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Original Article

Microbiology Section

# The Antibiotic Susceptibility Patterns of Uropathogenic *Escherichia Coli*, With Special Reference to the Fluoroquinolones

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## ABSTRACT

**Context:** The emergence of drug resistance to trimethoprim-sulfamethoxazole, the penicillins, cephalosporins, and fluoroquinolones by Uropathogenic *Escherichia coli* (UPEC) has limited the options for selecting the appropriate antibiotic for the treatment of urinary tract infections.

**Aims:** The The *E. coli* isolates, which were obtained from the culture of urine samples,were studied for their antibiotic resistance patterns, with special reference to the antimicrobial activity of the fluoroquinolones and the production of the extended spectrum ß-lactamases. (ESBL), Settings and Design: This was a hospital based, prospective study which was done for a period of eighteen months.

**Material and Methods:** This study was done by using the standard culture techniques for urine samples, the modified Kirby-Bauer disk diffusion method for the antibiotic susceptibility testing and the disk diffusion method to confirm the ESBL production by the clinical isolates of *E. coli* in urine. The sensitivity pattern was correlated with the clinical condition and the presence of the risk factors.

**The statistical analysis which was used:** The statistical analysis was done by using the proportions of sensitive, resistant and intermediates. Descriptive statistics like the total, mean and percentage were done by using the Statistical Package for the Social Sciences (SPSS), version 15.0.

**Results:** The hospital isolates showed high degrees of resistance to the penicillins, cephalosporins, nalidixic acid and the fluoroquinolones, with 59% of the isolates being ESBL producers.

**Conclusions:** The incidence of the multidrug resistant strains of *Escherichia coli* has been steadily increasing over the past few years. The knowledge on the resistance pattern of the bacterial strains in a geographical area will help in guiding the appropriate and the judicious use of antibiotics. Also, the formulation of an appropriate hospital antibiotic policy will go a long way in controlling these infections.

**Key Words:** ESBL, UPEC, Fluoroquinolones, *Escherichia coli*

## INTRODUCTION

*Escherichia coli*, the most prevalent facultative gram-negative bacillus in the human faecal flora, usually inhabits the colon as an innocuous commensal [1]. Urinary tract infections (UTIs) are the most common form of the extra-intestinal *Escherichia coli* infections and *Escherichia coli* is the most common cause of UTIs. At some point of their lives, at least 12% men and 10-20% women experience an acute symptomatic UTI [2-4], and even greater numbers develop asymptomatic bacteriuria [3].

*Escherichia coli* exhibits multi-drug resistance. The prevalence of antimicrobial resistance among the human clinical isolates of *Escherichia coli* has increased dramatically in the recent years. This emerging resistance limits the use of agents like trimethoprim-sulfamethaxole, which results in increased reliance on broad spectrum agents such as the fluroquinolones and extended spectrum cephalosporins. Unfortunately, this emerging resistance is now threatening these agents as well [5]. With the emergence of the resistance to the fluoroquinolones and the first, second and third generation cephalosporins, the treatment of the infections which are caused by this organism has become more challenging and it may even increase the morbidity or the mortality of a simple UTI.

## SUBJECTS AND METHODS

The present study was conducted for a period of eighteen months (2009-2010) in the Department of Microbiology, Kasturba Medical College (K.M.C.), Mangalore, India, after obtaining clearance from the institutional ethics committee.The study population included the patients of all the age groups, who attended the Government Lady Goschen Hospital, and KMC Hospitals, Mangalore, India. A detailed clinical history was obtained, which included the age, sex, duration of the hospital stay, the underlying disease, predisposing factors like catheterization, previous episodes of UTIs, the administration of antibiotics and any obstructive pathology like prostate hypertrophy or renal stones or renal stenosis, which were considered to see their effects on the antibiotic sensitivity pattern of the organism. The urine samples were examined macroscopically and microscopically by wet mount and gram staining. The urine culture was done by a semi-quantitative method on Mac conkey's agar and the CLED medium. A colony count of $\geq 10^5$CFU/ml of urine was considered as significant. The bacteria which had grown with significant counts were identified by their colony morphologies, Gram's smears, motilities and biochemical reactions.

