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Antibiotic resistance in *Escherichia coli* from clinical and drinking water sources of Northern India

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ABSTRACT

The distribution of antibiotic resistance rate to Ampicillin, Augumentin, Cephelexin Cefuroxime, Ceftriaxone, Gentamycin, Amikacin, Netilmycin, Ciprofloxacin, Ofloxacin, Chloramphenicol, Trimethioprim, Tetracycline & Furazolidone in *E. coli* isolated from drinking water sources and clinical sources have been investigated. The antibiotic resistance rate was significantly higher ($p < .05$, $< .01$ & $< .001$) in *E. coli* isolated from clinical sources in comparison to those from drinking water sources except for three antibiotics *i.e.* Cephelexin, Amikacin and Netilmycin. The Multidrug resistant (MDR) has also been observed in all these strains of *E. coli*. The prevalence of MDR was significantly ($p < .05$) higher in clinical sources of *E. coli* for (10-12), numbers of antibiotics when compared to those from drinking water sources. These results suggested that the antibiotics resistance is significantly higher in clinical sources in comparison to drinking water sources.

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KEY WORDS : Drug resistant, *E. coli*, Resistant pattern

Introduction

The population of Varanasi city is supplied with water either from the river Ganga after filtration and chlorination or from deep bore tube wells. In India as a whole and in the eastern part of Northern India, there is no control over the use and sale of antimicrobials. It will be interesting to discriminate the possible sources of these contaminating bacteria, by looking into the extent of faecal contamination in different water samples.

Escherichia coli is a pathogen in man and animals and causes both septic infection and diarrhea. This bacterium is considered one of the most important biological indicators of faecal contamination[25]. Before the start of the antibiotic era, none of the bacteria isolated from the clinical environment carried resistance genes to commonly used antimicrobials. Indeed, it has been suggested that the introduction of antibiotics has added 10 years of life expectancy[9]. But bacteria have responded to the development and use of antibiotics with a bewildering array of the resistance mechanism[23]. Despite the continuous development of newer antimicrobials, resistant strains have continued to emerge and it is not unlikely that in near future the battle against antibiotics resistance may be lost and the antibiotics era may prove to be only transitory[27]. However, this could be avoided or reduced if we gain knowledge not only of resistance mechanisms but also of the reservoir and sources of resistant strains. The resistance gene pool in clinical as well as veterinary environments is equally significant as antimicrobials used in both situations are important in India[27].

Antimicrobials are routinely used for disease prevention and growth promotion in the animal population. This practice leads to the intestinal tracts of food animals, which poses a public health threat[2]. It has been argued that antibiotic genes have an environmental origin and

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TABLE-1 : Number of water samples positive for total and faecal coliform bacteria and *E. coli* (faecal coliform).

Total no. of Samples collected (168)	No. of samples positive for total and faecal coliform	Percentages of samples positive for total and faecal coliform
No. of samples positive for total coliform	99	58.89 %
No. of samples positive for faecal coliform	72	42.85 %
Presence of *E. coli* in water samples	72	42.85 %

have evolved as a part of a defense mechanism against toxic compounds in the environment, such as plant microbial metabolites encountered in soil[20].

Therefore, the detection of *E. coli* is of great importance in estimating the sanitary index of water. *E. coli* has emerged as a public health threat, following its initial identification as a pathogen in a 1982 outbreak of illness associated with the consumption of undercooked ground beef[16]. The US center for disease control and prevention estimated that *E. coli* causes approximately, 73400 illnesses and 60 deaths in the United States[17]. Recent reports indicate that antimicrobial resistance of *E. coli* is rising day by day[11,19,31].

The frequency of occurrence of drug-resistant bacteria is particularly high among the gram-negative enteric bacilli. A study[22] showed that 80.86% of strains of *E. coli* isolated from clinical sources were recorded to be resistant to 5 to 12 antibacterial drugs tested. In another study at Japan[28,] 81% of strains of *E. coli* have been found resistant to one or more antibiotics (up to six) in various combinations and 935 of them carried multiple drug resistance against 3- 4 antibiotics.

In India, a sensitivity survey at Lucknow on 218 strains of *E. coli* of animal origin reported only 13.8% of strains resistant to various antibiotics[1], while another study in New Delhi indicates as much as 76.5% of human strains of *E. coli are* resistant to multiple drugs[13].

