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Antimicrobials Resistance Pattern of *Escherichia coli* Collected from Various Pathological Specimens

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Abstract: Irrational use of antibiotics is common in Bangladesh. The purpose of the present study was to know the effectiveness of various commonly used antimicrobials against *Escherichia coli*. Antimicrobial susceptibility test was done by Disc Diffusion method. In this study, antimicrobial resistance pattern of 163 isolates of *Escherichia coli* collected from various pathological specimens were determined. Most of the isolates (77%) were collected from urine sample. The highest numbers of isolates were resistant to cloxacillin (96.93%) and the lowest number isolates were resistant to imipenem (5.52%). Out of 163 isolates 141 (86.5%) were resistant to ampicillin, 89 (54.60%) to ceftazidime and 88 (53.99%) to ceftriaxone. From this study, it also appears that third generation cephalosporins (ceftazidime and ceftriaxone) were more effective against the test isolates in comparison to penicillin. The present study also revealed that 113 (69.33%) isolates were resistant to ciprofloxacin, 92 (56.44%) to chloramphenicol, 121 (74.23%) to co-trimoxazole and 128 (78.53%) to nalidixic acid. To the aminoglycoside drug 58 (35.58%) isolates were resistant to gentamicin and 74 (45.40%) to netilmicin. In this study 138 (84.66%) isolates were resistant to doxycycline, 126 (77.30%) isolates were resistant to tetracycline. Four isolates showed resistance to all the antimicrobials used except to imipenem. In the present study imipenem was found to be the most effective and 154 out of 163 isolates (94.48%) were found to be sensitive and cloxacillin was least effective and only 5 out of 163 isolates (3.07%) were sensitive to this penicillinase resistant drug.

Key words: Antimicrobials, resistance, multidrug, indiscriminate use of antibiotics

INTRODUCTION

Bacterial isolates are highly resistant to antimicrobials throughout the world specially in the developing countries (Calva *et al*., 1996). Routine monitoring of antimicrobial resistance provides data for antibiotic therapy and resistance control. Normal intestinal flora may contain several antibiotic resistance genes and presence of multidrug resistant *Escherichia coli* in a community indicates the presence of antimicrobial resistance genes in the bacterial population of the area in question (Levy *et al*., 1988). Bacteria can develop a variety of cellular mechanisms for acquiring resistance against antimicrobials, including genetic mechanisms. Unfortunately, both pathogenic and non pathogenic bacteria develop resistance to newly introduced antibiotics within few years. Moreover, improper use of antibiotics resulted in the spread antibiotic resistant bacterial strains in nature.

The presence of antibiotic-resistant species of the Enterobacteriaceae family in aquatic animal and human environments has given rise to considerable concern over the recent years. Antimicrobials resistance of bacteria is increasing worldwide and may result in significant public health problems. Actions to control the problem include the development of new antimicrobials, infection control programs and more appropriate use of existing anti-infective drugs. As a consequence antimicrobials to become more expensive, less effective and more prone to cause iatrogenic complications surveillance of bacterial resistance to antimicrobials may advice on appropriate therapy. Multiple antibiotic-resistant bacteria have been recognized as a serious threat to the national public health. Advantage of therapeutic use of antibiotics is greatly hampered due to the emergence of drug resistant strains. Bacterial infectious diseases account for much of the mortality among children (Reley *et al*., 1983). Every year about 11 million children below five years died of infectious diseases in the world, of which about five million children died in the developing countries (WHO, 1997). Antibiotic resistance of bacteria is increasing world wide and may result in significant public

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health problems (Shears, 1993). Antibiotic abuse is a problem that results in an increase of resistant bacterial strains (Hart and Kariuki, 1998).

The use of third-generation cephalosporins like ceftriaxone and ceftazidime have increased substantially since their introduction in Bangladesh, due to their efficacy, favorable Pharmacokinetics and low side-effects. These agents are more active than the earlier generation Cephalosporins like cephalothin, cephalexin, or cefuroxime, against Gram-negative bacilli with modest activity against Gram-positive bacteria. Use of these antibiotics have led to resistance among Gram-negative bacilli mediated by different beta-lactamase enzymes, e.g., extended-spectrum beta-lactamases and chromosomal (class 1) β-lactamases. Among the third generation cephalosporins, ceftriaxone is the most commonly used antibiotic in Bangladesh, followed by ceftazidime and cefotaxime. Despite being introduced quite recently in Bangladesh, incidences of resistance against these drugs are not uncommon among gram-negative bacilli. So, in this study survey of the susceptibility pattern of commonly isolated Gram-negative bacilli pathogens (collected from all body sites) to Cephalosporins, with emphasis on the third generation antibiotics are determined.

