Research Article

Trends in Antibiotic Resistance Pattern among *Escherichia coli* Isolates from Patients with Urinary Tract Infection in Tertiary Care Hospital, Bellary

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ABSTRACT

To study the prevalence rate and to determine the resistance pattern among *Escherichia coli* isolates to commonly used antibiotics from patients having Urinary tract infections (UTI) and to determine Extended spectrum beta lactamase (ESBL) producers among them and their sensitivity pattern. Out of 580 urine samples processed from patients with urinary tract infection during a study period of six months, 303 samples yielded growth on culture out of which *Escherichia coli (E. coli)* was found to be the commonest isolate accounting for 39.6% (n=120), whereas 183 samples yielded bacteria and fungi other than *E. coli*. All the *E. coli* isolates were subjected for antibiotic sensitivity testing by Kirby Bauer disk diffusion method on Muller Hinton Agar in accordance with CLSI guidelines. Out of the 12 antibiotics tested against *E. coli* isolates, amoxycillin (100%) , fluoroquinolones [ciprofloxacin (95.8%), norfloxacin (91.7%), ofloxacin (96.7%)], third generation cephalosporins [ceftazidime (91.7%), ceftriaxone (94.2%), cefotaxime (97.5%)], showed the highest resistance, followed by cotrimoxazole (66.7%), piperacillin-tazobactum (55.8%) and amikacin (39.2%), while least resistance was shown by imipenem (5.9%) and nitrofurantoin (21.7%). Moreover our study identified 26 *E. coli* isolates (21.6%) to be extended spectrum beta lactamases (ESBL) producers by Double disc diffusion synergy test (DDST). All ESBL *E. coli* were resistant to ceftazidime, cotrimoxazole and amoxicillin whereas sensitive to imipenem. Highest number of ESBL producers was detected by ceftazidime. *E. coli* were found to be the commonest causative agent of UTI in our study showing co-resistance to many classes of antibiotics. The study concluded that nitrofurantoin could be recommended for empirical therapy in UTI patients in our region.

Keywords: Antimicrobial susceptibility, Double disc diffusion synergy test (DDST), Empirical therapy, ESBL, *Escherichia coli*, Urinary tract Infection.

INTRODUCTION

Urinary tract infection (UTI) is one of the commonest bacterial infections in humans affecting all age groups and both genders in the community and hospital setup.[1] Urinary tract infection is the major cause of morbidity associated with high mortality and economic loss accounting for approximately 150 million cases annually and as many as 40-50% of nosocomial infections.[2] It is the second most common cause of bacteraemia in hospitalized patients.[3] The impact of disease is even worst in developing countries due to UTI caused by multidrug-resistant (MDR) pathogens and the possibility of transfer of MDR traits between them.[4] In almost all cases of UTI, empirical antimicrobial treatment is initiated before the laboratory results of urine culture are available; resulting in increase in antibiotic resistance due to frequent misuse of antibiotics.[5,6] Therefore, determining the etiological agents of UTI and their antimicrobial resistance patterns in specific geographical locations may aid clinicians in choosing the appropriate antimicrobial empirical therapy. Among both outpatients and inpatients, *Escherichia coli* (*E. coli*) is the most common etiological agent, accounting for 75% to 90% of UTI isolates.[5, 7] Worldwide data showed that a significantly high proportion of the urinary *E. coli* isolates have already developed resistance to the currently prescribed empirical antibiotics making the therapeutic options very limited.[4,8] Moreover, extended spectrum beta lactamases producing *E. coli* is also on the rise which has contributed to high prevalence of MDR *E. coli* in UTI thus creating difficulties in treating UTI.[2, 9] In Asian countries, isolation of ESBL *E. coli* varies from 10 to 46.5% whereas its prevalence in India ranges from 41.0 to 63.6 per cent.[10] Thus, knowledge of the antibiotic resistance patterns of uropathogens is an important factor for selection of appropriate empirical antimicrobial treatment.

Thus, the present study was undertaken to determine the prevalence of the commonest causative agent of urinary tract infection and its antibiotic resistance pattern including ESBL producers among patients attending our tertiary health care centre in Bellary which can guide the physician in selecting antibiotics for empirical therapy.

