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Research Article

ANTIMICROBIAL SUSCEPTIBILITY PROFILE AND DETECTION OF EXTENDED SPECTRUM BETA-LACTAMASE PRODUCTION BY GRAM NEGATIVE UROPATHOGENS

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ABSTRACT

Objective: Rates of resistance have undergone considerable variation, and consequently the treatment of urinary tract infection (UTI) requires constant updating of the antibiotic sensitivity profile of important implicated etiological agents in an area, country or institution. The aim of this study was to determine the distribution and antibiotic susceptibility pattern of bacterial strains isolated from patients suffering from UTI at a hospital in Mumbai, as well as identify extended-spectrum β-lactamases (ESBL) producers.

Method*:* A retrospective study was conducted of 112 Gram negative pathogens isolated from symptomatic UTI cases at a tertiary care hospital in central Mumbai. Automated identification and susceptibility (AST) system that analyzed MIC patterns was used. ESBL producers and their phenotypes were identified. Results were analysed using computer software, specifically designed to evaluate the results generated by the automated system.

Results: Of the 112 tested samples the most prevalent pathogens were *E. coli* (80%) followed by *Klebsiell*a spp (16.07%). Majority of the isolates (59%) were from females. Results indicated that ampicillin, ampicillin/sulbactam, and third generation cephalosporins like ceftriaxone and cefpodoxime should no longer be considered as first line of drugs for UTI. Instead, amikacin, carbapenem drugs, combinations of piperacillin/tazobactum, cefopar/tazobactum and cefoperazone-sulbactum could be preferred for the treatment of complicated UTI. 27.6% of isolates were found to produce ESBL*s* among which 70.9% were *E. coli* isolates. CTX-M like was the most common ESBL (51.6%). 12.9% of ESBL producers were found to produce carbapenemase enzyme. Production of AmpC β-lactamase was also detected. ESBL producers showed around 70 % resistance to cefepime, ampicillin, ampicillin /sulbactum and ciprofloxacin.

Conclusion: The current study reveals an increasing resistance to third generation cephalosporins and quinolone drugs. The data highlights a serious need to monitor patients for ESBL-producing *Enterobacteriaceae,* particularly the highly prevalent, CTX-M like ESBL, in general practice. The present investigation could prove to be useful for clinicians to ensure improved treatment as well as serve as a platform for further studies across the city.

Keywords: Urinary tract infection, Extended-spectrum beta-lactamase, Antibiotic resistance, Uropathogens, Carbapenemase.

INTRODUCTION

Urinary tract infection is considered to be the most common bacterial infection and is said to be responsible for about five percent of all visits to primary care physicians [1]. Approximately 40 percent of women and 12 percent of men experience at least one symptomatic infection during their lifetime. Nevertheless, it is difficult to accurately assess the incidence of UTIs, because they are not considered reportable diseases in most countries. Specific subpopulations at increased risk of UTI include infants, pregnant women, patients with spinal cord injuries, diabetes, multiple sclerosis, acquired immunodeficiency disease syndrome or underlying urologic abnormalities [2]. Catheter-associated UTI is the most common nosocomial infection, accounting for more than one million cases in hospitals and nursing homes. In non institutionalized elderly population, UTIs are the second most common form of infection, accounting for nearly 25% of all infections. There are important medical and financial implications associated with UTIs [2]. Uncomplicated cases are easily treated with a short course of antibiotics, although resistance to many of the antibiotics is increasing. Due to the rapidly evolving adaptive strategies of microorganisms, the etiology of UTI and the antibiotic resistance profile of uropathogens has changed considerably over the past years, both in community and nosocomial infection [3].

