Original Research Article

Antibiotic resistance profile of gram negative uropathogen isolated from a tertiary care center in Kolkata

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Abstract

Background: Urinary tract infections are one of the most common infectious disease in India. Resistance pattern of common antimicrobials to uro-pathogens are changed drastically over last few years due to widespread use of antibiotics. **Aim and Objectives:** The aim of this study is to determine the bacterial etiology of UTI and the antimicrobial resistance pattern of gram-negative uro-pathogens isolated among the patients attending in a tertiary care hospital in Kolkata.**Material and methods:**The study was conducted to determine the distribution and antibiotic sensitivity pattern of gram-negative uro-pathogen in the Department of Microbiology, NRSMC&H, Kolkata from March 2019 to February 2020.Freshly voided clean catch midstream urine specimens were collected from suspected hospitalized and OPD patients and processed bacteriologically as per standard procedures. Antimicrobial susceptibility and resistance was detected phenotypically as per CLSI guidelines. **Results:**From 5930 urine specimens, 36.42% had positive results for bacterial cultures. Growth of gram negative uro-pathogens was 80.32%. Female (65.37%) was outnumbered and most of the cases belongs to the age group of 30-50 years (42.60%). *E. coli* was the most prevalent uro-pathogen (61.78%). High degree of resistance was noted in *Klebsiella* spp.Most of the isolates of our study were ESBL producer (68.54%).**Conclusion:**This study reinforced that Treatment for UTIs should always be considered based on current local antimicrobial susceptibility patterns of uro-pathogens to reduce therapeutic failures and prevent antibiotic misuse.

Keywords: ESBL, Resistance, Uro-pathogen, Urinary tract Infections.

Introduction

Urinary tract infections (UTI) are one of the most common infections diagnosed in outpatients as well as in hospitalized patients, and can lead to significant mortality [1]. UTI accounts for a large proportion of antibacterial drug consumption and have large socio-economic impacts [2].UTIs are the most common infections after upper respiratory tract infections. Although several different microorganisms can cause UTIs, including fungi and viruses, bacteria are the major causative organisms and are responsible for >95% of UTI cases.[3,4]Although UTI can be caused by both gram-positive and gram-negative bacteria, gram negative pathogens are more common. Due to the rapidly evolving adaptive strategies of bacteria, the etiology of UTI and antibiotic resistance profile of bacterial uro-pathogens have changed considerably over the past years, both in community and health-care associated infections [5]. Apparent shift in the etiological agents of urinary tract infection and associated problem of antibiotic resistance amongst bacterial uro-pathogens from time to time and from one institution to another have initiated health institutions to carry out continuous evaluation of UTI from the view point of their spectrum and drug susceptibility testing. [6]Hence, it becomes important to regularly monitor the resistance or susceptibility patterns of uro-pathogens, so that the guidelines for empirical antibiotic therapy can be improved to include antibiotics with low resistance, aiding clinicians in proper management of UTIs with minimal therapeutic failures. [7,8]With this background, the aim of the study is to determine the antimicrobial resistance pattern of gram-negative uro-pathogens isolated among the patients attending in a tertiary care hospital in Kolkata.

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Material and Methods

Study design: This cross-sectional observational study was conducted to determine the bacterial etiology of UTI and the antibiotic sensitivity pattern of gram-negative uro-pathogen in the Department of Microbiology, Nil Ratan Sircar Medical College & Hospital, Kolkata from March 2019 to February 2020. All urine samples collected from admitted patients in different wards or outpatient basis, were included in this study.

Specimen collection: All patients from outpatient department (OPD) were instructed about the method of collection of mid-stream clean catch urine. Freshly voided 5–10 ml of clean catch midstream urine specimens was collected using sterile, graduated, wide mouthed plastic container. Clean catch mid-stream urine samples or those obtained by aspiration from catheter tube or suprapubic aspirate collected from suspected hospitalized patients, as per ICMR guideline [9].The specimen was labeled and transported to the microbiology laboratory for processing within 2 h.

