

Original Research Article

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Changing Antimicrobial Susceptibility Pattern of *E. coli* Isolated from Urine and Pus Samples

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ABSTRACT

Antimicrobial resistance in *E. coli* is of major concern worldwide due to its increasing resistance to several commonly prescribed antibiotics. The aim of this study was to determine the antimicrobial susceptibility pattern of *E. coli*. A retrospective analysis of *E. coli* and their antimicrobial susceptibility was done on urine and pus samples at Subbaiah Institute of Medical Sciences, Shimoga. Antibiotic susceptibility was tested on Mueller Hinton agar by Kirby Bauer disc diffusion method. Total of 73 *E. coli* isolates from urine sample were studied. Females 55 (75%) were more susceptible to urinary tract infections (UTIs) than males 18 (25%). Majority of the isolates were sensitive to amikacin 69 (95%), gentamicin 55 (75%) and nitrofurantoin 65 (89%). Out of 28 isolates from pus sample, 19 (68%) were from males and 9 (32%) from females. Most of the isolates were sensitive to amikacin 25 (89%), gentamicin 21 (75%), meropenem 21 (75%) and chloramphenicol 20 (71%). Resistance rates to ampicillin and cephalosporins were high. Since most isolates were sensitive to amikacin and gentamicin in both samples, they are considered as appropriate antibiotics for treatment of *E. coli* infections.

Keywords

E. coli,
Antimicrobial
susceptibility,
Amikacin,
Gentamicin.

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Introduction

Escherichia coli (*E. coli*) is the most significant species in the genus *Escherichia* (Naheed Akhtar Khan *et al.*, 2002). *E. coli* is a common inhabitant of the human and animal gut, but can also be found in water, soil and vegetation (Kibret *et al.*, 2011). It is the leading pathogen causing urinary tract infections and is among the most common pathogens causing blood stream infections, wound infection, otitis media and other complications in humans (Gautam *et al.*, 2013).

Urinary tract infection (UTI) is defined as presence and active multiplication of

microorganisms within the urinary tract and is one of the commonest bacterial infections seeking treatment in clinical practice (Banerjee *et al.*, 2009). Pyogenic infection is characterized by several local inflammations, usually with pus formation, generally caused by one of the pyogenic bacteria, which can produce the accumulation of dead leukocytes and infectious agent commonly known as pus (Vijeta Sharma *et al.*, 2015). Most of these infections are caused by Gram-negative bacteria like *Escherichia coli* (*E. coli*), *Klebsiella* spp., *Proteus mirabilis*, *Pseudomonas aeruginosa*, *Acinetobacter* spp., and *Serratia* spp. and Gram-positive bacteria

such as *Enterococcus* spp. and *Staphylococcus* spp. (Mulugeta Kibret *et al.*, 2014). *E. coli* is one of the most frequently isolated organisms from urine and pus samples. These strains are often multi drug resistant, i.e. refractory to 3 or more different classes of antibiotic agents (Sayan Bhattacharyya *et al.*, 2015). They are the frequent cause of morbidity in outpatients as well as most frequently involved in the cause of nosocomial infection in many hospitals (Shanthi *et al.*, 2012).

Production of beta-lactamases is the principal mechanism of resistance to beta-lactam antibiotics among members of the *Enterobacteriaceae* family (Mohanalakshmi *et al.*, 2014). Three major groups of such enzymes are usually distinguished, class C cephalosporinases (AmpC), extended spectrum beta-lactamases (ESBLs) and metallo-beta-lactamases (MBLs), are of great concern (Fam *et al.*, 2006). These enzymes hydrolyze the amide bond of the four membered characteristic beta - lactam ring, thus rendering the antimicrobial ineffective (Prashant Durwas Peshattiwari *et al.*, 2011). Bacteria carrying such enzymes are the important cause of multi-drug resistant infections throughout the world and also the incidence of such organisms appear to be increasing in the community (Nwosu *et al.*, 2014).

