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Bacteriological Profile and Antimicrobial Sensitivity Pattern of the Uropathogens in a Rural Hospital

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Abstract

Urinary tract infections are gaining much importance among community acquired infections. Poor personal hygiene may act as a contributory factor in such frequent infections in developing countries. The condition becomes serious with antimicrobial resistance among uropathogens. Specifically, surge of Extended Spectrum beta Lactamase (ESBL) producers poses much concern in the available treatment options. Present work was aimed to observe the bacteriological profile and antimicrobial susceptibility pattern of uropathogens with special reference to ESBL producing strains. This study was carried out in the microbiology department, SGT University, Gurugram, Haryana. Total 600 samples were processed as per standard bacteriological procedures i.e, microscopy, culture and biochemical reactions followed by antibiotic susceptibility testing and interpreted as per CLSI guidelines. ESBL producer strains were spotted by double disc synergy testing (DDST). Out of 600 specimens, 128(21.3%) showed culture positivity. Females contributed more compared to males. 21-40 years age group showed highest isolation rate. E.coli was predominant organism. Highest resistance towards amikacin was shown by gram negative organisms. Gram positive organisms demonstrated high resistance towards gentamicin, cotrimoxazole, ampicillin and ciprofloxacin. 34.4% ESBL producing E.coli strains were observed. Alarming rate of antibiotic resistance as well as beta lactamase production by strains and increasing urinary tract infections should be considered as potential threat to the community. Routine investigation should be done to see the burden in order to implicate advance treatment policies in order to treat such infections.

Keywords: Urinary Tract Infection (UTI), Enterobacteriaceae, Community Acquired Infections, Rural, ESBL

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INTRODUCTION

Urinary tract infection (UTI) is considered as a very commonly encountered clinical problem among all community acquired infections. Lack of sanitation and unhygienic personal habits in developing countries contribute a major part for such infections.[1]

Pathogenic microorganisms present in the kidney, bladder, urethra or urine can cause UTI with or without symptoms. In addition, UTI may be considered as a contributory factor for morbidity among rural population and a second most common cause for frequent hospital visits of the population.[2]

Bacterial spectrum causing complicated UTI is much broader compared to those causing uncomplicated UTI. Complicated UTI may be given a thought in situations with treatment failure and repeat infections and they will lead to significant morbidity and mortality with a poor outcome.[3] However uncomplicated UTI may be defined among healthy nonpregnant women, premenopausal having no abnormal urinary tract anomaly previously.

The most commonly encountered microorganisms associated with complicated UTI are Gram negative (GN) bacteria i.e, *Escherichia coli* (*E.coli*), *Pseudomonas aeruginosa, Citrobacter* spp*, Proteus vulgaris* and *Enterobacter aerogenes* whereas Coagulase Negative *Staphylococcus* (CoNS), *Acinetobacter* spp. and *Salmonella* spp. are observed less commonly.[4]

E.coli, a commonly isolated bacteria, is causative agent of more than 80% of all the community associated UTI. The organism is responsible for range of UTI's, including symptomatic cystitis, acute prostatitis, prostatic abscess, uncomplicated urethritis and urosepsis. Primarily sexually active females who are colonized by uropathogenic strains of *Escherichia coli* complaining of uncomplicated cystitis.[5,6] Extensive injudicious use of antimicrobials may result into emergence of multidrug resistance (MDR) in different pathogens. In addition many healthcare systems have initial outbreaks of infections which are majorly contributed by surge of ESBL in pathogenic microorganisms which in turn result into high mortality rate as antibiotics are found inactive in response towards ESBL producing strains. Hiked multidrug resistance (MDR) in bacterial strains responsible for uropathies are an important and emerging public health concern these days.[7]

Most common antibiotics in the cure of bacterial ailments belong to β-lactam group however β-lactamases production makes bacterial strains resistant to this group. β-lactamases are extracellular enzymes produced by bacteria breaking the amide bond of β-lactam ring of Penicillin and capable of inactivating Oxyimino-cephalosporins group and Aztreonam group but are inactive against Cephamycin and Carbapenem.[8]

Extended Spectrum Beta Lactamases

(ESBL) are majorly plasmid or chromosomal mediated. It has been observed that the pre-existing broad spectrum β-lactamases (TEM-1, TEM-2, SHV-1) had been mutated to result inESBL as a sequel for use of Aztreonam and 3rd generation Cephalosporins.[9] These enzymes arecoded by plasmids and their ability to spread toother bacteria led to dramatic increase in theirprevalence worldwide in a very short span of life.[10]

ESBL Production in Bacteria Causing

UTI leave physicians with very limited options of antibiotics for treating such patients. ESBL enzymes are found predominantly in *Escherichia coli* and *Klebsiella* spp. Therefore the study was designed and conducted to find out the profile of bacteria causing UTI and the antibiotic sensitivity patterns with special emphasis on the presence ofESBLs.