Determination of Infection: To define 'positive UTI', microscopic enumeration of urine leucocytes (WBCs) and semiquantitative urine culture were done. For microscopic enumeration, 10-15 ml of mid-stream urine sample was centrifuged at 1500 to 3000 rpm for 5 min. Supernatant was decanted using pipette, leaving 1 ml urine at the bottom of the test tube. Sediment in bottom of the test tube was re-suspended with the retaining 1 ml of urine. Then 15 µl of re-

suspended sediment was applied on a glass slide using calibrated pipette. Cover slip was used to avoid any possible contamination. Then the slide was examined with a light microscope using the high dry objective (40x). At least greater than 5 leucocytes per high power field (HPF), were considered as positive UTI. All positive samples were further subjected to urine culture [10].

Isolation and identification of Uro-pathogens: Semi quantitative urine culture was done using a calibrated loop. A loopful (0.001 mL) of well mixed un-centrifuged urine was inoculated onto the surface of cysteine lactose electrolyte deficient medium. The culture plates were incubated aerobically at 37°C for 18-24 h and count were expressed as colony forming units (cfu) per milliliter (ml). For this study, significant bacteriuria was defined as culture of a single bacterial species from the urine sample at a concentration of 105 cfu/ml. [11] Only patients with significant bacteriuria (≥105 cfu/ml) were included for microbiological analysis. The culture isolates were identified by standard microbiological methods. [12]All culture media were procured from Hi-Media Laboratories, Mumbai, India.

Antimicrobial susceptibility testing: All gram negative isolates were subjected for antimicrobial susceptibility testing by Modified Kirby Bauer Disc Diffusion method on Muller Hinton Agar as per the CLSI Standards.[13] The antibiotics tested were amikacin (30 µg), ampicillin (10 µg), cefepime(30 µg), ceftazidime (30 µg), cefuroxime (30 µg),cefotaximen(30 µg),Cefoperazone(75 µg), co-trimoxazole(25 µg), ciprofloxaci(5 µg), gentamicin (10 µg), imipenem(10 µg), levofloxacin(5 µg), meropenem (10 µg), nitrofurantoin (300 µg), piperacillin + tazobactam (100 + 10 µg), polymyxin B (300 µg) and Cefoperazone + Sulbactam. *E. coli* ATCC 25922 and *Pseudomonas aeruginosa* ATCC 27853 were used as quality control strain. [13]

Detection of antimicrobial resistance: All Gram-negative uropathogens resistant to 3rd generation cephalosporins were screened for extended-spectrum β-lactamases (ESBLs) including Amp C detection phenotypically by disc diffusion method followed by confirmatory test as per CLSI guideline [13].Carbapenemase production was detected from carbapenem resistant gram-negative isolates phenotypically by Carba NP test as per CLSI guideline [13].

Statistical analysis: Calculations were done using Microsoft Excel. Data were analyzed using Graph Pad Prism 7.

Results

A total of 5930 urine samples from outdoor and indoor patients were collected and processed during a period of one year. 2160 (36.42%) urine samples showed the significant growth which were comprised of 748 (34.63%) samples from males and 1412 (65.37%) from females. A total of 2160 uro-pathogens comprised of 1735 (80.32%) gram negative, 261 (12.08%) gram positive and 164 (7.59%) *Candida* were isolated from positive urine samples [Figure 1]. Pediatric patients (0–14 years) and elderly patients (≥50 years) accounted for 11.48% and 25.32% of the total number of UTI, respectively. Urinary tract infection was the highest (42.6%) in patients of age group 30-50 followed by age groups of >50 (25.32%) [Table 1].