MATERIALS AND METHODS

The study was conducted at the Department of Microbiology, Vijayanagara institute of Medical sciences (VIMS), Bellary, Karnataka, India.

Study population

The study included 623 urine samples collected from patients with UTI. 43 samples that showed contamination and insignificant bacteriuria were excluded from the study. The analysis of remaining 580 urine samples included both outpatients and inpatients of all age groups, of both genders presenting with symptoms of UTI like burning micturition, fever, pyuria, haematuria, dysuria, increased frequency, flank pain, suprapubic discomfort, attending VIMS, Bellary.

Study Duration

6 month period from January 2011 to June 2011.

Collection of Urine Samples

The patients were asked to submit early morning mid-stream urine samples in sterile, wide mouthed containers with screw cap tops along with requisition forms. On the urine sample bottles were indicated name, age, sex, and time of collection. The samples were analyzed and processed according to standard protocol within 1 hour of collection.[11]

Sample processing

Microscopy: Wet Mount preparation

50 µl of well-mixed uncentrifuged urine was taken on a slide and a cover slip placed on it and viewed under high-power objective. Microscopy findings of more than 10 WBC per high power field were considered significant.[12] Jensen's modification of gram staining was employed. At least 20 fields were examined and detection of one or more morphologically similar bacteria per oil immersion field was treated as significant.[12]

Culture

Culture of urine samples was done using a sterile calibrated bacteriological loop of diameter 4.0mm designed to deliver 0.01ml. A loopful of the well mixed urine sample was inoculated into duplicate plates of Blood agar and MacConkey agar media. After allowing the urine to be absorbed into the agar, the plates were then inverted and incubated at 37°C for 18-24 hours.[6] The plates were then examined macroscopically and microscopically for bacterial growth. The colony count was done using semi-quantitative method and multiplied by 100 to give an estimate of the number of bacteria present per milliliter of urine. A significant bacterial count was taken as count equal to or in excess of 10^5 bacteria per milliliter. 11 Such isolates were further processed for identification and antibiotic susceptibility pattern was determined. If CFU was less than 10^5 it was considered as negative for culture or insignificant bacteriuria. In cases of mixed growth the patients were asked to submit repeat samples with early morning fresh urine specimens.

Identification of uropathogens

Identification of the isolated bacterial pathogens was done on the basis of gram staining, morphology and biochemical characters by standard methods.[11]

Antimicrobial Susceptibility Testing

Antimicrobial sensitivity of the isolated pathogens was determined by using a panel of 12 antibiotics by Kirby Bauer Agar Disc Diffusion method on Muller Hinton agar (MHA) according to Clinical and Laboratory Standards Institute guidelines (CLSI, 2010).[13] The plates were incubated at 37^0C for 18-24 hours and results read. Negative cultures were reincubated for another 24 hours and report was given as no growth at the end of 48 hours of incubation. The antibiotics tested were amikacin (AK), nitrofurantoin (NF), co-trimoxazole (CO), amoxycillin (AMX), cefotaxime (CE), ceftriaxone (CI), ceftazidime (CAZ), norfloxacin (NX), ciprofloxacin (CF), ofloxacin (OF), piperacillin-tazobactum combination (PIT) and imipenem (IPM). Those isolates suspected to be ESBL producers were tested with amoxycillin/clavulanic acid combination disc. The results were interpreted as per CLSI guidelines.[13] The antibiotic discs used were purchased from Himedia, Mumbai, India.

ESBL screening test

Based on the routine antibiotic disc sensitivity test, isolates showing inhibition zone size for any of the 3 third generation cephalosporins (3GC), of ≤ 22 mm with Ceftazidime (30 µg), ≤ 25 mm with Ceftriaxone (30 µg), and ≤ 27 mm with Cefotaxime(30 µg) were identified as ESBL producers based on CLSI guidelines.[2,14]

ESBL detection by Double Disc Diffusion synergy test (DDST)

To confirm the production of ESBL, Double disc diffusion synergy test (DDST) was performed. This was done by placing a combination disc of 20mcg of amoxycillin and 10mcg of clavulanic acid and 30mcg of each of 3GC at a distance of 15 mm apart on a lawn culture of the resistant isolate under test on MHA.[2,9,15] After overnight incubation at 37^0C, the test organism was considered to produce ESBL if the zone size around the test antibiotic of any of 3GC increased towards the amoxycillin/clavulanate disc showing synergy. This increase occurs because the clavulanic acid inactivates the ESBL produced by the test organism.

