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Research Article

One year surveillance of antimicrobial sensitivity pattern of different antibiotics on clinical isolates of *Escherichia coli* with comparison of resistance against different antibiotics in four major cities in Bangladesh

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ABSTRACT

Background: The aim of study was to provide supportive implications for proper treatment of *E. coli* induced infections and related complications regarding antibiotic resistance.
Methods: Total 600 isolates of *E. coli* from four major cities of Bangladesh were included in studies which were isolated from clinical diagnostic facilities in Dhaka, Chittagong, Rajshahi and Khulna. The antimicrobial sensitivity test of each *E. coli* isolate to 16 antimicrobial agents was carried out by the Kirby-Bauser disc diffusion method.
Results: In the present investigation, the most effective antimicrobial against *E. coli* was found to be antibiotics of Carbapenem group; Doripenem 98.67% sensitive in Dhaka and Chittagong, 100% sensitive at Rajshahi and Khulna, Meropenem 89.33, 92.67, 95.33 and 97.33%, Imipenem 84.00, 86.00, 95.33 and 97.33 in Dhaka, Chittagong, Rajshahi and Khulna respectively. Apart from these third generation antibiotics like Cefixime 80.00% (D), 78.67% (C),78.00% (R), 82.00% (K) and Ceftriaxone 78.00% (D), 76.67(C), 65.33(R), 66.67(K) were shown satisfactory amount of sensitivity against *E. Coli.* Our research reveals that in Bangladesh commonly used conventional older, common, cheaper antibiotics used against *E. coli* were shown alarming rate of resistance to *E. coli* strains. Among them Amoxicillin, Tetracycline, Cloxacillin and Nalidixic Acid showed more than 80% resistance in most of the areas. Most widely used antibiotic Ciprofloxacin has become 39.99 to 49.99% resistant.
Conclusion: These findings suggest urgent need for creating greater public awareness about antibiotic. It is also important that healthcare providers effectively communicate with their patients, to improve treatment compliance and health outcomes.

Keywords: Antibiotic Susceptibility, Resistance, *E coli*

INTRODUCTION

Multiple antibiotic-resistant bacteria have been recognized as a serious threat to the nation's public health. The increasing resistance of microbes now jeopardizes the tremendous therapeutic advantage afforded by antibiotics. Two- thirds of the world's population lives in the developing nations of Asia, Africa and South America. Most of these populations exist under conditions of poverty, inadequate medical care, poor sanitation and nutrition.[1] Bacterial infectious diseases account for much of the morbidity and mortality, particularly among young children. Effective antimicrobial therapy is limited by a large reservoir of antibiotic- resistant bacteria. This appears to be related to selective pressure exerted by extensive use of antibiotics.[2] According to the 1996 WHO report (Mihill, 1996) old and new infectious diseases are coming back worldwide within the past twenty-five years. Resistance to the commonly available broad-spectrum antibiotics may be a problem. In a broader sense, the resistance problem is ecological. High resistance in Asian countries is possibly due to uncontrolled use of antibiotics while relatively low rates in Scandinavia and in the UK are likely to be attributable to conservative antibiotic usage (Resistance to antimicrobial agents). Recently developed drugs are

scarce in developing countries. Even in the tertiary referral hospitals first line drugs are ampicillin, chloramphenicol, erythromycin, gentamicin, penicillin, and tetracycline. Antibiotic use is unregulated and may be purchased in pharmacies or general stores with and without prescriptions. Locally produced antibiotics in some cases have low potency. Because laboratory facilities are not widely available, most patients are treated empirically using antibacterial in acute diarrheal diseases and respiratory tract infections even though in a good number of cases these are viral infections. Increasingly, problems of resistance in pneumococcal infections, tuberculosis, typhoid fever, sexually transmitted diseases, and nosocomial infections occur.[3] Managing the problem of antimicrobial resistance in developing countries should include the following, 1) Improved access to diagnostic laboratories, 2) Surveys to detect the emergence of resistance, 3) Regulation of the use of antibiotics, 4) Train prescribers of antibiotics, and 5) Education of the public. Data on resistance, prevalence and antimicrobial use is lacking in developing countries. In addition, there are constraints to the development of effective surveillance because of lack of laboratory facilities among other factors. Antimicrobial resistance in developing countries require improved surveillance of antimicrobial resistance that will contribute to providing relevant data to prescribers and aid in developing disease control strategies.[4-5] Antibiotic resistance to pathogens is identified as a major contributing factor. Resistance may be developed due to widespread empiric use of broad-spectrum antibiotic. Improper dose and easy availability of antibiotic from pharmacy may cause the development of bacterial resistance. As a consequence, treatment tends to become more expensive, less efficient and more prone to cause iatrogenic complication.[6]