While several pathogenic bacteria have become resistant to first line broad spectrum antibiotics, fresh resistant strains have resulted from the introduction of new drugs [4, 5, 6]. Gram negative pathogens like Proteus, Pseudomonas, Escherichia coli and Klebsiella have been associated with cases which are increasingly challenging to treat. India has witnessed a rise in the number of antibiotic-resistant microorganisms, primarily due to administration of drugs without any preceding isolation or sensitivity test, coupled with poverty and illiteracy [7]. Among the emerging resistant pathogens, spread of infections caused by Gram negative multidrug resistant and extended-spectrum β-lactamases (ESBL) producing hospital isolates is of major concern. ESBL*s* are defined as beta-lactamases capable of hydrolyzing oxyiminocephalosporins [8]. ESBL*s* are chromosomal or plasmid mediated β-lactamases which have mutated from pre-existing broad-spectrum β- lactamases (TEM-1, TEM-2, SHV-1) as a consequence of widespread use of 3rd generation cephalosporins as well as aztreonam [9]. Production of ESBL enzymes confers multiple drug resistance, making infections difficult to treat [10]. Study of drug resistance among uropathogens has recently gained importance since the mechanism of resistance of ESBL production can vary. This along with the vast number of species included in the family Enterobacteriaceae adds to the diagnostic and clinical complications associated with such infections [11]. Due to considerable variability in different countries, local epidemiological data along with local resistance patterns is critical for clinicians for the effective management of UTI*s*.

The current investigation attempts to examine the incidence of community acquired UTI among patients admitted for treatment at a tertiary care hospital in central Mumbai. Further, the study reports the antibiogram pattern and the prevalence of ESBL production among the clinical isolates. Such area specific monitoring studies would assist in documenting the type of pathogen responsible for causing UTI*s* and help in the selection of an effective empirical treatment [12].

MATERIALS AND METHODS

Samples

A total of 112 clinical isolates from UTI patients were analysed at the microbiology laboratory of a tertiary care hospital located at central Mumbai (Maharashtra, India) during the first six months of the year 2013.

Bacterial Isolation

Urine samples (mid-stream urine) were cultured using a 10µml calibrated loop onto blood agar, MacConkey agar and CHROMagar™ plates. These were incubated at 37 °C for 18-24 hours and the number of colonies was counted. Significant bacteriuria was defined as greater than 10^5 colony forming units/ml of a single pathogen.

Identification and determination of antimicrobial susceptibility

Direct inoculation of urinary pathogens from CHROMagar media was done for identification using VITEK® 2 compact system, an automated ID and susceptibility (AST) system (bioMe´rieux, USA). The system included an Advanced Expert System (AES) that analyzed MIC patterns and detected the phenotype of organisms. Pure subcultures of QC and clinical organisms were suspended in aqueous 0.45% (wt/vol) NaCl to achieve a turbidity equivalent to that of a McFarland 2.0 standard (range, 1.80 to 2.20), as measured by the DensiChek (bioMe´rieux) turbidity meter. Strain characterization and antimicrobial susceptibility testing were performed with the VITEK 2 automated system using the ID-GNB and AST-037 cards, in accordance with the manufacturer's instructions. The VITEK 2 instrument automatically filled, sealed, and incubated the individual test cards with the prepared culture suspension. Cards were held at 35.5°C for 18 h, with optical readings taken automatically every 15 min. Based on these readings, an identification profile was established and interpreted according to a specific algorithm. Final profile results were compared to the database, generating identification of the unknown organism. The antimicrobial susceptibility testing card comprised of various antibiotics including ceftriaxone, aztreonam, gentamycin,imipenum and ciprofloxacin. Final results were analysed using version 3.02 software, an AES specifically designed to evaluate the results generated by the VITEK 2 system. Testing was repeated wherever suggested by the AES.

ESBL Testing

Each isolate was tested using the VITEK 2 system with the ESBL test panel with six wells containing three third generation cephalosporins, alone and in combination with clavulanic acid (CA). Growth in each well was quantitatively assessed by means of an optical scanner. The proportional reduction in growth in wells containing cephalosporin plus CA compared with those containing the cephalosporin alone was considered indicative of ESBL production. Quality control strains were included in each run. All phenotypic interpretations of ESBLs were reported as a positive ESBL screening result. Strains were reported as ESBL-negative whenever phenotypic interpretations other than ESBLs were proposed by the AES.

