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Antibiotic Sensitivity Test of *E. coli* Isolated from Drinking Water from Different Sources in and Around Ranchi, Jharkhand

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The present study was undertaken to isolate and determine the antibiotic sensitivity of *E. coli*, from drinking water samples collected from different sources in and around Ranchi, Jharkhand, India. The coliform count was done using most probable number (MPN). Isolation and identification of *E. coli* were done by morphological characteristics, enrichment media, selective media, and biochemical tests. Antimicrobial sensitivity testing by the disc diffusion method was conducted for 11 antibiotics: Amikacin, Amoxycillin, Ampicillin, Cephalothin, Chloramphenicol, Ciprofloxacin, Colistin, Cotrimoxazole, Enrofloxacin, Nalidixic acid and Oxytetracycline against isolated E.coli. The mean values of coliform MPN/100ml of water ranged from 0 to 1800+. Estimation of coliform in drinking water revealed that of 100 samples examined 47% samples had no coliform count, while in 6% samples the coliform count ranged between 1 to 10, in 7% samples the coliform count ranged between 11 to 20, in 19% the coliform count ranged between 21 to 100 and in 21% samples the coliform count was above 100 coliform per 100 ml of water. The isolates were highly sensitive to drug such as Amikacin (96.1%) and Colistin (96.1%) followed by Ciprofloxacin (92.3%), Chloramphenicol (88.4%) and Cotrimoxazole (84.6%). Intermediate sensitivity was shown for drugs such as Cephalolthin (34.6 %), Oxytetracycline (26.9 %) and Nalidixic acid (15.3 %). Maximum resistant was shown for drugs such as Enrofloxacin (73%), Oxytetracycline (65.3 %) and Cephalolthin (26.9 %). Although most strains of *E. coli* are not pathogenic, their presence is indicative of the possible presence of pathogenic organisms and the antibiotic sensitivity testing proved that the isolated *E.coli* are sensitive to many antibiotics and resistant to few of them.

Key words: Antibiotic sensitivity Test, Coliform count, *E.coli*, Water.

The World Health Organization estimated that up to 80% of all sicknesses and diseases in the world are caused by inadequate sanitation, polluted water or unavailability of water. The pollution of drinking water is responsible for a large number of mortalities and morbidities due to water-borne diseases like typhoid, cholera, dysentery, hepatitis as well as many protozoan and helminthic infestations (WHO, 1997). The availability of drinking water is an indispensable feature for preventing epidemic diseases and improving the quality of life (Borchard *et al.*, 2004). In the present study 100 water samples were collected from different sources like handpumps, wells, taps, ponds and rivers to estimate the faecal pollution. The coliform count was done using most probable number (MPN). Isolation and identification of *E. coli* were done by morphological characteristics, enrichment media, selective media, and biochemical tests. Antimicrobial sensitivity testing by the discdiffusion method was conducted for 11 antibiotics: Amikacin, Amoxycillin, Ampicillin, Cephalothin, Chloramphenicol, Ciprofloxacin,

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Colistin, Cotrimoxazole, Enrofloxacin, Nalidixic acid and Oxytetracycline. Theantimicrobial susceptibility revealed that isolated *E. coli* strains were sensitive against Amikacin (96.1%), Colistin (96.1%) and Ciprofloxacin (92.3 %) but resistant to Enrofloxacin (73%),Oxytetracycline (65.3 %) and Cephalolthin (26.9%).

MATERIALS AND METHODS

A total number of 100 water samples from different sources like hand-pumps, wells, taps, ponds and rivers were collected in and around Ranchi at varying interval for the present study. Water samples were randomly collected from different sources in thoroughly washed and sterilized container. The water sampling bottles meant for bacteriological examination were added with small crystal of sodiumthiosulphate. The bacteriological analysis was conducted within 4 to 6 hours of sample collection. From running taps, the water was collected after allowing the tap to run for 5 minutes. Prior to this the nozzle were cleaned and then washed with an appropriate disinfectant like ethyl alcohol. It was more convenient to flame the nozzle for few seconds to disinfect it. A little space was left while collecting samples to allow mixing as and when required.

The bacteriological quality of water was assessed as per method recommended by APHA (1995).

Most Probable Number (MPN) of Coliform

The presumptive test was performed by inoculating 10 ml of water from each sample into a set of 5 tubes each containing 10 ml of double strength MacConkey broth with inverted Durham's tube. 1 ml and 0.1 ml of water from each sample were inoculated into set of 5 tubes each containing 5 ml single strength MacConkey broth with inverted Durham's tube. All tubes were incubated for 24 hours at 37^0C, after which production of gas in the Durham's tube were noted. The production of gas was considered as positive where as absence of gas production in 24 hours was taken negative.

Confirmed test was applied to the presumptive positive tubes of two highest dilutions by streaking a loopful from each tube on a Eosin Methylene Blue Agar plates and the plates were incubated at 37^0C for 24 hours. The development of typical nucleated colonies with or without metallic sheen after 24 hours incubation was taken positive.

