Isolation, Identification and Antimicrobial Susceptibility Pattern of Escherichia Coli Isolated From Urine Samples at Tertiary Care Hospital

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Abstract: Urinary tract infection (UTI) is the most common bacterial infection caused by both gram negative bacilli and gram positive cocci among which E. coli remains the leading uropathogens involving both men and women of all age groups. To isolate, identify and study the Antimicrobial Susceptibility Pattern of E. coli isolated from urinary tract infected patients of both sex of all age group. A sterile urine samples were collected from various clinical departments with suspected urinary tract infection and transported to central laboratory. The samples were subjected to direct microscopy, culture and biochemical tests for isolation of bacterial pathogens. Organisms were identified and Antimicrobial susceptibility testing was performed on Muller-Hinton agar by Kirby-Bauer disk diffusion method following CLSI (Central Laboratory Standard Institute) guidelines. A battery of antibiotics includes Gentamycin (10µg), Tobramycin (10µg), Ciprofloxacin (5µg), Ceftazidime (30µg), Piperacillin-Tazobactam (100/10µg), Ceftriaxone (30 µg), cefuroxime (30µg), Imipenem (10µg), Trimethoprim-sulfamethoxazole (1.25/23.75 µg), Ampicillin (10µg), Amoxicillin-clavulanic acid (20/10µg) and Nitrofurantoin (300 µg). A total of 486 urine samples were processed in central laboratory out of which 88 were isolated and identified as E. coli in both sex of all age groups. The E. coli isolates, were highly susceptible to Imipenem (95%), Piperacillin-Tazobactam (90%), Tobramycin (80%), Gentamicin (75%), nitrofurantoin 94%, with maximum resistance to Ampicillin (75%) and Amoxicillin-Clavulanic acid (68%). In this study the antimicrobial susceptibility to uropathogenic E.coli were studied and most classes of antibiotics were found to be sensitive.
Keywords: Urinary tract infection (UTI), E.coli, Antimicrobial susceptibility pattern.

INTRODUCTION

Escherichiae coli a gram negative, lactose fermenting, motile, anaerobic bacilli a most common UTI causing organism of family Enterobacteriaceae. UTI is one of the most frequently occurring nosocomial infections ranging from asymptomatic to severe sepsis. Approximately 35% of all the hospital acquired infection are contributed by UTI [1]. Urinary tract infection is caused by pathogenic invasion into urinary tract leading to inflammatory response of the uroepithelium. UTI is defined as persistence of actively growing microorganism within urinary tract. The clinical manifestation of UTI depends on the portion of urinary tract involved, the etiological organism, severity of infection and patient's ability to mount on immune response to it. The most susceptible group of patients is neonates, young women's and men. Signs and symptoms include fever, chills, dysuria, frequency, urgency and cloudy urine. Infection to female is most common due to shorter urethra and close to anus. In males, secretion of prostrate contains the bactericidal substrates which plays a vital role in encountering with E. coli and prevents infection in men. Though antibiotics are widely available, urinary tract infection is still a most common clinical complication. Distribution of urinary pathogens and their susceptibility to antibiotics varies regionally. Due to irrational use of antibiotics in practice the prevalence of antimicrobial resistance pattern among urinary pathogen is increasing worldwide. The aim of the study is to identify the uropathogenic E. coli and to evaluate the susceptibility pattern of commonly used antibiotics in our tertiary care hospitals. This may help the clinician to manage and treat the patients with symptoms of urinary tract infections.

MATERIALS AND METHODS

The present study was conducted over a period of four months in the microbiology lab, at Sree Balaji Medical College and Hospital, Chrompet. A total of

486 sterile urine samples obtained from inpatients of various clinical departments were sent to central laboratory for processing, out of which 88 were isolated and identified as E. coli. Escherichia coli a clinically important UTI causing organism was isolated from both sex and of all age groups.

Collection of samples

Sterile urine samples were collected in a wide mouthed container and sent to central lab of microbiology department for isolation of UTI causing bacterial pathogens. The processing involved direct gram stain and culturing onto MacConkey agar, Blood agar plate by streaking method. The inoculated plates were incubated at 37°C for 24 hours. Colonies grown on agar plates were further subjected to morphological and biochemical identification such as motility, catalase test, and triple sugar iron test, indole test, citrate utilization test, urease test, methyl red, voges proskauer (table 1).

Antimicrobial susceptibility testing

Antimicrobial susceptibility testing was performed on Muller-Hinton agar by Kirby-Bauer disc diffusion method following CLSI (Central Laboratory Standard Institute) guidelines. A battery of Enterobacteriaceae drugs were used which includes Gentamycin (10µg), Tobramycin (10µg), Ciprofloxacin (5µg), Ceftazidime (30µg), Piperacillin-Tazobactam (100/10µg), Ceftriaxone (30 µg), Cefuroxime (30µg), Imipenem (10µg), Trimethoprim-sulfamethoxazole (1.25/23.75 µg), Ampicillin (10µg), Amoxicillin-clavulanic acid (20/10µg) and Nitrofurantoin (300 µg).

RESULTS

Out of 88 E. coli positive isolates, maximum age group infected were 21-40 years of age (37.5%), followed by > 60 years (30.7%), 41-60 years (28.4%) and <20 years (3.4%) (Table-3).