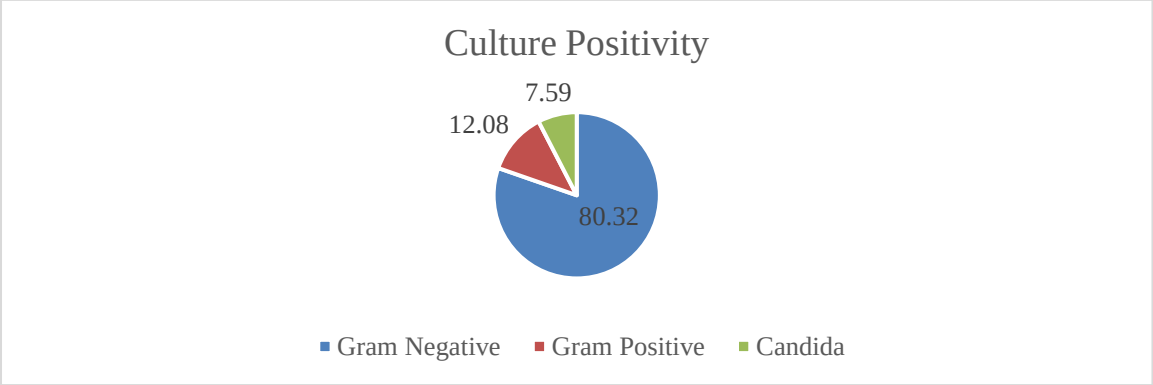

Fig 1: **Showing distribution of isolated uro-pathogens (n=2160)**

Table 1: **Showing Sex and age wise distribution of cases (n=2160)**

Variables		Number	Percentages (%)
Gender	Male	748	34.63
	Female	1412	65.37
	Total	2160	100
Age	<14 years	248	11.48
	14-30 years	445	20.60
	30-50 years	920	42.60
	>50 years	547	25.32
	Total	2160	100

UTI in the out patients was 5.79% which was less as compared to the indoor patients. Most of the indoor cases were from Medicine ward (51.34%) [Figure 2].

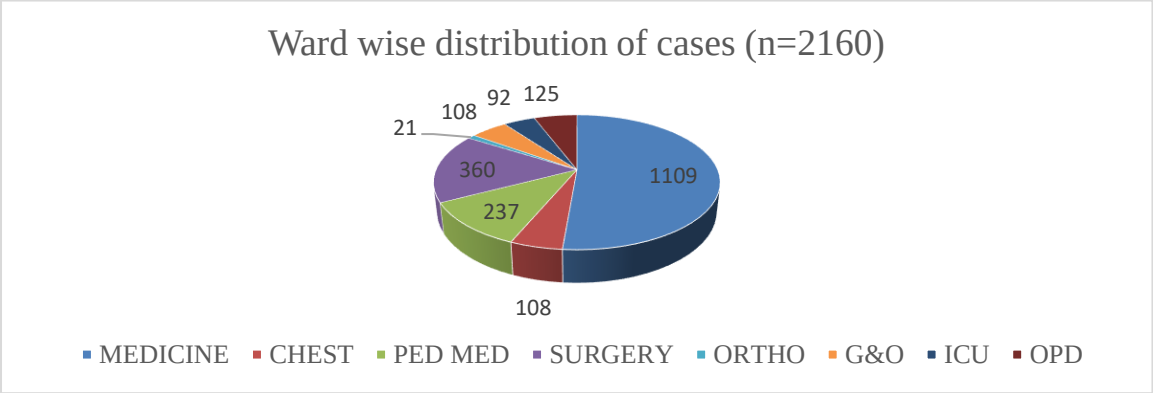

Fig 2: **Showing ward-wise distribution of cases (n=2160)**

Table 2: **Showing distribution of gram negative uro-pathogens (n=1735)**

Gram negative Organism(s) isolated	Number	%
E.coli	1072	61.78
Klebsiella	384	22.13
Enterobacter	48	2.76
Citrobacter	26	1.49
Pseudomonas	120	6.91
Acinetobacter	51	2.94
Proteus	19	1.09
P.mirabilis	14	
P.vulgaris	5	
Morganella	9	0.58
Serratia	2	0.11
Alcaligenes	1	0.05
Burkholderia	2	0.11
Strenotrophomonas	1	0.05

Escherichia coli was found the dominant bacteria among all isolated uro-pathogens with the prevalence rate of 61.78%. [Table 2] The second most prevalent isolate was *Klebsiella spp* (22.13%) followed by *Pseudomonas aeruginosa* (6.91%).

