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PREVALENCE AND ANTIBIOTIC SUSCEPTIBILITY PATTERN OF *E. COLI* ISOLATES FROM CLINICAL SAMPLES OF GULBARGA CITY, INDIA

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ABSTRACT

The misuse and overuse of antibiotics has led to the selection of new strains of bacteria that resist to antibiotics, a situation which is found in the case of *Escherichia coli* strains. There are many types of *E. coli* and most of them are harmless. But some stereotypes can cause food poisoning, bloody diarrhoea and gastrointestinal infections. *E. coli* is the most common cause of urinary tract infections (UTIs) in humans and leading cause of enteric infections. Some of the strain can also cause kidney failure, which can lead to death. In this study we have evaluated the ability of *E. coli* strains to resist antibiotics isolated from infections of the gastrointestinal system and diarrhoea. The objective of this study was to determine the sensitivity of *E. coli* to antimicrobial drugs. A total of 285 strains of *E. coli* were evaluated for their antibiotic resistant pattern against eight selected antibiotics. The antibiotic sensitivity test was performed using the disc diffusion method prepared according to the standards of the Clinical and Laboratory Standard Institute (CLSI). The results showed over 53.33% of the strains were resistant to ampicillin and 69.47% were resistant to Ciprofloxacin and most of the strains were sensitive to Chloromphenicol (92.98%), Amikacin (76.49%) and Nalidixic acid (70.53%).

KEYWORDS: ***Escherichia coli*, Urinary Tract infections (UTI), Multi Drug Resistance (MDR), Minimal Inhibitory Concentration (MIC), Antibiotics.**

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INTRODUCTION

Escherichia coli is a rod-shaped, Gram-negative, facultative anaerobic bacterium that is commonly found in the intestine of warm-blooded animals. In humans, they are the major aerobic organism residing in the intestine, typically 10^6 to 10^9 colony forming units per gram of stool[1]. There are many types of *E. coli* and most of them are harmless. But some stereotypes can cause food poisoning, bloody diarrhea and infections of the gastrointestinal system[2]. *E. coli* is the most common cause of urinary tract infections (UTIs) in humans and leading cause of enteric infections and systematic infections[3]. The systemic infections include bacteremia, nosocomial pneumonia, cholecystistis, cholangitis, peritonitis,osteomyelitis and infectious arthritis[4,5]. Infections of *E. coli* can also cause kidney failure, which can lead to death. Every year 130–175 million patients suffer uncomplicated UTI worldwide and more than 80% them are due to *E. Coli*[6]. UTI due to multi drug resistant (MDR) *E. coli* increases the cost of treatment, morbidity and mortality especially in developing countries like India[7,8]. The types of *E. coli* that can cause infections can be transmitted through contaminated water or food, or through contact with animals or people. Antibiotic resistance in *E. coli* has been reported worldwide and increasing rates of resistance among *E. coli* is a growing concern in both developed and developing countries[9]. The aim of this study was to determine antibiotic susceptibility of *E. coli* from clinical samples of selected antimicrobial drugs by the disc diffusion method.

MATERIALS AND METHODS

Bacterial isolation

In our study we have isolated 285 strains of *E. coli* from different clinical samples collected at various hospitals and diagnostic centers of Gulbarga viz. Government Hospital, Basaveshwar Hospital, Mediscan Diagnostic and Pooja Diagnostic. A total of 310 specimens (stool and urine) have been collected. The stool samples were homogenized and inoculated into tryptic soya broth (TSB) and incubated at 37°C for 24 hours[10]. The tubes showing turbidity and gas in Durham tubes were selected. The cultures from these tubes were inoculated into Luria broth (LB) and were incubated for 24 hours at 37° C. The LB tubes showing turbidity were streaked on plates of eosin methylene blue (EMB) agar and MacConkey agar and kept for incubation at aerobic atmosphere at 37°C for 24 hours. Based on the characteristic colony morphology and staining characteristic of *E. coli* on selective and differential media were isolated as pure culture.

Identification

The serotypes were identified according to the standard operational procedures as per the standard microbiological methods by Farmer et al.,[1]. The slides have been prepared and microscopic observations were done followed by Gram's staining. Motility test was performed by using cavity slide by Hanging drop method[12]. For the confirmation Nitrate Reduction, Catalase, Oxidase and IMViC tests[11] were performed.