Quality Control

Escherichia Coli ATCC 25922 was used for the quality control of Kirby-Bauer disc diffusion method and ESBL testing methods.

RESULTS

Out of the 580 urine samples that were processed, 277 samples yielded no growth on aerobic culture. Out of 303 urine samples that yielded growth, *Escherichia coli* was the commonest isolate with 120 samples (39.6%) yielding *E. coli* (n=120), 183 samples (60.4%) yielded bacteria and

fungi other than *E. coli*. (Fig. 1) Out of the 120 patients whose urine samples yielded *E. coli*, 78 were males (65%) and 42 (35%) were females (Figure 2).

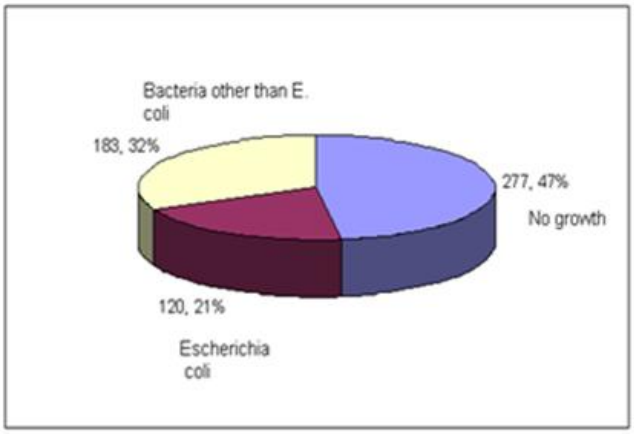

Figure 1: Distribution of Urinary Isolates

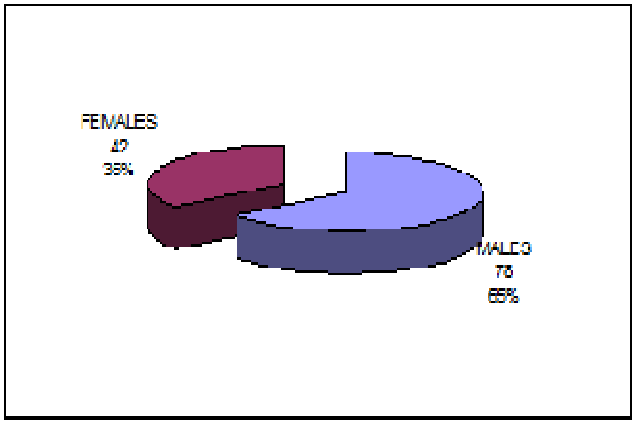

Figure 2: Sex Distribution of UTI Patients

Table 1: Age Distribution of Patients

Age group in years	Number of patients	Percentage
0-10	9	7.5%
11-20	10	8.3%
21-30	19	15.8%
31-40	26	21.7%
41-50	21	17.5%
51-60	15	12.5%
61-70	9	7.5%
71-80	11	9.2%
TOTAL	**120**	**100%**

Our study revealed that maximum number of UTI cases were found in the age group 31-40 years (21.7%), followed by 41-50 years (17.5%), 21-30 years (15.8%), 51-60 years (12.5%), 71-80 years (9.2%), 11-20 years (8.3%), and 0-10 & 61-70 years contributing 7.5% each. The study demonstrated that the highest incidence of UTI was found in age group between 21-50 years (55%).

