STUDIES ON ANTIBIOTICS AND HEAVY METAL RESISTANCE PROFILING OF *ESCHERICHIA COLI* FROM DRINKING WATER AND CLINICAL SPECIMENS

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ABSTRACT

Bacterial resistance to antibiotics and heavy metals is an increasing problem in today's society. Antibiotics resistances in the clinical isolates were high as compare to *E.coli* from drinking water. The drinking water and clinical *E.coli* showed more or less equal resistance to antibiotic: metronidazole, penicillin, clindamycin, cephoxithin and heavy metals; copper, mercury and lead, except cadmium metal ions. Multiple antibiotic resistance (MAR) indices in the clinical isolates were high as compare to MAR indices of drinking water. *E.coli* isolates showed higher MAR indices to cephalothin, cephoxithin, clindamycin, metronidazole, penicillin and vancomycin indicated its human origin in drinking water. No significant variation in heavy metal tolerance (HMT) was recorded from both types of isolated. Thus MAR indices were much more reliable indicator to differentiate origin of *E.coli.*

Key words: E.coli, MAR, Heavy metal tolerance

INTRODUCTION

Bacterial resistance to antibiotics and heavy metals is an increasing problem in today's society. Resistance to antibiotics is acquired by a change in the gene makeup of bacterium, which can occur by either a gene mutation or by transfer of antibiotic resistance genes between bacteria in the environment. The increasingly use of antibiotics in health care, in agriculture and animal husbandry is in turn contributing to the growing problem of antibiotic resistant bacteria (Dhanorkar and Tambekar, 2004). Heavy metals used in industry and in household products are, along with antibiotics creating a selective pressure in the environment that leads to the mutations in microorganisms that will allowed them better survive and multiply (Calomiris *et al.,* 1994).

A correlation exists between metal tolerance and antibiotic resistance in bacteria because of the likelihood that resistance genes to both (antibiotics and heavy metals) may be located closely together on the same plasmid in bacteria and are thus more likely to be transferred together in the environment. Bacteria isolated from drinking water showed high resistance to metals and to antibiotic (Kawane and Tambekar, 2004). Krumperman (1983) showed that the multiple antibiotic resistance indices of *Escherichia coli* from wild animals was generally low, while human and poultry isolates had higher MAR indices. Kaspar and Burgess (1990) reported that there were larger multiple antibiotic resistance of *Escherichia coli* isolated in urban areas than from rural areas. Bacterial resistance to toxic heavy metals is a widespread phenomenon and reported to enhance the antibiotic resistance ability of microorganisms (Edlund *et al,* 1996). Ramteke (1997) studied on the antibiotic resistance of 448 coliforms isolated from drinking water and their tolerance to heavy metals. More than 90% of isolates showed resistance to one or more antibiotics tested and expressed tolerance to multiple metals. Parveen *et al.*, (1997) studied total 765 *Escherichia coli* isolates from point and nonpoint sources and their multiple-antibiotic resistance (MAR) profiles with 10 antibiotics and stated antibiotics resistance pattern influenced by geographical areas. Pathak and Gopal (1994) studied on antibiotics resistance and metal tolerance among coliform from drinking water in a hilly area. Karbasizaed *et al.,* (2003) studied the antimicrobial, heavy metal resistance patterns and plasmid profiles of coliforms isolated from nosocomial infections and healthy human feaces and showed 72% stains isolated from nosocomial infections posses MAR, compared to 45% of strains from healthy faeces. Most strains isolated from hospital were more tolerant to heavy metal than those from healthy persons.

Tambekar and Charan (2004) had reported antibiotic sensitivity indexing of *Escherichia coli* to identify source of faecal contamination in drinking water in Purna Valley of Vidarbha. Very little information is available to differentiate drinking water and clinical *E.coli* isolates on the basis of MAR and HMT hence this study was undertaken to investigate any relationship between the previously proved antibiotics resistance and heavy metal tolerance to *Escherichia coli* isolated from drinking water and clinical specimen.

MATERIALS AND METHODS

Escherichia coli, 30 each strains were isolated from contaminated drinking water and clinical specimen collected in Amravati. The isolated strains of *Escherichia coli* were identified and confirm on the basis of standard techniques. These 30 + 30 *Escherichia coli* cultures then studied for their antibiotic and heavy metal resistance pattern by disc diffusion and cup method. Zone of inhibition of the growth counted as sensitive to the antibiotics and heavy metals.