Drug resistance of pathogens is a serious medical problem, because of very fast arise and spread of mutant strains that are insusceptible to medical treatment. Microorganisms use varied mechanisms to acquire drug resistance viz. horizontal gene transfer (plasmids, transposons and bacteriophages), recombination of foreign DNA in bacterial chromosome and mutations in different chromosomal locus (Jahangir Alam *et al.*, 2013). Infections due to multi drug resistant (MDR) *E. coli* increases the

cost of treatment, morbidity and mortality especially in developing countries like India (Siddarth Surwonsa *et al.*, 2017). Hence the present study was conducted to detect such resistant *E. coli* and to know their susceptibility pattern.

Materials and Methods

Data analysis

A retrospective analysis of *E. coli* isolates of urine and pus samples was performed at Subbaiah Institute of Medical Sciences, Shimoga. The sex, age and outpatient/ Inpatient details of patients, number of *E. coli* isolates and their antimicrobial susceptibility profiles were collected from the records. The data were entered into Excel for analysis. As the study was based on secondary data there were no ethical issues.

Sample collection and processing

As per the standard operation procedures, pus sample and clean-catch midstream urine specimens were collected. Samples were plated on MacConkey agar and, blood agar and then incubated aerobically at 37 °C for 24 h. From positive cultures, pathogens were identified according to the standard operational procedures as per the standard microbiological methods (Collee *et al.*, 2007). In urine samples, a significant bacterium was considered if culture yield $\geq 10^5$ CFU/mL.

Antimicrobial susceptibility testing

According to the standard operational procedures, antimicrobial susceptibility tests were done on Mueller-Hinton agar using Kirby-Bauer disk diffusion method. The antimicrobial agents tested were: ampicillin (10µg), amoxycylav (20/10µg), gentamycin (10µg), amikacin (30µg), ciprofloxacin (10µg), norfloxacin (10µg), cotrimoxazole

(1.25/23.75 µg), ceftazidime (30µg), ceftriaxone (30µg), cefotaxime (30µg), cefuroxime (30µg), cefoxitin (30µg), cefipime (30µg), aztreonam (30µg), meropenem (30µg), piperacillin (100µg), chloramphenicol (300µg), tetracycline (30µg). Resistance data were interpreted according to Clinical laboratory Standards Institute (CLSI, 2017).

Results and Discussion

Of 73 *E. coli* isolates from urine sample, 55 (75%) were from females and 18 (25%) from males and 49 (67%) isolates were from outpatient department and 24 (33%) from inpatient. The age 18-45 years 37(51%) in case of urine isolates and age of > 45 years 18(64%) in case of pus isolates were the most affected group. Majority of the isolates were sensitive to amikacin 69 (95%), gentamicin 55 (75%) and nitrofurantoin 65 (89%). Out of 28 isolates from pus sample, 19 (68%) were from males and 9 (32%) from females and 7 (25%) isolates were from outpatient department and 21 (75%) from inpatient. Most of the isolates were sensitive to amikacin 25 (89%), gentamicin 21 (75%), meropenem 21 (75%) and chloramphenicol 20 (71%). In both the samples, resistance rate to ampicillin and all cephalosporins were high (Table 1-4).

Indeed, members of *E. coli* are widely distributed in the environment and cause a variety of infections in the hospital and community settings (Marwa Aly *et al.*, 2012). UTIs and pyogenic infections are among the most common diseases diagnosed worldwide. About 150 million people are diagnosed with UTI each year and about 80% of UTIs are caused by *E. coli* (Reem Fouad Polse *et al.*, 2016). Availability of new antimicrobials has improved the management of UTIs. However, the management of UTI infections has been jeopardized by increase in emergence of antimicrobial drug resistance (Mulugeta Kibret *et al.*, 2014).