All 120 E. coli isolates were subjected to antibiotic sensitivity testing with a panel of 12 antibiotics as mentioned. The study revealed that none of the isolates in our study were sensitive to all the antibiotics tested. Out of total E. coli isolates (n=120), 40 (33.3%) showed sensitivity to trimethoprim-sulpahmethoxazole whereas 80 showed resistance (66.7%). However, resistance to nitrofurantoin was detected in 26 (21.7%) isolates whereas 94 (78.3%) were found to be sensitive. Among the fluoroquinolones, norfloxacin, ciprofloxacin, and ofloxacin showed resistance of 110 (91.7%), 115 (95.8%), and 116 (96.7%) isolates respectively. Out of the third generation cephalosporins that were used, 110 (91.7%), 113(94.2%), 117(97.5%) of the isolates were found to be resistant to ceftazidime, ceftriaxone and cefotaxime respectively. 47 (39.2%) and 73 (60.8%) of the total isolates were found to be resistant and sensitive to aminoglycoside amikacin respectively. In case of piperacillin-tazobactum combination, 53 (44.2%) were sensitive and 67 (55.8%) were found to be resistant. Imipenem showed the highest sensitivity with 113 isolates (94.1%) and least resistance with only 7 isolates (5.9%). The highest resistance rate was to amoxycillin (100%), cefotaxime (96.7%) and ofloxacin (96.7%) and lowest resistance rate was to imipenem (5.9%).

Those isolates that showed resistance to any one of the third generation cephalosporin in our study (117 isolates) was suspected to be ESBL producer and further subjected to DDST. This method detected 26 of total E. coli isolates to be ESBL producers (21.6%) out of which 19 were males and 7 were females. The maximum number of ESBL E. coli were found in patients belonging to age groups 21 -40 yrs. The mean age was found to be 38.6. All the ESBL producers were resistant to ceftazidime, cotrimoxazole, amoxycillin, ciprofloxacin and ofloxacin. High rates of resistance were obsereved with ceftriaxone and cefotaxime too. However, 11.5% of isolates were sensitive to norfloxacin. Most of the isolates were sensitive to nitrofurantoin (77%), amikacin (73%) and piperacillin-tazobactum (61.5%). All ESBL producers were found to be sensitive to imipenem (100%). This study shows that the most effective antibiotic in the detection of ESBL was ceftazidime (100%) followed by ceftriaxone (96.2%) and cefotaxime (80.8%).

DISCUSSION

Urinary tract infections (UTIs) are one of the most frequently encountered clinical conditions in medical practice requiring antimicrobial therapeutic intervention which has become more difficult in recent times, due to increased resistance rates to commonly used antibiotics.[1] Worldwide data reveals E. coli to be the major cause of UTI.[1,3,7,16-18] In the present study, E. coli predominated amongst urinary isolates in patients attending our health care centre (39.6%), alike studies conducted in Poland (38.3%)[19], Salem (58.2%)[16], Kathmandu (81.3%)[4], Nigeria (34.6%)[7]. Mexico (71.3%)[20], Makati (60.7%)[21], Diyarbakir(38%)[1], Chandigarh (70.7%)[6], Barielly (33.1%)[12], Ghana (46.7%)[22] and Tehran (68.8%).[5]

The selection of antibiotics against any urinary tract pathogen depends on the antibiotic resistance pattern. Those organisms which showed resistance to at least three or more antibiotics of different structural classes were considered MDR by possessing the antibiotic resistant genes in its transferable R-plasmid (s).[4,16,23] Antimicrobial therapy of UTI caused by E. coli is often impaired due to the high rates of resistance to commonly used antimicrobial agents indicating spread of multi drug resistant strains in the community due to misuse of antibiotics and inadequate surveillance. A very high rate of MDR E. coli has been reported in USA from 2000-2003 (76%). [23]

