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Original Research Article

Susceptibility of E. coli to Commonly Used Antimicrobials isolated From Urine Samples

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Abstract: Urinary tract infections (UTIs) are among the most common infectious diseases all over the world. Recent studies reported an increased antibiotic resistance in Escherichia coli, primary causative agent of UTI. The resistance has emerged even to more potent antimicrobial agents like fluoroquinolones. Details of 204 urine culture positive reports for E.coli and their antibiotic sensitivity pattern pertaining to the study period of 4 years from Jan 2012 to Dec 2015 were collected from Central Microbiology Laboratory of Tertiary care Hospital and the results were statistically analysed. The sensitivity pattern of E.coli to antibiotics in UTI were Nitrofurantoin (89.68%), Amikacin (88.25%), Co-trimoxazole (27.44%), Gentamycin (49.90%), Ceftazidime (22.5%), Ciprofloxacin (10.78%), Levofloxacin (31.85%) Cefotaxime (15.78%), Cefuroxime (14.21%), Ceftriaxone (16.17%), Piperacillin-Tazobactum (85.27%). The isolates showed high levels of resistance to Ampicillin, Amoxicillin-Clavulanic Acid, Norfloxacin, Cefuroxime, Ceftriaxone and Co-trimoxazole. The study shows that the organism E.coli in UTI is resistant to commonly prescribed drugs like Quinolones. This resistance was seen more in the in-patients, elderly males and females. The drug Quinolone is commonly prescribed because it achieves high concentration in urine. Over use of Quinolone has led to increased prevalence of E.coli resistance to Quinolones E.coli has developed resistance to third generation Cephalosporins, Quinolones, and cotrimoxazole and so they cannot be considered for empirical treatment in UTI caused by E.coliin this group of population.
Keywords: *E. coli*, Urinary Tract Infection, Antibiotic sensitivity.

INTRODUCTION

Urinary Tract Infections [UTI] are most commonly encountered infections in clinical practice all over the world with high rate of morbidity and economic burden on health care systems. UTI are commonly caused by Gram-negative bacteria such as Escherichia coli, *Klebsiella species, Enterobacter species, Proteus species* and Gram-positive bacteria like *Enterococcus* species, and *Staphylococcus saprophyticus*. *E. coli* is the most common organism causing both community as well as hospital acquired UTIs [1]. The incidence of UTI is greater in female as compared to male that may be due to anatomical predisposition or urothelial mucosa adherence to mucopolysaccharides lining or other host factors [2]. In men as the age advances the incidence of UTI increases which may be due to enlargement of prostate gland or neurogenic bladder [3]. Recurrent UTI can result in irreversible damage to the kidneys with renal hypertension or frank renal failure in severe cases [3]. Despite the widespread availability of antibiotics, UTI remains the top most bacterial infection in human population. [4] Antibiotics are generally prescribed empirically before the laboratory results of urine culture are available [5] to ensure proper antimicrobial therapy the knowledge of antimicrobial resistance pattern of common uropathogens is essential. Therefore there exists a need for antimicrobial surveillance at various levels. With this aim we in the present study tried to evaluate the antibiotic resistance pattern of E.coli in urinary tract infections in a tertiary care hospital.

MATERIALS AND METHODS

The study was conducted in a tertiary care hospital, detail of 426urine culture samples out of which 205 positive reports for E.coli and their antibiotic sensitivity pattern pertaining to the study period of 4 years from Jan 2012 to Dec 2015 were collected from Central Microbiology Laboratory. Ethical permission for the study was obtained from Institutional Ethical committee. URICHROM was used for the analysis of samples because of the ability of URICHROM to support the growth of the organisms was concurrent with that of the conventional media. In addition it also provided the advantage of rendering colour differentiation to the various isolates for preliminary identification, which were further confirmed by standard tests using Micro Scan walkaway 96 s fully automated instrument

