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Patterns of antimicrobial resistance among Multi-Drug resistant *Escherichia coli* in a tertiary care centre in South Karnataka

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Abstract

Introduction: Emergence of multi-drug resistant (MDR) organisms is an important public health concern especially in developing countries. The present study was carried out to assess the current antimicrobial pattern of multi-drug resistant *Escherichia coli*.

Materials and Methods: 346 consecutive, non-duplicate isolates of *E. coli* isolated during a period of two years were included in the study. The numbers of multi-drug resistant isolates were determined. Their antimicrobial resistance phenotypes and Multiple Antibiotic Resistance (MAR) index were determined.

Results: Among the 346 isolates of *E. coli*, 297 (85.84%) were multi-drug resistant with 61.3% of the isolates being resistant to drugs in more than five antimicrobial categories. The Multiple Antibiotic Resistance index of 82.7% of isolates was found to be greater than 0.2. Carbapenems, aminoglycosides, Chloramphenicol and Nitrofurantoin were found to be the most effective agents against MDR *E.coli*.

Conclusion: Antimicrobial resistance profiles of bacteria are ever-changing and periodic evaluation of resistance phenotypes of isolates is essential for the formulation of appropriate antibiotic policy and initiation of pertinent empirical therapy.

Keywords: Antibiogram, *Escherichia coli*, Multi-drug Resistance, Multiple Antibiotic Resistance Index, Antibiotic Resistance Phenotypes.

Introduction

Escherichia coli is a normal commensal of the gastrointestinal tract on one hand and on the other, is capable of causing a vast array of human infections ranging from the intestinal tract to blood stream, urinary tract, central nervous system, etc.[1] It possesses great genetic flexibility and is capable of efficiently acquiring and transferring genetic material coding for resistance to other enteric pathogens like *Salmonella*, *Vibrio*, *Yersinia* and *Shigella*.[2,3] Consequently, commensal *E. coli* can act as a repository of resistance genes.[4] In India, from 2008 to 2013, resistance in *E. coli* to third generation cephalosporins, fluoroquinolones and carbapenems increased from 70% to 83%, 78% to 85% and 10% to 13% respectively.[5] The rapid dissemination of drug resistant bacteria is a crucial public health concern especially in developing countries.[6] This has been attributed to over-the-counter access to antibiotics, self-medication, lack of patient compliance to antibiotic regimen, indiscriminate use by prescribing doctor and antibiotic use in animal husbandry to name a few.[7] A marked difference in antibiotic resistance pattern has been noted in different geographical areas and from time to time.[8] In spite of the increased demand for new antimicrobial agents, the pace of their discovery has considerably slowed down in the recent past.[9] The antibiotic armamentarium available for the treatment of drug resistant organisms is diminishing. Hence regular surveillance, measuring antibiotic resistance in bacteria is essential for appropriate and timely initiation of empirical therapy. The present study intends to assess the current prevalence of multi-drug resistant (MDR) *E. coli* in our region and to describe its resistance and sensitivity trends.

Materials and Methods

The present study was conducted in the Microbiology department of Shridevi Institute of Medical Sciences and Research Hospital, Tumkur after obtaining approval from the Institutional Ethical Committee. All isolates of *Escherichia coli* isolated from various clinical samples including urine, pus, sputum, stool, vaginal swab, blood and miscellaneous samples during a period of two years from January 2014 to December 2015 were included in the study. Processing of samples and the identification of the isolates were performed by conventional methods.[10]