In this study, the susceptibility of *E. coli* strains isolated in four major cities of Bangladesh was investigated against different antimicrobial agents to provide supportive implications for the proper treatment of these microbial induced infections and related complications. This study also revealed the irrational uses of antibiotic in different city with investigational comparison.

METHODS

Patients were referred by community practitioners, clinics, and hospitals throughout Dhaka, Chittagong, Khulna and Rajshahi four major cities of Bangladesh to the Popular Diagnostic Centre (Dhaka, Chittagong and Rajshahi) and City imaging Centre (Khulna) private laboratory and clinical diagnostic facility for evaluation of possible serious bacterial infection, among them isolates of *E. coli* were chosen for this study. The study includes clinical isolates of *E. coli from* urine, pus or stool of patient. The specimens and identification of isolates was done by standard microbiological method according to Cheesbrough[7] and Cowan. The antimicrobial sensitivity test of each isolate was carried out by the Kirby-Bauser disc diffusion method.[8] and as per recommendation of National Committee for Clinical Laboratory Standards.[9] The results were expressed as susceptible/resistant according to criteria developed by NCCLS and Manual of Antimicrobial Susceptibility Testing guidelines (Coyle, 2005; Cheesbrough, 2006; Okonko et al., 2009 a, b).[10-11] The discs used contained following antibiotics: amoxicillin (AMX) 30 mcg, ampicillin (AMP) 25mcg; Ceftazidime (CAZ) 30mcg; ceftriaxone (CRO) 30mcg; chloramphenicol (CHL) 30mcg; ciprofloxacin (CIP) 5mcg; Cloxacillin, (CXL) 5mcg; co-trimoxazole, (CRX) 25mcg; Doxycycline DOX (30mcg); Gentamicin (GEN) 10 mcg; Nalidixic acid (NAL) 30 mcg; Imipenem (IPM) 10 mcg; Netilmicin (NET) 10 mcg; Tetracycline (TET) 30 mcg; Doripenem (BCTM, Dickinson) 10 mcg; Meropenem 10 mcg, Cefixime (Thermo scientific) 5 mcg.

General Procedure & Equipment

All media were sterilized by autoclaving at 121°C for 15 min. in an autoclave (Equirtron, India). Glassware such as pipette**,** petri dish etc. were sterilized at 160°C for 1 hour in a hot- air oven (Gallenkamp, UK) prior to use. Agar slant was prepared, kept at room temperature and all inoculations & subculturing was 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 by using an analytical balance (Sartorius, Model BL-150, USA). Constant heating was done using a water bath (Memmert, Germany)

Bacterial identification

Isolates of *Escherichia coli* were identified by catalase test, Motility Indole Urea test and Triple sugar Iron Agar test.

Culturing of E. coli

Those isolates whose Antimicrobial Susceptibility were to be performed were at first sub cultured in freshly prepared MacConkey (MC) agar medium (MAC) under laminar air flow cabinet & the organism were allowed to incubate overnight at 37°C. The media were from Himedia Laboratories Pvt. Limited, Mumbai (Bombay)-400086, India. The composition of MC agar medium (in gram per liter) was given Peptone-17, Protease Peptone-3, Lactose-10, Bile salts no 3-1.5, Sodium chloride-5, Neutral red-0.03, Crystal violet-0.001, Agar-13.5, Distilled Water-1000 ml.

Data Analysis: Data were analyzed by using Microsoft excel.