Following antibiotics and heavy metals were used in the study.

Antibiotics	Quantity	Antibiotics	Quantity
Carbenicillin (Cb)	100mcg	Gentamycin (G)	10mcg
Cephoxithin (Cn)	30mcg	Oflaxacin (OF)	10mcg
Chloramphenicol (C)	30mcg	Cephalothin (Ch)	30mcg
Clindamycin (Cd)	20mcg	Vancomycin (Va)	30mcg
Erythromycin (E)	15mcg	**Heavy metals**	
Metronidazole (Mt)	15mcg	$HgCl_2$	Concentration varies from 50μg to 500μg
Penicillin (P)	10mcg	$CuSO_4$	
Co-trimoxazole (Co)	25mcg	$CdSO_4$	
Tetracycline (T)	30mcg	$PbNO_3$	

MAR Index:

$$\text{The MAR index for a antibiotic} = \frac{\text{Number of antibiotics resistance isolates}}{\text{No. of antibiotics tested X no. of isolates}}$$

$$\text{The HMT index for a heavy metal} = \frac{\text{Number of heavy metal tolerant isolates}}{\text{No. of heavy metal tested X no. of isolates}}$$

RESULTS AND DISCUSSION

To assess on antibiotic and heavy metal resistance profile of *Escherichia coli* isolated from drinking water and clinical specimen, in all 30 + 30 = 60, clinical and drinking water isolated were screened for antibiotic sensitivity against thirteen antibiotics and four heavy metal ions (Hg^{2+}, Cu^{2+}, Pb^{3+} and Cd^{2+}). Thirty each *Escherichia coli* isolated from drinking water and from clinical specimen showed resistance to most of antibiotics a high frequency of resistance among *Escherichia coli* strain isolated from clinical and drinking water to antibiotics was observed (Fig 1 and 2). The drinking water and clinical isolates showed more or less equal resistance to antibiotic; Metronidazole, Penicillin, Clindamycin, Cephoxithin and heavy metals; copper, mercury and lead. In case of cadmium, the HMT was 86.6% in drinking water where as 70 % in clinical *Escherichia coli* (Fig 3). Least tolerance was recorded in mercuric (20%) in both clinical and drinking water *Escherichia coli* isolates. More than 46% of isolates showed resistance to one or more antibiotics tested in drinking water isolates and 64% in clinical isolates (Fig. 1). Similarly large number (62%) of drinking