Antimicrobial susceptibility testing was performed by Kirby-Bauer disc diffusion method and the susceptibility to antibiotics was assessed based on the Clinical Laboratory Standards Institute guidelines.[11] The antibiotics tested were Ampicillin (10µg), Cephalexin (30µg), Cefuroxime (30µg), Cephotaxime (30µg), Cefoperazone (75µg), Ceftazidime (30µg), Ceftriaxone (30µg), Cefepime (30µg), Amoxycillin/Clavulanic acid (20µg/10µg), Ampicillin/Sulbactam (10µg/10µg), Gentamicin (10µg), Amikacin (30µg), Netilmicin (30µg), Tetracycline (30µg), Ciprofloxacin (5µg), Cotrimoxazole (Trimethoprim 1.25µg/Sulfomethoxazole 23.75µg), Choramphenicol (30µg), Imipenem (10µg), Piperacillin/Tazobactam (100µg/10µg) and Meropenem

(10µg). For isolates obtained from urine Nitrofurantoin (300µg) and Nalidixic acid (30µg) disc were added and Chloramphenicol disc was excluded. All isolates showing intermediate susceptibility were counted as resistant. All antibiotic discs were procured from Himedia Laboratories Pvt. Ltd. *Escherichia coli* ATCC 25922 was used for quality control.

Isolates of *Escherichia coli* resistant to at least one agent in three or more antimicrobial categories were classified as Multi-drug resistant (MDR) and were further grouped based on the number of categories to which the isolate was resistant.[12] The antimicrobial resistance pattern of the MDR *Escherichia coli* isolates were assessed and the predominant antimicrobial resistance phenotypes were determined. Multiple Antibiotic Resistance (MAR) index was also calculated by dividing the number of antibiotics to which the isolate was resistant by the total number of antibiotics to which the isolate was tested.[13] Statistical significance was assessed using the Chi-square test.

Results

Of the 1281 positive cultures obtained in the Microbiology department, *Escherichia coli* was the most predominant, adding up to 346 isolates.

Majority of the *Escherichia coli* isolates were obtained from patients in the age group of 21 to 70 years (75.14%) with paediatric patients accounting for 10.4% of the isolates. Maximum numbers of isolates were acquired from patients with Urinary tract infection (56.07%) as shown in Table 1.

Table 1: Distribution of *Escherichia coli* obtained from various samples

Sample	Total isolates	% of total	No. of MDR isolates	% of MDR isolates
Urine	194	56.07	156	52.52
Pus	118	34.10	107	36.03
Sputum	14	4.05	14	4.71
Stool	7	2.02	7	2.36
Vaginal swab	6	1.73	6	2.02
Miscellaneous	4	1.16	4	1.35
Blood	3	0.87	3	1.01
Total	346	100	297	100

Multi-drug resistant (MDR) *Escherichia coli* accounted for 85.84% (n=297) of the isolates. Of the MDR isolates, 112 (37.71%) were from out-patients, 168 (56.57%) from in-patients in general wards and 17 (5.72%) from the Intensive care units. The increased occurrence of multidrug resistant isolates among inpatients was not found to be statistically significant at p value <0.05 (Chi square = 0.172, p value = 0.678381).

Majority of the MDR isolates showed high level resistance with 23.23% of the isolates being resistant to 7 groups and 21.21% being resistant to 8 groups of antimicrobial agents as shown in Fig. 1.