RESULTS

Resistance and sensitivity patterns of *Escherichia coli* in 4 major cities to several antimicrobial agents are given in

Table 1 and graphical representation of resistance pattern are given in Figure 1. A total of 600 samples (150 from each division) were used. Number of isolates found in different specimen is given in Table 2.

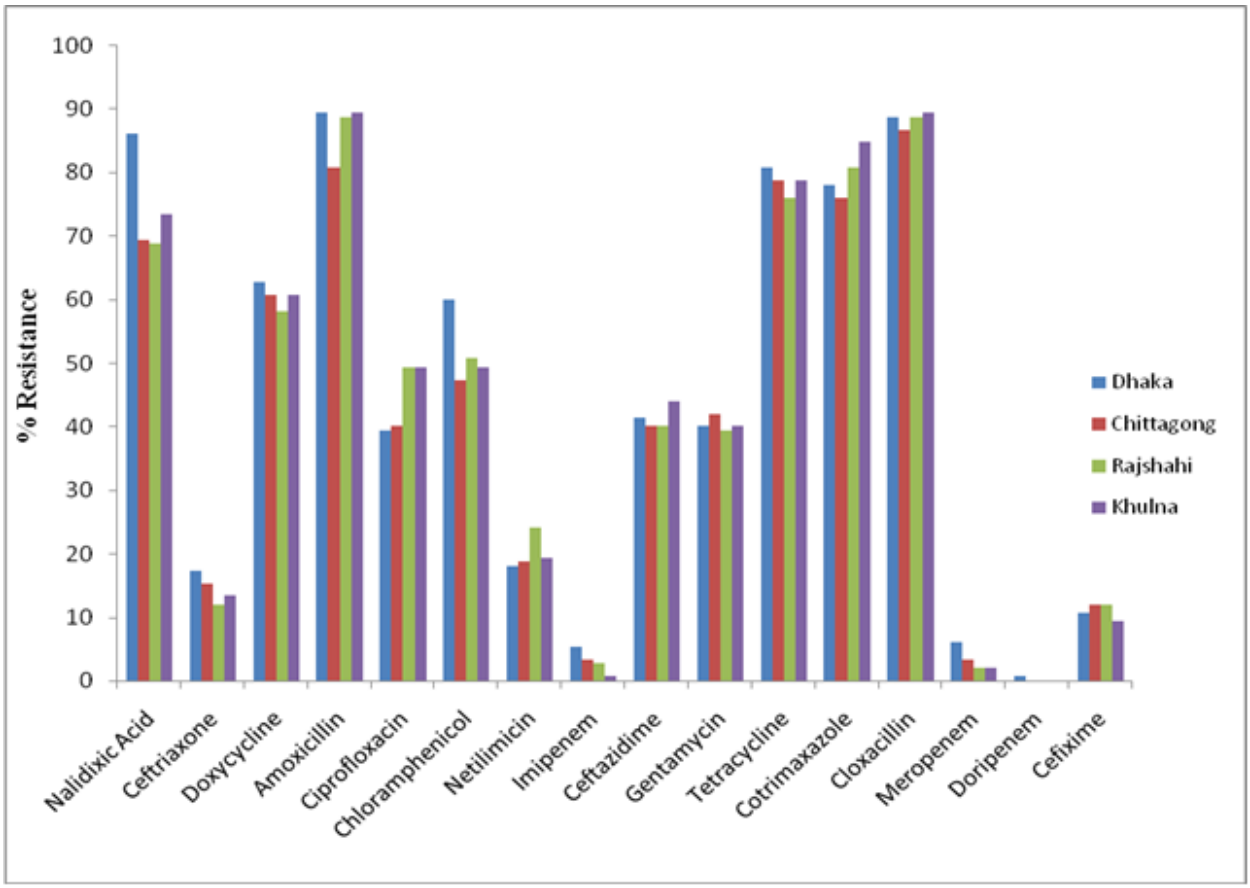

Figure 1: Graphical representation of resistance pattern against *E. coli* in four cities of Bangladesh.

Table 1: Resistance and sensitivity patterns of *Escherichia coli* in 4 major cities; Here Dhaka, Chittagong, Rajshahi and Khulna is denoted as D, C, R and K respectively.