- Escherichia coli – mostly Pink to burgundy
- Klebsiella – mucoid green
- Enterobacter spp – light mucoid green
- Proteus, Providencia - mostly brown
- Morganella species - mostly brown
- Pseudomonas aeruginosa -Green
- Staphylococcus -Colourless
- Staphylococcus aureus – golden yellow for first 24 hours , later greenish opaque after 48 hours
- Streptococcus -Colourless
- Enterococcus spp – mostly pin point green to pin point violet
- Candida – Colourles

Hence preliminary identification of Escherichia coli was done within first 18 – 24 hours, thesamples were then inoculated on culture plates, inoculated plates were incubated at 37°C over night (16-20 hr) and examined the next day morning. The organisms isolated from urine culture were identified by standard conventional methods and negative breakpoint combo 42 and identification by walkaway 96 s fully automated for identification and sensitivity by MIC values. Comparatively the antibiotic sensitivity test was done on Mueller-Hinton agar by Kirby-Bauer disc diffusion test as per Clinical and Laboratory Standard Institute (CLSI) guidelines The isolates were tested for Ampicillin (10 µg), Cefuroxime (30 µg), Ceftriaxone (30 µg), Norfloxacin (10 µg), Nitrofurantoin (300 Mg), Amoxicillin-Clavulanic Acid (10/20 Mg), Co-Trimoxazole (1.25/23.75 Mg), Cefepime (30 Mg), Ciprofloxacin (5 Mg), Amikacin (30 Mg), Piperacillin-Tazobactam (100/10 µg) and imipenem (10 µg) (Hi-mediA). After adding inoculums of 0.5 mcfarland turbidity standards, specified antibiotic discs placed 2 cm apart from each other with sterile forceps and were incubated for 16-18 hours at 37°C aerobically The degree of sensitivity was determined by measuring zone of growth inhibition around the disc. The growth of bacterium would be inhibited around the discs containing antibiotics to which the bacterium is susceptible, while no inhibitory zone around is observed for resistant ones. The results were interpreted as sensitive, intermediately sensitive and resistant to the different drugs. The zone of inhibition was interpreted according to the Kirby-Bauer antibiotic sensitivity chart An isolate was considered as MDR if found resistant to three or more antimicrobials belonging to different classes/groups of antimicrobials. The data regarding the urine culture and sensitivity pattern were obtained from the Microbiology laboratory registers. The patients' details were collected from case sheets in the Medical Records Department and wards.

RESULTS

A total of total 425 urine positive cultures - 205 isolates were E.coli, 48.23 % and 13.65 % were Klebsiella pneumonia,0.47% were Klebsiella oxytoca , 11.7% were Pseudomonas aeruginosa, 1.40% % were Proteus mirabilis , 1.17% were Morganella , 7.25% were Enterococcus spp,3.51% were Enterobacter spp, 9.61% were Candida spp Among the various organisms isolated from urine culture are shown in *E. coli* was the commonest accounting for 48.23 % of the uropathogens given in table 1.

Table 1: Proportion of the uropathogens among the urinary isolates

Organism	Total number of positive samples (n= 425)	Percentage
E.coli	205	48.23%
Klebsiella pneumonia	58	13.65%
Klebsiella oxytoca	2	0.47%
Pseudomonas aeruginosa	50	11.76%
Burkholderia cepecia	3	0.70%
Stenotrophomonas maltophila	2	0.47%
Acinetobacter spp	2	0.47%
Proteus spp	6	1.41%
Morganella	5	1.17%
Enterobacter spp	15	3.51%
Citrobacter spp	5	1.17%
Enterococcus spp	31	7.29%
Candida spp	41	9.64%
Total	425	100

Of these 205 *E. coli* isolates, 91 (76.51%) were multi drug resistant (MDR). The isolates showed high levels of resistance to Ampicillin (96.6 %), Amoxicillin-Clavulanic Acid (71.7%), norfloxacin (92.159%), Cefuroxime (85.8%), Ceftriaxone (83.83%) and Co-Trimoxazole (72.56 %), Gentamycin (48.84 %), Ceftazidime (77.6 %), Ciprofloxacin (88.8 %), Evofloxacin (67.86 %) , Norfloxacin (92.2 %), Cefotaxime (84.22 %).