Table 2: Antibiotic Sensitivity pattern of Escherichia coli

Name of Antibiotic	Percent sensitive	Percent resistant
Amoxycillin (AMX) 10 µg	0; n=0	100; n=120
Cotrimoxazole(CO) (sulphamethoxazole/trimethoprim 23.75/1.25 µg)	33.3; n=40	66.7; n=80
Nitrofurantoin (NIT) 300 µg	78.3; n=94	21.7; n=26
Norfloxacin (NX) 10 µg	8.3; n=10	91.7; n=110
Ciprofloxacin (CF) 5 µg	4.2; n=5	95.8; n=115
Ofloxacin (OF) 5 µg	3.3; n=4	96.7; n=116
Ceftazidime (CAZ) 30 µg	8.3; n=10	91.7; n=110
Ceftriaxone (CI) 30 µg	5.8; n=7	94.2; n=113
Cefotaxime (CE) 30 µg	2.5; n=3	97.5; n=117
Amikacin (AK) 30 µg	60.8; n=73	39.2; n=47
Piperacillin-tazobactum (PT) 100/10 µg	44.2; n=53	55.8; n=67
Imepenem (IPM) 10 µg	94.1; n=113	5.9; n=7

Table 3: Antibiotic sensitivity pattern of ESBL producing Escherichia coli

Name of antibiotic	Percent sensitive	Percent resistant
Ceftazidime (CAZ) 30 µg	-; n=0	100; n=26
Ceftriaxone (CI) 30 µg	3.8; n=1	96.2%; n=25
Amikacin (AK) 30 µg	73; n=19	27; n=7
Ciprofloxacin (CF) 5 µg	-; n=0	100; n=26
Nitrofurantoin (NIT) 300 µg	77; n=20	23; n=6
Ofloxacin (OF) 5 µg	-; n=0	100; n=26
Norfloxacin (NX) 10 µg	11.5; n=3	88.5; n=23
Cefotaxime (CE) 30 µg	19.2%; n=5	80.8%; n=21
Piperacillin-tazobactum (PT) 100/10 µg	61.5; n=16	38.5; n=10
Imepenem (IPM) 10 µg	100; n=26	-; n=0
Cotrimoxazole(CO) (sulphamethoxazole/trimethoprim 23.75/1.25 µg)	-; n=0	100; n=26
Amoxycillin (AMX) 10 µg	-; n=0	100; n=26
Amoxycillin/ clavulanic acid (AC) 20/10 µg	100; n=26	0; n=0

In the present study, the isolates showed low degree of susceptibility to fluoroquinolones similar to studies done elsewhere.[7,18,21,23-25] Fluoroquinolone resistant E. coli from urine were frequently multidrug resistant indicating that they can no more be opted for treating UTI.[26,27] This could be related to their enhanced use, easy access, use in animal feed and subsequent transmission of resistant strains from animals to humans. However, various studies conducted in Aligarh[28], Tehran[5] and Nigeria[7] have shown high rate of sensitivity of quinolones to E. coli.

Extended Spectrum Beta Lactamases (ESBL's) are defined as β- lactamases produced mainly by Escherichia coli, Klebsiella species, capable of hydrolyzing oxyimino cephalosporins, conferring broad resistance to penicillin, cephalosporin and monobactam but inactive against cephamycins and carbapenem and are inhibited by β-lactamase inhibitors The incidence of ESBL producers worldwide has been steadily increasing over the past years with co-resistance to many classes of antibiotics resulting in limitation of therapeutic options.[10,19] Urine (70.4%) was the main source of ESBL producing isolates from patients.[25] It is obvious from our study that there is increased resistance for 3GC showing resistance rate of 97.5%, 94.2%, and 91.7% for cefotaxime, ceftriaxone and ceftazidime respectively. Alike studies also report high

resistance rate to 3GC (76%). [2] Iraj et al reported 100% resistance to both third and fourth generation cephalosporins, which is an indication that many of the organisms are ESBL and Amp C producers.[25] This was unlike studies conducted in Senegal which showed high sensitivity to them.27 Our study detected 30.4% of E coli isolates to be ESBL producers with DDST which was comparable with studies conducted elsewhere.[1,19] Ceftazidime detected the maximum number of ESBL producers (100%) like similar studies.[2,25] A study conducted in Punjab says that cefepime, a fourth generation cephalosporin is a more reliable detection agent for ESBL especially in the presence of AmpC enzymes.[9] ESBL producing isolates in our study were found to show co resistance to many classes of antibiotics like similar studies conducted elsewhere.[2,4,9,19] Reports say the incidence of Urinary tract infections by ESBL producing E. coli was found highest in India (60%) followed by Hongkong (48%) and Singapore (33%).[2] High rates of third generation cephalosporin use have been implicated as a major cause of this problem, resulting in prolonged hospital stay, increased morbidity, mortality and health care expenses. The National Committee for Clinical Laboratory Standards (NCCLS) recommends that Microbiology laboratories reported ESBL-producing isolates. In our study imipenem showed sensitivity to all E. coli esbl producers which was concordant with other studies.[13,25,29] However, overuse of carbapenems may lead to resistance of other gram-negative organisms.