The antibiotic susceptibility testing was done by using the modified Kirby-Bauer disk diffusion method. The antibiotic disks which were used were ampicillin, ampicillin/sulbactum, ceftazidime, cefotaxime, cefuroxime,cefaperazone/sulbactum, nitrofurantoin, co-trimoxazole, gentamicin, amikacin, netillin, norfloxacin, ciprofloxacin, piperacillin, piperacillin/tazobactum, imipenem and meropenem. The zone size around each antimicrobial disk was interpreted as sensitive, intermediate or resistant according to the CLSI criteria.

The ESBL production by the *Escherichia coli* strains was tested by the disk diffusion method by using ceftazidime(30µg) vs ceftazidime/clavulunic acid (30/10µg) and cefotaxime (30µg) vs cefotaxime/clavulunic acid (30/10µg). Regardless of the zone diameters, a ≥5mm increase in the zone diameter of an antimicrobial agent which was tested with clavulunic acid, vs its zone size when it was tested alone, indicated ESBL production [6]. The *Klebsiella pneumoniae* ATCC 700603 strain was used as a positive control for the ESBL production and the *Escherichia coli* ATCC 25922 strain was used as a negative control for the ESBL production.

The statistical analysis was done by by using the proportions of sensitive, resistant and intermediates. Descriptive statistics like the total, mean and percentage were done by using the statistical package, SPSS, version 15.0.

## RESULTS

During the study period, a total of 8833 urine samples were collected from the patients with suspected UTIs, amongwhich 2674 were positive by culture (30%). Various organisms like *Escherichia coli*, *Klebsiella spp., Citrobacter spp., Proteus spp., Pseudomonas spp., Enterococcus spp., Staphylococcus spp. and Candida spp.* were isolated. *Escherichia coli* formed 48.3% (1292) of the organisms which were isolated in the culture. 58.5% of the isolates were obtained from female patients. 62% of the samples were obtained from hospitalized persons. Among the ICU urinary tract infections, only 8.9% were caused by *E. coli*. The patients had been admitted to the ICU for various co-morbid conditions like the complications of Diabetes mellitus, postoperative complications, cerebrovascular insufficiency, hepatic dysfunction, neutropaenia or trauma.

The isolates showed the best sensitivity to antibiotics like the carbepenems 100% cefaperazone/sulbactum 95.6%, piperacillin/ tazobactum 92.2% and ampicillin/sulbactum 62.3% [Table/Fig-1]. Cotrimoxazole showed a sensitivity of 41.6%. The isolates showed high degrees of resistance to the pencillins and the cephalosporins. The most common risk factor for acquiring multidrug resistant organisms was a prior exposure to antibiotics and admission to the ICU [Table/Fig-2].

59% of the isolates were ESBL producers. The ESBL producing isolates were also studied for the presence of a co-resistance to ciprofloxacin. 65.4% of the ciprofloxacin disk resistant strains were also ESBL producers [Table/Fig-3].

Similarly, the isolates were resistant to fluoroquinolones like ciprofloxacin, with a sensitivity of only 27% and with a sensitivity of 28.2% for norfloxacin. The 801 isolates which were isolated from the hospital showed 97.7% resistance to ciprofloxacin, 94.7% resistance to norfloxacin and 0.2% resistance to nitrofurantoin. The 491 community acquired isolates showed 32.5% resistance to ciprofloxacin, 34.4% resistance to to norfloxacin and 16.4% resistance to nitrofurantoin [Table/Fig-4].