Table 2 – Resistance pattern of commonly used urinary antimicrobials

Antibiotic	Percentage resistance
Ampicillin	96.60%
Amox-clav	71.70%
Norfloxacin	92.16%
Cefuroxime	85.80%
Ceftriaxone	83.83%
Cefotaxime	84.22%
Ciprofloxacin	88.80%
Levofloxacin	67.86%
Nitrofurantoin	10.32%
Amikacin	10.71%
Pip-tazo	36.21%

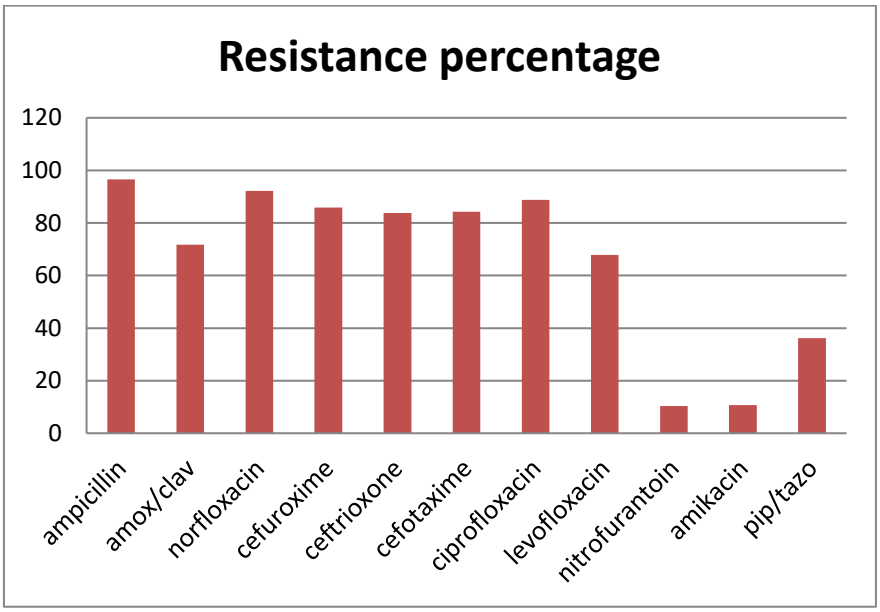

Fig-1: Showing the resistance percentage to various antibiotics

The isolates were sensitive to Amikacin (89.29%), Piperacillin-Tazobactum (63.79%), Nitrofurantoin (89.68%) and Imipenem (98.9%).