ESBL detection has gained high attention among uropathogens from the suspected cases of UTI as urine specimen may be considered as a tool for consideration of epidemiological markers in the assessment of community spread of pathogenic drug resistant organisms.[11]

In recent storyline, antibiotic resistance is increasing alarmingly with emergence of new multidrug resistant bacteria which in turn results into enhanced mortality and morbidity rate.

The present work gives an insight to the burden of urinary pathogens and their antimicrobial resistance rate and enhanced ability to produce ESBL enzymes.

MATERIALS AND METHODS

Study Area

The current research was accomplished in the Microbiology department, SGT University, Gurugram, Haryana. Total 600 urine samples received in the microbiology laboratory were taken into consideration for the study after approval from Institutional ethics committee.

Sample Collection and Processing

Appropriate sterile collection of mid-stream urine samples was ensured from all patients. All the specimens were received in sterile, disposable universal containers within one hour of collection.

Processing

All the collected specimens were processed during sixty minutes of receiving.

Direct Microscopy

Urine samples were examined microscopically for significant pyuria, RBCs and crystals.

Standard loop method (Semi quantitative method)

Significant bacteriuria was considered as a marker of positive urine culture bacteriuria i.e, isolation of 1 or 2 pathogens with $>10^5$ CFU (colony forming units)/ ml, for pyuria detection of >5 pus cells/ high power field (HPF).

Culture

Urine samples were inoculated on to CLED medium (Cystine Lactose Electrolyte Deficient medium) and the Culture plates were subjected to incubation at 37°C for overnight or 18-24 hrs. The isolates were identified on the basis of colony morphology, gram's reaction, motility testing, oxidase test and catalase test. These primary testing methods were further followed by chain of biochemical reactions for further identification of organisms as per standard guidelines.[12]

Antimicrobial Sensitivity Testing

Antimicrobial sensitivity of bacterial strains was conducted by disk diffusion method on MHA (muller-hinton agar). The susceptibility result will be interpreted as per the CLSI (Clinical Laboratory Standard Institute).[13]

Antibiotics used Gram Negative Bacilli

Amikacin (30µg), Amoxyclav (30µg), Levofloxacin (5µg),Ampicillin (10µg), Cefotaxime (30µg), Azithromycin (15µg), Ceftriaxone (30µg), Ciprofloxacin (5µg), Imipenem (10µg), Cefaclor (30µg),Pipracilin /Tazobactum (100/10µg), Gentamycin (10µg).

For Gram Positive Cocci

Amikacin (30µg), Cefotaxime (30µg), Amoxyclav (30µg), Ampicillin (10µg), Azithromycin (15µg), Cefoxitin (30µg), Cephalexin (30µg), Clindamycin (2µg), Cotrimoxazole (25µg), Erythromycin (15µg), Gentamycin (10µg), Levofloxacin (5µg), Linezolid (30µg), Ofloxacin (5µg), Ciprofloxacin (5µg), Vancomycin (30µg).

ESBL Detection

DDST (Double-Disc Synergy Test)

Strains of *E. coli* were checked for ESBL production by placing a ceftazidime disc at 20mm distance centre to centre from the combined disc (clavulanic acid +ceftazidime). An increase in zone of inhibition more than 5mm towards ceftazidime disc was considered as the ESBL production.

Table 1. Age group distribution of all the study subjects

Age group	Samples	Number of positive samples			
		Total	Males	Females	Statistical analysis
<20	120 (20%)	20(6.6)	6 (30%)	14(70%)	
21-40	306 (51%)	75(24.5)	16 (21.3%)	58(77.3%)	
41-60	142 (23.6%)	28 (19.7%)	12(42.8%)	16(57.1%)	
61-80	32 (5.3%)	5 (16.1%)	1 (3.2%)	4 (12.9%)	NC
Total	600 (100%)	128 (21.3%)	35(27.3%)	92 (71.8%)	

Table 2. Distribution of Uropathogens

No.	Isolates	Total number of isolates
1.	*E.Coli*	58
2.	*S.aureus*	23
3.	*Klebsiella* spp.	21
4.	*Enterococcus* spp.	11
5.	*Micrococcus*	8
6.	*Acinetobacter*	4
7.	*Pseudomonas*	2
8.	*Proteus*	1
	Total	128

RESULTS

A total of 600 mid-stream urine specimens were analysed in the laboratory throughout the study duration. Out of 600 samples, 128 (21.3%) showed culture positivity.