In this study, more isolates from urine samples were recovered from females 55(75%) as compared to males 18(25%) due to a short urethra and its proximity to the anal opening, which correlated with the study of Yashwant Kumar *et al.*, (2013). In case of pus samples, highest isolates were observed in male 19(68%) as compared to female 9(32%). It was correlated with Vijeta Sharma *et al.*, (2015) study which shows male preponderance (55%). Maximum number of patients belonged to age group 18-45 years 37(51%) in case of urine isolates and to age group of > 45 years 18(64%) in case of pus isolates. Findings were compared to other authors like Mulugeta Kibret *et al.*, (2014) showed similar results. In urine sample, OP isolates were 49(67%) and IP 24(33%) and in pus sample, OP isolates were 7(25%) and IP 21(75%).

Antimicrobial resistance among *E. coli* shows considerable variations during different time periods and in different areas. The problem becomes further complicated with the emergence of beta-lactamase producing *E. coli*. It is of major concern worldwide due to its increasing resistance to several commonly prescribed antibiotics. *E. coli* isolates varied in their susceptibility to different antibiotics belonging to different groups. From the result of our study, most of the urine isolates were sensitive to amikacin 69 (95%), gentamicin 55 (75%) and nitrofurantoin 65 (89%) and pus isolates to amikacin 25 (89%), gentamicin 21 (75%), meropenem 21 (75%) and chloramphenicol 20 (71%). Hence, aminoglycosides and nitrofurantoin in case of UTI and meropenem, chloramphenicol in case of pyogenic infections could be considered as a choice for treatment. This result correlated with study done by Rebecca Naveen *et al.*, (2005). Susceptibility to nitrofurantoin has remained practically unchanged since its introduction into clinical practice more than 50 years ago.

Table.1 Age, sex and OP/IP distribution of *E. coli* isolates from urine sample

Age (years)	No. of isolates NO. (%)	Sex		OP NO. (%)	IP NO. (%)
		M NO. (%)	F NO. (%)		
1 – 18	6(8)	2(3)	4(6)	3(4)	3(4)
18 -45	37(51)	5(7)	32(43)	28(38)	9(12)
> 45	30(41)	11(15)	19(26)	18(25)	12(17)
Total	73(100)	18(25)	55(75)	49(67)	24(33)

Table.2 Antibiotic sensitivity pattern *E. coli* isolates from urine sample

	AMP NO. (%)	AMC NO. (%)	G NO. (%)	AK NO. (%)	NET NO. (%)	CIP NO. (%)	NX NO. (%)	COT NO. (%)	NIT NO. (%)	CAZ NO. (%)	CTR NO. (%)	CTX NO. (%)	CXM NO. (%)	CN NO. (%)	CPM NO. (%)	AT NO. (%)	MRP NO. (%)
OP isolates(49)	8 (16)	11 (22)	39 (80)	47 (96)	42 (86)	17 (35)	15 (31)	24 (49)	44 (90)	13 (27)	13 (27)	13 (27)	13 (27)	22 (45)	12 (24)	13 (27)	31 (63)
IP isolates(24)	2 (8)	5 (21)	16 (67)	22 (92)	20 (83)	4 (17)	6 (25)	10 (42)	21 (88)	3 (13)	3 (13)	3 (13)	3 (13)	11 (46)	9 (38)	3 (13)	14 (58)
Total (73)	10 (14)	16 (22)	55 (75)	69 (95)	62 (85)	21 (29)	21 (29)	34 (47)	65 (89)	16 (22)	16 (22)	16 (22)	16 (22)	33 (45)	26 (36)	16 (22)	45 (62)

Table.3 Age, sex and OP/IP distribution of *E. coli* isolates from pus sample

Age (years)	No. of isolates NO. (%)	Sex		OP NO. (%)	IP NO. (%)
		M NO. (%)	F NO. (%)		
1 – 18	2(7)	-	2(7)	2(7)	-
18 -45	8(29)	6(22)	2(7)	4(14)	4(14)
> 45	18(64)	13(46)	5(18)	1(4)	17(61)
Total	28(100)	19(68)	9(32)	7(25)	21(75)

