Research Journal of Microbiology

ISSN 1816-4935

Academic
Journals Inc.

www.academicjournals.com

Research Journal of Microbiology 1 (5): 458-462, 2006
ISSN 1816-4935

Prevalence of Thermotolerant *Escherichia coli* in Drinking Water and its Multidrug Resistance

D.H. Tambekar, N.B. Hirulkar, M.V. Kalikar, Y.S. Patil and S.R.Gulhane
Department of Microbiology, S.G.B. Amravati University,
Amravati 444 602, India

Abstract: Waterborne diseases are among the leading causes of morbidity and mortality in developing countries and every year around 2.2 million people die due to basic hygiene related diseases like coliform diarrhoea. The total 1000 sample from various sources such as tube wells (340), open wells (340) and hotels and restaurants (320) were analyzed for the presence of *Escherichia coli* contamination in drinking water and recorded 425 water samples contaminated with total coliform by MPN technique. Out of them 85 strains of thermotolerant *Escherichia coli* 51 (60%) from *open* well, 23 (32%) from tube well and 11 (13%) from hotels and restaurants were isolated and identified. These isolates showed maximum resistance to ofloxacin followed by novobiocin, cefdinir and ciprofloxacin. The azithromycin; gentamycin, amikacin, chloramphenicol, co-trimoxazole and tetracycline were the most effective while the ofloxacin, novobiocin, cefdinir and ciprofloxacin were the least effective against the *E. coli* isolated strains. Thus findings recommended that amikacin and azithromycin, gentamicin, chloramphenicol and tetracyclin are the best choice of drugs, while the ofloxacin, cefdinir, ciprofloxacin and novobiocin antibiotics should be avoided against *E. coli* diarrhoeal infections in the region.

Key words: MAR index, *E. coli*, gentamycin, amikacin, azithromycin, chloramphenicol

Introduction

Waterborne diseases are among the leading causes of morbidity and mortality in developing countries and every year around 2.2 million people die due to basic hygiene related diseases like coliform diarrhoea. Interventions in hygiene, sanitation and water supply proved to control these diseases. Universal access to safe drinking water and sanitation has been promoted as an essential step in reducing these preventable diseases (Tambekar and Banginwar, 2005). The normal inhabitant of human intestine, *Escherichia coli* has central place in water microbiology as an indicator of faecal pollution whereas certain strains of pathotype *E. coli* can also cause diarrhoea (Nataro and Kaper, 1998). About 50% deaths (4.6 million) in children under 5 years of age occur due to diarrhoea disease caused by drinking polluted water (Myder and Merson, 1982; Kudan and Zenyoji, 1977). The use of antibiotics to combat these infections is a common practice. The drug resistances displayed by these *E. coli* are indicated indiscriminate use of antibiotics, which warrants the initiation of steps to prevent public health hazard (Tambekar and Charan, 2004).

Pandey and Mussarat (1993) worked on antibiotic resistant coliform bacteria in drinking water in the urban area of Aligarh City and revealed the presence of multiple drug resistance *E. coli*. Parveen *et al*. (1997) recorded association of multiple antibiotic resistance profiles with point and non point sources of *E. coli* in Apalachicola Bay. Shukui Guan *et al*. (2002) developed a procedure to discriminate among *E. coli* isolated from animal and human sources. The reliability and repeatability

Corresponding Author: D.H. Tambekar, Department of Microbiology, S.G.B. Amravati University, Amravati 444 602, India Tel: 917212530141

Table 1: Antibiotics used in the study

Antibiotics	Quantity (mcg disc^{-1})	Antibiotics	Quantity (mcg disc^{-1})
Amikacin	30	Co-trimoxazole	23.75
Azithromycin	15	Gentamicin	10
Cefazolin	30	Kanamycin	30
Cefdinir	05	Nitroflurantoin	300
Ceftazidime	30	Norfloxacin	10
Ceftriaxone	30	Novobiocin	30
Chloramphenicol	30	Ofloxacin	05
Ciprofloxacin	30	Tetracyclin	30

of antibiotic resistance indexing of *Escherichia coli* were used to identify the source of faecal contamination in drinking water in Purna Valley of Vidarbha (Tambekar and Kalbende, 2004). In view of its public health implication, we undertook to determine the incidence of drug resistant *E. coli* in drinking water. No attempt has so far made to study the presence of antibiotic resistant bacteria in local drinking water and unfortunately very little attention has been paid for the same. Therefore, the study was aimed to evaluate the presence of thermotolerant *E. coli* in drinking water available in various source such as tube well, open well and hotel and restaurants in Amravati city and determined their multiple antibiotic resistance indexing for proper use of antibiotics.

Materials and Methods

To study the antibiotic resistance of *E. coli* in drinking water, the total 1000 drinking water samples were collected from open wells (340), tube wells (340) and hotels and restaurants (320) from different localities of Amravati city by using sterilized plastic water sample collection bottle. All the water samples were examined by MPN test (9 tube-test; containing double and single strength MacConkey purple broth with Durham's tube) for its potability and the coliform contamination in water was recorded within 24 h. The MPN positive test broth was further processed for the presence of thermotolerant *E. coli* by sub-culturing in Tryptone broth for indole test and Brilliant Green Lactose Bile Broth (Eijkman test) and incubated at 45.5°C. Identification of *E. coli* was made on the basis of standard test.