RESULTS AND DISCUSSION

Urinary tract infection is among the most prevalent infectious disease in general population. Associated symptoms are burning micturation, pain during voiding and increased frequency of urination, the alleviation of which in uncomplicated urinary tract infection has been explored by several investigators [13]. The effectiveness of an antibiotic administered to a patient depends on the site and severity of the infection, liver and renal function, presence of implants and local (geographic) resistance patterns. It is also believed that age and pregnancy in the patient determine the effectiveness of the antibiotic used [14]. Recently, with increased rates of antimicrobial resistance, treatment of complicated UTIs (cUTIs) has become increasingly challenging for clinicians.

Amoxycillin (a β-lactam antibiotic) was traditionally used in the first line therapy for UTIs, but with the spread of drug resistance, treatment options have now changed. Complicated cases of UTI usually require a longer course or intravenous antibiotics, and in case symptoms do not improve in two or three days, further diagnostic testing is needed. Since bacterial resistance to antibiotics represents a serious problem for clinicians and pharmaceutical industry, efforts have been made recently to reverse this trend by exploring alternate methods [15, 16].

For the current investigation, a total of 112 urine specimens received and processed in a hospital located in central Mumbai were studied. More than 105 colony forming units (cfu) of bacteria/mL of urine was considered significant bacteraemia. Gram-negative isolates were identified up to species level by VITEK 2 automated microbiology system. Of the total 112 isolates, the most commonly isolated bacteria was Escherichia coli 90 (80%). Other isolates included Klebsiella pneumonia 18 (16.07%), Pseudomonas aeruginosa 2 (2.67%) and Proteus mirabilis 1 (0.8%). Other studies have also similarly reported a high frequency of UTI caused by Escherichia coli [17, 18].

Data analysis revealed a much higher percentage of women (59%) to be suffering from UTI as compared to men (41%).It is known that UTI occur more commonly in women, with half of them having at least one infection at some point in their lives. It is believed that bacteria are usually transmitted to the urethra from bowel, with females at greater risk due to their anatomy. During pregnancy, high progesterone levels elevate the risk of decreased muscle tone of the ureter and bladder, which leads to a greater likelihood of reflux, towards the kidneys.

For the study, the antibiogram pattern of the 112 isolates was checked against 25 antibiotics belonging to different groups and possessing varied modes of action. A high number of cultures showed resistance to most of the drugs, as shown in Figure1.