| Antibiotics n (%) | Hospital n (%) | Community n (%) |
|---|---|---|
| Ampicillin 224 (17.3) | 57 (7) | 167 (34) |
| Ampicillin sulbactum 805 (62.3) | 388 (48) | 417 (85) |
| Cefaperazonesulbactum 1235 (95.6) | 759 (94.7) | 476 (97) |
| Cefotaxime 512 (39.7) | 237 (29.7) | 275 (56) |
| Ceftazidime 771 (59.7) | 481 (60) | 290 (59) |
| Cefuroxime 465 (36) | 185 (14.3) | 280 (57) |
| Cotrimoxazole 537 (41.6) | 238 (29.7) | 299 (61) |
| Amikacin 1212 (93.8) | 730 (91) | 482(98) |
| Gentamicin 808 (62.6) | 381 (47.6) | 427 (87) |
| Netillin 1210 (93.7) | 734 (91.6) | 476 (97) |
| Nalidixic acid 158 (12.2) | 7 (1) | 151 (30) |
| Ciprofloxacin 349 (27) | 18 (2) | 331 (67.5) |
| Norfloxacin 364 (28.2) | 42 (5) | 322 (65.6) |
| Nitrofurantoin 1209 (93.6) | 799 (99.7) | 410 (83.6) |
| Piperacillin 324 (25.1) | 26 (3) | 298 (60.6) |
| Piperacillintazobactum 1191 (92.2) | 725 (90.5) | 466 (95) |
| Imipenem 1292 (100) | 801 (100) | 491 (100) |
| Meropenem 1292 (100) | 801 (100) | 491 (100) |
| ESBL producers 764 (59) | 629 (78.5) | 135 (27.5) |

**[Table/Fig-1]:** Antibiotic sensitivity of E.coli isolates from hospital and community

| Variables | Number |
|---|---|
| Risk factor | |
| Present | 571 |
| Absent | 721 |
| Pregnancy | 153 |
| Catheterization | 198 |
| Prior antibiotics | 403 |
| ICU admissions | 256 |
| Previous episodes of UTI | 107 |
| Obstruction (prostate hypertrophy etc.) | 68 |

**[Table/Fig-2]:** Risk factors in patients with UPEC

| | | Ciprofloxacin | |
|---|---|---|---|
| | | Resistance | Sensitive |
| ESBL | Yes | 611 | 153 |
| | No | 332 | 196 |

**[Table/Fig-3]:** Comparison of resistance pattern of Ciprofloxacin and ESBL production

| Site of isolation and number | Ciprofloxacin | Norfloxacin | Nalidixic acid |
|---|---|---|---|
| Hospital (801) | 783 (97.7%) | 759 (94.7%) | 794 (99%) |
| Community (491) | 160 (32.5%) | 169 (34.4%) | 340 (69.2%) |

**[Table/Fig-4]:** Comparison of fluoroquinolone resistance in isolates from hospitalized and community acquired UTI

## DISCUSSION

Among the suspected cases of urinary tract infections, only 30% were positive on culture. 48.3% of the positive cultures were of

*E.coli*. This percentage was slightly less as compared to that in other studies, as most of the isolates were from hospitalized patients. In case of the community acquired or the outpatient cases, 63.2% of the isolates were of *E.coli*, followed by those of *Enterococcus spp.* TMP-SXT has been considered as the first-line empirical treatment for more than 30 years. The increasing frequency of the TMP-SXT resistance is worrisome, since this agent is being frequently prescribed for uncomplicated UTIs in many developed and developing countries. In this study, a 58.4% resistance rate to TMP-SXT was observed. The resistance of *E. coli* to TMP-SXT is a significant problem in our region. As was demonstrated by this study, TMP-SXT does not seem to be appropriate as an empirical treatment for community acquired UTIs, as a 39% resistance was seen, without the consideration of the antimicrobial susceptibility results [7].