Antibiotic	Resistant				Sensitive				%R				%S			
	D	C	R	K	D	C	R	K	D	C	R	K	D	C	R	K
Nalidixic Acid	129	104	103	110	15	35	43	39	86.00	69.33	68.67	73.33	10.00	23.33	28.67	26.00
Ceftriaxone	26	23	18	20	117	115	98	100	17.33	15.33	12.00	13.33	78.00	76.67	65.33	66.67
Doxycycline	94	91	87	91	44	48	59	55	62.67	60.67	58.00	60.67	29.33	32.00	39.33	36.67
Amoxicillin	134	121	133	134	12	23	17	12	89.33	80.67	88.67	89.33	8.00	15.33	11.33	8.00
Ciprofloxacin	59	60	74	74	76	76	74	71	39.33	40.00	49.33	49.33	50.67	50.67	49.33	47.33
Chloramphenicol	90	71	76	74	52	70	70	72	60.00	47.33	50.67	49.33	34.67	46.67	46.67	48.00
Netilmicin	27	28	36	29	117	114	110	117	18.00	18.67	24.00	19.33	78.00	76.00	73.33	78.00
Imipenem	8	5	4	1	126	129	143	146	5.33	3.33	2.67	0.67	84.00	86.00	95.33	97.33
Ceftazidime	62	60	60	66	79	76	79	71	41.33	40.00	40.00	44.00	52.67	50.67	52.67	47.33
Gentamicin	60	63	59	60	84	83	84	86	40.00	42.00	39.33	40.00	56.00	55.33	56.00	57.33

Tetracycline	121	118	114	118	21	36	32	29	80.67	78.67	76.00	78.67	14.00	24.00	21.33	19.33
Cotrimoxazole	117	114	121	127	29	31	29	20	78.00	76.00	80.67	84.67	19.33	20.67	19.33	13.33
Cloxacillin	133	130	133	134	12	13	15	12	88.67	86.67	88.67	89.33	8.00	8.67	10.00	8.00
Meropenem	9	5	3	3	134	139	143	146	6.00	3.33	2.00	2.00	89.33	92.67	95.33	97.33
Doripenem	1	0	0	0	148	148	150	150	0.67	0.00	0.00	0.00	98.67	98.67	100.00	100.00
Cefixime	16	18	18	14	120	118	117	123	10.67	12.00	12.00	9.33	80.00	78.67	78.00	82.00

Table 2: Number of isolates found in different specimen.

Region	Specimen			
	Stool	Urine	Blood	Unknown
Dhaka	46	69	19	16
Chittagong	43	79	21	7
Rajshahi	54	70	15	11
Khulna	49	75	20	6

DISCUSSION

In our one-year study it was observed that the isolates of *E .coli* showed different degree of resistance to different antimicrobials. Maximum numbers of isolates were collected from urine indicating that urinary tract was more prone to infection by *E. coli*, which corresponds, to finding by authors (Iqbal et al., 2002).[12] In this study, the less resistant drug found was Doripenem >Imipenem> Meropenem. From the result of Table 1 it is clear that these three antibiotics are more resistant in Dhaka city than other rural area. The possible cause behind this would be – as Dhaka is the capital city of Bangladesh most of the specialized doctors reside here and practicing in specialized hospitals that are mostly located in Dhaka. Critical conditioned patients requiring emergency attention come to these specialized hospitals in Dhaka for treatment. Doripenem, Imipenem and Meropenem are the last line therapy which is costly and mostly given to critical conditioned patient as the last resort to combat fatality. Unfortunately if the patient died the antibiotic course left untreated and resistant strain are come back to the environment. Another cause that may contribute to lesser availability of these sensitive strains in rural regions is that people of the rural and suburban area seek medications from RMP, Quack even Chemists they usually refrain from prescribing latest advance molecules as they tend to be expensive. In rural Bangladesh, for example, 95% of drugs consumed for 1 month by more than 2,000 study participants came from local pharmacies; only 8% were prescribed by physicians.[13] People are encouraged to buy from unofficial distributors because drugs often are not available in government hospitals.[14]

In the present study percentages (average) of isolates showed sensitivity to Cefixime, Ceftriaxone and Ceftazidime were 79.66%, 71.66% and 50.83%. In contrast Rahman et al (1990) observed percentages of *E. coli* isolates sensitive to Ceftriaxone to be 95%. Hence our findings suggest significant increase in resistance against these drugs by *E. coli* during the last 13 years in Bangladesh. Loss of both Ompc porins in laboratory mutants of *E. coli* has been reported to determine an 8 to 16-fold increase in the MICs of Cephalosporins (Jocoby and sutton, 1985).

