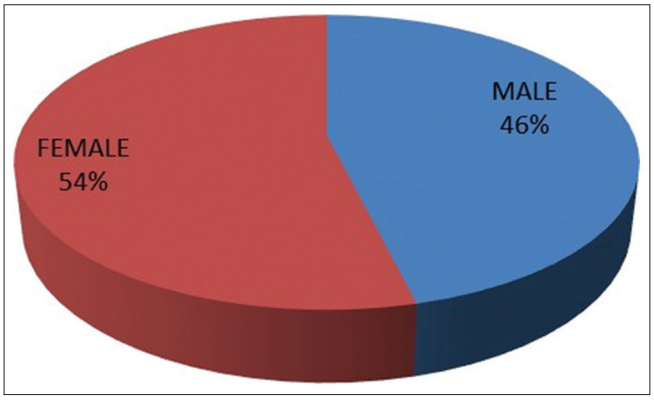

Fig. 3: Sex Wise Distribution of Patients with Gram-Negative Bacilli Causing Urinary tract infections

(Table 5). The finding shows *E. coli* as the core of the predominant bacterial pathogen causing urinary tract infection in cancer patients.

The antibiotic susceptibility pattern of all ESBL producers showed that mostly all ESBL producers (*E. coli*, *K. pneumoniae*, and *P. aeruginosa*) were multi-drug resistant (Table 6). However, significant isolates of *E. coli* (58.82%), *Klebsiella pneumonia* (80.81%), and *P. aeruginosa* (50%) were sensitive to carbapenem. Hence it can be considered as the drug ofchoice.

DISCUSSION

The emergence and rapid spread of MDR isolates are of great concern worldwide [4] among them, ESBL producing Enterobacteriaceae has been a major concern [7]. During the past decades, ESBLs producing

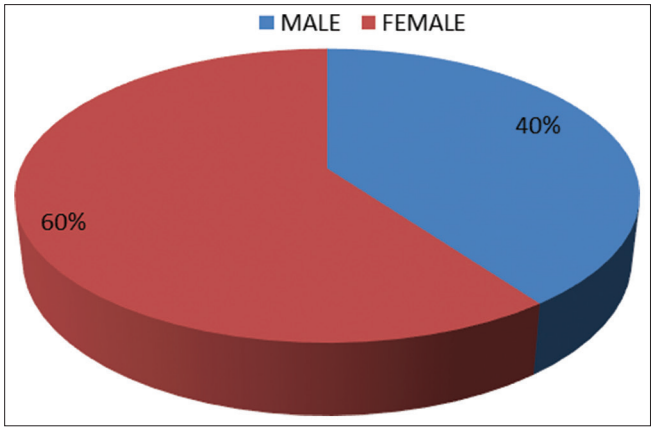

Fig. 4: Sex-wise distribution of patients with Extended-Spectrum Beta-Lactamase producers causing Urinary tract infections

Table 5: Etiology of ESBL producers

Pathogen	Total number of isolates (n=30)	Percentage
Escherichia coli	17	56.6
Klebsiella pneumoniae	11	36.6
Pseudomonas aeruginosa	2	6.66

ESBL: Extended-spectrum beta-lactamase

Table 6: Antibiotic Susceptibility Testing of ESBL-producing GNB

Antibiotics	*Escherichia coli*		*Klebsiella pneumoniae*		*Pseudomonas aeruginosa*	
	S	R	S	R	S	R
Amikacin	58.82	41.17	18.18	81.81	50	50
Amoxyclav	29.41	70.58	18.18	81.81	-	100
Cefepime	5.88	94.11	9.09	90.90	-	-
Cefuroxime	-	100	-	100	-	100
Ceftriazone	-	100	-	100	-	100
Co-trimoxazole	11.76	88.23	18.18	81.81	50	50
Gentamicin	29.41	70.58	18.18	81.81	50	50
Imipenem	58.82	41.17	81.81	18.81	50	50
Levofloxacin	23.52	76.47	18.18	81.81	50	50
Minocycline	58.82	41.17	18.18	81.81	-	-
Pip-taz	58.82	41.17	18.18	81.81	100	-
Cefoperazone	23.52	76.47	9.09	90.90	-	-
Colistin	58.82	41.17	81.81	18.18	50	50
Tigecycline	58.82	41.17	27.27	72.72	-	100

ESBL: Extended-spectrum beta-lactamase, GNB: Gram-negative bacteria

GNB especially *E. coli* and *K. pneumonia* have emerged as serious pathogens both in hospital and community infection worldwide [8]. In the present study, the prevalence of ESBL Producing GNB is 57.69%.