Table.4 Antibiotic sensitivity pattern *E. coli* isolates from pus sample

	AMP NO. (%)	AMC NO. (%)	G NO. (%)	AK NO. (%)	CIP NO. (%)	NX NO. (%)	COT NO. (%)	CAZ NO. (%)	CTR NO. (%)	CTX NO. (%)	CXM NO. (%)	CN NO. (%)	CPM NO. (%)	AT NO. (%)	MRP NO. (%)	PI NO. (%)	C NO. (%)	TE NO. (%)
OP isolates (7)	1 (14)	3 (43)	5 (71)	7 (100)	2 (29)	3 (43)	4 (57)	4 (57)	3 (43)	3 (43)	3 (43)	5 (71)	4 (57)	3 (43)	6 (86)	4 (57)	4 (57)	3 (43)
IP isolates (21)	3 (14)	9 (43)	16 (76)	18 (86)	8 (38)	7 (33)	8 (38)	4 (19)	5 (24)	5 (24)	5 (24)	9 (43)	6 (29)	5 (24)	15 (71)	9 (43)	16 (76)	10 (48)
Total (28)	4 (14)	12 (43)	21 (75)	25 (89)	10 (36)	10 (36)	12 (43)	8 (29)	8 (29)	8 (29)	8 (29)	14 (50)	10 (36)	8 (29)	21 (75)	13 (46)	20 (71)	13 (46)

AMP – AMPICILLIN, AMC – AMOXYCLAV, G – GENTAMYCIN, AK – AMIKACIN, CIP – CIPROFLOXACIN, NX –NORFLOXACIN, COT –
COTRIMOXAZOLE, CAZ – CEFTAZIDIME, CTR – CEFTRIAZONE, CTX – CEFOTAXIME, CXM – CEFUROXIME, CN – CEFOXITIN, CPM –
CEFIPIME, AT – AZTREONAM, MRP – MEROPENEM, PI – PIPERACILLIN, C – CHLORAMPHENICOL, TE – TETRACYCLINE

Since standard medication with nitrofurantoin does not achieve therapeutic concentrations in the bloodstream, its use is advised only for complicated cystitis (Gautam *et al.*, 2013). Most isolates were resistant to beta lactum antibiotics like ampicillin and cephalosporins. This result was comparable to Eze *et al.*, (2015) study. This high resistance may be due to the spontaneous and uncontrollable use of these antibiotics. Most cases of multiple antibiotics resistant strains have been demonstrated to be due to transferable, extrachromosomal circular DNA, plasmids (Charles Okechukwu Esimone *et al.*, 2010). Drug resistance is also common in the elderly which most likely reflects greater comorbidities, hospitalizations, and antimicrobial exposure among older patients (Sanjeev Swami *et al.*, 2013). Several studies have documented a positive relationship between the use of third-generation cephalosporins, other β -lactams or fluoroquinolones and the acquisition of antimicrobial resistance by ESBL-producing organisms. In recent years, use of fluoroquinolones has increased in many countries and emergence of resistance of bacterial isolates to fluoroquinolones has been observed (Saghir Ahmad Jafri *et al.*, 2014). In our study, both urine and pus isolates showed only 25% to 35% sensitivity to fluoroquinolones. Tailoring antibiograms and treatment recommendations to better match specific patient populations could improve patient care, simplify treatment regimens, improve antimicrobial stewardship, and reduce health care costs (Christian Smith *et al.*, 2015).

In conclusion, many variations exist among *E. coli* in terms of antibiotic sensitivity pattern. Periodic formulation and review of antimicrobial policies are therefore required for controlling the development and dissemination of drug resistance. Rationales for prudent antimicrobial use, followed by

discrete adherence to the same, are needed. Moreover, due to the high prevalence of variability among antibiograms, empirical selection should be based on the knowledge of local prevalence and individual sensitivity rather than on universal guidelines.

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