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Characterization and Antibiogram Study of *E.coli* Clinical Isolates

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Hospital environment is most suitable reservoir for nosocomial pathogens. Indiscriminate use of antibiotics has led to the global drug resistance in these microbes leading to several health hazards. *E.coli* is one such bacterial pathogen. It is therefore important to isolate and identify it from various clinical samples and study its antibiogram. In present study a total of one hundred samples of blood, urine, pus and stool collected from various pathology laboratories were used for isolation of *E.coli*. Twenty eight samples were reported positive for the occurrence of *E.coli* in them. The highest rate of incidence was found in stool samples whereas all the pus samples analyzed were negative. Antibiogram study of these *E.coli* clinical isolates showed resistance to ampicillin and cephalothin followed by amoxycillin and ciprofloxacin. Absolute sensitivity to chloramphenicol and tetracycline followed by norfloxacin, imipenem, streptomycin, moxifloxacin and ofloxacin was observed. All most all isolates showed multiple drug resistance.

Key words: Antibiogram, *E.coli*, Nosocomial infections.

Hospital borne infection are quite common and a significant cause of morbidity and mortality throughout the world. Amongst various pathogens responsible for nosocomial infection, *Escherichia coli* is the well known[1,2]. This uropathogen which is normal inhabitant of human colon and which was earlier considered to be sensitive to drugs of choice has now surprisingly acquired resistance to them. Drug resistance by microbes has now become a global problem. Jones in 1992 have reported such increasing drug resistance especially in nosocomial bacterial pathogens[3]. In India too several reports have highlighted such drug resistance in hospital pathogens[4,5]. Multiple drug resistance in *E.coli* is being reported frequently[1,2,6,7,8]. *E.coli* is common bacterial species found in hospital environment. Such environment may act as a reservoir of antibiotic resistant coliforms and is thus a warning signal and may be responsible for epidemic outbreaks[9]. The present study therefore was undertaken to find out the rate of incidence of *E.coli* in various pathological samples and its antibiogram pattern.

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MATERIAL AND METHODS

Dehydrated growth media and selective media, various analytical grade chemical ingredients and standard antibiotic discs were purchased from Hi-media Laboratory, Mumbai. Various clinical samples viz. blood, pus, urine and stool were collected from the local pathology laboratories. Asceptically, they were brought to the microbiology laboratory and immediately processed.

Isolation of *E.coli* from the clinical samples was done by inoculating a loopful of the test sample on Eosin Methylene Blue agar and incubating at 37^0C for 24-48 hrs. Black centered colonies showing blue green metallic sheen were suspected to be *E.coli*. Such colonies were picked up and transferred asceptically onto the fresh sterile nutrient agar slants. All isolates were maintained at 4 - 6^0C. The isolates were confirmed by their morphological, cultural and biochemical tests.

Antibiogram pattern of these isolates was studied according to the method of Bauer – Kirby (10). Inoculum for the study was prepared by transferring a loopful of 24 hrs nutrient agar slant culture of *E.coli* test clinical isolate into 5 ml sterile Trypticase Soy Broth and incubating at 37^0C for 5-10 hrs until light to moderate turbidity developed due to growth. The desired turbid culture comparable with Mcfarland 0.5 Standard Barium Chloride turbid solution was used for inoculation. One tenth millilitre of the test *E.coli* broth was spread thoroughly on the surface of sterile Mueller – Hinton (MH) agar asceptically with a sterile bent glass rod spreader. After 15-20 minutes, standard antibiotic discs of known concentration were placed onto the inoculated MH agar surface. All the plates were then incubated at 37^0C for 24-36 hrs. Zone of inhibition of confluently growing *E.coli* culture around the antibiotic disc to the nearest millimeter was measured in diameter for each antibiotic and compared with standard zone size interpretative chart to find out its sensitivity or resistance to the particular antibiotic. *E.coli* ATCC 25922 was used as a quality control organism.

