Antibiotic Susceptibility Pattern of *Escherichia coli* Isolated from Urinary Tract Infections and Commensal *Escherichia coli* from Gastrointestinal Tract of Apparently Healthy Adult - A comparative study

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Abstract

Introduction: This study has been under taken to find out the drug resistance patterns exhibited by Escherichia coli (E. coli) originating Urinary Tract Infection (UTI) in comparison to intestinal commensal E.coli.

Materials and Method: One hundred UTI patients who had E. coli isolates were selected for this study. A whole of 100 isolates of E. coli from stool samples of apparently healthy individuals were included as controls. Antibiotic susceptibility testing (AST) was carried out by Kirby Bauer disk diffusion method on Mueller Hinton agar (Hi media).

Result: In the present study E. coli showed more resistance to commonly used antibiotics such as ciprofloxacin, norfloxacin, cefotaxime, ceftriaxone. There is also increasing resistance to gentamicin. In comparison, the antibiotic resistance between cases and controls showed a small difference.

Conclusion: E. coli is the most common cause of UTI, and microscopic examination of the urine is an important screening method for UTI cases. High resistance to widely used antibiotics for treatment may be attributed to inadvertent and indiscriminate use.

Keywords: Escherichia coli, Urinary Tract Infection, antimicrobial susceptibility testing, antibiotic resistance, commensal *E. coli*

Introduction

Escherichia coli (E. coli) is a Gram negative, motile, lactose fermenting, indole positive enterobacterium. In the intestinal tract, it resides harmlessly and poorly adapted to cause disease in healthy individuals. However, there exist a plethora of pathotypes that can cause a particular type of illness both in healthy hosts and those with compromised nonspecific defence mechanisms. E. coli causes intestinal infections and extraintestinal infections. E. coli frequently infects extraintestinal sites like urinary tract and are the most frequent pathogen isolated from uncomplicated urinary tract infections.[1,2]

The ubiquity of antibiotic resistance of E. coli strains is increasing. Earlier they were highly susceptible to a broad range of antimicrobial agents. In indefinite, the regularity of ampicillin use precludes its practical use. The prevalence of resistance to first generation cephalosporins and trimethoprim-sulfamethoxazole is on the increase.[3] The resistance of E. coli to fluoroquinolones has raised over the last decade. Among quinolone resistant strains, a significant prevalence of co-resistance to amoxicillin/clavulanic acid and piperacillin has been reported. The occurrence of co-resistance to second, third and fourth generation of cephalosporins, monobactams, piperacillin-tazobactam and the non-amikacin aminoglycosides is increasing.[4]

In India, antimicrobial susceptibility design of euro pathogens varies extensively by region. High resistance rates to oral antibiotics have been observed, probably due to uncontrolled consumption of these antibiotics. The global trend of empirically handling community procured UTI may not apply for precise geographical regions such as India, where limited susceptibility rates are documented for common urinary pathogens. In the Indian setting, routine urine cultures may be necessary, since treatment failure with empirical therapy is likely to occur.[5]

The pervasiveness of antibiotic resistance in commensal E. coli is also increasing. Commensal E. coli may be used as the indicator organisms for detecting antimicrobial resistance. Commensal E. coli can play as stories of resistance genes that easily transfer to other commensal E. coli as well as other potentially pathogenic bacteria.[6] Hence it is necessary to know the antibiotic susceptibility pattern of these commensals.

This study was conducted in KVG Medical College & Hospital, Sullia, Karnataka. No studies have been carried out on antibiotic susceptibility pattern of E. coli in this locality. Hence this study has been initiated to compare the antibiotic susceptibility pattern of E. coli isolated from UTI with that of E. coli isolated from the apparently healthy adult gastrointestinal tract.

Materials and Method

This study was conducted in KVG Medical College & Hospital in 2011. A total of 600 urine samples from patients with UTI were processed by microscopy and culture, of these 100 patients had E. coli isolates. A total of 100 isolates of E. coli from stool samples of

apparently healthy individuals were included as controls.

