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Original Research Article

Antibiotic susceptibility pattern of *Escherichia coli* isolated from urine samples in Pattukkottai, Tamilnadu

C.Manikandan[1*] and A.Amsath[2]

[1]Gangasaras Diagnostic and Research Centre, Pattukkottai-614601, Tamilnadu, India,
[2]P.G. and Research Department of Zoology, Khadir Mohideen College, Adirampattinam-614701, Tamil Nadu, India
**Corresponding author*

A B S T R A C T

Urinary tract infections (UTIs) are serious health affecting problems worldwide. Antibiotic resistance has emerged due to its frequent use. Resistance has emerged even to more potent antimicrobial agents. The study was conducted to isolate and determine the antibiotic resistance in *E. coli* from urinary tract infections in various hospitals, Pattukkottai, Tamilnadu. Urine samples were collected from patients with signs and symptoms of Urinary tract infections. Samples were screened for significant bacteriuria and pus cells counts. Bacteria were isolated and identified by conventional biochemical profile. Antibiotic resistance pattern of *E. coli* against different antibiotic was determined by Kirby-Baur method. Bacterial etiological agent was isolated from 650 samples with highest prevalence of *E. coli* (54.6%) was the maximally isolated UTI causing bacterium, followed by, *K. pneumoniae* (11.2%), (87%), tobramycin (76.9%), ciprofloxacin (69.0%), norfloxacin (67.6%), ofloxacin (64.5%), gentamicin (62.5%), cefotaxime (58.0%), ceftazidime (54.7%) and ceftriaxone (47.0%) respectively. All isolated *E.coli* strains were multidrug resistant each to 4, 5, 6, 7 and 8 of the selected antibiotics used. The present study concludes that a high number of *E.coli* strains isolated from both female and male patients showed high resistant to the antibiotic amoxicillin followed by ampicillin, cephalexin, nalidixic acid and cotrimoxazole respectively.

Keywords

UTIs, Uropathogens *E.coli*, Antibiotics, Multidrug resistant

Introduction

Urinary tract infection (UTI) is one of the most important causes of morbidity in the general population, and is the second most common cause of hospital visits (Kolawale *et al.*, 2009). Urinary tract infections (UTIs) are among the most common infectious diseases of humans, with *Escherichia coli* being responsible for >80% of all cases. Urinary tract infections (UTIs) are more common among women than men, although the prevalence in elderly men and women is similar. Most of the research on UTI has focused on young, sexually active women who are at high risk for developing an infection (Harrington and Hooton, 2000). Worldwide, about 150 million people are diagnosed with UTI each year (Gupta, 2001; Amin *et al.*, 2011). Urinary tract infections (UTIs) is one of the common infectious diseases, and nearly 10% of people will experience a UTI during their life-time. UTI is the leading cause of morbidity and health care expenditures in persons of all the ages (Prabhu and Selvaraj, 2012).

Escherichia coli are a bacterium commonly found in the large intestine of humans and other warm blooded animals. *Escherichia coli*, the most prevalent facultative Gram-negative bacillus in the human fecal flora, usually inhabit the colon as an innocuous commensal. Strains of *E.coli* that cause disease outside of the gastrointestinal tract are referred to as extraintestinal pathogenic *E.coli* (ExPEC) and are divided into uropathogenic *E.coli* (UPEC) strains causing neonatal meningitis and septicemic *E.coli*. UPEC is the most common pathotype of ExPEC and is found in patients with urinary tract infections (Katouli, 2010).

The infection of urinary tract by *Escherichia coli* are important and serious problems in the clinical field. Moreover the bacterial urinary tract infection is the common danger disease in Pattukkottai area. Therefore, the present investigations were undertaken to study the prevalence and antibacterial susceptibilities of *Escherichia coli* isolated from patients with urinary tract infections in Pattukkottai area, Tamil Nadu, India.

Materials and Methods

Specimen collection: The study was conducted for a period of 18 months from January 2012 to June 2013 among urinary tract infection patients in various hospitals in Pattukkottai. A total of 2400 urine samples were collected from patients who had visited the private hospital in Pattukkottai area to see doctors with various complaints which were diagnosed tentatively as symptoms of urinary tract infections (UTIs). The consulting doctors had then referred the patients to the Gangasaras Diagnostic and Research Centre for urine mcs (microscopy, culture and sensitivity) investigation for the purpose of making definite diagnosis. Recruited patients were instructed on how to collect the samples. The specimens were processed according to standard bacteriological methods and identified by standard conventional methods.

