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ANTIMICROBIAL RESISTANCE ANALYSIS AMONG *ESCHERICHIA COLI* ISOLATED FROM EXTRAINTESTINAL SITES: A CROSS-SECTIONAL STUDY

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ABSTRACT: Background: *Escherichia coli (E. coli)* is one of the most common agents of bacterial infections, the emergence of multidrug resistance in *E. coli* has posed major threat to global health resulting in failure of treatment that leads to increased mortality and cost of healthcare facilities. **Material and Methods:** For this study, a total of 500 specimens like urine, blood, pus, stool, sputum and different body fluids received in the Clinical Microbiology Laboratory were processed and inoculated on culture plates (Blood agar, MacConkey agar, Cysteine Lactose Electrolyte Deficient Agar) and incubated at 37°C for 18-24 hours. After identification, *E. coli* was processed for Antibiotic susceptibility testing on Muller Hinton Agar using Kirby Baeur disk diffusion method. *E. coli* ATCC 25922 was used as reference strain. **Results:** Of 500 specimens received in clinical laboratory from different age groups, 180 were culture positive, of which 88 were *E. coli* isolates. 48(54.5%) *E. coli* isolates were obtained from female patients while 40(45.5%) were from male patients. Isolation of *E. coli* was highest in Urine (80.68%) followed by Pus and other samples. *E. coli* were 100% resistant to Ampicillin followed by ceftriaxone (85.2%) and ciprofloxacin (63.6%) whereas least resistance was observed in imipenem (21.6%), piperacillin-tazobactam (29.5%). **Discussion:** *E. coli* isolates exhibited high resistance to ampicillin &ceftriaxone. Consequently, an obligate need exists for antimicrobial resistance surveillance to provide clinically appropriate and cost effective therapy.

INTRODUCTION: *Escherichia coli* a Gram-negative, rod-shaped bacilli typically colonizes the gastrointestinal tract of human infants within a few hours after birth. *Escherichia coli* is one of the principal pathogens causing urinary tract infections (UTI) in more than 80 percent of cases. It is also amongst the most common pathogens causing bloodstream infections, otitis media, wound infections, neonatal meningitis and nosocomial pneumonia [1-5]. *E. coli* is also considered as a leading cause of waterborne and food borne human diarrhoea mainly in developing countries peculiarly among children under five years of age [6].

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Extraintestinal infections caused by *E. coli* depend on virulence factors that help *E. coli* survive under hostile conditions at those sites. Antimicrobial resistance among *E. coli* is a major global public health issue of great concern due to lack of availability or affordability of second-line therapies [7, 8]. Commensal *E. coli* acts as storage of resistance genes in the human intestine which get transferred to other commensal or pathogenic organisms [9, 10].

Extended-spectrum beta-lactamases (ESBLs) are responsible for resistance to β-lactam antibiotic, which are plasmid-mediated and easily transmitted among Enterobacteriaceae members [11, 12]. Carbapenem drugs have been introduced in clinical settings to overcome resistance to ESBLs. However, resistance to carbapenem among the members of the Enterobacteriaceae family has been increasingly reported around the world due to the production of carbapenem hydrolyzing enzymes

called carbapenemases, which are encoded by KPC, VIM and IMP genes [13]. Antimicrobial susceptibility patterns of *E. coli* exhibit significant topographical variation among diverse populations. This study aims to isolate and identify E. coli from various clinical samples and determine its antibiotic susceptibility profile.

MATERIAL AND METHODS: The present prospective study was carried out in the Microbiology Laboratory of Guru Gobind Singh Medical College, Faridkot, from January 2021 to June 2021 post ethical committee permission vide letter no UIPMS/2022/4042-43. A total of samples like urine, blood, pus, sputum and different body fluids collected using the standard aseptic techniques received in the Clinical Microbiology Laboratory were included in the study. The received samples were processed and inoculated on culture plates (Blood agar, MacConkey agar and Cysteine Lactose Electrolyte Deficient Agar for urine) and incubated at 37°C for 18-24 hours. All media were prepared according to manufacturer's specifications and sterilized at 121°C for 15 minutes at 15Ib pressure. Colonies obtained after culture were identified on the basis of their morphology on various differential culture media and gram staining. Further identification was done using standard microbiological techniques with the help of biochemical test.

Inclusion Criteria: All the isolates of *Escherichia coli* isolated from various clinical samples were included in the study.

Exclusion Criteria: All other bacteria except *Escherichia coli* were excluded from the study. From urine, *E. coli* isolates that showed significant colony count were included in the study.

After identification, *E. coli* isolates were further processed for Antibiotic susceptibility testing on Muller Hinton Agar using KirbyBaeur disk diffusion method. The antimicrobial agents tested were:

Ampicillin (10µg), Co-trimoxazole (25µg), Genta-micin (30µg), Amikacin (30µg), Cipro-floxacin (5µg), Cefotaxime (30µg), Ceftriaxone (30µg), Imipenem (10µg), Piperacillin- tazobactam (100/10µg), Colistin (10µg). Norfloxacin (10µg) and Nitrofurantoin (300µg) for urine isolates.