Table 3: **Showing antimicrobial resistant profile of isolated uro-pathogens**

	***E.coli* (n=1072)**	***Klebsiella* (n=384)**	***Enterobacter* (n=48)**	***Citrobacter* (n=26)**	***Pseudomonas* (n=120)**	***Acinetobacter* (n=51)**	***Proteus* (n=19)**
Ampicillin	1008(94.02%)	384(100%)	48(100%)	24(92.30%)	NA	NA	18(94.7%)
Amoxyclav	862(80.41%)	315(80.03%)	45(93.75%)	22(84.61%)	NA	NA	16(84.2%)
3rd gen cephalosporin	745(69.49%)	267(69.53%)	34(70.83%)	22(84.61%)	55 (45.83%)	36(70.5%)	15(78.4%)
Cefoperazone-sulbactam	393 (36.66%)	223(58.07%)	30(62.50%)	8 (30.76%)	49(40.83%)	22(43.1%)	8(42.1%)
Piperacillin-tazobactam	452(44.03%)	236 (61.46%)	29(60.41%)	8 (30.76%)	37 (30.83%)	29(56.8%)	7 (36.84%)
Amikacin	307(28.63%)	158 (41.14%)	17(35.41%)	9 (34.61%)	52 (43.34%)	26(50.9%)	8(42.10%)
Gentamicin	474(44.21%)	197 (51.30%)	22(45.84%)	10(38.46%)	61(50.83%)	32(62.7%)	12(63.1%)
Levofloxacin	821(76.58%)	247 (64.32%)	34(70.83%)	12(46.15%)	50 (41.67%)	33(64.7%)	9(47.36%)
Ciprofloxacin	875(81.62%)	271(70.57%)	44(91.66%)	16 (61.53%)	64 (53.34%)	38(74.5%)	11(57.8%)
Nitrofurantoin	182(16.97%)	191(49.74%)	37(77.08%)	10(38.46%)	NA	NA	NA
Imipenem	310 (28.91%)	194(50.52%)	30(62.50%)	5 (19.23%)	40 (33.34%)	18(35.2%)	6 (31.57%)
Meropenem	325 (30.31%)	203(52.86%)	31(64.58%)	6(23.07%)	35(29.16%)	16(31.3%)	6 (31.57%)
Co-trimoxazole	683(63.71%)	247(64.32%)	25(52.08%)	22(84.61%)	NA	35(68.62%)	NA
Polymyxin B	38 (3.54%)	24(6.25%)	3(6.25%)	0%	4(3.34%)	1(1.96%)	19(100%)

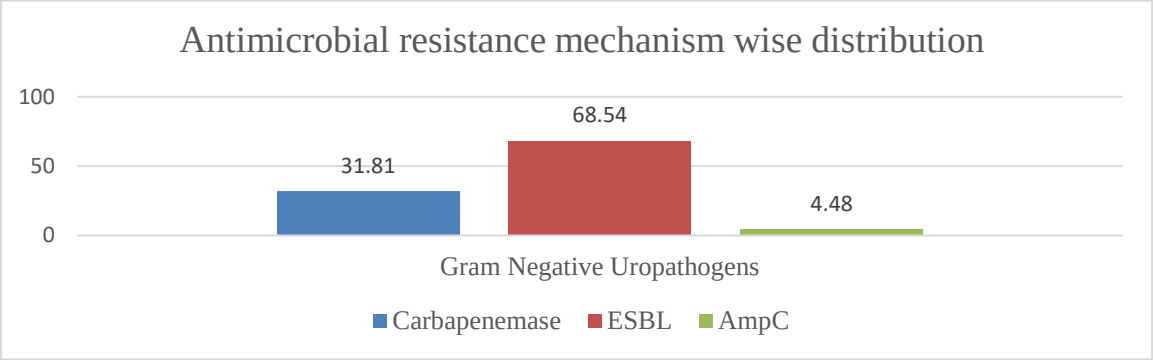

Fig 3: Showing anti-microbial resistance mechanisms of isolated uro-pathogens

The comparison of the resistance pattern of organisms to various antimicrobial agents from all the specimens was shown in Table 3. High degree of resistance was noted in *Klebsiella* spp. *Pseudomonas* spp. and *Acinetobacter* spp. that were resistance more than 30% to most of the commonly used antibiotics.Most of the isolates of our study were ESBL producer (68.54%), 4.48% were AmpC producer and Carbapenemase was produced in 31.81% cases. Among 648 carbapenem resistant isolates, 552 (85.18%) were Carbapenemase producer.