Confirmation of significant bacterial count by microscopy: All samples that recorded significant bacterial counts were subjected to urine microscopy test to detect presence of five pus cells per high power focus using X40 objective microscopically. All samples that were positive for significant bacterial count and also recorded five pus cells and above were then cultured on laboratory media. Similarly presence of bacteria, casts, crystals, RBCs was noted. Another drop of uncentrifuged urine sample was placed on a clean slide and was allowed to air dry. This smear was Gram stained and examined under oil immersion. Presence of at least one organism per field was considered significant.

Urine nitrite: The use of dipstick designed to detect the presence of urine nitrite. The nitrate test of urine has been used as rapid screening test for significant bacteriuria. A positive nitrite test indicates that cause of the UTI is a Gram negative organisms, a most commonly *E.coli*. The reason for nitrites existence in the presence of UTI is due to a bacterial conversion of endogenous nitrates to nitrites.

Bacteriology: Urine was mixed by rotating the container and was inoculated on Nutrient agar, McConkey agar, Blood agar and UTIchrom agar. The plates were then aerobically incubated at 37°C for overnight. A specimen giving $\geq 10^5$ cfu/ml or forming at least 20 colonies will be considered as positive for UTI. All the bacteria were isolated and identified using morphological, microscopy and biochemical tests following standard procedures described by Sharma (2008).

Antibiotic susceptibility test: Antibiotic sensitivity test was carried out by disc diffusion technique on Muller Hinton agar plates (Bauer *et al*., 1966). Antibiotic susceptibility testing of *Escherichia coli* was carried out by the disk diffusion technique using a commercially available disc. The following antibiotics such as amoxicillin (AX, 30mcg), ampicillin (AM, 10mcg), cefotaxime (CTX, 30mcg), ceftazidime (CAZ, 30mcg), ceftriaxone (CT,30mcg), cephalexin (CL,30mcg), ciprofloxacin (CIP, 5mcg), nalidixic acid (NA,30mcg), norfloxacin (NX, 10mcg), ofloxacin (OF, 5mcg), amikacin (AK, 30mcg), gentamicin (GEN, 10mcg), tobramycin (TB, 10mcg), imipenem (IPM, 10mcg), nitrofurantoin (NIT, 300mcg) and cotrimoxazole (COT, 30mcg) were used to determine antibiotic susceptibility pattern. Isolated colonies were picked up from a fresh isolation plate, inoculated on Trypticase Soya broth medium and incubated for 2 to 6 hrs at 37°C until good visible growth. A lawn of test pathogen was prepared by evenly spreading with the help of a sterilized spreader onto the entire surface of the agar plate. The plates were allowed to dry before applying antibiotic disc. The antimicrobial discs were placed at equi-distance and the discs were pressed gently with forceps. After 16-18 hrs incubation of the plates at 37°C, the zone of inhibition were read with metallic rulers in mm and interpreted using standard zone of inhibition charts.

Statistical analysis: The statistical analysis was performed with the Statistical Package for Social Sciences version 17 for Windows software and Microsoft Excel 2010. Descriptive analysis was done by calculating frequencies and percentages. Chi-square test was applied to evaluate the incidence of disease with gender, to observe the correlation between the prevalence of organism and gender, to observe the correlation between the prevalence of organism and the age groups. Significance of results was calculated at 95% confidence level ($P \leq 0.05$).

Results and Discussion

Causative Agents and their Prevalence in UTIs

A total of 2400 urine samples yielded 650 (27.1%) strains of pathogens belonging to 12 species with seven Gram negative bacteria, four Gram-positive, and *candida* spp., during the span of 18 months. The most common isolates in this study have been the Gram negative bacilli which accounts for 85.4% of the total positive isolates. *E.coli* (54.6%) was the maximally isolated UTIs causing bacterium, followed by *K.pneumoniae* (11.2%), *P.aeruginosa* (10.5%), *Proteus* spp., (6.8%), *CONs* (4.1%), *Enterococcus* spp., (3.7%), *Staph.saprophyticus* (2.3%), *Enterobacter* spp., (1.5%), *S. aureus* (1.1%), *Citrobacter* spp., (0.6%), and *Acinetobacter* spp., (0.3%). Figure 1 shows the detailed frequency of all the isolates identified.

Prevalence and characterization of *E.coli*

Out of total 2400 urine samples which were processed for screening of Urinary tract infection (UTI), 650 (27.1%,$p<0.05$) samples were found positive for UTI. Prevalence of UTI in females (69.8%) is higher than males (30.2%) and it is statistically significant (P <0.05) in females.