In the present investigation attempt was made to know the degree of effectiveness of several commonly used antimicrobials against *Escehrichial coli* isolated from different clinical specimens.

MATERIALS AND METHODS

All investigations related to this investigation performed in the Pharmaceutical Microbiology Laboratory of the Department of Pharmacy, University of Asia Pacific (UAP), Dhaka.

General Procedure and equipment: All media were sterilized by autoclaving at 121°C for 15 min. in an autoclave (Equitron, India). Glassware such as pipette, petridish etc., were sterilized at 160°C for 1 h in a hot- air oven (Gallenkamp, UK) prior to use. Agar slant was prepared, kept at room temperature and all inoculations and subculturing were done under aseptic condition in a laminar air flow cabinet (manufactured by SAARC, Bangladesh). The inoculated cultures were incubated in incubator (Binder, Germany) at 37°C. Accurate weights were measured using an analytical balance (Sartorius, Model BL-150, USA). Constant heating was done using a water bath (Memmert, Germany).

Sample: One hundred and sixty three isolates of *Escherichia coli* were collected from the microbiology department of two famous diagnostic centers in Dhaka. The samples were obtained from the pathological specimens of urine, pus, high vaginal secretion, blood and wound swab. All isolates were brought to the laboratory for culture without delay and identified on the basis of their colony morphology and biochemical tests were performed now and then. Diagnostic centers also identified the organism.

Antibiotic disc: The antibiotic sensitivity of *Escherichia coli* isolates to 14 antibiotics (ampicillin, aztreonam, cloxacillin, chloramphenicol, ceftazidime, ceftriaxone, ciprofloxacin, co-trimoxazole, doxycycline, gentamicin, imipenem, nalidixic acid, netilmicin and tetracycline) were determined by disc diffusion method. The disc were purchased from Himedia Laboratories, Mumbai, India and their efficacy were tested with the standard strain of *Escherichia coli* 25922 ATCC.

Antimicrobial susceptibility test: Antimicrobial susceptibility testing was done by agar disk diffusion method according to the National Committee for Clinical Laboratory Standards. Nutrient agar medium for sub-culturing the bacteria was prepared by boiling the constituents at 100°C with agitation until the agar melts. It was then autoclaved at 121°C in screw-capped test-tube for 15 min. Slants were prepared by keeping the test tubes at about 30°C until the agar solidified. Similarly, Mueller Hinton agar was prepared and autoclaved and after autoclaving the media was poured into previously sterilized petridishes in aseptic condition in such a way that the thickness of media remains around 2 mm. A Mueller Hinton Agar plate was inoculated with the test isolate and the antibiotic discs were placed on it at sufficient distance apart from one another. Inoculation was performed in aseptic condition. After inoculation the plate was incubated at 37°C for 24 h. Slide caliper was used to measure the zone of inhibition. Precise placement of the discs and performance of appropriate control tests are critical (Moland and Thomson, 1994) . Moreover, the thickness of the medium 2 mm and uniformity of the thickness were maintained to avoid false reading. Isolates were preserved in milk medium and stored in the freezer for possible reuse when needed, such as contamination of the isolates during the culture sensitivity test.

Data analysis: Data were analyzed by using Microsoft excel version 7.

RESULTS

In this study, the sensitivity patterns of 163 isolates of *Escherichia coli* to 14 different antimicrobial agents

Table 1: Correlation of antimicrobial sensitivity pattern of *Escherichia coli* isolated from various pathological specimens

		Source					
Antimicrobial agent	Interpretations	Urine	Pus	Blood	HVS	W/S	No. of isolates
Ampicillin	S	15	3	0	4	0	22
	R	111	14	9	3	4	141
Aztreonam	S	37	6	2	1	2	48
	R	89	11	7	6	2	115
Ceftriaxone	S	56	10	4	4	1	75
	R	70	7	5	3	3	88
Ciprofloxacin	S	38	6	2	3	1	50
	R	88	11	7	4	3	113
Chloramphenicol	S	53	5	6	3	4	71
	R	73	12	3	4	0	92
Ceftazidime	S	58	8	3	4	1	74
	R	68	9	6	3	3	89
Co-trimoxazole	S	33	1	1	5	2	42
	R	93	16	8	2	2	121
Cloxacillin	S	4	1	0	0	0	5
	R	122	16	9	7	4	158
Doxicycillin	S	21	0	2	2	0	25
	R	105	17	7	5	4	138
Gentamicin	S	76	13	6	6	4	105
	R	50	4	3	1	0	58
Imipenem	S	119	16	8	7	4	154
	R	7	1	1	0	0	9
Nalidixic acid	S	27	3	2	2	1	35
	R	99	14	7	5	3	128
Netilmicin	S	73	6	5	1	4	89
	R	53	11	4	6	0	74
Tetracycline	S	25	5	3	2	2	37
	R	101	12	6	5	2	126