Clean catch midstream urine specimens were collected in a sterile wide mouthed universal container, and stools samples were solicited in a sterile wide mouthed leak-proof container. All the samples were transported to the laboratory within 1 hour and processed.

Urine samples were subjected to wet film examination (uncentrifuged), and cultured semi quantitatively by standard loop method on Sheep blood agar and Mac Conkey agar. Stools were inoculated on Mac Conkey agar. Identification of E. coli was made by conventional methods and susceptibility by Kirby Bauer method as per CLSI guidelines.

Antibiotic susceptibility testing (AST):[7,8,9] AST was carried out by Kirby Bauer disk diffusion method on Mueller Hinton agar (Hi media). The media was prepared as per the instructions of the manufacturer by suspending 38g of dehydrated media in 1000ml of distilled water, autoclaved at 121° C for 15 min and poured into Petri dishes to a depth of 4 mm. A broth culture of the isolate with turbidity equivalent to 0.5 McFarland turbidity standards was lawn cultured on the Mueller Hinton agar and allowed to dry. The antibiotic disks contained in cartridges taken out from the refrigerator half an hour earlier to bring to room temperature were placed on the surface seeded with E. coli and incubated at 37ºC overnight. The E. coli isolates were examined for susceptibility to following antibiotics using commercial antibiotic disks (Hi media).

Amikacin (30 µg)
Amoxiclav (20/30 µg)
Ampicillin (10 µg)
Cefotaxime (30 µg)
Ceftriaxone (30 µg)
Chloramphenicol (30 µg)
Ciprofloxacin (5 µg)
Cotrimoxazole (1.25 / 23.75 µg)
Gentamicin (10 µg)
Nalidixic acid (30 ug)
Nitrofurantoin (300 µg)
Norfloxacin (10 µg)
Piperacillin – Tazobactam (100/10µ)

The antibiotic susceptibility was interpreted as sensitive, moderately sensitive or resistant as per CLSI guidelines. E. coli ATCC 25922 was used as control to check the antibiotic disks.

Results

Details of some urine specimens processed and some cultures are showing E. coli with significant bacteriuria is given in Table 1. Age wise distribution of UTI cases (n=100) is shown in Table 2. A total of 100 patients with UTI were included in the study of which 47 were males, and 53 were females. Female to male ratio 1.12:1.Clinical features of UTI cases are given in Table 3. Microscopy of urine samples is shown in Table 4.

Table 1: Occurrence of *E. coli* in UTI

Total no of urine specimens processed	Number of no growth or no significant growth	Number of cultures yielded 2 pathogens	Number of cultures showing bacteria other than E.coli	Number of cultures showing *E. coli* with significant bacteriuria
600	356	16	128	100

Table 2: Age wise distribution of UTI cases (n=100)

Age group(in years)	Males (n=47)	Female (n=53)
0-9	10	7
10-19	1	1
20-29	7	6
30-39	2	4
40-49	6	5
50-59	4	10
60-69	9	9
70-79	7	8
80-89	1	3

Table 3: Clinical features of UTI cases (n = 100)

Clinical features	Present	Absent
Fever	62	38
Frequency / Urgency / Dysuria	84	16
H/O Diabetes mellitus	30	70

Table 4: Microscopy of urine samples (n=100)

Finding	Present
Pus cells	81%
Bacteria	54%

Table 5: The antibiotic susceptibility of the 100 *E. coli* from UTI cases

Name of the antibiotic	Sensitive %	Moderately Sensitive %	Resistant %
Ampicillin	7	00	93
Amikacin	68	18	14
Amoxyclav	14	06	80
Cefotaxime	21	07	72
Ceftriaxone	23	04	73
Chloramphenicol	73	07	20
Ciprofloxacin	21	07	72
Cotrimoxazole	29	03	68
Gentamicin	50	11	39
Nalidixic acid	11	02	87

Nitrofurantoin	75	08	17
Norfloxacin	18	00	82
Piperacillin-Tazobactam	70	14	16

The antibiotic susceptibility of the 100 E. coli from UTI cases is given in Table 5. Maximum no of isolates showed resistance to ampicillin (93%) and sensitivity to nitrofurantoin. Higher resistance to commonly used antibiotics like ciprofloxacin(72%), norfloxacin (82%) and cefotaxime(72%) was seen. Nitrofurantoin and amikacin still show excellent sensitivity.