The diameters of zone of inhibition of antibiotics were then measured with the help of Vernier caliper and interpreted as per CLSI criteria. Isolates with intermediate susceptibility were considered resistant and these isolates are nonsusceptible. *E. coli* ATCC 25922 was used as reference strain.

RESULTS: Of the 500 clinical samples, 182 (36.4%) had significant bacterial growth and 318 (63.6%) were sterile. Of 182 positive bacterial culture, 88 (48.35%) showed growth of *E. coli*. Majority of the *E. coli* was isolated from urine 71 (80.68%) followed by pus 14 (15.9%), blood 2(2.27%) and ascitic fluid 1(1.13%).

TABLE 1: DISTRIBUTION OF *ESCHERICHIA COLI* AMONG IN VARIOUS CLINICAL SAMPLES (N=88)

Sr. no.	Types of Clinical specimen	No. of culture positive clinical specimen	no. of *E. coli* isolated (n=88)
1	Urine	108	71(80.68%)
2	Pus	42	14(15.91%)
3	Blood	20	2(2.27%)
4	Ascitic Fluid	6	1(1.14%)
5	Pleural Fluid	0	0(0%)
6	Sputum	6	0(0%)
7	CSF	0	0(0%)

TABLE 2: DISTRIBUTION OF 88 *ESCHERICHIA COLIISOLATES* AMONG PATIENTS IN DIFFERENT AGE GROUPS AND THEIR RELATION TO GENDER

Age Group in years	Gender		Total	
	Female (n=48)	Male (n=40)	Frequency	%age
0-15	9	5	14	15.9
16-30	17	7	24	27.37
31-45	16	11	27	30.6
46-60	6	9	15	17.04
>60	6	2	8	9.09

More female patients (54.54%) suffered *E. coli* infections than male counterparts (45.45%) in age group of 16-45 years

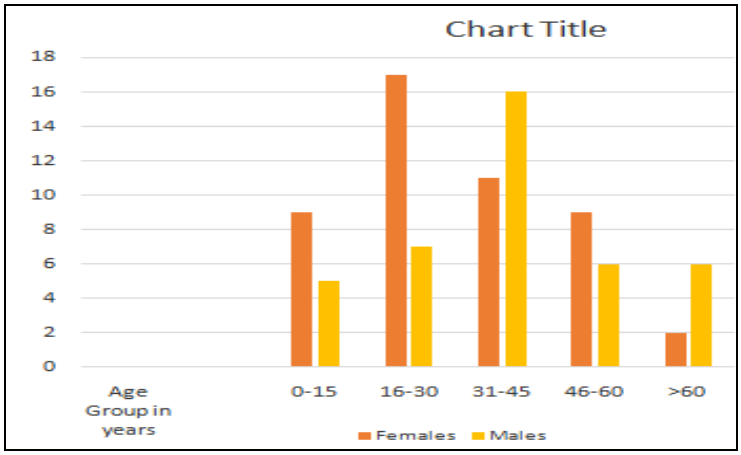

FIG. 1: DISTRIBUTION OF *ESCHERICHIA COLI* ISOLATES AMONG MALES AND FEMALES OF DIFFERENT AGE GROUP

TABLE 3: ANTIBIOTIC SUSCEPTIBILITY PROFILE OF *ESCHRECHIA COLI*AMONG VARIOUS CLINICAL SAMPLES (N=88)

Antimicrobials	Resistant (%)	Susceptible (%)
Ampicillin	88(100%)	0(100%)
Cefotaxime	76(86.36%)	12(17%)
Co-trimoxazole	57(64.7%)	31(35.3%)
Ciprofloxacin	56(63.6%)	32(36.4%)
Gentamycin	49(55.7%)	39(44.3%)
Amikacin	27(30.7%)	61(69.3)
Piperacillin-tazobactam	26(29.5%)	62(70.5)
Imipenem	19(21.6)	69(78.4%)
Colistin	0(0%)	88(100%)
Nitrofurantoin	21(29.6%)	50(70.4%)
Norfloxacin	68(81.7%)	13(18.3%)

E. coli isolates showed 100% susceptibility to colistin followed by imipenem (78.4%) and piperacillin-tazobactam (70.5%). 100% resistance to ampicillin was shown by *E. coli*, followed by cefotaxime (86.3%) and cotrimoxazole (63.4%). nitrofurantoin and norfloxacin tested in urine samplesshowed resistance of 29.6 and 81.7% respectively.