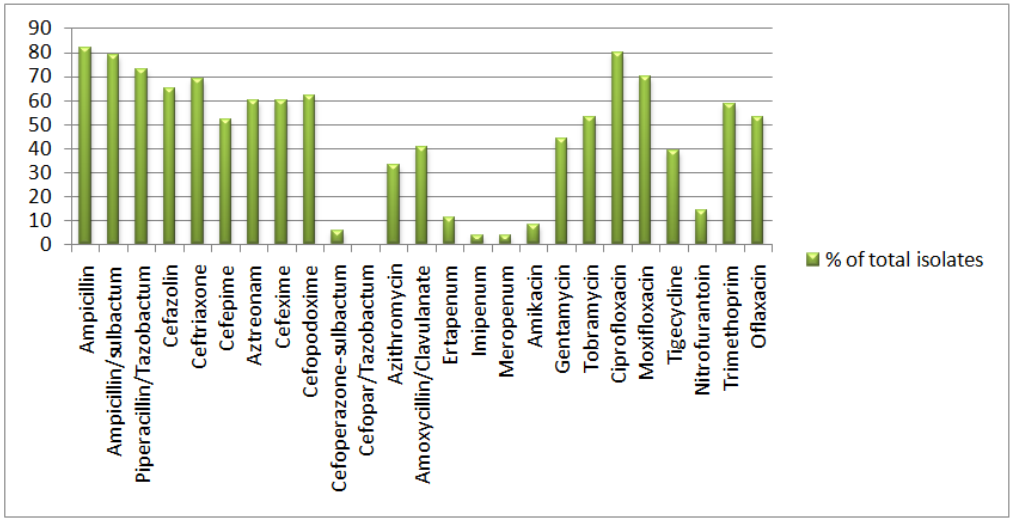
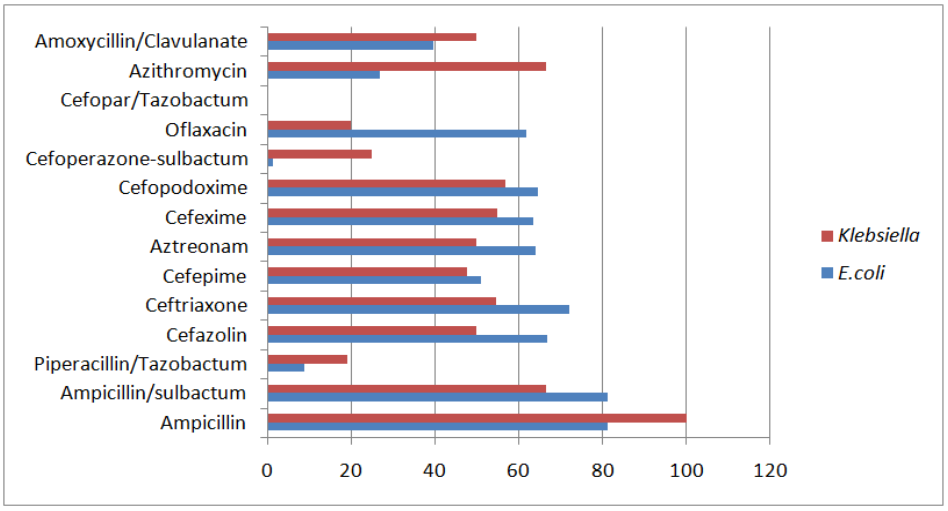
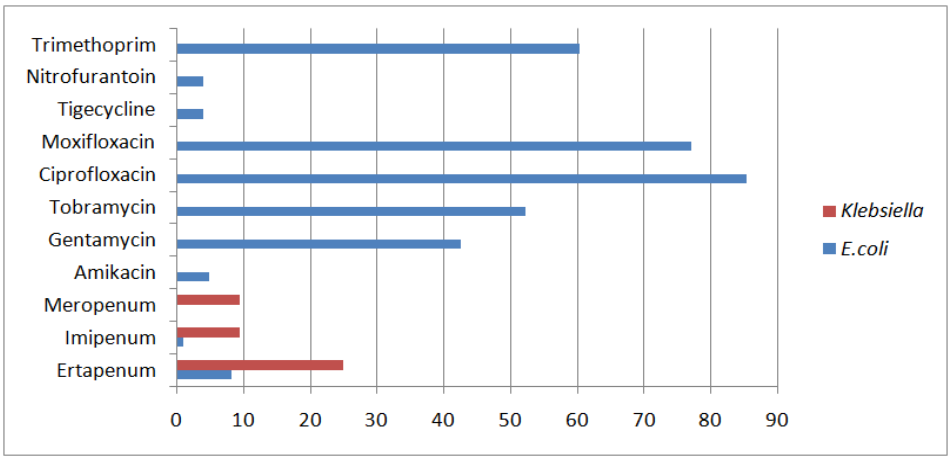


Fig. 1: Percentage of total isolates resistant to the drugs tested (n= 112).