The susceptibility pattern of Nitrofurantoin is satisfactory in our study with 78.3% of isolates being sensitive and could be used for empirical therapy for E. coli uropathogens in our region in contrast to study conducted in Aligarh where 80% of isolates were resistant.[28] The Infectious Diseases Society of America recommends an antibiotic for empirical therapy only if <10-20% of the urinary pathogens are resistant to it.[30] Nitrofurantoin has been recommended for empirical therapy by many studies since its multiple mechanisms of action seem to have enabled it to retain potent activity against E. coli despite nearly 50 years of use.[5,18,23] The resistance rate of amikacin was found to be 39.2% in our study but was found to be effective against ESBL E. coli. Studies conducted in Taiwan and Bangladesh revealed that amikacin had high rate of sensitivity towards E. coli isolates.[1,25] Significant increase in resistance of uropathogenic strains to cotrimoxazole and amoxycillin has been found worldwide what agree with the results reported in the present study, therefore alternative agents need to be considered in empirical therapy of UTI.[21,31] Highest sensitivity was seen towards imipenem in our study similar to other studies.[2,28]

In the present study it was found that all the strains were at least resistant to five classes of antibiotics. Our study shows that majority of our isolates showed high resistance rates to different classes of antibiotics like penicillins, fluoroquinolones, third generation cephalosporins, amikacin and cotrimoxazole thus being multidrug resistant. Out of the 12 antibiotics tested for E. coli isolates, least resistance was observed to imipenem (5.9%) followed by nitrofurantoin (21.7%). Moderate sensitivity was observed to amikacin (60.8%). Comparison of antibiotic resistance profiles revealed that most of the ESBL producers were multidrug resistant.

Our study demonstrated that the highest incidence of UTI was found in age group between 21-50 years (55%) as it is the sexually active group and child bearing age. In this study, yet another small peak of incidence was observed in the age group 51-80 years which is vulnerable to risk factors like urinary tract obstruction, poor bladder emptying and chronic age related diseases as noted by Oluremi et al with highest incidence of UTI in the elderly.[7]

Higher resistance rate of E. coli to various antibiotics with the exception of imipenem and nitrofurantoin in this study reflects the need for accurate and updated population surveillance data of regional antimicrobial susceptibility patterns which will guide in the formation of a strict antibiotic policy and empirical therapy. Prevention remains a significant priority in controlling the development and spread of ESBL producers, hence, regular detection of ESBLs, judicious use of antibiotics and appropriate infection-control measures should be undertaken.

CONCLUSION

To conclude, the present study reveals the major isolate among patients with Urinary tract infections in our health care centre to be Escherichia coli. The findings of the present study showed high prevalence of resistance of E. coli to commonly used antibiotics. Many isolates were found to be resistant to atleast five classes of antibiotics. Nitrofurantoin may be considered as first line empiric agent against UTI isolates of E. coli in and around Bellary. The use of third generation cephalosporins, amoxycillin, fluoroquinolones, aminoglycosides and cotrimoxazole should be discouraged as they showed high rate of resistance in our study. The study clearly highlights that the isolated ESBL producers were resistant to the third generation cephalosporins, fluoroquinolones, amoxycillin, cotrimoxazole but are still sensitive to carbapenems, nitrofurantoin, amikacin and amoxycillin-clavulanic acid.

Due to the emerging antimicrobial resistance, it is strongly suggested that the antibiotic therapy in UTI should be commenced only after the sensitivity report from the Microbiology laboratory and empirical antibiotic selection should be based on the knowledge of local prevalence of causative organisms and their antimicrobial sensitivities rather than on universal guidelines.

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