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Antibiotic Susceptibility of Thermo-Tolerant *Escherichia coli* 2 Isolated from Drinking Water of Khairpur City, Sindh, Pakistan

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Abstract: A total 72 drinking water sample were collected and analyzed by membrane filtration method during 1 year study from various points in Khairpur City . Out of these 58 (80.55%) samples were found to be contaminated with thermo-tolerant *Escherichia coli* 2. The susceptibility of these isolates to 35 antibiotics was studied by disc diffusion method and the organism was highly sensitive to levoflaxin, cefipime, enoxobid, noroxin, tarivid, ciproxin, avelox, amikacin, kanamycin, rocifin, pipenedic acid and slightly sensitive to cravit, naladixic acid, neomycin, cefizox, fortum cefotaxime, cefizox, fortum, tobramycin and cefoperoxone. The resistance against 16 antibiotics such as meropenem, linkomycin, fusidic acid, orbenin, penicillin, streptomycin, bacitracin, minocin, zinacef, amoxil, ceclor, claracid, cephalexin, augmentin, cephradin and dalacin was shown by these isolates. We report the presence of multi-drug resistance in thermo-tolerant *Escherichia coli* isolated in municipal water with different levels of prevalence in Khairpur City. In this study a higher number of positive results were obtained in all sampling points indicating the more fecally polluted municipal water.

Key words: Drug-resistance, drinking water, contamination, treatment, developing countries

INTRODUCTION

The World Health Organization (WHO) says that every year more than 3.4 million people die as a result of water related diseases, making it the leading cause of disease and death around the world. Most of the victims are young children, the vast majority of whom die of illnesses caused by organisms that thrive in water sources contaminated by raw sewage. At any given time, close to half the population of the developing world is suffering from waterborne diseases associated with inadequate provision of water and sanitation services (WHO, 2005). The effect of these diseases vary in severity from an upset stomach to death, every year around 2.2 million people die because of coliform diarrhea (Sonu *et al*., 2007). In 1990 an epidemic of cholera caused wide spread suffering and deaths in South America, (Daniel and Edward, 2005). At present, 9 countries in the Southern Africa Region are reporting cholera cases. Trans-border infections have been recorded and cholera is becoming endemic (recurrent throughout the year) in most of the affected countries. Socio-economic and political factors, growing urbanization, population movement and lack of appropriate response are aggravating factors. There are concerns that, with the rainy season, the disease will spread even further (OCHA, 2009).

Efforts are being taken by all technological advancements including antibiotic usage to control transmission of waterborne diseases, but multi-drug resistance by these *Escherchia coli* warrant the beginning of steps to prevent the public health hazards (Tambekar *et al*., 2006; Pandey and Musrat, 1993; Parveen *et al*., 1997). Resistance to antimicrobial drugs is increasing day by day worldwide in almost all bacterial genera and to almost all drug classes. The use, misuse and abuse of antibiotics are held to be responsible for this development (Austin *et al*., 1999; Bronzwaer *et al*., 2002). Keeping in view the public health effects of waterborne pathogens i.e., *E. coli*, since it has been used as an indicator of water quality and to assist the control of water borne diseases (Ejaz and Ahmad, 2001; Kjrschner *et al*., 2004; Araujo *et al*., 1997). We aimed to evaluate the bacteriological quality of drinking water by isolating thermo-tolerant *Escherichia coli* (indicator of fecal contamination) in municipal water of Khairpur City and their possible association with drug-resistance.

MATERIALS AND METHODS

Sampling: Drinking water samples were collected from different points of Khairpur City i.e., main reservoir (n = 24), distribution line (n = 24) and consumer taps (n = 24) total (n = 72) samples were collected fortnightly

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for 1 year to analyze the source of *E. coli* and their response to various antibiotics, in municipal water used for drinking; all points were supplied by same water network which distributes water, originating from surface water sources. Samples were collected in sterilized screw cap (500 mL) white glass flasks (Pyrex), after a flow time of 5 min to eliminate any contaminant present. In order to neutralize the residual free chlorine, 10% solution sodium thiosulfate was added in sterile bottles and then samples were placed in ice boxes and brought to laboratory. An analysis was carried out after 2 h of collection keeping in view the standards of World Health Organization (WHO) (Luby *et al*., 2001).

Bacteriological analysis: *Escherichia coli* was isolated from drinking water of Khairpur City by membrane filtration method using membrane filter white, with pore size (0.45 μm) and diameter (47 mm) (Millipore) on Eosine Methylene Blue (EMB) agar by filtering (100 mL) of sample the membrane filter placed on EMB agar and incubated at 44.5°C the colonies with green metallic sheen were selected as *E. coli* and confirmed by performing oxidase test and API 20E (Biomerieux) (Table 2). Susceptibility range of antibiotics was determined by a disc diffusion method on nutrient agar (Oxoid), total 35 antibiotics were used in the study (Table 3).