Isolation of uropathogens vary significantly between samples from male and female population.

Uropathies were observed in high frequency among females compared to males. Major number of patients were observed from the age group of 21-40 years, as demonstrated by highest isolation frequency in them as compared to other age groups. (Table 1) Of the total 128 bacterial isolates, Gram negative bacteria accounted for 67%, maximum being members of *Enterobacterales* (62.5%). *E. coli* was most frequently isolated (72.5%) uropathogen among total *Enterobacterales.* Gram positive bacteria contributed well as 32.8% Gram positive cocci isolated from culture positive samples from clinically suspected population. (Table 2)

Overall *E. coli* was isolated as the most frequent uropathogen compared to *Klebsiella* Spp, *S.aureus, Enterococcus* spp, *Proteus* spp, *Micrococcus, Acinetobacter* and *Pseudomonas* (Table 2)

E.coli and *Klebsiella* showed higher resistance towards amikacin compared to other classes of antibiotics. 3 (75%) of *Acinetobacter* isolates were not found susceptible towards carbapenem. (Table 3) 100% *Pseudomonas* strains were found resistant towards ampicillin & cefaclor. All Proteus isolates showed resistance towards amikacin and gentamycin in the current research work. *S. aureus* strains were not found sensitive towards gentamycin and cotrimoxazole however all the isolates found sensitive towards vancomycin. *Enterococcus* spp. showed a high resistance towards ampicillin and ciprofloxacin. (Table 3)

Out of total 58 *E.coli* isolates, 20(34.4%) isolates were observed as ESBL producers (Figure).

DISCUSSION

Urinary tract infections are the commonly occurring infections having potential to produce ailments among all age groups throughout the

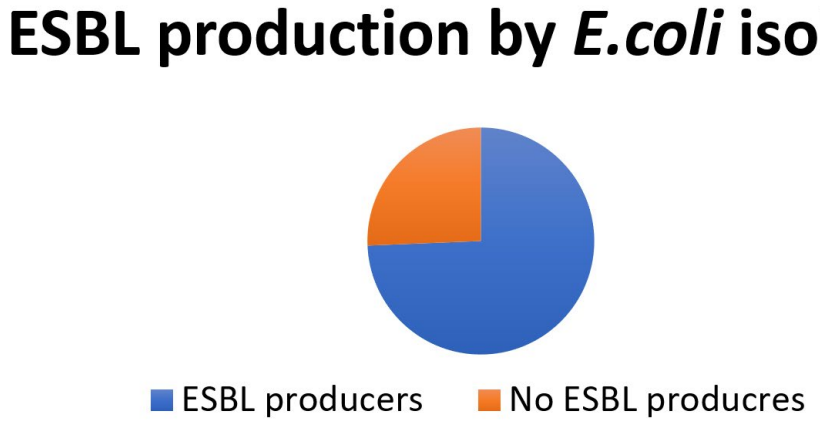

Figure. ESBL production by *E.coli* isolates.

world.[14] Urinary tract infections (UTI) impose a high load on hospitals due to high occurrence of infection in both community and nosocomial settings. Variety of pathogens including *P. aeruginosa, E.coli* and *K. pneumonia* are found responsible for UTI. Surveillance programs for antimicrobial resistance of urinary pathogens is not helping with this surge in antimicrobial resistance rate, in turn provides an in sight for management and empirical treatment of patients suffering from UTIs.[15]

Out of 600 symptomatic patients, included in the present study, urine culture was positive in 128 subjects (21.3%). The positivity was found to be lower than other reports in the year 2014[15] and 2015 (41.18% and 43.07% respectively). Female population contributed 22.5% of the total study group, which is a concordant finding with another study (24.5%).[16]

High isolation rate of uropathies among 21-40 yrs age group may be justified due to that females being more susceptible to develop UTI than males due to certain anatomical and physiological factors such as absence of prostatic secretions, easy contamination of the urinary tract with faecal normal flora and short urethra and it has been well documented in some studies also.[17-19] These facts also supported by clinical studies and findings of present study are in total agreement.[20] About 40-50% of females belonging to reproductive age group had gone through at least a single episode of UTI in their lifetime.[21] Uropathies are most commonly occurring among sexually active females. Among males, high incidence of urinary tract infections was observed in 41 to 60 years age group which could be explained due to co-morbid conditions like prostate hypertrophy and propensity to have high blood sugar levels in the male population of this age group.

