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Incidence and Antibigram Patterns of *Escherichia coli* Isolated from Various Clinical Samples from Patients at N.I.H. Islamabad

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Abstract: A total number of 355 samples including urine (179), pus (55), throat (43), stool (31), sputum (22), eye & ear (12) swabs, high vaginal swabs (10) and semen (3) were collected from patients to determine the presence or otherwise of *Escherichia coli* and to ascertain their antibiogram sensitivity pattern. The incidence of *E. coli* was highest in urine samples (22), followed by pathogenic *E. coli* (3) from stool samples and 2 isolates from wound and 1 from HVS, whereas the other samples did not yield any. In case of urine samples *E. coli* isolates were found to be highly susceptible to amikacin and norfloxacin while resistant to ampicillin. All *E. coli* isolates of stool samples were resistant to ampicillin, chloramphenicol and tetracycline whereas aztronam, cefotaxime and ofloxacin were 100% effective against *E. coli*. In case of pus samples, *E. coli* isolates were found to be susceptible to amikacin and tobramycin, but completely resistant to cefuroxime, ampicillin and cephradine. Incidence of *E. coli* was reported to be high in urine samples, with females more susceptible than males.

Key words: *Escherichia coli*, clinical samples, antibiotic sensitivity

Introduction

Escherichia coli is the most significant species in the genus *Escherichia* recognized as important potential pathogen in humans (Mahon and Manuselis, 1995). *E. coli* forms part of the normal microbial flora of the intestinal tract of humans and animals, yet can be found in water, soil and vegetation. It is not normally pathogenic, but may be referred to as an opportunistic pathogen, often associated with urinary tract infection (including cystitis, pyelitis and pyelonephritis), wound infection, appendicitis, peritonitis, infection of gall bladder, bacteremia, neonatal meningitis, diarrheal diseases and sepsis (Cheesebrough, 1991). *E. coli* is the main causative agent of uncomplicated urinary tract infection (UTI) and accounts for more than 85% of recurrent cystitis, and at least 35% of recurrent pyelonephritis (Barnett and Stephens, 1997). The reservoir for uropathogenic *E. coli* is faecal flora, from which bacteria spread to the urogenital mucosa, ascend into the bladder, and adhere to bladder epithelium. Once established in the bladder, the bacteria multiply and develop a local infection (cystitis) and may further ascend to involve the ureters and kidneys (pyelonephritis) (Langermann and Ballou, 2001). Urinary tract infection (UTI) is the most common bacterial infection in women, and it occurs with much greater frequency among elderly than among the younger women with increasing frequency among postmenopausal women (Raz, 2001).

Diarrheal *Escherichia coli* strains are divided into six categories including enteropathogenic, enterotoxigenic, enteroinvasive, enterohemorrhagic and more recently, diffused adherent and enteroaggregative *E. coli* (Mahon and Manuselis, 1995).

Enteropathogenic *E. coli* strain is known to cause infantile diarrhea and enterotoxigenic *E. coli* is associated with diarrhea of infants and adults in tropical and sub-tropical regions, especially in developing countries. Enteroinvasive strains produce dysentery, with direct penetration, invasion and destruction of intestinal mucosa. Enteroadherent *E. coli* and enteroaggregative *E. coli* causes diarrhea by adhering to and accumulating on the mucosal surface of the intestine (Mahon and Manuselis, 1995).

Most of the *E. coli* strains are normally sensitive to many of the antibiotics and chemotherapeutic agents, but in recent years resistance has been encountered in numerous cases (Hameed *et al.*, 1995). Resistance to cephaloridin, cephalosin and kanamycin has been reported, while a few strains of *E. coli* have been found to be susceptible to most of antibacterial drugs except ampicillin.

Therefore, it is advisable to perform antibiotic sensitivity test to minimize the hazards of potential drug resistance and to avoid economic losses on treatment (Hameed *et al.*, 1995).

