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Antibiotic Resistance of Coliforms Isolated From Fresh Drinking Water of Nanded District (MS), India

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ABSTRACT

Water-borne disease outbreaks associated with the drinking of unsafe water, containing pathogenic bacteria, are common in densely populated countries like India. The present study was attempted to detect indicator bacteria from drinking water samples for the presumptive occurrence of contaminations that are responsible for health-associated problems. Therefore, a laboratory-scale qualitative analysis through the most probable number (MPN) method was employed.A total of six coliform bacteria isolated from fresh drinking water samples were tested against four antibiotics to determine the prevalence of antibiotic resistance and also to find out the high-risk source of contamination. Almost all of the identified coliform bacteria showed resistance against commonly used antibiotics which is of significant health concern.

Keywords: Drinking water, Most probable number (MPN), Coliform, Contamination.

I. INTRODUCTION

Coliforms are a group of indicator bacteria in water, soil, and other environments, often considered as a measure of water quality. They represent major contaminants in surface and groundwater in developing countries. Recently, the widespread use of antibiotics in agriculture and medicine is accepted as a major selective force in the increasingly high incidence of antibiotic resistance among bacteria [1,2]. High antibiotics resistant bacteria are found in environments such as hospital effluents, sewage, and wastewater [3].Coliforms, generally regarded as non-pathogenic indicators ofpollution [4]but eventually used to study the bacteriological quality of water and foods. It hasbeen demonstrated that antibiotic-resistant coliform bacteria from effluents and land runoffeventually may enter into waters[5]. River water is the main reservoir of antibiotics and antibiotic-resistant bacteria in the environment. They are directly introduced into surface water through fisheries, animal farms, and agricultural practices [6]. A large number of sewage and effluent containing antibiotic-resistant bacteria arereleased into rivers, streams, lakes, and seawater [7]. The antibiotic resistance bacteria in drinking water are a prime concern to public health [8].

The present study was designed to investigate multiple antibiotic resistance of coliform bacteria isolated from the fresh drinking water of the Nanded district.

II. MATERIALS AND METHODS

2.1 Study area and sampling

The drinking water samples used by the residents of Umri and Nanded (Vishnupuri and University campus) were tested in the current study. Three watersamples were collected from separate household and commercial points which were used to consume after pre-treatments during Feb. 2018. Samples were collected aseptically in sterile screw-capped bottles and transported to the laboratory under cold storage within 24 hours for microbiological analysis [9].

2.2 MPN (Most Probable Number)

The most probable number (MPN) techniquewas used[10] for the enumeration of total coliform. The evaluation procedure included three tests namely presumptive, confirmative, and completed test.

2.2.1 Presumptive Test

For each water sample; 5 tubes of each 10, 1, and 0.1 ml were used. 10 ml sample was inoculated in double strength of MacConkey broth media and rest 1 and 0.1 ml was inoculated in single strength MacConkey broth media. All the inoculated tubes were incubated at 37°C for 24–48 hrs. Tubes which showing the presence of growth (turbidity) with or without gas were submitted to the confirmatory phase.

2.2.2 Confirmative Test

All tubes which show positive results from presumptivetest were lightly shaken and using a micropipette and sterile tips culture were added to BGBL (Brilliant Green Bile Lactose Broth) and incubated at 37°C for 48 hrs. The gas formation within 48 hrs was taken for the completed test. Then the Numbers of positive tubes were recorded.

2.2.3 Completed Test

The cultures from the positive tubes were streaked on EMB (Eosin Methylene Blue) agar and these plates were placed at 37°Cfor 24 hrs in an inverted position. The presence of green metallic sheen colony confirms the presence of coliforms. Some of these isolated colonies from plates were transferred on the non-selective media such as nutrient agar slants for further biochemical testing of coliforms. These isolates were also confirmed by Gram's staining and biochemical tests [11].

2.3 IMViC Test

Allthe selected isolates were subjected to the IMVIC Test (Indole, Methyl red, Voges-Proskauer and Citrate) for the identification ofselected Isolates[14].