The highest resistance overall was observed against ampicillin, ciprofloxacin and ampicillin/sulbactum combination while all isolates showed sensitivity towards cefopar/tazobactum combination. Figure 2(A) and (B) depict the antibiotic susceptibility profile of the two main clinical isolates, *E.coli* and *Klebsiella,* observed during the study.



(A)



(B)

Fig. 2: Percentage of *E.coli* and *Klebsiella* isolates resistant to the drug tested. (*E.coli* n= 90, *Klebsiella* n=18).

Mandal J. et al, in 2012 have reported very similar patterns of resistance among uropathogens isolated from a tertiary hospital at Puducherry, India [19]. They too quoted the percentage of isolates of *E. coli* resistant to ampicillin to be as high as 80.6 per cent and resistance to ceftriaxone as 60.5% and 59.3 % in all isolates of *E.. coli* and *K. Pneumonia* respectively Compared to the other Gram-negative uropathogens, resistance to nitrofurantoin was comparatively less among isolates of *E. coli*.

Data analysis showed that all *Ps. aeruginosa* isolates were resistant to ampicillin/sulbactum combination, tigecycline and ceftriaxone, while 66.6% exhibited increased resistance to cefepime, aztreonam, gentamycin, tobramycin and ciprofloxacin. However a high level of sensitivity was exhibited toward piperacillin/tazobactum combination.

The *proteus* isolate showed resistance against cefepime, cefpodoxime, azithromycin, gentamycin, amikacin, tobramycin as well as to ciprofloxacin. A significantly high resistance was noted to β-lactam group of antimicrobials by all the Gram negative bacilli (GNB).

The accurate detection of extended-spectrum β-lactamases is a major clinical problem, particularly in invasive infections, frequently leading to therapeutic failure and adverse clinical outcome. In typical circumstances, ESBLs derive from genes for TEM-1, TEM-2, or SHV-1 by mutations that alter the amino acid configuration around the active site of these β-lactamases. This extends the spectrum of β-lactam antibiotics susceptible to hydrolysis by these enzymes. Successful spread of ESBL-encoding genes within the microbial genome can be attributed to their common localization on self-transmissible or easily movable broad-range plasmids [20, 21]. Their reliable detection is prerequisite for the successful management of infection and implementation of valid therapeutic strategies. However, often they are not detected during routine susceptibility testing, as the expression of phenotypic resistance is multifactorial, depending on the bacterial carrier and test conditions [22].

The Advanced Expert System (AES) in conjunction with the VITEK 2 automated antimicrobial susceptibility test system is widely used in clinical microbiology laboratories for the identification and evaluation of the susceptibility profiles of bacteria and helps in the detection of extended-spectrum β-lactamases (ESBLs) produced by organisms such as Escherichia coli, Klebsiella pneumoniae and Klebsiella oxytoca . It possesses the ability to identify β-Lactam phenotypes in isolates of Enterobacteriaceae and Pseudomonas aeruginosa [23]. The phenotypic data generated in the current study, using this system, indicates a considerably significant prevalence of ESBL producers in the region of central Mumbai, where a total of 31 out of 112 (27.67%) uropathogens were found to be ESBL producers (Table 1).

Table 1: Number of ESBL producing uropathogen isolates.

Pathogens	No of isolates	ESBL producers
Escherichia coli	90	22
Klebsiella pneumoniae	18	7
Pseudomonas aeruginosa	3	2
Proteus mirabilis	1	0
Total	112	31

Many countries share a high prevalence of extended-spectrum-β-lactamase and carbapenemase-producing GNB, most of which are associated with nosocomial infections. β-lactamase genes such as those for CTX-M-15, OXA-48, and NDM-1 are well-known and have become widespread. The β-Lactum phenotypes of the isolates observed during the current study are shown in Table 2. It was noted that CTX-M like was the most common ESBL (51.6%).