RESULTS AND DISCUSSION

Incidence of *E.coli* in different clinical samples is shown in Table 1. Stool and urine samples showed highest incidence rate of this organism, while pus samples were found negative. All the clinical isolates of *E.coli* were found to be Gm –ve, coccobacilli exhibiting sluggish motility. Glucose, lactose, mannitol and sucrose was fermented with acid-gas production by all the isolates under test exhibiting their aerogenic character. Indole and MR test was positive. Acetyl-methyl carbinol from glucose was not formed and sodium citrate was not utilized. None of the isolates hydrolyzed urea. Table 2 depicts the antibiogram result of clinical *E.coli* isolates. This study showed that all the isolates under test were sensitive to chloramphenicol and tetracycline. One isolate was found to resist norfloxacin. Highest resistance was observed in amplicillin (83.71%) and cephalothin (78.57%) followed by amoxycillin (67.85%) and ciprofloxacin (57.14%). The result of antibiotic sensitivity is comparable with the work of Sahm *et al*.,[2] and Khan *et al*., [11]. Moreover the multiple antibiotic resistance was also observed in our study but the percent isolates exhibiting multiple drug resistance was found more as compared to the findings of Sahm *et al*.,[2].

Number of organisms becoming resistant to various antibiotics and the prevalence of resistant infection in community are increasing enormously[11-14]. Jones[3] and Neema *et al*.,[4,5] reported that rising multiple drug resistance is the worst problem in hospital practice. It is very important to know the drug sensitivity and drug resistance pattern of pathogen so as to choose and suggest appropriate antibiotic treatment to the patient in such a way that the therapy will not favour the development of antibiotic resistance in that pathogen.

Multiple drug resistance in *E.coli* isolates has been reported frequently (6,15,16,17). This multiple drug resistance in *E.coli* is posing problem in the treatment of urinary tract infection. Such uropathogenic strains are becoming very common (9,18,19,20, 21). Various clinical samples carrying

Table 1. Incidence of *Escherichia coli* in various clinical samples

Sample	Total Samples	Positive	Negative	% Incidence
Blood	25	01	24	04
Stool	25	19	06	76
Pus	25	00	25	00
Urine	25	08	17	32
Total	100	28	72	28

Table 2 . Antibiogram of *Escherichia coli* clinical isolates (n = 28)

Antibiotic (conc. / disc)	Resistant	Sensitive
Amoxycillin (10 mcg)	19	09
Ampicillin (10 mcg)	24	04
Cephalothin (30 mcg)	22	06
Ciprofloxacin (5 mcg)	16	12
Choloramphenicol (30 mcg)	ND	28
Imipenem (10 mcg)	03	25
Moxifloxacin (5 mcg)	08	20
Norfloxacin (10 mcg)	01	27
Ofloxacin (10 mcg)	10	18
Tetracycline (30 mcg)	ND	28
Streptomycin (10 mcg)	05	23
Gentamycin (10 mcg)	12	16

ND – Not detected

such strains may spread the infection and is a warning signal. In the present study the most effective antibiotic against *E.coli* clinical isolates was found to be chloramphenicol and tetracycline followed by norfloxacin, imipenem and streptomycin. It was also found that all most all *E.coli* clinical isolates exhibited multiple antibiotic resistance (Table 3). Five isolates showed resistance against only two antibiotics while one isolates was resistant to seven different antibiotics under test. Nine *E.coli* isolates registered resistance against five different antibiotics whereas five isolates were found resistant to six different antibiotics under study. Our study on MAR thus comply with the report that nosocomial pathogens are becoming more and more antibiotic resistant and needs strict attention from medical world[3,4,5,11-14].

Table 3. MAR pattern of *E.coli* clinical isolates (n = 28)

Isolates (no.)	5	4	4	9	5	1
Resistant to antibiotics (no.)	2	3	4	5	6	7

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