Table-1: Colony characteristics and Biochemical identification of E. coli:

Features	E.coli
Colony on blood agar	Non-hemolytic, large, grey, moist colony
Colony on MacConkey agar	circular, low convex, smooth, translucent, lactose fermenters colony
Gram staining	Gram negative, rod shaped, pink color
Voges-Proskauer	Negative
Methyl red	Positive
Indole	Positive
Motility	Positive
H_2S production	Negative
Triple sugar iron	Alkaline/Alkaline
Oxidase	Negative
Citrate utilization	Negative
Catalase	Positive
Urease	Negative

Table-2: Sex wise distribution

Gender	No Of Cases (%)
Male	15(17.1%)
Female	73(82.9%)
Total	88(100%)

Table-3: Age wise distribution

Age in years	Total isolates	Percentage (%)
<20	3	3.4%
21-40	33	37.5%
41-60	25	28.4%
>61	27	30.7%
Total	88	100%

Table-4: Distribution of samples in various departments

S.No	Department	No Of Cases (%)
1.	ICU	8(9.1%)
2	Medical ward	24(27.3%)
3	Surgical ward	26(29.5%)
4	Obstetrics and gynaecology	30(34.1%)
	Total	88(100%)

Table-5: Antimicrobial susceptibility pattern of E. coli from urine samples

S.no	Antibiotics	Sensitivity (%)	Resistant (%)
1	Ampicillin	22(25%)	66(75%)
2	Amoxicillin clavulanic acid	28(32%)	60(68%)
3	Ciprofloxacin	50(57%)	38(43%)
4	Cefuroxime	47(53%)	41(47%)
5	Ceftazidime	49(56%)	39(44%)
6	Ceftriaxone	56(64%)	32(36%)
7	Trimethoprim-sulfamethoxazole	52(59%)	36(41%)
8	Gentamicin	66(75%)	22(25%)
9	Imipenem	84(95%)	4(5%)
10	Nitrofurantoin	83(94%)	5(6%)
11	Piperacillin-tazobactam	79(90%)	9(10%)
12	Tobramycin	70(80%)	18(20%)

DISCUSSION

A total of 486 urine samples were processed. Among the cultures screened, 88 (18%) samples showed positive growth for E. coli. A similar study done by Mahajan *et al*. showed Prevalence rate of E. coli to be 18.2% [2]. The positive growth of E. coli was confirmed by cultural, microscopic, and various biochemical tests presented in (Table 1).

Of these 88 positive cases, 15(17%) isolates were males and 73(82.9%) were females (Table 2). The result indicates that female patients had higher Prevalence of UTI than in males of which predominant isolates were from Obstetrics and Gynecology ward (34%). The factors associated with high prevalence of infection in females is due to shorter and wider urethra. This result is consistent with other study done by Tanzina *et al*. [3]. In our study, the most susceptible age group of patients to UTI were 21-40 years (37.5%), followed by > 60 years (30.7%), 41-60 years (28.8%), <20 years (3.4%). This study suggests that UTI is commonly encountered in the age group between 21-60 years. This occurrence may be due to frequent sexual intercourse, diaphragms, use of contraceptive spermicidal agents, and menopause for women and enlargement of prostate gland for men. A study done by Santhosh john *et al*. showed 18.6% of patients infected by E.coli were around age group of 26-35 years which was comparatively less than our study [4]. In the present study, the isolates were further subjected to antimicrobial susceptibility to commonly used antibiotics by disc diffusion method (Kirby-Bauer Method) in which, E.coli isolates were highly susceptible to Imipenem (95%), Piperacillin-Tazobactam (90%), Tobramycin (80%), Gentamicin (75%). A study done by Tanzina *et al*. reported 100% sensitive to Gentamicin, Imipenem, Piperacillin-Tazobactam and 96% sensitivity to Tobramycin [3]. A study done by sadaf guldin *et al*. showed 89.2% of Imipenem, 83.3% Gentamicin and 78% of Piperacillin-Tazobactam were sensitive to E. coli isolates which was less close to our present study [5]. Our study showed, sensitivity to Nitrofurantoin was 94% which is close to another study done by Shalini *et al.* where Nitrofurantoin was found to be 93.48% sensitive [6]. High level susceptibility of E. coli to Nitrofurantoin may reflects limited indication, narrow spectrum of activity, narrow tissue distributions, and limited contact of this antibiotic with bacteria present outside the urinary tract [7]. Antimicrobial susceptibility to third generation Cephalosporins such as Ceftriaxone, Ceftazidime and Cefuroxime was found to be 64%, 56% and 53% respectively. This result is similar to another study by Faraji. R *et al*. where more than 60% isolates were sensitive to third generation cephalosporins. In our study, 57% and 59% of Ciprofloxacin and Trimethoprim-Sulfamethoxazole were sensitive respectively. A controversy to our study by shanthi *et al.* who reported cotrimoxazole to be only 24.20% sensitive to UTI causing E. coli [8]. Highest resistance was seen against Ampicillin (75%) and Amoxicillin-Clavulanic acid (68%). Our study shows that majority of isolates were highly sensitive to many classes of antibiotics.

CONCLUSION

The study demonstrated that E.coli remains the most common UTI causing pathogens with majority of the isolates from female patients. The antimicrobial susceptibility pattern reveals that Imipenem, Piperacillin-Tazobactam, Tobramycin, Gentamicin and Nitrofurantoin are highly effective whereas Ampicillin and Amoxicillin-Clavulanic acid found to be less effective. Though carbapenems are found to be highly effective they should be used only in emergency and life threatening situations and not as a part of routine use, so that the therapeutic value of these drugs is preserved and available if absolutely necessary. Our study suggests that Nitrofurantoin should be used as empirical therapy for UTI. Therefore, routine monitoring of antimicrobial susceptibility pattern is mandatory to treat and to evaluate any resistance to third generation cephalosporins that cause ESBL production among strains of E. coli that is emerging worldwide. This will help the clinician to treat urinary tract infection and also to prepare antibiotic policy for individual institute.

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