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In the Gram negative bacilli, the most predominant isolate from UTI were 355 strains of *E.coli* (54.6%) included for this study. All Gram negative, raised, entire, circular, motile, lactose, glucose fermenting, indole positive, methyl red positive, voges-proskauer negative, citrate negative and urease negative bacilli strains were identified as *Escherichia coli.* On the TSI test *E.coli* strains produced acids both in butt and slant along with gas production. *E.coli* presumptively identified on Chromagar media according to pigment reactions. *E.coli* grown on chromagar, when *E.coli* forms small, pink-purple colonies.

Antibiotic susceptibility pattern

This experiment was carried out to study the susceptibility of the bacterial isolates collected from urine specimens toward different 16 antibiotics. The lowest percentage of susceptibility was manifested against amoxicillin (8.2%) followed by ampicillin (11.3%), cephalexin (11.5%), nalidixic acid (22.8%), cotrimoxazole (23.7%) (Table 1; Figure 2), whereas more susceptibility was observed with imipenem (93.5%) and amikacin (88.2%) followed by nitrofurantoin (87%), tobramycin (76.9%), ciprofloxacin (69.0%), norfloxacin (67.6%), ofloxacin (64.5%), gentamicin (62.5%), cefotaxime (58.0%), ceftazidime (54.7%) and ceftriaxone (47.0%) respectively.

Three aminoglycoside antibiotics, amikacin (11.8%), tobramycin (23.1%) and gentamicin (37.5%) were moderately resistant to species of pathogens used. Similarly, resistance patterns of the beta-lactam group, ampicillin (88.7%) and amoxicillin (91.8%) antibiotics were almost equally resistant to the isolated *E.coli*. Imipenem resistance patterns were 6.5% of *E.coli.* Further, the cephalosporin antibiotics, ceftriaxone (53%), ceftazidime (45.3%) and cefotaxime (42.0%) were moderately resistant and cephalexin (88.5%) was highly resistant to species of pathogens used. Similarly, the fluoroquinolone group, ofloxacin (35.5%), norfloxacin (32.4%) and ciprofloxacin (31%) were low resistant and nalidixic acid (77.2%) were highly resistant to species of pathogens used. Among these four antibiotics, nalidixic acid was recorded to be more resistant to these pathogens. Lastly, detailed antibiograms of two stand-alone antibiotics, cotrimoxazole and nitrofurantoin were recorded. Nitrofurantoin was found very low resistance for 13% of *E.coli*. The resistance percent values of the cotrimoxazole were 76.3%.