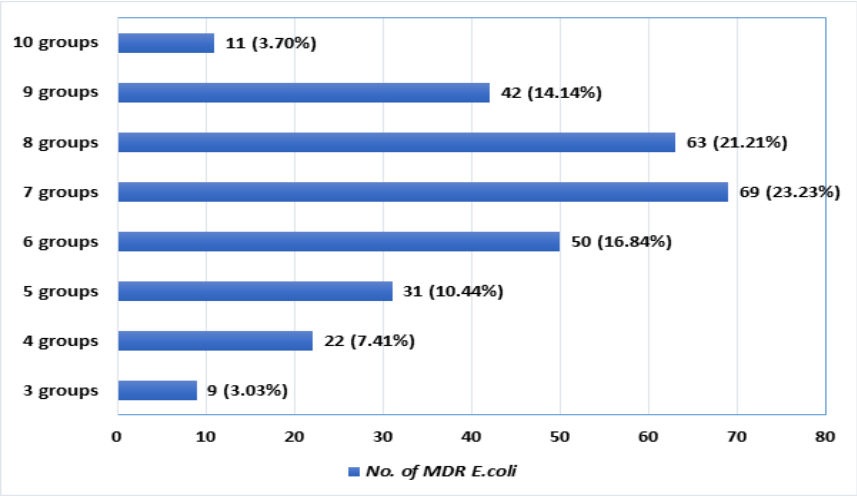

Fig. 1: Distribution of MDR *Escherichia coli* isolates based on resistance to different antimicrobial groups

The MDR isolates showed maximum resistance to β-Lactam agents. 100% resistance was exhibited against Ampicillin and Cephalexin. Resistance against Cefuroxime was 97.64%, Amoxicillin/Clavulanic acid and Ampicillin/Sulbactam 90.25% followed by third and fourth generation Cephalosporins showing 87.54% and 82.83%

resistance respectively. Maximum sensitivity was demonstrated for Carbapenems. Most isolates showed high degree of sensitivity to Chloramphenicol (81.56%), Nitrofurantoin (81.41%), Amikacin (77.1%) and Piperacillin/Tazobactam (77.1%). Table 2 shows the resistance pattern of *Escherichia coli*.

Table 2: Antimicrobial resistance patterns of *Escherichia coli* isolates against different classes of antibiotics

Antibiotic class	Drugs tested	No. of isolates tested	No. of resistant MDR isolates	No. of resistant non-MDR isolates	Overall resistance
Penicillins	Ampicillin	346	297(100)	36(73.5)	333(96.2)
Non-extended spectrum Cephalosporins (1st & 2nd generation)	Cephalexin	346	297(100)	27(55.1)	324(93.64)
	Cefuroxime	346	290(97.64)	27(55.1)	317(91.62)
Extended spectrum Cephalosporins (3rd & 4th generation)	Cephotaxime	346	260(87.54)	0(0)	260(75.14)
	Cefoperazone	346	260(87.54)	0(0)	260(75.14)
	Ceftazidime	346	260(87.54)	0(0)	260(75.14)
	Ceftriaxone	346	260(87.54)	0(0)	260(75.14)
	Cefepime	346	246(82.83)	0(0)	246(71.1)
Penicillins+β-Lactamase inhibitors	Amoxicillin/Clavulanic acid	346	268(90.25)	1(2.04)	269(77.75)
	Ampicillin/Sulbactam	346	268(90.25)	1(2.04)	269(77.75)
Anti-Pseudomonal Penicillins+β-Lactamase inhibitor	Piperacilin-Tazobactam	346	68(22.9)	0(0)	68(19.65)
Carbapenems	Imipenem	346	9(3.03)	0(0)	9(2.60)
	Meropenem	346	11(3.70)	0(0)	11(3.18)
Aminoglycosides	Gentamicin	346	159(53.54)	0(0)	159(45.95)
	Netilmicin	346	84(28.28)	0(0)	84(24.28)
	Amikacin	346	68(22.9)	0(0)	68(19.65)
Fluoroquinolones	Nalidixic acid	196	146(93.59)	23(57.5)	169(86.22)
	Ciprofloxacin	346	245(82.5)	3(6.12)	248(71.68)
Folate pathway inhibitors	Trimethoprim-Sulphamethoxazole	346	228(76.77)	5(10.20)	233(67.34)
Phenicols	Chloramphenicol	150	26(18.44)	2(22.22)	28(18.67)
Tetracyclines	Tetracycline	346	224(75.42)	4(8.16)	228(65.9)
Nitrofurans	Nitrofurantoin	196	29(18.59)	4(10)	33(16.84)

A total of 69 resistance phenotypes were exhibited by the 297 MDR isolates. Most frequently encountered resistance phenotype (n=34) demonstrated resistance to Ampicillin, Amoxicillin/ Clavulanic acid, Ampicillin/ Sulbactam, Cephalosporins, Gentamicin, Cotrimoxazole, Ciprofloxacin and Tetracycline. Table 3 shows the predominant resistance phenotypes of the MDR isolates.