Table 2: **β-Lactum phenotypes of the isolates**

Species (no. of isolates)	β-Lactam phenotype (no. of isolates)
E. coli (22)	CTX-M like (16), ESBL (3), acquired penicillinase (2), high level cephalosporinase (AmpC)* (1)
Klebsiella pneumoniae (7)	CTX-M like, ESBL + impermeability (cephamycin)** (2), ESBL carbapenemase(2), acquired penicillinase + impermeability SHV1 hyperproduction(1), acquired penicillinase + cephamycin (1), ESBL+ impermeability + cephamycin (1)
Ps. aeruginosa(2)	carbapenemase(2)

*The phenotype results from mutation in regulatory genes that control the amount of the basal level of AmpC β-lactamase and the inducibility of enzyme expression.

**The phenotype involves reduced susceptibility to cephamycins, cefepime, and imipenem as well as other β-lactam antibiotics.

CTX-M beta-lactamases are named for greater activity against cefotaxime than other oxyimino-beta-lactam substrates (e.g., ceftazidime, ceftriaxone, or cefepime). Rather than arising by mutation, they represent examples of plasmid acquisition of beta-lactamase genes These enzymes are not very closely related to TEM or SHV beta-lactamases in that they show only approximately 40% identity with these two commonly isolated beta-lactamases. More than 80 CTX-M enzymes are currently known. Emergence of CTX-M, producing *Enterobacteriaceae* isolates is of major concern and highlights the need for further surveillance in this area.

Though carbapenems are considered the drug of choice for serious infections, carbapenem-resistant isolates have recently been reported. In the current study 12.9% of ESBL producers were found to produce carbapenemase. AmpC β-lactamases are clinically important cephalosporinases encoded on the chromosomes of many of the Enterobacteriaceae and a few other organisms. In contrast to ESBLs, they hydrolyse broad and extended-spectrum cephalosporins (cephamycins as well as oxyimino-β-lactams) but are not inhibited by β-lactamase inhibitors such as clavulanic acid. They mediate resistance to cephalothin, cefazolin, cefoxitin, most penicillins, and β-lactamase inhibitor-β-lactam combinations. Resistance due to plasmid-mediated AmpC enzymes are less common than extended-spectrum β-lactamase production in most parts of the world but are usually both harder to detect and broader in spectrum [24]. In the current study too, only one isolate (3.2%) was detected as resistant due to Amp C enzyme.

The antibiogram pattern for the ESBL-producing isolates towards antibiotics that are routinely prescribed against urinary tract infections is shown in Figure 3.

ESBL producers showed around 70 % resistance to antibiotics like cefepime, ampicillin, ampicillin /sulbactum combination and ciprofloxacin. The most effective antibiotic against ESBL producers was found to be cefopar/tazobactum, with all the isolates showing sensitivity to this drug combination.