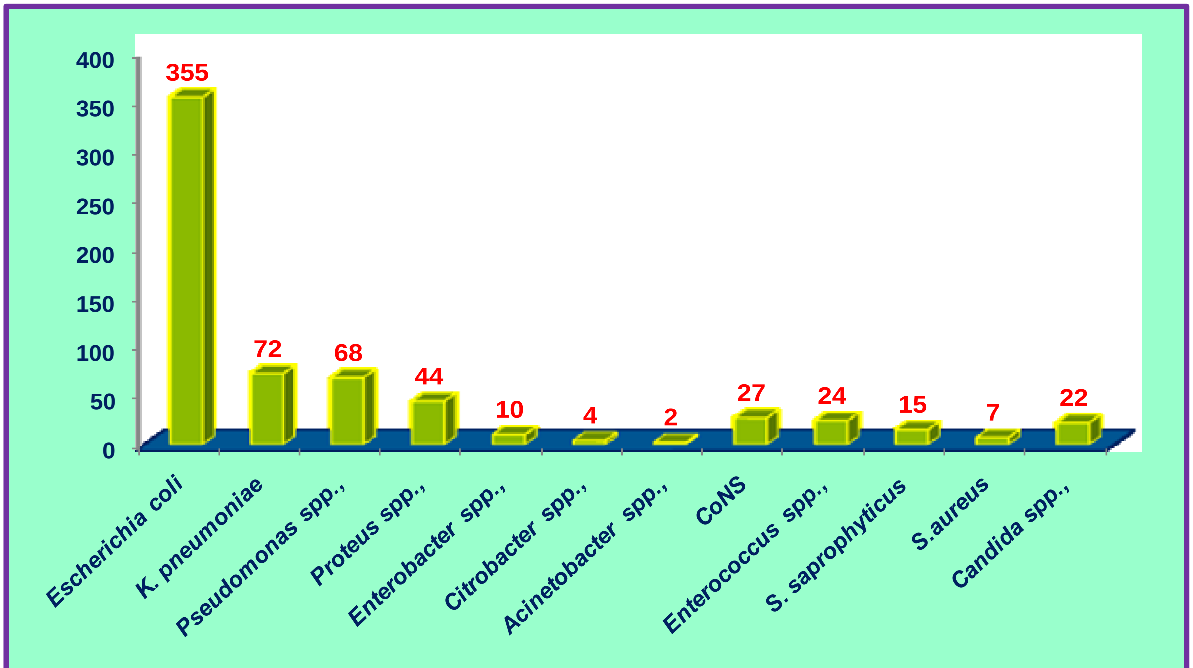

Figure 1: Frequency of all the isolates identified

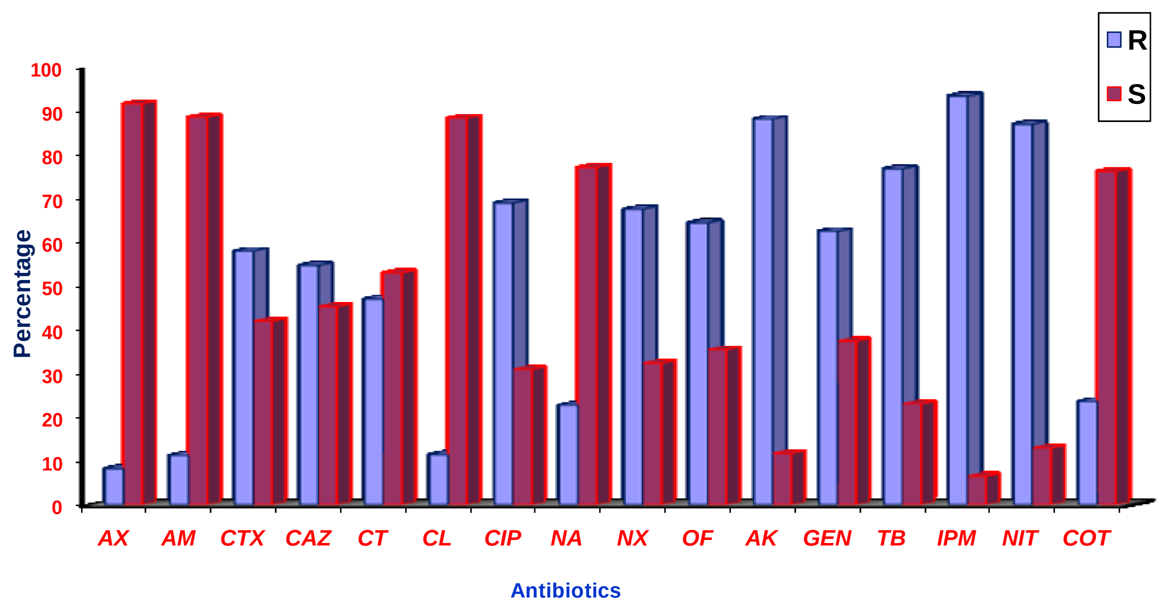

Figure 2: Antibiotic susceptibility pattern of *E. coli*

Table.1 Antibiotic susceptibility pattern of *E. coli*

Antibiotic	Sensitive No (%)	Moderately Sensitive No (%)	Total Sensitive (%)	Total Resistant No (%)
Amoxicillin	20 (5.6)	9 (2.6)	29 (8.2)	326 (91.8)
Ampicillin	30 (8.5)	10 (2.8)	40 (11.3)	315 (88.7)
Cefotaxime	190 (53.5)	16 (4.5)	206 (58.0)	149 (42.0)
Ceftazidime	188 (53.0)	6 (1.7)	194 (54.7)	161 (45.3)
Ceftriaxone	163 (45.9)	4 (1.1)	164 (47.0)	188 (53.0)
Cephalexin	39 (10.9)	2 (0.6)	41 (11.5)	314 (88.5)
Ciprofloxacin	239 (67.3)	6 (1.7)	245 (69.0)	110 (31.0)
Nalidixic acid	78 (22.0)	3 (0.8)	81 (22.8)	274 (77.2)
Norfloxacin	237 (66.8)	3 (0.8)	240 (67.6)	115 (32.4)
Ofloxacin	227 (63.9)	2 (0.6)	229 (64.5)	126 (35.5)
Amikacin	297 (83.7)	16 (4.5)	313 (88.2)	42 (11.8)
Gentamicin	212 (59.7)	10 (2.8)	222 (62.5)	133 (37.5)
Tobramycin	269 (75.8)	4 (1.1)	271 (76.9)	82 (23.1)
Imipenem	327 (92.1)	5 (1.4)	332 (93.5)	23 (6.5)
Nitrofurantoin	303 (85.3)	6 (1.7)	309 (87.0)	46 (13.0)
Cotrimoxazole	81 (22.8)	3 (0.9)	84 (23.7)	271 (76.3)

Results are expressed as a percentage of 355 *E.coli* isolates susceptible, moderately susceptible and resistant, respectively, for each antimicrobial.