2.3.1 Indole Test

The bacterial cultures inoculated into a test tube of 5ml peptone water and then incubated at 37^0C for 24 hours and after that 5 drops of Kovac's indole reagent was added and shaken gently. A positive reaction was indicated by the development of a red color formation on the top layer.

2.3.2 Methyl Red

Isolate were inoculate in 5mlof MR-VP broth and incubated for 48hrs at 35^{0}C and after incubation1ml of the broth was transferred into a test tube and 2-3 drops of methyl red was added. Formation of red color indicates positive methyl red test, a yellow color indicates negative test.

2.3.3 Voges-Proskauer

The culture inoculate in MR-VP broth and incubated for 48 hrs, after incubation 15 drops of 15% alpha–napthal in alcohol was added and 5 drops of 40% KOH was also added then shake gently red color formation within 1hr indicates a positive test

2.3.4 Citrate Test

The isolates were inoculated into Simmon's citrate agar and incubated for 24-48 hrs. Development of a deep blue colour indicates a positive reaction.

2.4 Antibiotic susceptibility test of the identified bacteria

The pathogenic isolates were examined for antibiotic susceptibility traits (either drug-resistant or sensitivity) by well diffusion method on Mueller-Hinton agar against commonly used antibiotics following the standard protocol [13]. Antibiotics used in the study included cefotaxime, ceftriaxone, ciprofloxacin,and Metronidazole.

III. RESULTS AND DISCUSSION

3.1 Bacteriological quality of the drinking water samples tested

All of the water samples used in the present study were highly contaminated with lactose fermentation positive bacteria which determined by the formation of gas in the Derhum tube after 48 hrs of incubation period at 37°C.Sample number 1and 2 showed maximum counts of positive results for each of the three test tubes by looking at the formation of gas resulting in MPN index 920 MPN/100 ml and >1800/100ml of the sample. Sample no. 3 showed the lowest count as 8 MPN/100 ml of sample (Table-3.1).

Table-3.1: MPN index and Confirmed test results of drinking water samples.

Sample No.	Sample collection area	Water samples			MPN INDEX (MPN/100 ml)	Growth on EMB agar	Production of green metallic sheen	Result
		10ml	1ml	0.1ml				
1	Umri	5+	5+	3+	920	+	+	Nonpotable
2	Vishnupuri	5+	5+	5+	>1800	+	+	Nonpotable
3	S. R. T. M. University campus	3+	0+	0+	8	-	-	Potable

Confirmed results showing that samples no. 1 and 2 exhibited green metallic sheen on EMBagar plates (Fig.3.1)showing the presence of fecal coliform i.e., *E. coli*that makes a water sample non-potable (Table 3.1).