With the growing concern over numerous environmental factors related to diarrheal outbreaks in Pakistan, being further complicated with the increase in antimicrobial resistivity, this study was designed to ascertain the incidence of *Escherichia coli* in various clinical samples and to determine their antibiogram patterns, so as to identify the best therapeutic regime and to control the spread of resistant strains.

Materials and Methods

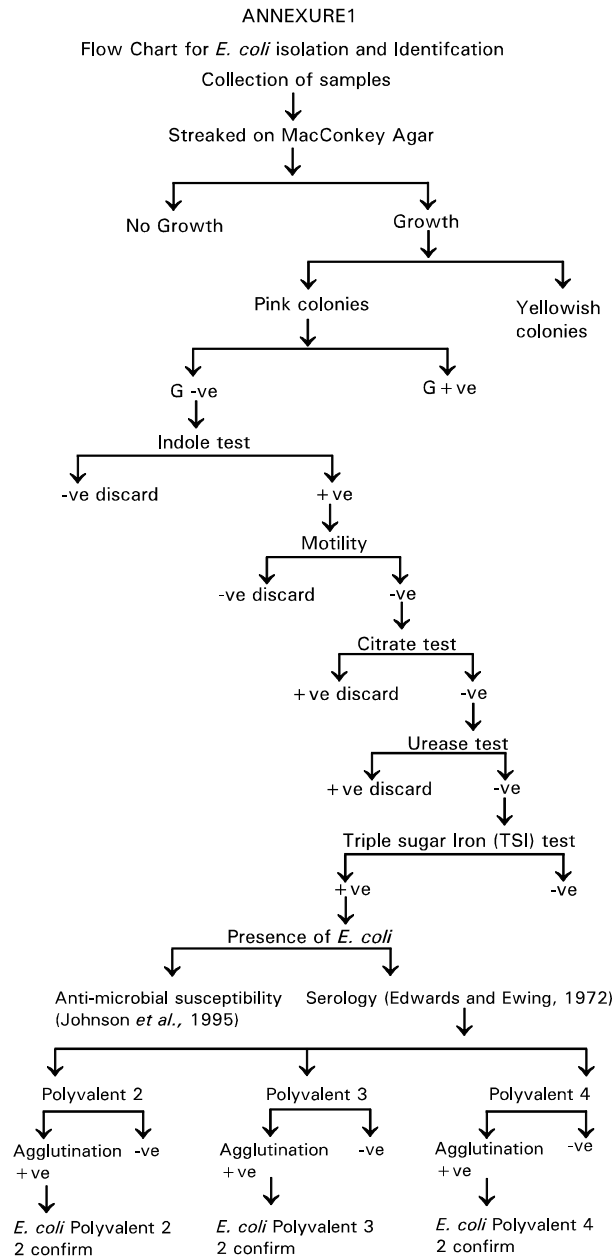
The work was conducted at the Bacteriology Laboratory, Public Health Division, NIH, Islamabad, between January and June 2001. Three hundred and fifty-five clinical samples; 179 urine, 55 pus, 43 throat, 31 stool, 22 sputum, 10 high vaginal swab (HVS), 12 ear and eye swabs, and 3 semen were collected and streaked onto MacConkey agar and incubated at 37°C for 24 hrs. Characteristic colonies were identified based on the ability of *E. coli* to ferment lactose, giving rise to pinkish colonies. Species verification was completed by biochemical tests and serologic typing, at the bacteriology laboratory, N.I.H., as illustrated in the flow chart (Annexure 1).

Serological testing: Biochemical tests alone are inadequate in making attempts to differentiate pathogenic *E. coli* from non-pathogenic ones. Pathogenic *E. coli* may possess different antigenic identities but they may have identical biochemical characteristics. So differentiation within species was made on serological grounds (Edwards and Ewing, 1972).