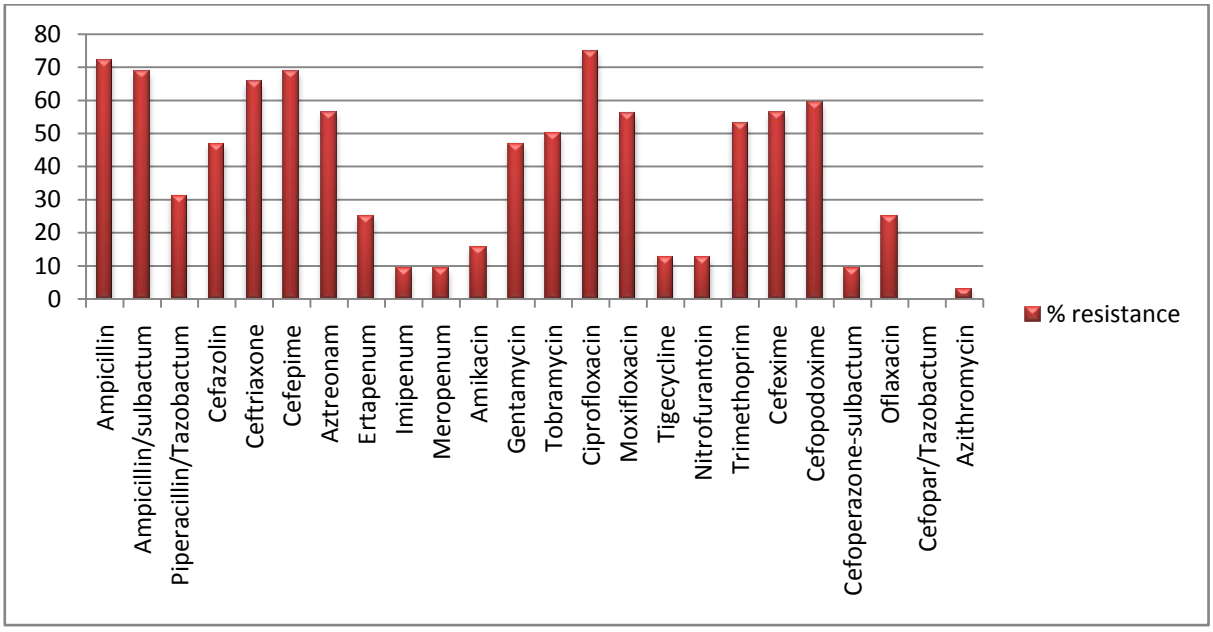


Fig. 3: Percentage of total ESBL producers (n= 31) resistant to different drugs tested.

CONCLUSION

In the current study, *E. coli* was found to be to be the most predominant bacterial pathogen responsible for community acquired UTIs among patients admitted at the hospital.

More than half of the isolates of GNB were resistant to ceftriaxone or cefpodoxime, both third generation cephalosporins. However, cefoperazone-sulbactam (a combination of third generation cephalosporin with β lactamase inhibitor) and cefopar/tazobactum

were found to be effective drugs against these organisms. Quinolones including third generation drug, moxifloxacin had poor activity against *E.coli* and *Klebsiella* whereas a high degree of sensitivity was seen with carbapenum drugs towards all *Enterobacteriaceae* isolates. A similar pattern of resistance by Gram negative uropathogens has been reported recently by investigators [18, 25]. A significantly high number of isolates were found to be producing ESBL, especially the CTX-M like ESBL.

The current trend of growing antibiotic resistance indicates that it is imperative to rationalize the use of antimicrobials and employ them conservatively. Surveillance must be strengthened to monitor and control the emergence of multi-drug resistant organisms. However since the present investigation was restricted to a specific healthcare centre, additional studies can be carried out with a larger sample size from various hospitals across the city to obtain a more representative picture.