Statistical analysis: The descriptive statistics was done by using SPSS software.

RESULTS AND DISCUSSION

Of the total 72 samples tested, 58 were found to be positive for the presence of thermo-tolerant *E. coli* on Eosine Methylene Blu e (EMB) agar when incubated at (44.5°C) indicating a prevalence of 80.55%. Over all, 80.55% (58 of 72 samples were positive for *E. coli* after isolation and biochemical identification (Table 2). The prevalence was 54% from main storage reservoirs, 92% from main distribution line and 96% from consumers tape i.e, households, hotels and hospitals (Table 1).

Antibiotic susceptibility testing: Fifty eight isolates of thermo-tolerant *Escherichia coli* indicator of fecal contamination analyzed were resistant to sixteen antibiotics, such as meropenem, linkomycin, fusidic acid, orbenin, penicillin, streptomycin, bacitracin, minocin, zinacef, amoxil, ceclor, claracid, cephalexin, augmentin, cephradin and dalacin and sensitive to nineteen different antibiotics. The organism was highly sensitive to levoflaxin, cefipime, enoxobid, noroxin, tarivid, ciproxin, avelox, amikacin, kanamycin, rocifin, pipenedic acid and slightly sensitive to cravit, naladixic acid, neomycin, cefizox, fortum cefotaxime, cefizox, fortum, cefotaxime, tobramycin and cefoperoxone (Table 3).

A very high percentage of samples tested (80.55%) were positive for the presence of thermo-tolerant *Escherichia coli* by membrane filtration method (Table 1) which is the indicator of fecal contamination, when incubated at elevated temperatures 44.5°C. Such a high prevalence (80.55%) of *E. coli* in drinking water strengthen the earlier observation by Shar *et al.* (2008). The prevalence of *E. coli* was the highest (96%) in consumer taps and (92%) in distribution line. This is to be expected because of cross contamination by sewage water due to leakage of water distribution pipes at various points where contamination with fecal material is often recorded. It is of significance that water from municipal

Table 1: Prevalence of thermo-tolerant *Escherichia coli* in municipal water collected from different points in Khairpur City

Source of water	Sampling point	No. of samples	No. of positive for *E. coli*	Prevalence of *E. coli* (%)
Surface water	Municipal reservoir	24	13	54.00
Surface water	Distribution line	24	22	92.00
Surface water	Consumer's tap	24	23	96.00
Total		72	58	80.55

Table 2: Biochemical reaction of thermo-tolerant *Escherichia coli* 2 on API 20 E

Reaction	Result	Reaction	Result
OPNG	+	GLU	+
ADH	-	MAN	-
LDC	+	INO	-
ODC	-	SOR	+
CIT	-	RAH	+
H_2S	-	SAC	-
URE	-	MEL	-
TDA	-	AMY	-
IND	-	ARA	+
VP	-	OX	-
GEL	-		

+: Present, -: Absent, OPNG: Ortho nitrophenyl-βD-Galctopyrenoside, ADH: Arginine dihydrolase, LDC: Lysine decarboxylase, ODC: Ornithine decarboxylase, CIT: Citrate, H_2S: Hydrogen sulphide, URE: Urease, TDA: L-tryptophane deaminase, IND; Indole, VP: Voges proskauer, GEL: Gelatinase, GLU: Glucose, MAN: Maunitole, INO: Inositol, SOR: D-sorbitol, RHA: L-rhaminose, SAC: D-saccharose, MEL: Melibiose, AMY: Amygdalin, ARA: L- Arabinose, OX: Oxidas

Table 3: Multi-drug resistance of thermo-tolerant *Escherichia coli*

Antibiotic	Quantity (μg)	Sensitivity	Antibiotic	Quantity (μg)	Resistant
Cefipime	5	S	Meropenem	10	R
Imepenem	30	S	Lyncomycin	10	R
Naladixic acid	10	S	Fusidic Acid	10	R
Neomycin	30	S	Orbenin	5	R
Cefizox	30	S	Penicillin	10	R
Enoxobid	30	S	Streptomycin	10	R
Fortum	30	S	Bacitracin	10	R
Cefotexime	30	S	Monocin	30	R
Noroxin	30	S	Zinacef	30	R
Tarivid	10	S	Amoxil	10	R
Tobramycin	5	S	Ceclor (BBL)	30	R
Ciproxin	10	S	Clarid	15	R
Cefoperoxone	5	S	Cephalexin	30	R
Avelox	75	S	Augmentin	30	R
Amikacin	5	S	Cephradime	30	R
Kanamycin	30	S	Dalacin	2	R
Rocifix	30	S			
Pipenedic Acid	20	S			
Levofloxin	5	S			