Antibiotic Sensitivity Test

All the isolates of *E.coli* were tested against various antibiotics by paper disc diffusion method given by Bauer *et al.* (1966).

Cultures were incubated overnight in broth media and inocula were prepared. Then the inocula were spread evenly over the entire surface of nutrient agar plates with the help of sterile L-shaped glass rod. The disc of given potency were then applied on the nutrient agar plates with the help of sterile forceps and incubated at 37^0C for 24 hours. The results were recorded as zone of inhibition from the standard table supplied by Himedia Laboratory Private Ltd.

Table 1. Sample collected from different sources in and around Ranchi

Place	Handpump	Well	Tap	Pond	River	Total samples
1.Hatia	5	3	2	-	-	10
2.Doranda	4	2	1	-	-	7
3.Satellite colony	4	3	2	-	-	9
4.Ratu road	4	2	2	-	1	9
5.HEC	2	2	2	2	-	8
6. Main road	3	1	2	-	-	6
7.Bariatu	5	3	1	-	-	9
8. Namkum	2	2	1	1	1	7
9.Kanke	6	3	2	-	1	12
10.RVC	3	1	1	3	-	8
11.Jamshedpur	2	2	1	-	-	5
12.Koderma	4	3	3	-	-	10
Total	44	27	20	6	3	100

RESULTS AND DISCUSSION

The mean values of coliform MPN/100 ml of water ranged from 0 to 1800^{+}. Estimation of coliform in drinking water revealed that of 100 samples examined 47% samples had no coliform count, while in 6% samples the coliform count ranged between 1 to 10, in 7% samples the coliform count ranged between 11 to 20, in 19% the coliform countranged between 21 to 100 and in 21% samples the coliform count was above 100 coliform per 100 ml of water. The study revealed 26 isolates of *E. coli*were subjected to drug sensitivity test which were compared against the standard mention and results are depicted in the Table 2.

The isolates were highly sensitive to drug such as Amikacin (96.1%) and Colistin (96.1%) followed by Ciprofloxacin (92.3%), Chloramphenicol (88.4%) and Cotrimoxazole (84.6%). Intermediate sensitivity was shown for drugs such as Cephalolthin (34.6 %), Oxytetracycline (26.9 %) and Nalidixic acid (15.3 %).Maximum resistant was shown for drugs such as Enrofloxacin (73%), Oxytetracycline (65.3 %) and Cephalolthin (26.9 %).

E. coli is able to acquire resistance easily therefore it is considered as a good bio-indicator model for surveillance studies of antimicrobial resistance. (Von and Marre, 2005)

Similar observation has earlier been reported by Carrosco *et al.* (1997) who found that the coliform were sensitive to Chloramphenicol (94%), Tetracycline (94%) and Sulfathiazole (83%).

Likewise, Tambekar *et al.* (2006) reported that the isolates were sensitive to Amikacin (92%) followed by Chloramphenicol (85%) and Co-trimoxazole (82%).

The findings of the study are also in accordance with the findings of Shar *et al.* (2009) who reported the organism was highly sensitive to Amikacin (99.8%).

Similarly the isolates showed sensitivity against Amikacin (99.8%) and resistance against Nalidixic acid (92.6%) and Ampicillin (88.89%) in the study conducted by Patoli *et al*. (2010).

Summary

Although most strains of *E. coli* are not pathogenic, their presence is indicative of the possible presence of pathogenic organisms. Water is considered safe when it is free of *E. coli* (Wanke, 1990). The indiscriminate use of antibiotics in chemotherapy should be avoided to prevent the development of more resistant strains of *E. coli* and other pathogenic organism. From the coliform count results, 47% of the water samples met the WHO standard fordrinking water which states that the coliform count indrinking water should bezero/ 100ml.

Table 2. Antibiotic resistance patterns of *E.coli* isolated from drinking water

Organism	No. of isolates	Antibiotics	Sensitivity		
			Resistant	Intermediate	Sensitive
E.coli	26	Amikacin	-	1 (3.8)	25 (96.1)
		Amoxycillin	3 (11.5)	2 (7.6)	21 (80.7)
		Ampicillin	5 (19.2)	2 (7.6)	19(73)
		Cephalothin	7 (26.9)	9 (34.6)	10 (38.4)
		Chloramphenical	2(7.6)	1 (3.8)	23 (88.4)
		Ciprofloxacin	1 (3.8)	1 (3.8)	24 (92.3)
		Colistin	1 (3.8)	-	25 (96.1)
		Cotrimoxazole	2 (7.6)	2(7.6)	22 (84.6)
		Enrofloxacin	19 (73)	1 (3.8)	6 (23)
		Nalidixic acid	3 (11.5)	4 (15.3)	19 (73)
		Oxytetracycline	17 (65.3)	7 (26.9)	2 (7.6)

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