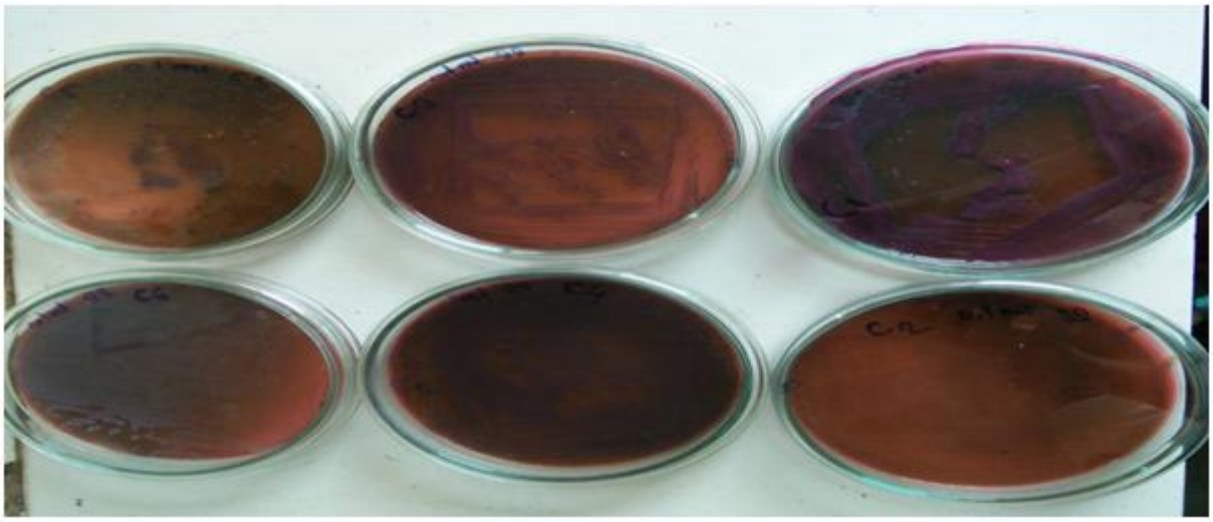


Fig. 3.1: Results of confirmed tests.

In the completed test, water sampleswere tested (1 and 2), bacterial sp. (Fig.3.1) isolates were further confirmed by their Gram reaction as Gram-negative. The presence of the indicator bacteria indicated the possible occurrence of fecal contamination.IMViC test results are shown in table-3.2.

Table-3.2: IMViC Test Results.

Test isolates	Indole	Methyl Red	VP	Citrate	Isolates Result
C1	Negative	Positive	Negative	Negative	*Shigella*
C2	Negative	Positive	Negative	Positive	*Salmonella*
C3	Positive	Positive	Negative	Negative	*E. coli*
C4	Negative	Negative	Positive	Positive	*Klebsiella*
C5	Negative	Positive	Negative	Positive	*Citrobacter*
C6	Negative	Positive	Negative	Positive	*Proteus*

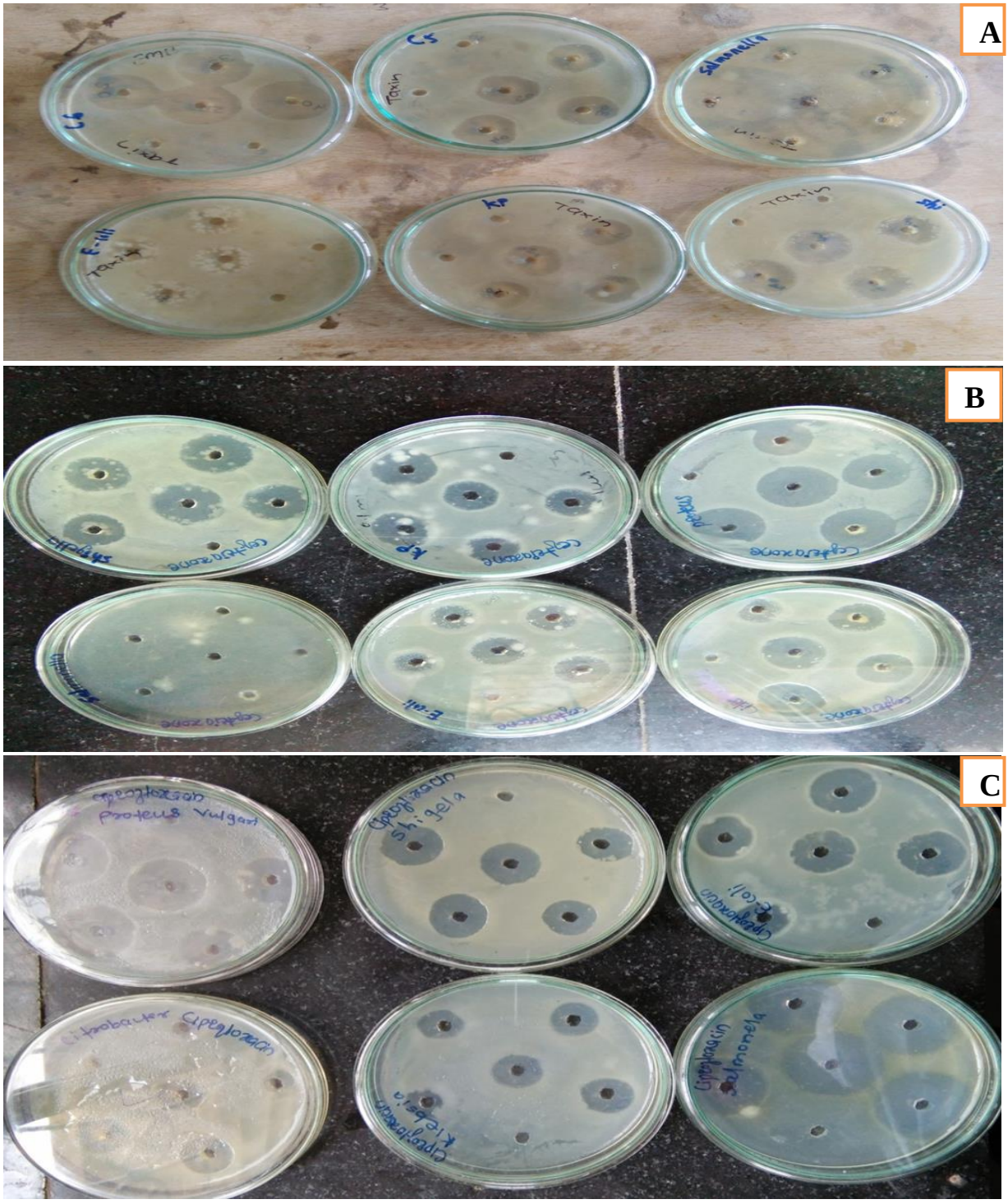
Antibiotic resistance in isolates from fresh drinking water samples