We found Ciprofloxacin most widely used antibiotic against *E. coli* showed average resistance in four major cities is significantly greater than the findings of Winokur et al (2001)[15] who found 15.2% of isolates were resistant to ciprofloxacin. In another study (Ahmed et al., 2001)[16] observed that 78 to 98 % of isolates were found to be sensitive to ciprofloxacin and Gentamycin whereas in our study 49.5% (Average) and 56.16% (Average) of isolates were sensitive to ciprofloxacin and Gentamycin respectively. Our findings indicate that the resistance of these antibiotics has been alarmingly increased in the last few years in Bangladesh. McDonald et al. (2001)[17] suggests that mutation in gyrA gene have relationship with ciprofloxacin resistance. In another study Ena et al, (1998)[18] observed the increase in the use of fluoroquinolones was associated with a significant increase in ciprofloxacin resistant *E. coli* isolated from urinary tract sources 3 to 20% against Ciprofloxacin.

The most resistant drugs found were Amoxicillin-89.33%(D), 80.67% (C), 88.67%(R), 89.33%(K); Cloxacillin-88.67% (D), 86.67%(C), 88.67%(R), 89.33(K); Nalidixic Acid- 86.00% (D), 69.33% (C), 68.67% (R), 73.33% (K); Tetracycline- 80.67% (D), 78.67% (C), 76.00% (R), 78.67% (K); Cotrimoxazole-78.00% (D), 76.00%(C), 80.67(R),84.67(K). One of the major causes was described by Calva et al[19], that drugs more commonly affected by bacterial resistance in developing countries are generally inexpensive and popular broad-spectrum agents. Moreover drug vendors in rural area usually have little or no knowledge of the

required dosage regimen, indications, or contraindications and They often suggest conventional, cheaper antibiotics owing to people's affordability which make these antibiotics more resistant in rural areas.[20,21] However, the relationship between antibiotic use and the emergence and spread of resistance is a complex one. Resistance of pathogens to these available, cheap, older and commonly abused drugs would definitely result in high cost of treatment, longer hospital stay and therapeutic failure, which might lead to life threatening diseases and more deaths (Lau et al., 2004).[22] In many African, Asian, and Latin American countries, common antibiotics readily available on demand from hospitals, pharmacies, patent medicine stalls, road side stalls and hawkers are cheaper and old generic antibiotics.[23] Another observation from our one year surveillance was that only costly and last line antibiotics become resistant in City areas rather than rural areas like Khulna and Rajshahi. Cheaper antibiotics are more resistant in rural areas than City areas.

CONCLUSION

A good, representative database on the current status of antibiotic resistance among common and important pathogens is essential for the proper treatment of infectious diseases in the Bangladesh. From the above discussion it is clear that the present situation of antibiotic resistance is alarming. Though Meropenem, Doripenem and Imipenem showed highest sensitivity but they are the last line of defense against *E. coli*. Restricted uses of these drugs are suggested in critical areas to avoid emergence of resistant strains. Apart from these Ciprofloxacin and Cephalosporin's still remains good option against *E. coli* but unfortunately ciprofloxacin lost its faith due to resistance which clearly indicates irrational use of this antibiotics widely in Bangladesh. This study reveals that Cefixime is most effective drug with highest sensitivity against *E. coli* among Cephalosporin's. As antibiotics are life saving drugs, therefore, it is very important to ensure rational use of antibiotics. Otherwise, it will develop drug resistant organisms. To avoid all those anomalies, prescription of antibiotics by quack should be prohibited, physician should not prescribe without pathological investigation as well as government, doctors, pharmacists, patients and all health related personnel should be alert and conscious to ensure rational use of antibiotics.

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