The multiplicity of resistance was found in increasing order in human isolates, while in decreasing order in animal isolates. It might be because these antimicrobial agents are commonly used for the treatment of the bacterial infection and consequently the appearance and spread of drug-resistant populations. However, the introduction of each new antibiotic was invariably followed by reports of resistant organisms. Levels of resistance in previously sensitive organisms rose with us, and this resistance was associated with treatment failure[8].

It is known that resistance rates vary markedly between countries[15] and even between local populations down to the size of the individual community practices[6].

The present study therefore, was planned to see the extent of the faecal contamination of drinking water in Varanasi, along with determination of drug resistance pattern in the *E. coli* isolates from drinking water & clinical sources. The extent of the drug resistance in *E. coli* may indicate the probable sources of contamination of drinking water in this city. The Multi drug resistant patterns were studied in both clinical as well as drinking water sources of *E. coli.*

Materials and Methods

Bacterial strains:

A total of 112 *E. coli* (70 from drinking water and 42 from clinical sources) were included in the present study. A two-stage sampling has been applied to select the specific muhallas from wards as first stage and specific households from the muhallas as second stage. To decide on the number of muhallas/ households from the wards/muhallas, method of stratified random sampling with proportional allocation has been used. In all 102 households were randomly- selected from the 12 wards of the Varanasi city. The drinking water samples were collected from each of the selected households from the available sources, hand pumps (10), jet pumps (8), water supply of the municipal corporation (13), wells (9) and ready to drink stored water(30) .The sources and number of *E. coli* isolated from the various clinical specimens were: urine (15), blood (8), stool (10), pus (9). These specimens were collected from the patients of Sir Sunder Lal Hospital referred to Dept. of Microbiology, Institute of Medical Sciences, Banaras Hindu University, Varanasi, India. These *E. coli* isolates were grown and identified by using standard morphological and biochemical parameters[25].

The Table-1 shows the number of water samples

TABLE-2 : Resistance pattern in *E. coli* isolates from drinking water and clinical sources and p –values for the significant differences in resistance rate

Antibiotic	Drinking Water sources (70)*	Clinical sources (42)*	P- value (H_2)
Amoxycillin	**33 (47.14)**	**39 (92.85)**	.001
Augumentin	23(32.85)	32(76.19)	**.001**
Cephelexin	22(31.42)	18(42.85)	**NS**
Cefuroxime	1(1.4)	11(26.19)	**.001**
Ceftriaxone	1(1.4)	15(35.14)	**.001**
Gentamycin	12(17.14)	16(38.09)	**.01**
Amikacin	4(5.71)	0(0)	**NS**
Netilmycin	2(2.85)	1(2.38)	**NS**
Ciprofloxacin	11(15.71)	29(69.04)	**.001**
Ofloxacin	9(12.85)	30(71.04)	**.001**
Chloramphenicol	21(30.00)	22(52.38)	**.01**
Trimethioprim	12(17.14)	30(71.14)	**.001**
Tetracyline	38(54.42)	32(76.19)	**.05**
Furazolidone	13(18.57)	14(33.33)	**.05**

Note: * Shows the total number of E. coli from different sources.

Numerical value in each row and in column 2 & 3 shows the number of resistant *E. coli.*

( ) in bracket shows the % of resistant *E. coli.*

positive for total and faecal coliform bacteria. This Table shows that 58.89% of the water samples are positive for coliform count and 42.85% of the water samples are positive for the faecal coliform.

Antimicrobial susceptibility testing:

Drug resistance against different antimicrobial agents was determined by modified Kirby Bauer Disc Diffusion method[18]. The following antibiotic agents included in the study with disc concentration shown in brackets of respective antibiotics were taken as per NCCLS guidelines[24]. These were Amoxycillin (AMX, 10mg), Augumentin (AUG, 10mg), Cephelexin (LEX, 30mg) Cefuroxime (FRX, 30mg), Ceftriaxone (CRO, 30mg), Gentamycin (GEN, 10mg), Netilmycin (NET, 10mg), Amikacin (AMK, 30mg), Ciprofloxacin (CIP, 5mg), Ofloxacin (OFL, 5mg), Chloramphenicol (CHL, 25mg), Trimethioprim (SXT, 23.75mg), Tetracycline (TET, 10mg) and Furazolidone (FDZ, 50mg). To decide whether a strain is resistant or intermediate sensitive or sensitive, the criteria given in NCCLS guidelines (1997) were strictly

followed.