Table 3: Predominant antimicrobial resistance phenotypes of MDR *E coli* isolates

Resistance phenotype	No. of isolates
AMP, A/S, AMC, CEP, CXM, CAZ, CPZ, CTR, CTX, CPM, GEN, NET, AK, COT, CIP, PIT, TE	15
AMP, A/S, AMC, CEP, CXM, CAZ, CPZ, CTR, CTX, CPM, GEN, NET, AK, COT, CIP, TE	21
AMP, A/S, AMC, CEP, CXM, CAZ, CPZ, CTR, CTX, CPM, GEN, NET, COT, CIP, TE	9
AMP, A/S, AMC, CEP, CXM, CAZ, CPZ, CTR, CTX, CPM, COT, NIT, CIP, TE	11
AMP, A/S, AMC, CEP, CXM, CAZ, CPZ, CTR, CTX, CPM, COT, CIP, PIT, TE	19
AMP, A/S, AMC, CEP, CXM, CAZ, CPZ, CTR, CTX, CPM, GEN, COT, CIP, TE	34
AMP, A/S, AMC, CEP, CXM, CAZ, CPZ, CTR, CTX, CPM, GEN, COT, CIP	9
AMP, A/S, AMC, CEP, CXM, CAZ, CPZ, CTR, CTX, CPM, COT, CIP, TE	31
AMP, A/S, AMC, CEP, CXM, CAZ, CPZ, CTR, CTX, CPM, COT, CIP	16
AMP, A/S, AMC, CEP, CXM, CAZ, CPZ, CTR, CTX, CPM, COT, TE	7
AMP, A/S, AMC, CEP, CXM, CAZ, CPZ, CTR, CTX, CPM, CIP, TE	16
AMP, A/S, AMC, CEP, CXM, CAZ, CPZ, CTR, CTX, CPM, CIP	10

Key - AMP: Ampicillin, A/S: Ampicillin/Sulbactam, AMC: Amoxycillin/Clavulanic acid, CEP: Cephalexin, CXM: Cefuroxime, CAZ: Ceftazidime, CPZ: Cefoperazone, CTR: Ceftriaxone, CTX: Cephotaxime, CPM: Cefepime, GEN: Gentamicin, NET: Netilmicin, AK: Amikacin, COT: Cotrimoxazole, CIP: Ciprofloxacin, PIT: Piperacillin/Tazobactam, TE: Tetracycline, NIT: Nitrofurantoin.

Multiple Antibiotic Resistance (MAR) index was calculated for the isolates. Majority of the isolates (n=91) showed an index of 0.7 followed by 0.6 (n=63) and 0.8 (n=60). MAR index was greater than 0.2 for 82.7% of the isolates as shown in Fig. 2.