S: Sensitive; R: Resistant

reservoir (main reservoir) had also a high prevalence of *E. coli* (54%), which indicates the in adequate disinfection treatment of water at municipal level. This suggests that water used for drinking purpose in Khairpur City is of poor quality and is the important source of waterborne diseases like coliform diarrhea, gastroenteritis and dysentery. The quality of drinking water in most cities of Pakistan is not good. Drinking water of many localities in Karachi was also found contaminated with high numbers of fecal coliform (Luby *et al*., 2001). Results of our study indicate that it is necessary to determine the prevalence *E. coli* from water intended for drinking in order to prevent the fecal contamination on regular basis. The isolation of multi-drug resistant thermo-tolerant *E. coli* will help further characterization of such strains. The presence of pathogenic strains in drinking water of Pakistan needs to be studied for the establishment of diseases burden from fecally contaminated water, which may be containing the etiologic agent of various waterborne diseases. In case of multi-drug resistance this study is supporting the results from other investigations of various scientists; the emergence of antimicrobial resistance in bacteria has been problem throughout the world (Cohen, 2000). Tambekar *et al.* (2006) reported the resistance of *E. coli* isolates to oflaxacin followed by novabiocin, cefdixin and ciprofloxacin. The azithromycin, gentamycin, amikacin, chloramphenicol, co-trimexazole and tetracycline were the most effective. It has been reported that beta-lactamase producing *E. coli* which had become resistant to ceftriaxome can become sensitive to the same antibiotic when the inhibitor sulbactam is added (Abdul *et al*., 2005). Alhussain *et al.* (2005) recorded the excellent susceptibility to meropenem and variable susceptibility to aminoglycoside and fluroquinolones but greatly reduced susceptibility to beta lactam beta lactamase inhibitors combination, trimethoprime and sulphamethoxazole in Extended Spectrum Bbeta Lactamase (ESBL) producing *E. coli.* During the work on *in vitro* susceptibilities of *E. coli* ampicillin-sulbactam and amoxicillin-clavulamic acid, Birgul and Nedim (2007) found that more organism were susceptible to amoxicillin-clavulamic acid than ampicillin-sulbactam. Wimmerstedt and Kahlmets (2008) investigated the trimethoprime resistance in ampicillin resistant than ampicillin susceptible isolates of *E. coli.* The results of the multi-drug resistance study (Table 3) indicate that *E. coli* resistant to sixteen different antibiotics is widespread in municipal water of Khairpur included in this study. The emergence of multi-drug resistance among these bacteria and their presence in drinking water is the matter of grave concern. In Khairpur the untreated domestic sewage is being disposed off in canals (source water). The presence of drug residues in such water can not be ruled out because that sewage also contains hospital wastes, further surface runoff during rainy season might introduce these bacteria into the fresh water environment. Hence it is possible that municipal water taken from surface water of river could be contaminated with *E. coli.* The presence of penicillin amoxil streptomycin and other antibiotic resistance in certain strains of *E. coli* might suggest a hospital or veterinary origin of such strains. *E. coli* in aquatic environment is exposed to sub-lethal doses of antibiotics present in that environment brought in by disposed waste. The use of antibiotic is widespread in animal industry. It i estimated that the use of antibiotics in animals is 100-1000 times more than that in human population (Feinman, 1998; Witte, 1998). No study so for has been done to evaluate the impact of frequent antibiotic use in animals as well as in human in Pakistan.

The uncontrolled usage of antibiotics in Pakistan contributes significantly antibiotic residues in fresh water environments. The antibiotic resistance patterns of strains of *E. coli* isolated from drinking water observed in present study suggests a greater risk in form of transfer of resistance to other pathogenic bacteria. The possible exchange of plasmids between *E. coli* and other bacteria has been previously reported (Grabow, 1976). The antibiotic resistance could be transferred from non-pathogenic to pathogenic bacteria by transfer of resistant genes in closely related bacteria such as members of Enterobacteriaceae family, It is remarkable that Alhussain and Naeem (2005) reported in their studies where *E. coli* was observed susceptible to meropenem, but the present study reported the resistance in *E. coli* to this antibiotic. The sources and routes of contamination in water are difficult to establish, as fresh water receives bacterial population from diverse sources. An effective surveillance infrastructure is needed to be made to determine the presence and distribution of antibiotic resistant strains of *E. coli* in municipal water in Pakistan.

CONCLUSION

In this study the detection of multi-drug resistant strains of *Escherichia coli* in municipal water indicates the necessity of regular appropriate monitoring by local health authority, to ensure the best possible control over the water treatment system. In fact, emergence of multi-drug resistance in *E. coli* is the matter of concern which makes the treatment difficult in case of waterborne diseases. The future studies could be focused to analyze the mechamism of exposure of bacteria to antibiotics and dissemination of antibiotic resistant bacteria in different fresh water environment.

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