The incidence of antibiotic resistance among the coliform bacteria from the fresh drinking water samples is presented in Tables-3.3 against selected antibiotics. All isolated coliforms from fresh drinking water were resistant to one or more of the antibiotics (Fig.3.2).

The frequency of antibiotic resistanceamong coliform bacteria from fresh drinking water sampleswas found to be highest againstmetronidazole(Fig. 3.2-D). Of the isolated coliforms,*Salmonella* showed resistance againstceftriaxone antibiotics (Fig. 3.2-B). Similarly, at 10 µl concentration coliforms shows resistance but on subsequent increasing the concentration, coliforms show a significant decrease in resistance against cefotaxime(Fig. 3.2-A).The incidence of metronidazole-resistant coliforms was in general higher (Fig. 3.2-D) than thatfound as compared to other antibiotics.Among obtained coliforms, a higher frequencyof multiple resistance was found highest against metronidazole, ceftriaxone, cefotaxime, and ciprofloxacin antibiotics.

Table-3.3: Antibiotic resistance among coliform bacteria from fresh drinking water.

Sr. No.	**Antibiotic resistanceresults**(mm)						
A.	**Cefotaxime (Taxim)**						
	Concentration	*Shigella*	*Salmonella*	*E. coli*	*Klebsia*	*Citrobacter*	*Proteus*
	50µl	22	13	20	21	22	25
	40µl	20	11	18	19	21	22
	30µl	19	11	17	17	18	21
	20µl	17	10	15	16	16	20
	10µl	-	-	-	-	-	-
B.	**Ceftriaxone (Troxane)**						
	Concentration	*Shigella*	*Salmonella*	*E. coli*	*Klebsia*	*Citrobacter*	*Proteus*
	50µl	23	10	18	20	21	23
	40µl	22	-	17	19	21	21
	30µl	21	-	17	17	19	21
	20µl	21	-	15	16	18	20
	10µl	18	-	14	14	16	19
C.	**Ciprofloxacin**						
	Concentration	*Shigella*	*Salmonella*	*E. coli*	*Klebsia*	*Citrobacter*	*Proteus*
	50µl	19	25	20	16	17	24
	40µl	19	24	19	16	15	23
	30µl	18	24	18	15	15	22
	20µl	16	23	16	14	10	19
	10µl	15	21	15	12	06	18
D.	**Metronidazole**						
	Concentration	*Shigella*	*Salmonella*	*E. coli*	*Klebsia*	*Citrobacter*	*Proteus*
	50µl	14	22	16	12	10	20
	40µl	10	20	10	-	-	10
	30µl	-	13	-	-	-	-
	20µl	-	-	-	-	-	-
	10µl	-	-	-	-	-	-