Antibiotic susceptibility pattern of control E. coli is given in Table 6. Maximum no of controls were sensitive to amikacin (86%), gentamicin (86%) followed by piperacillin-Tazobactam (83%). In our study controls were taken from stool samples of healthy adults. Antibiotic sensitivity in these isolates reflects sensitivity in the community.

Table 6: Showing antibiotic susceptibility pattern of control *E. coli* (n=100)

Name of the antibiotic	Sensitive Number (%)	Moderately Sensitive Number (%)	Resistant Number (%)
Ampicillin	08	00	92
Amikacin	86	06	08
Amoxyclav	26	00	74
Cefotaxime	32	06	62
Ceftriaxone	32	06	62
Chloramphenicol	81	00	19
Ciprofloxacin	45	00	55
Cotrimoxazole	39	00	61
Gentamicin	86	00	14
Nalidixic acid	45	00	55
Nitrofurantoin	52	18	30
Norfloxacin	15	15	70
Piperacillin-Tazobactam	83	16	01

Discussion

This study has been under taken to find out the drug resistance patterns exhibited by both the groups. In the present study, E. coli were seen in 41% of UTI isolates. Similar findings were recorded by Sonavane et al[10] and Taneja et al.[11] Even though UTI is more common in females[12] because of the shorter length of urethra and proximity of vagina and urethra to the anus; our study didn't show much of gender difference like that of Chatterjee et al.

In this study, 18 children with UTI were included out of which, 17 had a fever which correlates earlier finding.[13] Failure to thrive, vomiting, and fever seem to be the primary manifestation of UTI in children less than of 2 years of age. In the present study majority of the cases included were lower UTIs of which 84% had features of frequency/urgency/dysuria.

Significant pyuria is defined as[3] one pus cell /7 HPF in uncentrifuged urine (i.e., >104 pus cells/ml of urine). In the present study, significant pyuria was observed in 81% of culture positive cases. Finding of pyuria in excess than normal is indicative of UTI.[14] There is little value in the microscopical examination of the wet film for bacteria.[14]

In the present study, E. coli showed more resistance to commonly used antibiotics such as ciprofloxacin, norfloxacin, cefotaxime, ceftriaxone. There is also increasing resistance to gentamicin. Ampicillin resistance was seen in 93% of cases which correlates with previous reports.[15,16] There was 80% resistance to Amoxiclav, which is similar to the finding of Sonavane et al.[10] High resistance to cefotaxime (72%) and ceftriaxone (73%) was seen as reported by others.[10,17] Amikacin resistance was seen in 14% of cases, similar to Biswas et al[18] (11%) and Kausar et al[16] (8%). There was high resistance to nalidixic acid and fluoroquinolones which corroborate with earlier observations.[10,16,20] A little nitrofurantoin resistance (i.e. 17%) was observed and is comparable previous reports.[16,19,20] Cotrimoxazole resistance was seen in 68%. Similar findings were reported by others.[16, 20,21]

Mathai et al[6] reported that 18% of commensal E. coli were resistant to ampicillin, 24% to cotrimoxazole, 2% to cefuroxime, 2% to gentamicin, 6% to chloramphenicol, 26% to nalidixic acid and 4% resistance to ciprofloxacin.

In comparison, the antibiotic resistance between cases and controls showed a small difference. However molecular studies are needed to confirm this. Surveillance among commensal E. coli can be used as a criterion to monitor changes in the antimicrobial resistance over time.

Conclusion

From this study, it can be concluded that E. coli is the most common cause of UTI and microscopic examination of the urine is an important screening method for UTI cases. High resistance to widely used antibiotics for treatment may be attributed to inadvertent and indiscriminate use. Hence the unnecessary use of antibiotics must be avoided.

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