TABLE 4: COMPARISON OF ANTIBIOTIC SUSCEPTIBILITY PROFILE AMONG *E. COLI* ISOLATES OF URINE AND PUS SAMPLES

	Urine samples		Pus samples	
Antimicrobials	Resistant (%)	Susceptible (%)	Resistant (%)	Susceptible (%)
Ampicillin	71(100%)	0(0%)	14(100%)	0(0%)
Cefotaxime	59(83%)	12(17%)	14(100%)	0(0%)
Co trimoxazole	45(63.4%)	26(36.6%)	9(64.28%)	5(35.71%)
Ciprofloxacin	44(62%)	27(38%)	9(64.28%)	5(35.71%)
Gentamycin	39(55%)	32(45%)	8(57.14%)	6(42.85%)
Amikacin	19(26.8%)	52(73.2)	7(50%)	7(50%)
Piperacillin-tazobactam	19(26.8%)	52(73.2)	6(42.85%)	8(57.14%)
Imepenem	15(21.2%)	56(78.8%)	3(21.42%)	11(78.57%)
Colistin	0(0%)	71(100%)	0(0%)	14(100%)
Nitrofurantoin	21(29.6%)	50(70.4%)	ND	ND
Norfloxacin	58(81.7%)	13(18.3%)	ND	ND

E. coli isolated from urine and pus samples showed comparable susceptibility to colistin (100%) and imipenem (78%). Cefotaxime showed 100% resistance in pus samples. Cotrimoxazole, ciprofloxacin and gentamicin showed marginally more resistance in pus samples. *E. coli* in pus samples showed more resistance towards Amikacin and PCTZs which was not significant (p value > 0.05).

DISCUSSION: *E. coli*, a commensal of the intestinal flora is also involved in diseases such as septicemia, urinary tract infections or purulent infections. Apart from being isolated from the clinical samples, these isolates are also found in food, water and soil and thus can serve as reservoir for the spread of resistant determinants to man. *E. coli* is among one of the antibiotic-resistant organisms of serious clinical concern being responsible for failure in the treatment of infectious diseases, resulting in increased morbidity, mortality, and cost of healthcare services. In the present study, a total of 48.35% culture positive samples showed growth of *E. coli* similar to study done by Nepal K *et al* & V Bala G which showed 51.5% & 44.12% growth of *E. coli* among culture positive samples respectively [14-15].

The majority of *E. coli* were isolated from urine samples 71(80.68%) followed by pus 14(15.9%) in proportion with the maximum samples of urine followed by pus in our study. Our findings correspond with the study done by Nepal K *et al., which shows a similar finding of 84% prevalence of E. coli in urine but showed a* lesser prevalence of 8% in pus samples [14]. However, Kirbit *et al* showed a 45.5% prevalence of *E. coli* in urine samples and 18.7% among pus samples[16]. Maximum *E. coli* in our study were isolated in urine samples of female patients in the age group of 16-45 years of age. A study by Nadiq 1A & Rehman SU showed maximum prevalence of E. coli in urine samples of female patients and similar age groups, respectively [6, 17].

This could be due to the short urethra, which shortens the distance to be moved by bacteria to the bladder, and sexual activity increases the inoculation of bacteria into the bladder. Alteration in vaginal flora also encourages the colonization of the vagina with coliforms leading to UTI. In our study, the overall resistance of *E. coli* toward antimicrobial was high except colistin which showed 100% susceptibility. Various authors showed different susceptibility patterns in their study. Nadiq IA & Najmi *et al.* showed a susceptibility of 11.7% & 9.8% to ampicillin, respectively, in contrast to 100% resistance in our study [6, 18]. Susceptibility to cefotaxime was comparable to a study conducted by Najmi and Nepal [18, 14].

Resistance to cotrimoxazole was shown to be 45.7% & 51.1% by Nepal & Najmi respectively compared to 35.5% in our study which was comparable to study done by Kirbit M. Susceptibility to amikacin (92.8% & 93.9%), piperacillin-tazobactam (93.5% & 77%) & imepenem (96% & 93.9%) was high in study done by Nepal & Najmi. *E. coli* isolated from urine and pus samples also showed a difference in antibiotic susceptibility patterns, with more resistance towards antibiotics found among pus samples except for colistin, imipenem & cotrimoxazole among which susceptibility patterns were comparable. Similar findings were seen by Nadiq *et al* in their study. However, Kirbit et al. found that overall resistance patterns among urine and pus samples were comparable.

In our study, high resistance was observed to most antimicrobial agents except amikacin, piperacillin-tazobactam and imipenem to which *E. coli* showed moderate susceptibility. Colistin remains last resort of drug for treating severe infections for carbapenem-resistant *E. coli*.

CONCLUSION: The emergence of antimicrobial resistance to fluoroquinolones, cotrimoxazole, ampicillin and cefotaxime and the rise of ESβL producing organisms limit the use of these drugs as first-line treatment in *E. coli* infection. With regular surveillance regarding the susceptibility pattern of clinical isolates of the organism to different antibiotics, guidelines can be formulated to use antibiotics judiciously.

Ethical Approval: The study was performed after taking approval from the institution's Ethical committee.

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CONFLICTS OF INTEREST: None

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