Antibiotic susceptibility test

The antibiotic susceptibilities were tested to detect resistance to ampicillin (10 µg),tetracycline (30 µg), gentamicin (30 µg), amikacin (30 µg), ciprofloxacin (5 µg), amoxycillin (10 µg), chloramphenicol (30 µg) and Nalidixic acid (30 µg) by the using Kirby Bauer disk diffusion method[13] on Mueller-Hinton agar (Himedia Pvt Ltd, Mumbai,India) according to the Clinical and Laboratory Standards Institute (CLSI) guidelines[14,15]. The zone of inhibition was measured using standard antibiogram scale and results were interpreted.

Minimum Inhibitory Concentration:

MIC was determined by Broth dilution method following CLSI standard guidelines [14,15]for two selected antibiotics for which maximum number of strains were resistant: Ciprofloxacin and Ampicillin (commercially available as ciprofloxacin hydrochloride monohydrate). Stock solutions of Ciprofloxacin (2 mg/ml) and Ampicillin (1mg/ml) were prepared with reference to Andrews [16]. Significant MIC breaking point to Ciprofloxacin and Ampicillin were interpreted.

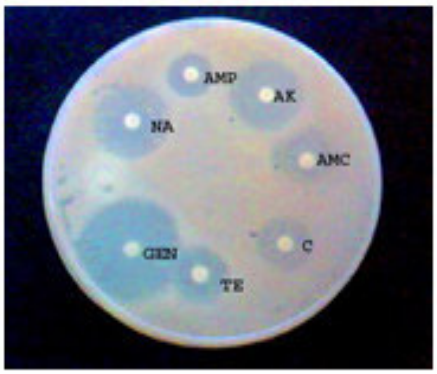
RESULTS AND DISCUSSION

In our research a total of 285 *E. coli* strains were isolated from Stool (87) and Urine (198) specimens collected from various hospitals and diagnostic centres of Gulbarga City.

Table 1
Microscopic and biochemical tests

Sl no	Tests	Results
1	Gram's staining	Negative
2	Motility test	Positive
3	Lactose fermentation	Positive
4	Oxidase test.	Negative
5	Catalase test	Positive
6	Nitrate Reduction	Positive
7	Indole Test	Positive
8	Methyl Red Test	Positive
9	Voges Proskaur Test	Negative
10	Citrate test	Negative

Microscopic characters of the isolates were Gram-negative, rod shaped and motile. *E. coli* produced pink colour colonies on MacConkey medium indicating positive test for lactose fermentation. On EMB agar medium *E. coli* colonies produced green metallic sheen. The isolates were confirmed up to special level by biochemical tests, showed positive results for Methyl Red, Catalase, Nitrate Reduction and Indole production and where as negative results for oxidase , Voges Proskaur and Citrate utilization (Table 1).



Interpretation by referring standard Zone Size Interpretative chart supplied by Himedia catlog. (GEN 26mm-S, NA 20mm-S, AMP 12mm-R, AK 19mm-S, AMC 15mm-R, C 14mm-I, TE 11.5mm-I)

Figure 1
Antibiotic Susceptibility test on Mueller-Hinton agar

The zone of inhibition produced by the each tested antibiotics against each isolates of *E.coli* were recorded and were grouped into Sensitive, Resistant and Intermediate based on their size of zone of inhibition compared with standard zone size interpretative chart supplied by Himedia Pvt.Ltd Mumbai. Percentage of *E.coli* isolates fall in Sensitive, Resistant and Intermediate groups is presented in Table.2.

Table 2
Antibiotics sensitivity pattern of E. coli isolates (285).

Sl.No	Antibiotics	Concentration in µg	Resistant % (n)	Sensitive % (n)	Intermediate % (n)
1	Ampicillin (AMP)	10	53.33(152)	40.35 (115)	06.32 (18)
2	Tetracycline (TE)	30	29.12 (83)	48.07(137)	22.81 (65)
3	Gentamicin (GEN)	30	39.30 (112)	58.95 (168)	01.75 (5)
4	Amikacin (AK)	30	22.81 (65)	76.49 (218)	0.70 (2)
5	Chloramphenicol (C)	05	4.56 (13)	92.98 (265)	2.48 (7)
6	Amoxycillin (AMC)	10	45.61 (130)	54.39 (155)	-
7	Ciprofloxacin (CIP)	05	69.47 (198)	30.18 (86)	0.35 (1)
8	Nalidixic acid (NA)	30	27.37 (78)	70.53 (201)	2.11 (6)