Data Analysis:

All the data were entered into computer sheet (EXCEL and MS DOS), which were used to generate descriptive statistics. Pair wise comparison of antibiotic resistance against different antibiotics was done using FORTRAN program.

The significant difference between p_1 & p_2 was tested where p_1 and p_2 were values of percentages of resistant *E. coli* from the two sources. The P-values have been obtained for two tests of H_0 with alternative hypotheses H_1 & H_2 given below:

H_0: $p_1=p_2$ against 1. H_1:$p_1>p_2$ 2.H_2: $p_1<p_2$

Table-2 shows resistance pattern in *E. coli* isolates from drinking water and clinical sources and p – values for the significant differences in resistance rates.

Table-3 gives the prevalence of multi-drug resistant *E. coli* for various numbers of antimicrobial agents isolated from drinking water and clinical sources.

Results

Table-2 shows the percentage of *E. coli* isolates from the drinking water sources resistant to various antimicrobial agents.

Of the â-lactam group, the two penicillin (AMX & AUG) agents tested, were found to have significantly higher ($p < .001$) resistance in the strains of clinical origin as compared to those of drinking water origin. Of the three Cephalosporins tested, the first generation agents LEX was observed with statistically comparable resistant isolates from both the sources, while the second generation FRX and third generation CRO had significantly higher number of resistant isolates from clinical sources as compared to that from drinking water sources ($p< .001$).

The *E. coli* from clinical sources (38.1%, 16/42) had significantly higher ($p <. 01$) resistance rate against GEN (17.14%, 12/70) than those from the drinking water. However, the other aminoglycosides *i.e.* AMK & NET had comparable resistant rates in the strains of the bacteria isolated from both the above sources.

Against CIP *E. coli* strains of clinical sources (69.04%, 29/42) were observed to have significantly higher ($p < .001$) resistant rate than that of the drinking water origin (15.11%, 11/70). OFL also showed significantly higher resistance ($p < .001$) in *E. coli* of clinical sources (71.04, 30/42) than that of the drinking water sources (12.85%, 9/70).

However, the most commonly used antibiotics like CHL, TRI, TET & FDZ were observed with significantly higher ($p < .001, .001, .05$ & $.05$ respectively) resistance in clinical as compared to that of from drinking water sources. (Table-2).

Table-3 shows the prevalence of MDR in *E. coli* from clinical and water sources for various numbers of combinations ranging from 2-14 antimicrobial agents in it. When drug resistances to single drug or up to 4 drugs in combination were analyzed, the isolates from water sources were found to be significantly higher in percentage as compared to clinical isolates. When number of drugs were 5-9 in different combinations prevalence of MDR isolates were observed statistically not significantly different in both the study groups. In contrast to above observation, percentages of resistant *E. coli* against 10-12 drugs in various combinations were significantly higher ($p < .05$) in clinical sources than those from drinking water sources.

Discussion

In the present study the strains of the *E. coli* isolated from different drinking water sources (well, hand pump, jet pump, stored water *etc.*) showed maximum resistance rate to those antibiotics, which are taken orally as well as used commonly in veterinary and poultry practices. These antibiotics included TET, AMX, AUG, LEX, CHL, and FZN in descending order of their rates of the resistance against the *E. coli*. In contrast to the above observation resistance rates in clinical isolates of *E. coli* antibiotics, > 78 % were resistant to at least 11 antibiotics. More than 92% of the clinical strains were resistant to AMX, such high level of resistance has already been reported from South and North India[21].

This â-lactam antibiotic is the most widely used antimicrobial through out the world[3,14]. Therefore resistance to this antimicrobial in clinical isolates of *E. coli* is a useful indicator of resistance arising from antibiotic selection of resistance strains in naturally susceptible species. Although 47% of the strains isolated from drinking water sources were observed to have resistance against AMX, which is significantly lower than that of the clinical isolates, but existence of such high rate in the latter sources deserve serious concern.