Several types of microorganisms may be the causative agents of UTI, including, viruses, fungi and protozoan parasites and among all the categories bacterial pathogens are considered as the major category of microorganisms accounting for much than 90% of all the UTI cases.[17]

The most commonly identified uropathogen was *E. coli* followed by *Klebsiella pneumoniae* in the present study. *E.coli* has been documented as a cause of UTI in over

Table 3. Antimicrobial sensitivity profile of uropathogens

Organisms (128)	Amikacin (30µg m)	Co- trimoxazole (25µgm)	Cefotaxime (30µgm)	Ampicill in (30µg m)	Cefaclor (30µgm)	Ciprofloxacin (5µgm)	Gentamycin (10µgm)	Imipenam (10µgm)	Vacomycin	Ofloxacin
E.coli (58)	50 (86.2%)	14 (24.1%)	19 (32.7%)	8 (13.7%)	12 (20.6%)	22 (37.9%)	33 (56.8%)	23 (39.6%)	NA*	NA
Klebsiella (21)	15 (71.4%)	6 (28.5%)	10 (47.6%)	3 (14.2%)	9 (42.9%)	10 (47.6%)	10 (47.6%)	11 (52.3%)	NA	NA
Acinetobacter (4)	2 (50.0%)	2 (50.0%)	2 (50.0%)	1 (25.0%)	1 (25.0%)	3 (75.0%)	2 (50%)	3 (75%)	NA	NA
Pseudomonas (2)	2 (100%)	2 (100%)	2 (100%)	-	-	2 (100%)	2 (100%)	2 (100%)	NA	NA
Proteus (1)	-	1 (100%)	1 (100%)	1 (100%)	1 (100%)	1 (100%)	-	1 (100%)	NA	NA
S. aureus (23)	13 (56.5%)	3 (13.0%)	11 (47.8%)	6 (26.0%)	NA	13 (56.5%)	2 (8.6%)	NA	23 (100%)	15 (65.2%)
Enterococcus (11)	6 (54.5%)	8 (72.7%)	4 (36.3%)	4 (36.3%)	NA	3 (27.2%)	6 (54.5%)	NA	5 (45.4%)	7 (63.6%)

80% of cases.[22,23] Many other studies have also demonstrated *E.coli* as their majorly encountered microorganism in UTI accounting for 66.9%, 61.02%, 39% and 37.95% respectively.[5,6,24,25] However the pathogenesis by uropathogenic *E. coli* (UPEC) include a range of factors i.e, vaginal, periurethral and urethral colonization, growth of planktonic cells in urine, interference with the bladder epithelium which is the primary defense system and biofilm formation. UPEC colonization in bladder and presence of various virulence factors i.e, pili, outer membrane proteins (OMP), lipopolysaccharide capsule, secretory toxins, also play condemnatory characters in urinary infections.

In the present study showed a countable involvement in the total uropathies. Besides *Enterobacteriaceae,* gram-positive organisms are also exerting countable impact in uropathies specifically among old age population, pregnant females or those who are immunocompromised. Gram positive bacteria include *S. aureus, Enterococcus* and *Micrococcus* specifically found associated with uropathies.

Our study supports this fact as 32.8% GPC were identified *S. aureus* as the predominant species over *Enterococcus* spp. and *Micrococcus.* (Table 2) Gram-positive organisms are associated with uncomplicated uropathies quite commonly seen in nonpregnant females, premenopausal and sexually active females. However, there's no doubt in the percentage of gram negative/gram positive association in uncomplicated uropathies, as gram negatives account for a major portion (70-75%) of the organisms associated with uncomplicated uropathies but the remaining proportion (25-30 %) is contributed by gram positives only. Similar order of isolation was also observed in another study.[26] Symptoms for uncomplicated uropathies resembles as that for complicated uropathies which include urinary frequency, dysuria, flank pain, nausea suggestive of upper UTI.[27]

In the present study, most frequent isolation of *S. aureus* is in contrast to findings of many studies where either *Enterococcus* spp or *S. saprophyticus* is most commonly isolated gram-positive bacteria from the samples of clinically suspected cases of UTI.[28,29]

Bacteremia due to *S. aureus* is strongly associated with bacteriuria supports the fact that bacteriuria is invariably preceded by bacteremia.[30] In patients with *S. aureus* bacteriuria (UTI), importance of history related to concomitant or preceding bacteremia cannot be overlooked.