Antimicrobial susceptibility testing: Antibiogram pattern of isolated strains were determined on Mueller – Hinton agar. A sterile cotton swab was dipped into a sample from well-mixed colonies in peptone water and applied onto a Mueller – Hinton agar plate. Commercially available anti-microbial disks were placed on the plate by means of multi-disks dispenser and pressed firmly onto the agar with sterile forceps and incubated at 37°C for 24 hours, before measuring the zones of inhibition to determine the susceptibility of the isolates.

For comparison, the anti-microbial susceptibilities of isolates from the standard cultures were determined by a standard disk method according to the guidelines of the National Committee

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for Clinical Laboratory standard (NCCLS). *E. coli* ATCC 25922 was included for quality control (Johnson *et al.*, 1995). Zones of inhibition were determined with the help of list of break points of antibiotics. If the zone of inhibition was less than the minimum value of break point then it was assumed to be ineffective and if the zone of inhibition was equal to the maximum value or greater than maximum value of break point, then the antibiotics were assumed to be effective against *E. coli*.

Results and Discussion

Of the 355 clinical samples, *E. coli* was isolated from 28 (8%). Of these positive cases, the incidence of pathogenic *E. coli* was highest in urine samples, 22 cases (78.5%), followed by 3 cases in stool, 2 cases in wound pus and 1 case in HVS samples (10.7%, 7.1% and 3.6%, respectively). No *E. coli* isolates were detected from sputum, semen, throat, eye and ear swabs in this study (Table 1).

Table 1: Incidence of *E. coli* in different clinical samples.

Samples	Male patients	Female patients	Total samples	Positive samples (Percentage of total positive cases)
Stool	16	15	31	3 (10.7%)
Urine	69	110	179	22 (78.5%)
Wound pus	35	20	55	2 (7.1%)
HVS	-	10	10	1 (3.6%)
Semen	3	-	3	-
Throat swab	14	29	43	-
Ear & eye swab	7	5	12	-
Sputum	17	5	22	-
TOTAL	161	194	355	28

Table 2: Sex-wise incidence of *E. coli* in clinical samples.

Samples	Male Patients	Female Patients
Stool	33.3%	66.6%
Urine	36.4%	63.6%
Wound pus	50.0%	50.0%
HVS	-	100.0%
Semen	-	-
Throat swab	-	-
Ear & eye swab	-	-
Sputum	-	-

Table 3: Antibigram sensitivity pattern in urine samples.

Antibiotics	Sensitivity	Intermediate	Resistance
Amikacin	100.0%	-	-
Norfloxacin	91.7%	-	8.3%
Ceftizoxime	87.5%	-	12.5%
Ofloxacin	83.3%	-	16.7%
Pipemidic acid	75.0%	-	25.0%
Nitrofurantoin	68.8%	6.25%	25.0%
Cefamendol	66.67%	-	33.3%
Cefotaxime	60.0%	-	40.0%
Chloramphenicol	55.6%	-	44.4%
Kanamycin	50.0%	25.0%	25.0%
Cefuroxime	50.0%	16.6%	33.3%
Nalidixic acid	50.0%	-	50.0%
Augmentin	41.7%	-	58.3%
Tobramycin	27.27%	9.1%	63.6%
Ampicillin	22.2%	-	77.8%

Table 4: Antibigram sensitivity pattern in stool samples.

Antibiotics	Sensitivity	Intermediate	Resistance
Cefotaxime	100.0%	-	-
Ofloxacin	100.0%	-	-
Aztreonam	100.0%	-	-
Amikacin	33.3%	33.3%	33.3%
Ampicillin	-	-	100.0%
Chloramphenicol	-	-	100.0%
Tetracycline	-	-	100.0%

Table 5: Antibigram sensitivity pattern in pus samples.