W/S: Wound swab, HVS: High vaginal secretion, S: Sensitive, R: Resistance

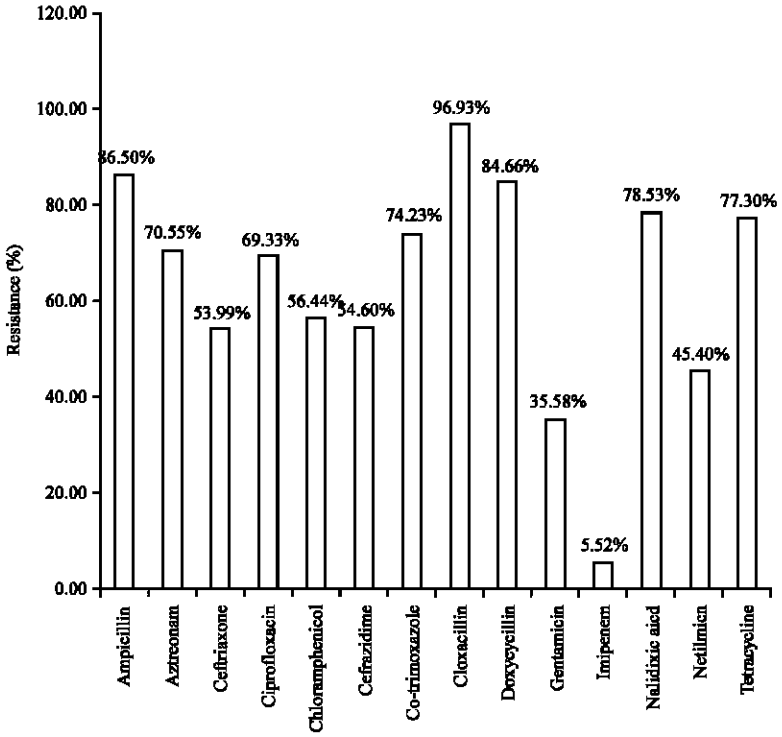

Fig. 1: Percentage resistance pattern of *Escherichia coli* to different antimicrobials

isolated from various pathological specimens were determined. Of the 163 isolated sample, 126 (77%) were from urine, 17 (10%) were from pus, 9 (5%) were from blood, 7 (4%) were from HVS, 4 (2%) were obtained from wound swab (Table 1). Figure 1 shows that of the 163 isolates 22 (13.50%) were sensitive and 141 (86.50%) were

resistant to ampicillin and 48 (29.45%) of isolates were sensitive and 115 (70.55%) were resistant to aztreonam. It was observed that 74 (45.40%) isolates were sensitive, 89 (54.60%) were resistant to 3rd generation cephalosporin, ceftazidime. Ceftriaxone, a third generation of cephalosporin group to which 75 (46.01%) isolates were sensitive and 88 (53.99%) was resistant. It was observed that 50 (30.77%) isolates were sensitive and 113 (69.33%) were resistant to ciprofloxacin. In this investigation it was found that 71 (43.66%) isolates were sensitive and 92 (56.44%) isolates were resistant to chloramphenicol. Present study also showed that 42 (25.77%) isolates were sensitive and 121 (74.23%) isolates were resistant to co-trimoxazole. Another powerful drug, nalidixic acid to which 35 (21.47%) isolates were sensitive and 128 (78.53%) were resistant. To the aminoglycosidic drug 104 (64.48%) isolates were sensitive and 58 (35.58%) isolates were resistant to gentamicin. In the case of netilmicin, another aminoglycosidic drug to which 89 (54.60%) isolates were sensitive and 74 (45.40%) isolates were resistant. It was also observed that 25 (15.44%) isolates were sensitive and 138 (84.66%) isolates were resistant to doxycycline, 37 (22.70%) isolates were sensitive and 126 (77.30%) isolates were resistant to tetracycline. Very high percentages (96.93%) of isolates were found to be resistant to cloxacillin, only 3. 07% were sensitive. Imipenem was found to be the most effective drug and 94.48% isolates were sensitive and 5.52% were resistant to this drug.