One recent observation showed the synergy of bacterial infections in urine with the mortality rate which demonstrated twice increased complication of death among the patients having *S. aureus* bacteremia as well as bacteriuria.[31] Enterococci are the normal flora of mouth, vagina and human gastrointestinal tract.[32]

E. faecalis may be isolated from 80% of human ailments as concluded by a survey.[33] In the samedirection it could be the major leading cause of urinary infections around the world.[34] *E. faecalis* was reported to have tropism for the kidneysin female C57BL/6J mice. If this finding can be extended to human beings, possibility of lower tract UTI caused by seeding from kidneys in earlier asymptomatic/ apparently symptomatic episodes, cannot be denied.[35]

Proteus spp. and *Pseudomonas* spp. are majorly encountered from health care associated UTIs compared to community associated UTIs. Few non fermenter bacteria are also isolated as *Pseudomonas* and *Acinetobacter* spp accounting 4.7% of total bacterial isolates. Similar finding have been observed in other studies.[22,23]

Antimicrobial resistance possess a worldwide threat to the community as well as healthcare system. Intense and overuse of antimicrobials is being the foremost cause for surge of antimicrobial resistance together. In turn the hike is responsible for the transmission of antimicrobial resistant strains among community and healthcare systems.

However there are some principles of antibiotic prescription also available, that must be followed in order to minimize the burden of AMR. Those principles include microbiological therapy guidelines wherever possible, Indications which should be evidence based, Narrowest spectrum requirement, appropriateness of the drug dosage towards site & type of infections and minimization of the therapy duration and to maintain monotherapy in most of the cases. Beside the above principal's antimicrobial surveillance,

teamwork and open pragmatic approach may also adopt to overcome the problem of AMR. Studies are required to monitor local resistance and surveillance for this emerging threat of resistance by means of antimicrobial susceptibility testing of the pathogens responsible for UTI, specifically for *E.coli* .[36] *Enterococci* are intrinsically resistant to a number of antimicrobial agents and can further easily acquire resistances which increase their potential to cause disease.[37] In the present study, increased resistance for 2nd and 3rd generation Cephalosporins like Cefaclor and Cefotaxime is seen. The resistance rates were 79.3%, 67.2% respectively. So the increasing resistance to Cephalosporins prompted us to search for ESBL producers. The incidence of ESBL strains among clinical isolates have steadily increased over the past few years resulting in major problem for clinical therapeutics.

Detection of ESBL isolates is a huge burden for microbiological setups because the ESBL producer GNB may be seen susceptible to some β-lactam antimicrobial agents during In vitro studies and in turn may result into treatment failure. So proper identification is necessary. In this study, the isolation rate of ESBL producers gram-negative bacterial strains were found as 20 (34.4%). Concordant results of ESBL producer microorganisms were observed in 2012-2015 (27.67%).[5,6,25,26]

There are some organisms which may carry genes coding for resistance towards different classes of antimicrobials and many of those termed as MDR (multi-drug resistant) organisms. Many of isolates were observed to be multidrug resistant. Hence this present study provides an insight towards the emergence of increased antimicrobial resistance among urinary pathogens in the rural community which is majorly a result of indiscriminate and overdose of antibiotics. In addition, the present data may give some assistance to the physician's towards treatment policies for urinary tract infections by avoiding misprescription of antimicrobial drugs.

CONCLUSION

High incidence of UTIs among females in 21-40 years age group is a great concern as in the reproductive age treating the infections with various drug resistant bacteria is very challenging. An increasing trend in production of ESBLs among UTI pathogens noted which is more prevalent in *E.coli.* ESBL producing bacteria make the treatment policies much complicated, In this regard, proper antimicrobial surveillance is needed in every healthcare system.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

AUTHORS' CONTRIBUTION

All authors listed have made a substantial, direct and intellectual contribution to the work, and approved it for publication.

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None.

DATA AVAILABILITY

The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

ETHICS STATEMENT

This study was approved by the Institutional Ethics Committee, Shree Guru Gobind Singh Tricentenary University, Gurgaon, India.

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