An alternative therapy for uncomplicated UTIs in settings with a >10-20% resistance to cotrimoxazole may include a fluoroquinolone, a nitrofurantoin or a fosfomycin. However, the data from the present study indicate that the fluoroquinolone resistance in *E. coli* was associated mostly with the isolates from complicated UTIs and / or the patients who had received previous antibiotic therapies. The results (8.5% resistance to the fluoroquinolones in the isolates from uncomplicated UTIs) have important clinical implications in the context of the empirical use of these antimicrobial agents. Based on the data from the present study, it has been seen that the quinolones are currently a valid option as an empirical therapy of uncomplicated UTIs, but a careful use is recommended, to avoid the selection and the spread of the resistant strains. The decline in the activity of ciprofloxacin correlates with a greater than 2.5 fold increase in the use of the quinolones (ciprofloxacin, levofloxacin and ofloxacin)-which are the popular agents for treating community-acquired pneumonia, urinary tract infections, and skin and soft tissue infections-over the past 10 years. Among the hospital isolates, the resistance to the fluoroquinolones was anywhere between 94-98%. It was seen that the risk factors like a previous hospitalization or treatment with the fluoroquinolones for various infections, were present in these cases. This calls for a warning of using fluoroquinolones judiciously and of preventing the use of these drugs for lowering the UTIs, so that the normal flora does not get exposed to these drugs.

In our study, 84.7% of the UPEC showed resistance to ampicillin. Ampicillin, as a single agent for the empirical treatment of the *E.coli* isolates, could not effectively treat a majority of the cases in our region. This is also true with the cephalosporins, which are the commonly prescribed drugs, even on an outpatient basis. The ESBL producing strains were also found among the outpatient samples (28%). The indiscriminate use of the cephalosporins is responsible for the high rate selection of the ESBL producing micro organisms. These results were consistent with those of previous studies from India [8]. The isolates which are ESBL producers are usually known to be multidrug resistant organisms. They show less susceptibility to the quinolones also [9]. In our present study, we found this correlation. 79.9% of the ESBL producers were resistant to ciprofloxacin. Since a plasmid or a transposon can carry several resistance indexes, the resistance to several antimicrobial agents may be acquired simultaneously and this can result in multiple drug resistant (MDR) organims [8].

The multidrug resistant organisms are generally acquired by a horizontal transmission from other patients, which may have been selected by antibiotic use. There is evidence that in many patients with *E.coli* UTIs, the uropathogenic clone colonizes the large intestine [10]. Just like any other *E.coli* strain, the uropathogenic *E.coli* clones can be excreted in faeces and carried in water, through which they can be ingested by other people. Once they are ingested, they can colonize the large intestine, from where they can initiate urinary tract infections in the new host. In fact, the *E.coli* clones that possess genes for the uropathogenic virulence factors, have been found in waste water and food [11, 12]. Although these studies have been done in western countries, there is a likelihood that the uropathogenic *E.coli* can spread through a faecal contamination of the ground water in India, where the sanitation facilities are poor. This problem can be reduced by a good infection control and a modification in the patterns of the antibiotic prescriptions [13].

Cefaperazone/sulbactum and piperacillin/tazobactum were the most common drugs which were given to the cases of drug resistance at our hospital. In our study, the sensitivity to nitrofurantoin was high (93.6%). This may be due to the less frequency of usage of this drug. It was usually prescribed only for the cases of uncomplicated lower urinary tract infections. In our setup, this drug was used for the cases of repeated UTIs and a complete course of two weeks was recommended. It was very heartening to note that all the isolates were sensitive to the carbepenems.

## CONCLUSION

Urinary tract infections are one of the common infections which are encountered in the clinical practice. The present study has revealed that UTIs are caused commonly by the organisms who belong to the Enterobacteriaceae family, *Escherichia coli* being the commonest aetiological agent of uncomplicated, community acquired UTIs. The emergence of drug resistance among the UPEC to trimethoprim-sulfamethoxazole, the penicillins, the cephalosporins and the fluoroquinolones has left the clinicians with limited options for selecting the appropriate antibiotics for the treatment of the infections which are caused by these multi-drug resistant organisms. The emergence of ESBL producing organisms among both the hospital and community isolates has forced the clinical microbiology laboratories to check for their presence compulsorily. Apart from this, treating the infections which are caused by these multi-drug resistant isolates by administering carbapenems, is not feasible for the common man in a developing country like ours. The judicious use of antibiotics and the proper implementation of an antibiotic policy in hospitals, will not only help in limitating the emergence of drug resistance, but also in limiting the spread of multidrug resistant strains.

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