DISCUSSION

In the present study year study it was observed that the isolates of *Escherichia coli* showed different degree of sensitivity to different antimicrobials. The samples were isolated from different pathological specimens. Maximum numbers of isolates were collected from urine indicating that urinary tract is more prone to infection by *Escherichia coli* which corresponds to the findings of other researchers (Sharafi *et al*., 1996). In another study, Ahmad (1982) found that the most common organism isolated from the cases of UTI was *E. coli* (55.6%). The least number of isolates were collected from HVS and wound swab. One isolate showed resistance to all the fourteen drugs used. Different resistance mechanisms are involved based on which drugs fail to kill different isolates. It is also difficult to cure these patients. Heterogeneous use of antibiotics may be helpful in managing the problem. In this study, four isolates were identified which were resistant to all the drugs, except imipenem. Among them three isolates were from urine, one isolate was from wound swab and one was from blood.

In this study, the most effective drug was found to be imipenem and the least effective drug was cloxacillin. Involvement of efflux system may result in such high percentage of β-lactamase inhibitor antibiotic resistance (Gotoh *et al*., 1995). The present study revealed that 69.33% E. *coli* isolates were resistant to ciprofloxacin (Fig. 1). In contrast, Wang *et al*. (2001) and Winokur *et al*. (2001) observed 60 and 15.2% *E. coli* isolates resistant to ciprofloxacin respectively suggesting that *E. coli* isolates are getting resistance to this drug with time. This study showed that 30.77 and 64.48% of isolates were sensitive to ciprofloxacin and gentamicin, respectively. It was observed that 94.48% isolates were sensitive to imipenem. Whereas, Aswapokee and Tiengrim (1998) found none was resistant to imipenem. Hence, imipenem appeared to be the most potent drug against *Escherichia coli*. Imipenem can be considered as the drug of choice for the treatment of *Escherichia coli* infection. Restricted use of imipenem in critical cases is suggested to avoid emergence of resistant strains. In this study, it was observed that 77.30% of isolates were resistant to tetracycline (Fig. 1), whereas, other workers (Aswapokee and Tiengrim, 1998) found that more than 50% of the isolates were resistant to tetracycline and co-trimoxazole. In contrast present study showed 74.23% of isolates were resistant to co-trimoxazole which indicates the more indiscriminate use of these antimicrobials in Bangladesh. Present study showed 54.60% of isolates were resistant to ceftazidime which is higher than the finding of Joks (2000) who observed 22.2% of isolates resistant to ceftazidime. In present study 35.58% of isolates were resistant to gentamicin and 45.40% of isolates were resistant to netilmicin, which is much higher than the findings of other worker (Joks, 2000) who found 22.2% isolates were resistant to gentamicin and 14.7% of isolates were resistant to netilmicin. Among the aminoglycosides drug, 65.48% of isolates were sensitive to gentamicin and 54.60% were to netilmicin. Iqbal *et al*. (2002) found 64% isolates were sensitive to gentamicin. Between them gentamicin was found to be more potent than netilmicin. In this study 70.55% of isolates were resistant to aztreonam which is much higher compared to Iqbal *et al*. (2002) who found 25% *E. coli* isolates were resistant to aztreonam.

In the present study percentages of isolates of *E. coli* showed sensitivity to ceftriaxone and ceftazidime were 46.01 and 45.40%, respectively and 78.53% of isolates were resistant to nalidixic acid. Other studies showed higher percentage (100%) of resistance (Kawser *et al*., 2001) to nalidixic acid. Present study showed 86.50% of isolates were resistant to ampicillin. This is consistent with the findings of Iqbal *et al*. (2002) who observed 86%

E. coli isolates were resistant to ampicillin. This study also revealed that high percentages of *E. coli* isolates were resistant to doxycycline (84.66%). Present study revealed that urinary tract is more prone to infection by *E. coli* and 77% isolates were collected from urine. Multi-drug resistance is on rise in Bangladesh and in case of many antimicrobials rise in resistance is significantly higher. Several studies relevant to these investigations suggest that an antimicrobial agent may be used for years before a gene expressing resistance to it emerges in a strain of bacteria somewhere. Progeny of that strain or others to which the gene is transformed, may then disseminated preferentially through global network of bacterial populations on people or animals treated with the agents as the gene become linked to genes expressing resistance to them.

CONCLUSION

In the present study, imipenem was found to be the most effective and 94.48% isolates of *E. coli* were found to be sensitive and cloxacillin was least effective and only 3.07% isolates were sensitive to this penicillinase resistant drug which is even less than the effectiveness of ampicillin and 14.5% isolates were sensitive to this drug. Four isolates showed resistance to 13 of the 14 antimicrobials except imipenem suggesting that they were exposed to several antimicrobials.

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