REFERENCES

1. Michael L. Wilson ML, Gaido L. Laboratory Diagnosis of Urinary Tract Infections in Adult Patients. Clin Infect Dis. (2004) 38 (8):1150-1158.
2. Foxman B. Epidemiology of urinary tract infections: incidence, morbidity, and economic costs. Am J Med. 2002;113 Suppl 1A:5S-13S.
3. Manges AR, Natarajan P, Solberg OD, Dietrich PS, Riley LW. 2006. The changing prevalence of drug-resistant Escherichia coli clonal groups in a community: evidence for community outbreaks of urinary tract infections. Epidemiology Infection 134, 425-431.
4. Kunin CM. Resistance to antimicrobial drugs - a worldwide calamity. Annals of Internal Medicine, 1993.118: 557 – 561.
5. Sack RB, Rahman M., Yunus M, Khan EH. Antimicrobial resistance in organisms causing diarrhoeal disease. Clin. Infect. Disease; 1997.24(1): S102- S105.
6. Rahal K, Wang F, Schindler J et al. Reports on surveillance of antimicrobial resistance on individual countries. Clin Infect. Disease, 1997.24(1): S69 – S 75.
7. Akram M, Shahid M, Khan AU. Etiology and antibiotic resistance patterns of community acquired urinary tract infections in JNMC Hospital Aligarh. Ann Clin Microbiol Antimicrob 2007; 6:4.
8. Ambler RP. The structure of Beta- lactamases. Philos. Trans. R. Soc. London, B, Biol Science 289 (1036), 1980, 321-31
9. Shukla I, Tiwari R, Agrawal M. Prevalence of extended spectrum β-lactamase producing Klebsiella pneumoniae in a tertiary care hospital. Indian J Med Microbiol.2004; 22:87-9.
10. Hassan SA, Jamal SA, Kamal M. Occurrence of Multidrug Resistance and ESBL producing E.coli causing Urinary tract infections. J of Basic and Applied Sci Vol 7, No 1, 2011, 39-43.
11. Aruna K, Mobashshera T. Prevalence of extended spectrum beta-lactamase production among uropathogens in south mumbai and its antibiogram pattern. Excli journal. 2012; 11:363-372.
12. Smith RD, Coast J. Antimicrobial resistance: a global response. Bull World Health Organ. 2002; 80:126–133.
13. Deepalatha C, Deshpande N. A comparative study of phenazopyridine (pyridium) and cystone as short- term analgesic in uncomplicated urinary tract infection. Int J Pharm Pharm Sci, 2011: 3 (2):224-226.
14. Aggarwal R, Chaudhary U, Sikka R. Detection of extended spectrum beta-lactamase production among uropathogens. J Lab Physicians. 2009; 1:7-10.
15. Shrikant, Sethi S, Gupta BL. Antimicrobial activity of medicinal plants on urinary tract pathogens , Int J Pharm Pharm Sci, 2012 ;4(2):626-628.
16. Borchert D, Sheridan L, Buchholz N. et al. Prevention and treatment of urinary tract infection with probiotics: Review and research perspective. Indian J. Urol, 2008; 24(2):139-144.
17. Pais P, Khurana R, George J. Urinary tract infections: a retrospective survey of causative organisms and antibiotics prescribed in a tertiary care setting. Indian J Pharmacol. 2002; 34:278–80.
18. Rizvi M, Khan F, Shukla I, Malik A, Shaheen. Rising Prevalence of Antimicrobial Resistance in Urinary Tract Infections During Pregnancy: Necessity for Exploring Newer Treatment Options. J Lab Physicians. 2011 Jul-Dec; 3(2): 98–103.
19. Mandal J, Acharya NS, Buddhapriya D, Parija SC. Antibiotic resistance pattern among common bacterial uropathogens with a special reference to ciprofloxacin resistant *Escherichia coli* .Indian J Med Res, 2012; 136: 842-849.
20. Jacoby GA, Sutton L. Properties of plasmids responsible for production of extended-spectrum β-lactamases. Antimicrob Agents Chemother .1991.35: 164–169.
21. Paterson DL, Bonomo RA. Extended-spectrum β-lactamases: a clinical update. Clin Microbiol Rev. 2005.18:657–686.
22. 22.Spanu T, Sanguinetti M, Tumbarello , D'Inzeo T et al. Evaluation of the new VITEK 2 extended-spectrum β-lactamase (ESBL) test for rapid detection of ESBL production in *Enterobacteriaceae* isolates. J Clin Microbiol .2006.44, 3257–3262.
23. Sanders CC, Peyret M, Moland EL,etal. Ability of the VITEK 2 Advanced Expert System to Identify β-Lactam Phenotypes in Isolates of *Enterobacteriaceae* and *Pseudomonas aeruginosa.* J. Clin. Microbiol. February 2000. 38 (2):570-574.
24. Jacoby GA. AmpC β-Lactamases. Clin. Microbiol. Rev. January 2009. 22 (1): 161-182.
25. Sasirekha B. Prevalence of ESBL, AMP C β- lactamases and MRSA among uropathogens and its antibiogram. EXCLI Journal. 2013; 12:81-88.