A
B
C

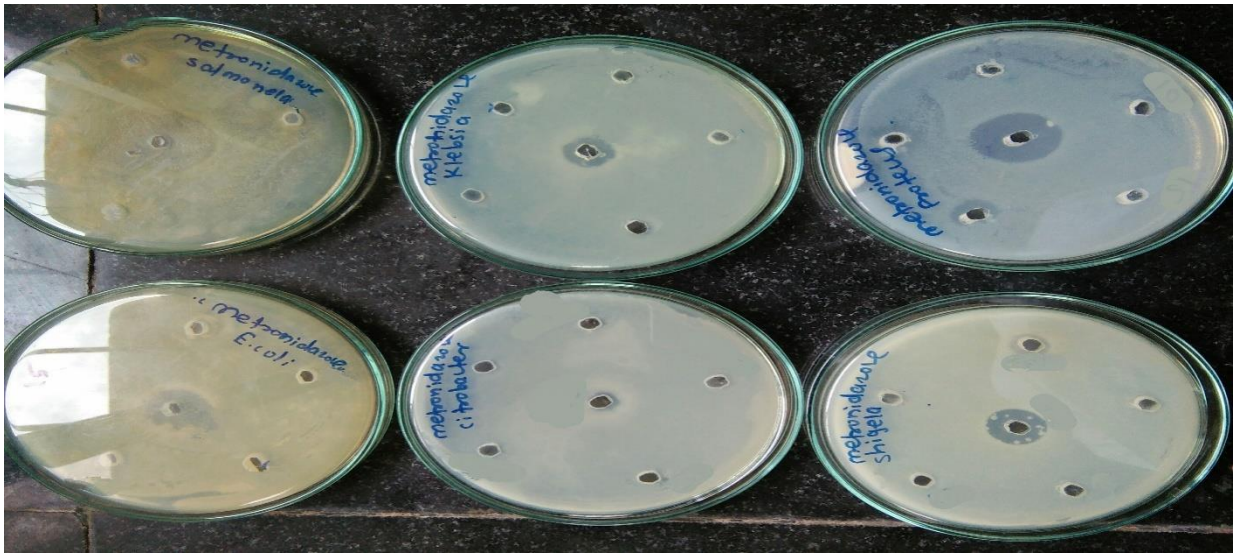


Fig. 3.2: Antibiotic resistance among coliform bacteria from fresh drinking water selected against antibiotics (A) Cefotaxime, (B) Ceftriaxone, (C) Ciprofloxacin, and (D) Metronidazole.

IV. CONCLUSION

The increasing pollution of drinking water sources and the presence ofantibiotic-resistant bacteriaincrease the risk to human health. Therefore, it is important to have detailed knowledge regarding suchissues.The present research shown that a sample of drinking water sources in Vishnupuri&Umrifrom Nanded district regionwere generallylow-qualitydrinking water andantibiotic resistance pools.Therefore,we advisethat this watershould not be consumed without further adequate treatment. Isolated coliformsare resistantto at least one antibiotictested and show multiple resistances.It indicates, the issueof antibiotic resistance is also critical in the water sources.Antibiotic-resistant isolatedcoliforms were still heavily seen in two studied source drinks of water. Asantibiotic resistance is difficult to eradicate, the prevalence of antibiotic resistanceof coliforms in source water it emergesindicates a potential public health hazard

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