ESBL has become a widespread serious problem [9]. The presence of ESBL compromises the activity of widespread antibiotics creating major therapeutic difficulties with a significant impact on the outcome of patients [4]. The continued emergence of ESBL presents diagnostic challenges to the clinical microbiology laboratories.

Its emergence becomes more prevalent in the case of *E. coli* [9]. The underlying risk factors which are associated with ESBL production include age. Studies have shown that the elderly (>60), females are more affected by UTI as compared to males.

In our study too, we found that overall *E. coli* (17%) wasthe predominant pathogen followed by *Klebsiella pneumonia* (11%). ESBL production was 56.6% in *E. coli* and 36.6% in *K. pneumoniae.* The widespread

application of beta-lactam antibiotics in most health care institutions and communities unarguably established problems thathave led to increased mortality, morbidity, and cost of health care. Understanding the antibiotic resistance profiles of urinary tract bacteria is very pertinent in helping clinicians to prescribe appropriate antibiotics as an evidence-based recommendation especially in empirical antibiotic treatment of UTI [10].

Resistant urinary tract infections, if not properly treated, can lead to complications that may lead to permanent or temporary infertility, pelvic inflammatory diseases, ectopic pregnancy, abscess formation, Fallopian tube obstruction, epididymitis, Orchids, and the involvement of kidneys causing nephritis [3]. The incidence of the ESBL producing GNB causing UTI among cancer patients was studied. The ESBL producers can cause infection at any stage, however; prevalence was high among the elderly age group. There was 46.6% UTI and 38.4% caused by ESBL producers in the elder population. In both the general UTI and UTI caused by ESBL producers, women were found to be more susceptible.

Among the ESBL producers, 56.6% were *E. coli*, while K. *pneumoniae* and *P. aeruginosa* were 36.74% and 6.66% respectively. The finding shows *E. coli* as a predominant bacterial pathogen causing urinary tract infection in cancer patients.

The widespread application of beta-lactam antibiotics in most health care institutions and communities unarguably established problems thathave led to increased mortality, morbidity, and cost of health care [7]. The antibiotic susceptibility pattern of all ESBL producers showed that mostly all ESBL producers (*E. coli*, *K. pneumoniae,* and *P. aeruginosa*) were multi-drug resistant. However, significant isolates of *E. coli* (58.82%), *Klebsiella pneumonia* (80.81%), and *P. aeruginosa* (50%) were sensitive to carbapenems. Hence it can be considered as the drug ofchoice.

CONCLUSION

The current study pointed the emergence and incidence of ESBL-producing GNB that are multi-drug resistant and causing UTI among cancer patients. Besides, the emergence of *E. coli* producing ESBL, in urine samples was notified through this study. Hence, further deeper investigation is the need of the hour at largescale to determine the resistance trend of ESBL producers and susceptibility of drugs in the specified geographical location to implement strict hospital infection control policies and prudent regiments for the use of antimicrobials and to improve the outcome of our patient management.

ETHICAL CLEARANCE

As this is a prospective study done with the samples received in microbiology lab, institutional review board clearance was got, there was no need of Ethical clearance.

ACKNOWLEDGMENT

Nil.

AUTHORS' CONTRIBUTIONS

Dr. Sajani Samuel, Ms. Vismaya p, and Dr. Parthiban R contributed substantially to the conception, design of the study, analysis, and interpretation of data. All authors discussed the results and commented on the manuscript. Dr. Sajani Samuel and Dr. Parthiban R drafted the final manuscript.

CONFLICTS OF INTEREST STATEMENT

None to declare.

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Nil.

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