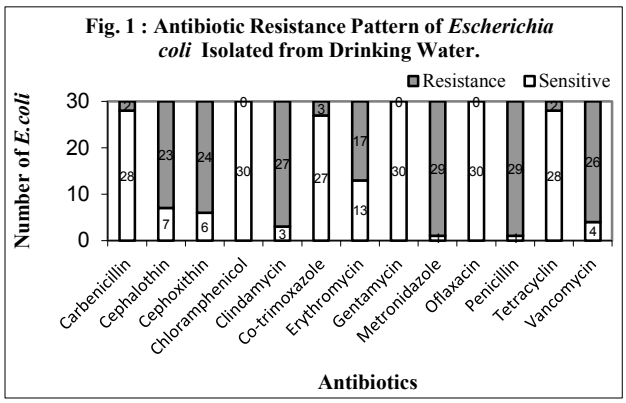
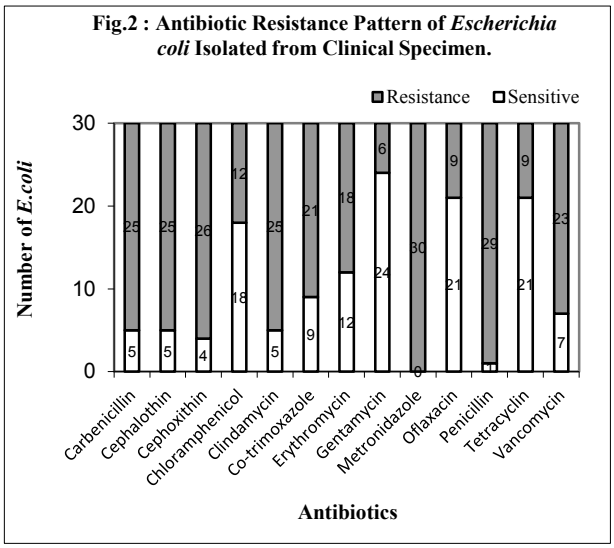
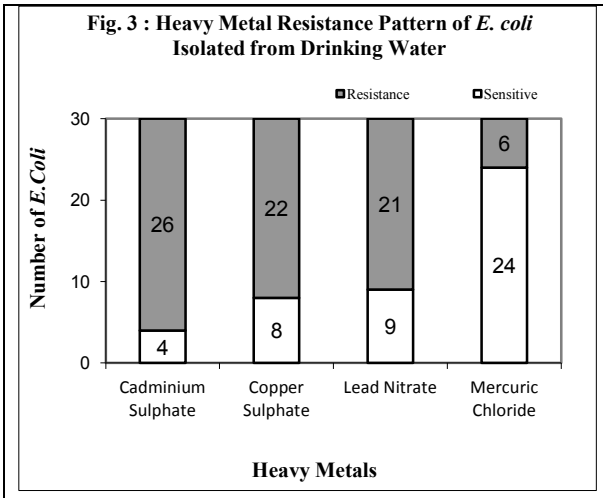
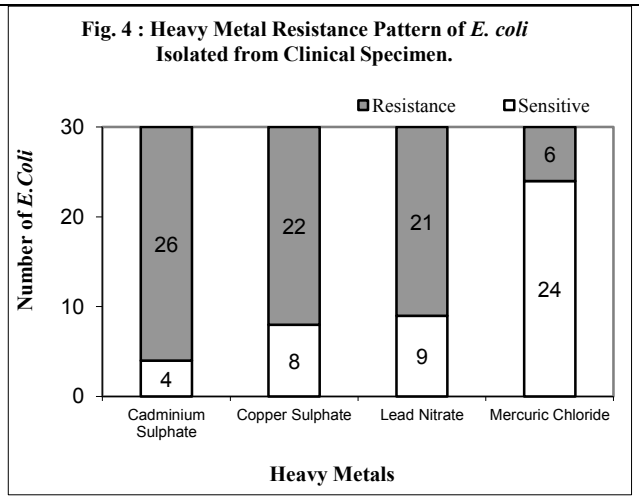


Fig. 1 : Antibiotic Resistance Pattern of *Escherichia coli* Isolated from Drinking Water.



water isolates was more tolerant than clinical isolates (57%) to Cu, Cd and Pb (Fig. 4). No variations were observed in metal tolerance pattern of clinical and drinking water isolates except cadmium metal ions where it 70% and 86.6% respectively indicating contamination of cadmium ions in drinking water. The incidence of high level of metal tolerance among bacteria could be attributed to release of metal ions in water bodies due to geochemical processes. No resistance was shown to three antibiotics Oflaxacin, Chloramphenicol and gentamycin by drinking water isolates where as it showed 30%, 40%, and 2% in clinical isolates. The resistant develop due to acquiring during environmental adaptability





Antibiotics resistances in the clinical isolates were high as compare to *E.coli* from drinking water (Fig. 1). Antibiotics resistance of *E.coli* from drinking water against chloramphenicol, gentamycin and oflaxacin were zero. Those isolates showed higher resistance against Cephalothin, Cephoxithin, Clindamycin, Metronidazole, penicillin and Vancomycin indicated the *E.coli* in drinking water of human origin. No significant variation in HMT was recorded in heavy metal from both types of isolated (Fig. 2). Thus MAR indices were much more reliable indicator to differentiate origin of *E.coli*.

Plate showing antibiotic and heavy metal resistance of *E. coli* isolated from clinical specimen

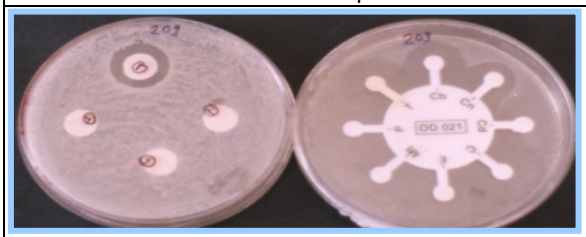


Plate showing antibiotic and heavy metal resistance of *E. coli* isolated from drinking water

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