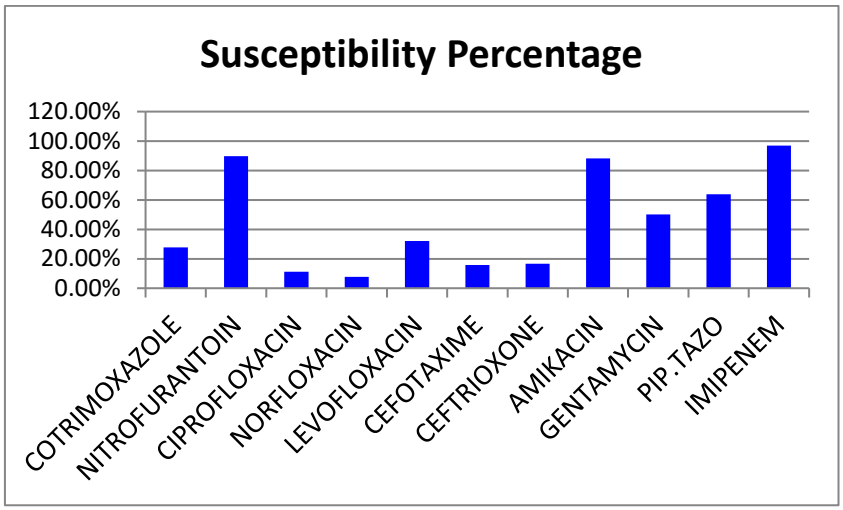

Fig-2: Sensitivity pattern of Escherichia coli

DISCUSSION

In the present study we found E.coli to be the commonest organism in urinary isolates 47.97%. Similar findings have been reported by Inabo and Obanibi [6]. The other common organisms were Klebsiella pneumonia 13.57% and Pseudomonas aeruginosa 11.7%given in table 1. The isolates showed high levels of resistance to Ampicillin (96.6 %), Amoxicillin-Clavulanic Acid (71.7%), Norfloxacin (92.159%), Cefuroxime (85.8%), Ceftriaxone (83.83%) and Co-Trimoxazole (72.56%). Tankhiwale et al; observed that maximum resistance was towards Ampicillin (79.6%), Co-Trimoxazole (82%), Nalidixic Acid (73.8%). They also reported that Nitrofurantoin

(62%), Cephotaxime (58.7%) and Norfloxacin (45%), constitute the reasonable option for treatment of UTI [7]. In the present study sensitivity was shown towards Amikacin (89.29%), Nitrofurantoin (89.68%) and highest sensitivity was towards Imipenem (98.9%).In a similar study by Das N et al; they found mean susceptibility was high towards Amikacin (87.2%), followed by Ciprofloxacin 74.8%, Ceftazidime 71.5% [8]. The sensitivity to Ampicillin, Cefuroxime, Ceftriaxone, Norfloxacin, Ciprofloxacin, & beta lactam / lactamase inhibitor combinations varied from 11-25 per cent.During the analysis it was observed that empirical therapy was started in 65 % of the cases. In most inpatient cases ceftriaxone was used, followed by Ciprofloxacin, Ofloxacin, Levofloxacin. In the study we found that 105 out of 205 (51.21%) E. coli isolates were ESBL producing and were Multi Drug Resistant [MDR]. A similar study by V Niranjan *et al*; found that 76.5 per cent of E. coliisolates from urine samples of inpatients were MDR [9]. The main reason for variations in resistance patterns depends up on the antibiotic usage in that area. Multiple Antibiotic Resistance [MAR] Indexing is a cost effective and valid method of bacteria tracking. Multiple antibiotic resistance index is calculated as the ratio of number of resistant antibiotics to which organism is resistant to total number of antibiotics to which organism is exposed [10]. MAR values of greater than 0.2 indicate high risk source of contamination where antibiotics are often used. In the current study we found that the MAR resistance of 51.21% were greater than 0.2 indicating high level of exposure of the organism to antibiotics. In one study by Ehinmidu *et al*; reported that the E.coli, S aureus and Ps aeruginosa strains were sensitive to ciprofloxacin and gentamicin and these isolates were rersistant to ampicillin, a finding similar to ours. They also reported that MAR resistance index of isolated bacteria was greater than 0.2 in agreement with our findings However in our study only 51.21% were having MAR indices greater than 0.2 [11]. In the present study E.coli has been found to be more sensitive to Nitrofurantoin than other antibiotics used in the sensitivity test. E.coli is highly resistant to third generation Cephalosporins, Quinolones and Aminoglycosides. Among the Aminoglycosides resistance is more to Gentamycin than to Amikacin Re-emergence of E.coli sensitivity to Nitrofurantoin is probably due to non-usage of the drug for a long period of time. Nitrofurantoin has been less commonly used in the treatment of uncomplicated UTI in recent years.

CONCLUSION

The study shows that the organism E.coli in UTI is resistant to commonly prescribed drugs like Quinolones in half of the cases. The drug Quinolone is commonly prescribed because it achieves high concentration in urine. Over use of Quinolone has led to increased prevalence of E.coli resistance to Quinolones E.coli has developed resistance to third generation Cephalosporins, Quinolones, and cotrimoxazole and so they cannot be considered for empirical treatment in UTI caused by E.coli in this group of population.

Conflict of interest: None

Source of Support: Nil

Ethical Permission: Obtained

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