Antibiotics	Sensitivity	Intermediate	Resistance
Amikacin	100%	-	-
Tobramycin	100%	-	-
Tetracycline	-	50%	50%
Ampicillin	-	100%	-
Cefuroxime	-	-	100%
Cephadrine	-	-	100%

According to sex-wise incidence pattern, *E. coli* was more prevalent in females than in males, as 66.6% of stool and 63.6% of urine samples were positive for *E. coli* from female patients. These findings are in conformity with those reported by other researchers (Sotto *et al.*, 2001). In wound pus samples, the incidence of *E. coli* was equal in both males and females. There was a lone isolate of *E. coli* from HVS samples (Table 2). The reason for high prevalence in case of females is that the females have open genitalia, predisposing it to fecal contamination, as compared to males whose relatively closed genitals prevents

the establishment of pathogens. *E. coli* easily spreads to vaginal passage through fecal contamination, where it invades and colonizes in the urinary tract leading to infection (Sotto *et al.*, 2001).

In case of urine samples, the antibiogram sensitivity pattern showed that all *E. coli* isolates were sensitive to amikacin and 91.7% of the isolates were sensitive to norfloxacin, followed by ceftizoxime and ofloxacin (87.5% and 83.3%, respectively). As for the resistivity pattern, this study revealed that 77.8% of the isolates were resistant to ampicillin followed by 63.6% to tobramycin (Table 3).

The resistance of *E. coli* to ampicillin, tobramycin and augmentin in case of urine samples in this study, also confirms similar findings reported by several workers (Johnson *et al.*, 1995; Gur *et al.*, 1999 and Samsyгина *et al.*, 2001).

All isolates of *E. coli* from stool samples were resistant to ampicillin, chloramphenicol and tetracycline, whereas aztreonam, cefotaxime and ofloxacin were 100% effective. As for the *E. coli* isolates from pus samples, all were sensitive to amikacin and tobramycin, but completely resistant to cefuroxime, ampicillin and cephradine and in case of lone HVS isolate, it was found to be resistant to ampicillin, yet sensitive to amikacin, ofloxacin and tobramycin (Tables 4 & 5).

This study also revealed a similar sex-wise distribution pattern amongst stool samples, where the prevalence of *E. coli* was higher in females (66.6%) than in males (33.3%). The possible reasons for this may lie in the fact that women are often associated with various household activities, such as cleaning of toilets / bathrooms, food preparation and changing diapers of babies, as well as potty training their children. This inevitably leads to contamination of hands and nails which may result in the introduction of the pathogens through the oral route (Chowdhary *et al.*, 1994). This may also explain the high incidence of UTI among women as well. However, because of the fact that hands are washed more frequently and that greater care is emphasized in this regard, the rate of infection through the fecal-oral route is far less than the genito-urinary tract (Chowdhary *et al.*, 1994, Sotto *et al.*, 2001).

The findings of this study revealed that *E. coli* strains are normally sensitive to most of the antibiotics and chemotherapeutic agents. About 55% isolates were susceptible to most of antibacterial drugs, while 15% showed intermediate susceptibility and only 33% were found resistant. These results are in conformity with those of other researchers (Hameed *et al.*, 1995; Johnson *et al.*, 1995; Gur *et al.*, 1999 and Samsyгина *et al.*, 2001).

In case of stool samples, cefotaxime was found to be the most active drug, while ampicillin was totally ineffective against *E. coli*. Similarly findings have been reported by other researchers (Hameed *et al.*, 1995; Sotto *et al.*, 2001). Such findings revealed that one drug is not effective against all isolates of *E. coli*. For this purpose, susceptibility tests should be carried out by clinicians, based on the sample, to ensure the prescription and use of the most effective antibiotics.

From this prospective study it is quite clear that the incidence of *E. coli* is higher in urine samples and amongst females than males. The high prevalence and spread of infection in females can be reduced by proper hygienic and medical care. The use of broad-spectrum antibiotics should be avoided, if the isolate is susceptible to the older drugs, in order to prevent the increase in resistance and, if one drug is found to be ineffective against all isolates of *E. coli*, susceptibility tests of the isolates become necessary.

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