The possibility of these strains coming to water from human, other mammals and birds cannot totally be excluded. The latter two sources contaminating water is the quite likely phenomenon. This suggestion can further be augmented by the fact that only < 16% of the *E. coli* isolates from drinking water sources were found to have resistance against Quinolone group which are still not frequently used in farming environment. Similarly, significantly lower resistance was also observed against the second and third generations of Cephalosporin, which are exclusively used in clinical environment. The higher resistance isolates from the clinical environment in the present study may be because of availability of

TABLE-3 : Prevalence of multi-drug resistant *E. coli* for various numbers of antimicrobial agents isolated from drinking water and clinical sources.

Various numbers of antibiotics	Number and % of resistant *E. coli* from Drinking water sources (70)	Number and % of resistant *E. coli* from clinical sources (42)	P -values
1	**14(20.00)**	**3 (7.41)**	.01
2	**16(22.85)**	**3(7.14)**	.05
3	**10(14.28)**	**1(2.38)**	.05
4	**7(10.00)**	**1(2.38)**	.05
5	3(4.28)	4(9.52)	NS
6	4(5.71)	5(11.90)	NS
7	2(2.85)	8(19.47)	NS
8	3(4.28)	2(4.76)	NS
9	0(0)	3(7.14)	NS
10	0(0)	6(14.28)	*.05*
11	1(1.42)	2(4.76)	*.01*
12	0(0)	4(9.52)	*.001*
13	**0(0)**	**0(0)**	-
14	**0(0)**	**0(0)**	-

Note: *1. Normal numbers are P –values for $H_{1.}$*

2. Italic bold is P –values for $H_{2.}$

3. NS means not significant.

antimicrobials over the counter without any control[4, 21]. Unlicensed productions of antimicrobial, poor sanitary condition and contaminated water supply have also added to antibiotic resistance[12].

The hypothesis that the *E. coli* isolates from drinking water sources were essentially not of the human origin may be proposed because significantly higher number of the isolates from this source (64%) were found to have resistance against lower number of combination (4or less) as compared to that of the clinical sources (17%). In contrast to this when higher numbers of combinations *i.e.* 10-12 drugs were evaluated, significantly higher percentage (28%) of the clinical isolates were found to have resistance as compared to only 1 isolate (1.42%) from water sources having resistance against 10 drugs. This single strain might be of the human origin contaminating the water.

Antibiotic use provides selective pressure favoring resistant bacterial strains; inappropriate use increases the risk for the selection and dissemination of antibiotic

resistant bacteria, which was placed at a competitive advantage. Therefore, one would expect that the drugs more commonly affected by bacterial resistance in developing countries are generally inexpensive and popular broad-spectrum agents and have caused resistance in bacteria[7,10,26]. However, relationship between antibiotic use and emergence and spread of resistance is complex. Antibiotics use in clinical practices alone cannot explain the high frequency of resistance as has been observed in the present study.

Developments of resistance in animal and poultry have been encouraged particularly through use of the antibiotics as at prophylactic and therapeutic levels in animal feed. This procedure is aimed at preventing infectious diseases caused by bacteria and protozoa are decreasing the amount of feed needed and increasing the rate of animal weight gain[30]. Further, the use of the antimicrobials in farming practices is for an indeterminable duration[5]. Therefore, the importance of this reservoir of antibiotic resistant gene coming to drinking water sources must not be underestimated. Aquatic environment has been reported to act not only as a reservoir of resistance but also as medium for the spread and evolution of resistant gene and their vectors[32].

The weakness of this study is that the Polymerase Chain Reaction (PCR) could not be used for identification and discrimination of different sources of *E. coli*. The strength of this study is the sampling design for collection of drinking water samples from all the wards of the Varanasi city. No such type of study has been done earlier in this region. The obtained result is therefore representing the resistant status in the whole of the Varanasi city.

However, this can be avoided if we gain knowledge not only of resistance mechanism but also of the reservoir and sources of resistant strains, to enable rational approach in the control and eradication of the resistant organism and thus to preserve the usefulness of antibiotics. Therefore, the observation made in the present study suggests that judicious use of the antimicrobials is not only essential in clinical environment but also in animals and poultry farming practices.

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