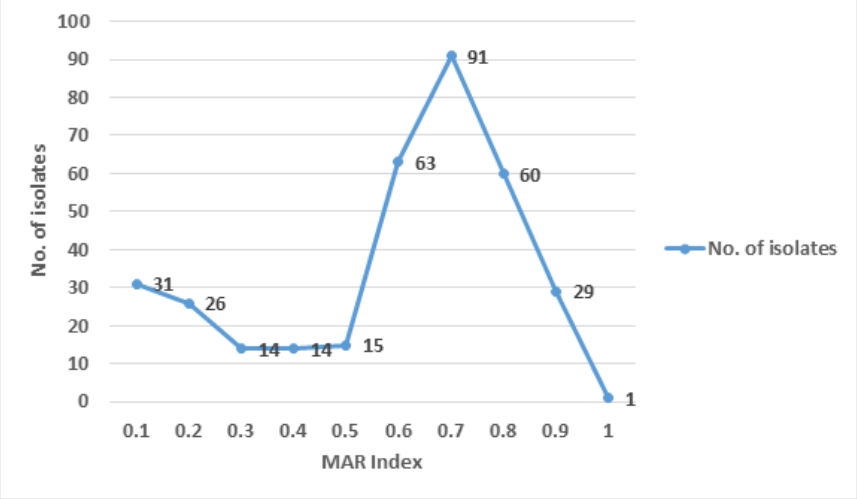

Fig. 2: Multiple Antibiotic Resistance (MAR) index of the isolates

Discussion

Antimicrobial susceptibility of bacteria is a constantly evolving phenomenon and varies with time and geographical area.[6] Periodic surveillance of the antimicrobial profile of pathogens is necessary for appropriate treatment of infections and for initiation of empiric therapy. *E. coli* is usually the most commonly isolated Gram negative pathogen.[14] It has been suggested that the extent of antibiotic resistance in *E. coli* can be used as an effective marker to gauge the drug resistance in the community.[2] The increasing incidence of multi-drug resistant bacteria is an important public health concern as they are susceptible to a very few and sometimes none of the antimicrobial agents available, prompting inadequate antimicrobial therapy and leading to poor patient outcomes.[12,15] The present study was undertaken to assess the extent of multi-drug resistant *E. coli* in patients attending our hospital and to elaborate their antimicrobial susceptibility pattern.

E. coli was the most frequent pathogen identified from various samples and a major fraction constituted uropathogenic *E. coli*. The incidence of multi-drug resistant strains defined as, isolates of *E. coli* resistant to at least one drug in three or more categories of antimicrobial agents was found to be alarmingly high accounting for 85.84% (n = 297) of the isolates. Moreover, majority of the multi-drug resistant isolates (n = 182, 61.3%) showed resistance to more than 5 groups of antimicrobial agents. Multi-drug resistant organisms are responsible for inadequate treatment, leading to increase in the duration and magnitude of morbidity, high mortality rates and an increased financial burden.[15] Table 4 shows the incidence of multi-drug resistant *E. coli* strains in various studies from India and abroad.

Table 4: Distribution of MDR *E coli* isolates from different studies

Author	Year	Region	Sample	Sample size	MDR (%)
Studies in India					
Shakya *et al*[4]	2013	Ujjain	Stool (commensal)	529	33
Mukherjee *et al*[8]	2013	Kolkata	Urine	40	92.5
Chaudhary *et al*[16]	2014	Mumbai	Samples from ICU	45	73.3
Niranjan *et al*[17]	2014	Puducherry	Urine	119	76.5
Ravishanker *et al*[18]	2015	Puducherry	Urine	72	83.3
Bijapur *et al*[19]	2015	Anjarakandy	Urine (Nosocomial)	96	84.37
Ranjini *et al*[7]	2015	Bangalore	Urine	179	82.6
Present study	2016	Tumkur	All samples	346	85.84
Other developing countries					

Ibrahim *et al*[15]	2012	Sudan	All samples	232	92.2
Yadav *et al*[20]	2015	Nepal	Urine	67	95.52
Dehbanipour *et al*[21]	2016	Iran	Urine	135	63
Developed countries					
Donk *et al*[22]	2012	UK	Urine	421	17.6
Alhashash *et al*[23]	2013	Nottingham, UK	Blood Urine	100 125	50.7 32
Khawcharoenporn *et al*[24]	2013	Chicago, USA	Urine	323	20.12

A dauntingly high incidence of multi-drug resistant isolates is demonstrated in developing countries in comparison to the developed countries. This has been ascribed to the lack of appropriate antibiotic policy leading to promiscuous use of antimicrobial agents especially in developing countries.[5]

Among the β-lactam antibiotics, the multi-drug resistant isolates showed 100% resistance to Ampicillin, conforming to the findings of Hoora S. *et al*[6] and Shafiyabi S. *et al.*[25] A greater than 80% resistance was also demonstrated against Cephalosporins, Amoxicillin/Clavulanic acid and Ampicillin/Sulbactam. A study conducted by Shafiyabi S. *et al*[25] showed similar results with 91.7% resistance to Ceftazidime, 95.8% to Ceftriaxone and 96.7% resistance to Cefotaxime. Our findings demonstrate the limited role of these antimicrobial agents in the treatment of infections with multi-drug resistant isolates.