***n-number of strains.**

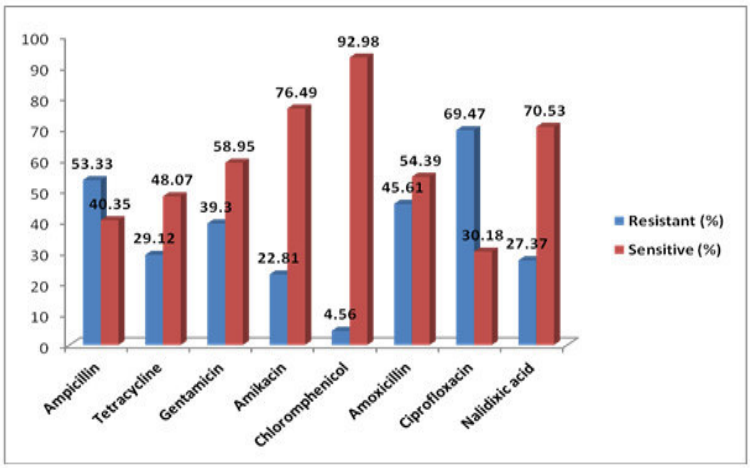


Figure 2
Prevalence of resistance to antibiotics among E. coli isolated from clinical samples.

As for the sensitivity to antibiotics, most of the isolates were sensitive to Chloramphenicol (92.98%), Amikacin (76.49%) and Nalidixic acid (70.53%). Over 53.33% of the strains were resistant to ampicillin and 69.47% were resistant to Ciprofloxacin (Table 2). High percentage of resistance to beta-lactams among enteric bacteria isolated from clinical and environmental origin had reported worldwide[17,18,19]. In India resistance to beta-lactams (ampicillin) in *E. coli* (83.3%) was also reported by Alam et al.[20] Resistance in *E. coli* to beta-lactams is due to chromosomally mediated genes[21]; still the beta-lactam group of antibiotics are the most common drugs used for the treatment of Gram-negative bacteria and they account for use around 50% for total antibiotic consumptions[22]. In India resistance to antibiotics which are usually recommended for empirical treatment for UTI with *E.coli*, such as ampicillin, cephalexin, cefpodoxime, norfloxacin, amikacin, nitrofurantoin, trimethoprim and imipenem had been evaluated[23]. In our research 53.33 % isolated strains were resistant to ampicillin and 22.81% to amikacin.

Table 3
Detection of MIC values with Ciprofloxacin and Ampicillin

Sl No.	Antibiotics	MIC Clinical Breaking point (µg/ml)	MIC level (in µg/ml)	No. of resistant isolates
01	Ciprofloxacin	1–4	8-12	22
			64-128	81
			128-256	78
			256-512	17
02.	Ampicillin	16–32	8-12	15
			64-128	60
			128-256	65
			256-512	12

Total 198 Ciprofloxacin resistant strains had been selected to evaluate MIC of Ciprofloxacin among them 17 strains have shown increased MIC in the range of 256-512 µg/ml, 78 strains have shown increased MIC in the range of 128-256µg/ml, 81 strains shown MIC of 64-128 µg/ml and remaining 22 strains have shown comparatively low MIC of 8-16 µg/ml and these results indicates the drastic increase in MIC of ciprofloxacin. Similarly 152 Ampicillin resistant strains have been selected to determine MIC of Ampicillin among them 12 strains have shown increased MIC in the range of 256–512 µg/ml, 65 strains have shown MIC of 128-256µg/ml, 60 strain have shown MIC in the range of 64-128 µg/ml and remaining 15 strain have shown MIC of 8-16 µg/ml (Table 3). We got relatively similar results in comparison with the previous study conducted by Alam *et al.*[20] and Shakti *et al.*[24]. Our results are compared with the analysis made by Vellinga et al.[21] for ciprofloxacin prescribing and resistance of uropathogenic *Escherichia coli* in general practice which revealed that in “mean” practices with one prescription per month, ciprofloxacin resistance was low (3%), whereas in practices with 10 prescriptions per month, ciprofloxacin resistance amounted to 5.5%[25].

CONCLUSION

A total of 285 strains of *E. coli* were isolated from hospitalized patients, out of which 198 strains were resistant to fluoroquinolone antibiotic and 152 strains were resistant to beta-lactam antibiotic i.e. Ciprofloxacin and Ampicillin respectively. Overall study gives systematic information on prevalence and antibiogram pattern for 8 commonly used antibiotics against MDR *E. coli* strains, isolated from different clinical samples. This study is anticipated to provide information for designing a specific antibiotics policy for combating multi drug resistance in *E. coli* strains. The MIC values of ciprofloxacin and ampicillin used in vitro could help the current treatment options. Patients with other bacterial infections had relatively higher chances of becoming infected with fluoroquinolone resistant *E. coli* strains.

CONFLICT OF INTEREST

Conflict of interest declared none.

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