Discussion

Urinary tract infection is caused by both Gram-negative and Gram-positive bacteria. However, the most commonly encountered bacteria are Gram negative in which *E. coli* consisting of the largest proportion of bacterial uro-pathogen worldwide [14]. This is evident in our study in which, out of 2160 bacterial isolates recovered, 1735 (80.32%) were Gram- negative bacteria. Positive culture rate or isolation rate in this study was 36.42%, which was close to that obtained by similar studies conducted across India[15-18]and outside India [19-21]. The members of the *Enterobacteriaceae* particularly *E. coli* and *Klebsiella* spp. are frequently reported as the most cause of UTI worldwide[22,23]. Similar findings were noted in our study showing *E. coli* was the most prevalent uro-pathogen (61.78%) followed by *Klebsiella* spp (22.13%). UTI was found to be highest (42.60%) in the ages between 30-50 years, and females were mostly affected (65.37%). This finding was consistent with George CE *et al* [15], Somashekara *et al* [17] and Singhal *et al* [18]. The reasons of increasing incidence of UTI in female in reproductive age group are associated with shorter urethra, high sexual activity, and a history of recurrent UTIs [24].In our study, 94% of E. coli isolates were found to be resistant to Ampicillin. High level of resistance to ampicillin was also reported in Gupta *et al*[25] (76%), Manjunath *et al* [26] (80.6%) and Murugan *et al* [27] (96.2%). The antimicrobial sensitivity pattern of the present study is similar to findings of Banerjee *et al*., [28] and Sharma *et al*. [29] The alarming finding was Ampicillin, Amoxicillin and clavulanic acid and most of the Cephalosporin were resistant to most of the strains. The possible explanation may be because these antibiotics have been in use for a long period and more often due to the misuse of antimicrobial drugs, which has today led to a general rise in the emergence of resistant bacteria. [30,31] Susceptibility of Nitrofurantoin was found maximum in *E. coli* (83.03%). Similar finding was also noted in George CE *et al* [15], Singhal *et al* [18] and Kulkarni *et al* [32]. Resistance to co-trimoxazole is around 67% in our study, which was almost similar with the study of Manjunath *et al* [26] (70.4%) and Murugan *et al* [27] (47.9%).The decreased resistance pattern to co-trimoxazole in Karnataka may be due to the decreased use of co-trimoxazole as empirical therapy for UTIs [33]. Higher resistance was noted to major oral as well as injectable antibiotic, instituted against uro-pathogens in our study. Similar observation was also noted in several studies across India [34].Most of the isolates of our study were ESBL producer (68.54%). Carbapenemase production was detected in 31.81% of gram-negative uro-pathogen and 85.18% of carbapenem resistant GNB. Several studies have shown almost similar findings [35-38].The emergence and spread of ESBL and Carbapenemase producing GNB are of significant clinical and public health concern. Because Carbapenemase genes are carried on plasmid, these genes can be spread horizontally by conjugation to naive bacteria, thus contributing to the reservoir of resistance in both environmental and clinical isolates of GNB[39].

Conclusion

The overall prevalence of urinary tract Infection is high and it is more common in female of reproductive age group. *E. coli* is the most prevalent uro-pathogen. Due to the fact that the pattern of sensitivity of bacteria to antibiotics varies over time and in different geographical regions, antibiotic treatment of infections should be based on local experience of sensitivity and resistance patterns. This study provides important data to monitor and compare with other studies, understanding the trend of antimicrobial susceptibility of uropathogens, helps us todecide empirical antibiotic policy according to local antimicrobial susceptibility pattern and thereby improving patient outcomes and minimizing antibiotic misuse.Early detection of resistance mechanism is essential for effective infection control practices to limit the spread of infection.

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