E. coli plays an important role in UTI; UTI is one of the most frequently encountered infectious diseases. In this study *E. coli* was the predominant uropathogens (54.6%) causing UTI. This was comparable to a retrospective study done at Bombay by Sonavane *et al.* (2008) in which *E. coli* was isolated from 41.31% of UTI cases. The pattern of isolation of organism in this study was similar to the results from various regions of India (Kothari and Sagar, 2008; Agarwal *et al.*, 2012; Patel *et al.*, 2012; Gautam *et al.*, 2013) and other countries (Amin *et al.*, 2011; Oladeinde *et al.*, 2011; Oluremi *et al.*, 2011; Yakubu *et al.*, 2012; Ghazi *et al.*, 2013), which indicate that *Escherichia coli* is the commonest pathogen isolated in patients with UTIs.

Antibiotic resistance was a major clinical problem in treating infections caused by these microorganisms. In the study, the highest percentage of resistance was noted against amoxicillin (91.8%) followed by ampicillin (88.7%), cephalexin (88.5%), nalidixic acid (77.2%), cotrimoxazole (76.3%) (Figure 2). The resistance to amoxycillin varies from place to place. While Kausar *et al.* (2009*)* from Hubli reported 91.5% resistance, Taneja *et al.* (2010) from Chandigarh observed 32.7% resistance. This was previously reported in other hospital as well as in other institutions in the various countries (Daoud *et al.*, 2006). Previously reported studies showed 60% of cotrimoxazole resistance and 25% from Pakisthan Medical University (Khan and Ahmed, 2001). In the present study, *E.coli* showed 31% and 32.4% resistance to ciprofloxacin and norfloxacin respectively. In this finding was at par with the observation made by Kausar *et al*. (2009) from Hubli (83% and 85% respectively), and much higher to the report of Arjunan *et al.* (2010) from Tirunelvelli (22.3%).

Third generation cephalosporins were the other commonly used antibiotics in the treatment of UTIs. In the present study *E, coli* exhibited 42% resistance to cefotaxime and 53% resistance to ceftrioxone. Cefotaxime resistance was similar to reports by Kausar *et al*. (2009) (59.5%) and ceftrioxone resistance was between the findings of Biswas *et al.* (2006) (22.6%) and Sonavane *et al.* (2008) (74.9%). Therefore, it appears that cephalosporins were still useful in the treatment of UTIs due to *E. coli* in this area.

E. coli showed a low resistance to nitrofurantoin (13%) in the present study. This is similar to the reports of Kausar *et al.* (2009) (15%) and Biswas *et al.* (2006) (9.3%). The reason for low resistance may be that nitrofurantoin was less frequently prescribed because of the requirement of sixth hourly dosage and gastrointestinal disturbances following oral administration. Nitrofurontoin resistance in western countries was as low as 0.4 % in the USA (Karlowsky *et al.,* 2002) to 5.7% in the Spain (Garcia *et al.*, 2007).

The resistance exhibited by *E.coli* to aminoglycosides varies. In this study, observed 37.5% resistance to gentamicin and 11.8% resistance to amikacin. Various other studies reported 5% resistance in non ESBL producing *E.coli* (Mahesh *et al.,* 2010) to 72.6% resistance in ESBL producing *E. coli* to gentamicin and 5% to 36.6% (Sonavane *et al.,* 2008) to amikacin. Gentamicin and tobramicin resistance has also increased as compared to other studies (Farooqi *et al.,* 2000), but amikacin has remained sensitive so far. Moreover, the result in this study of gentamicin and amikacin sensitivity against UTIs isolates was similarly to studies found in Karbala (Ali, 2011) and Lafi (Lafi *et al.*, 2012). As a whole *E.coli* in the study area appeared to be still resistance to ofloxacin (35.5%), ciprofloxacin (31%), and norfloxacin (32.4%), which are the common antibiotics prescribed for UTIs. The resistance to the antimicrobials has increased over the years. Resistance rates vary from country to country (Sharma *et al.*, 2005).

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