The MDR isolates showed high level of sensitivity to Piperacillin-Tazobactam. Similar results were documented by Niranjan V. *et al*,[17] Shafiyabi S. *et al*[25] and Kumar Y. *et al*.[26] The isolates were highly sensitive to Carbapenems. Similar findings have been reported by various studies from different parts of India.[6,7,17,25]

Ciprofloxacin is one of the most commonly used fluoroquinolone for the empiric treatment of urinary tract infections.[18,27] The MDR isolates in our study showed high levels of resistance against Ciprofloxacin. Many studies across India have reported similar findings.[6,8,25,27] An association has been established between increased use of quinolones and development of quinolone resistance.[27] These findings stress the need for antibiotic sensitivity testing before initiation of antibiotic treatment.

Cotrimoxazole is another antibiotic used in the empirical treatment of uncomplicated cystitis.[7] Resistance to Cotrimoxazole among our isolates was high, similar to Shafiyabi S. *et al*[25] and Mukherjee M. *et al*[8] who recorded 66.7% and 82.5% resistance respectively.

The urinary MDR *E. coli* isolates however showed high level of sensitivity to Nitrofurantoin. Nitrofurantoin has multiple mechanisms of action which have enabled it to retain its potency against *E. coli* and hence can be recommended as an effective agent for empirical therapy of urinary tract infection caused by *E. coli*.[20]

The aminoglycoside, Amikacin was found to be highly effective against our isolates. Being an injectable agent with high risk of neprotoxicity and ototoxicity, the restricted use of this drug leading to lower selective pressure has contributed to lower resistance level.[6,15]

Chloramphenicol was found to be highly effective against our isolates. Though a highly potent antimicrobial agent, it has fallen to disuse due to several toxic effects including aplastic anaemia.[9] Emergence of multi-drug resistant organisms has triggered interest in the use of older drugs like Chloramphenicol in the treatment of the infections caused by these organisms.[9]

Antimicrobial resistance phenotypes of the isolates were assessed. Of the 69 phenotypes recorded, majority showed resistance to Ampicillin with or without inhibitor combination and Cephalosporins. The most common antibiotics showing co-resistance with these agents were Gentamicin, Cotrimoxazole and Ciprofloxacin limiting their utility in the treatment of MDR *E. coli*.

A Multiple Antibiotic Resistance (MAR) index above 0.2 implies that a very large fraction of bacterial isolates have been exposed to several antibiotics.[6] Majority of the isolates in the present study exhibited a high MAR index suggesting that majority of our isolates have been exposed to high antibiotic pressure.

Conclusion

Antimicrobial profiles of bacteria are ever-changing. It has been suggested that there is a direct relationship between antibiotic use in the community and emergence of antibiotic resistant isolates.[28] Periodic evaluation of antibiograms of isolates from various samples is essential to determine the incidence of multiple drug resistant strains and to guide empirical therapy. The present study highlights the menace of antimicrobial resistance in developing countries like India. Therapeutic options available for the treatment of these infections are limited. A small percentage of isolates showed Carbapenem resistance in our study. Among the β-lactam agents, Carbapenems exhibit the broadest spectrum of activity and should be used as antibiotics of last resort.[29] Aminoglycosides and Chloramphenicol were also found to be highly effective, though risk of toxicity limits their use. Nitrofurantoin can be used as an effective drug for the empirical therapy of urinary tract infections.

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