Antimicrobial agents susceptibilities were determined according to the procedures of CLSI (formerly NCCLS). Total 16 combinations of antibiotics disc (Hi-media Pvt. Ltd., Mumbai) were used in the study (Table 1). The MAR indices for antibiotics were calculated as per Tambekar and Patil (2006).

Results and Discussion

In this study total 1000 sample from various sources such as tube wells (340), open wells (340) and hotels and restaurants (320) were analyzed for the presence of *Escherichia coli* contamination in drinking water. Out of these 425 water samples were found contaminated with total coliform by MPN technique. A total of 85 strains of thermotolerant *Escherichia coli;* open well 51 (60%), tube well 23 (32%) and hotels and restaurants 11 (13%) were isolated and identified (Table 2). These 85 isolates showed maximum resistance to ofloxacin (92%) followed by novobiocin (86%) and cefdinir (82%) and ciprofloxacin (79%). The antibiotics such as cefazolin (64%), ceftriaxone (58%) and nitroflurotoin (51%) were moderately effective against the isolates. It was also observed that azithromycin; gentamycin, amikacin, chloramphenicol, co-trimoxazole and tetracycline were the most effective while the ofloxacin, novobiocin, cefdinir and ciprofloxacin were the least effective against the *E. coli* strains. Several workers reported higher degree of sensitivity of *Escherichia coli* to gentamycin (Krumperman, 1983).

Table 2: *E. coli* contamination in drinking water

Source	No. of water samples	Contaminated water samples	No. of *E. coli* (%)
Open well	340	150	51 (60)
Tube well	340	150	23 (32)
Hotel and Restaurant	320	125	11 (13)
Total	1000	425	85 (20)

Table 3: Antibiotics response against isolated *E. coli* species

Antibiotics	Resistant (%)	Sensitive (%)
Azithromycin	01 (1)	84 (99)
Gentamicin	02 (2)	83 (98)
Amikacin	03 (4)	82 (96)
Chloramphenicol	07 (8)	78 (92)
Co-trimoxazole	13 (15)	72 (85)
Tetracyclin	15 (18)	70 (80)
Kanamycin	17 (20)	68 (80)
Norfloxacin	19 (22)	66 (78)
Ceftazidime	38 (45)	47 (55)
Nitroflurantoin	43 (51)	42 (49)
Ceftriaxone	49 (58)	36 (42)
Cefazolin	54 (64)	31 (36)
Ciprofloxacin	67 (79)	18 (21)
Cefdinir	70 (82)	15 (18)
Novobiocin	73 (86)	12 (14)
Ofloxacin	78 (92)	7 (8)

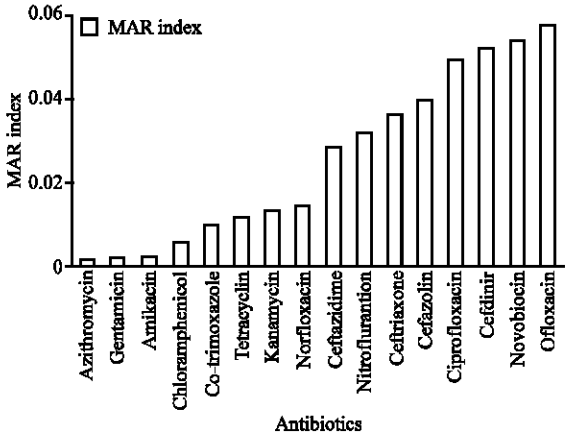

Fig. 1: MAR indices of the thermotolerant *E. coli*

In the present study MAR profile indicated that 84 (99%) isolates were sensitive to azithromycin followed by 83 (98%) to gentamycin, 82 (96%) to amikacin 72 (92%) to chloramphenicol, 72 (85%) to co-trimoxazole, 70 (82%) to tetracycline, 68 (80%) to kanamycin, 66 (78%) to norfloxacin and 47 (55%) to ceftazidime while their respective MAR indices were 0.0007, 0.0014, 0.002, 0.0051, 0.0095, 0.0110, 0.0125, 0.0139 and 0.0279 indicating non-human faecal contamination. Moreover the ofloxacin showed 78 (92%) resistance to antibiotics followed by 73 (86%) to novobiocin, 70 (82%) to cefdinir, 67 (79%) to ciprofloxacin, 54 (64%) to cefazolin, 49 (58%) to ceftriaxone and 43 (51%) to nitroflurantoin where as their respective MAR indices were 0.0573, 0.0536, 0.0514, 0.0492, 0.039, 0.0360 and 0.0316 indicating fecal origin of these strains (Table 3).

Tambekar *et al.* (2005) worked on multiple antibiotic resistance (MAR) indexing to discriminate the source of faecal contamination in drinking water and recorded 100% susceptibility to ofloxacin,

gentamycin and chloramphenicol (MAR-0), 93.3% to carbenicillin and tetracycline (MAR-0.0051), 90% to co-trimoxazole (MAR-0.0076) and 43.3% to erythromycin (MAR-0.033). A low MAR index indicated the non-human source of faecal *E. coli*. Begum *et al*. (2004) reported drug sensitivity patterns of *E. coli*, isolated from drinking water sources and reported highest susceptibility to ciprofloxacin, gentamycin, norfloxacin and trimethoprim and lowest to cefotaxime. The present study also showed consistent finding as with the other reports and indicated that the MAR indices of faecal *E. coli* from non-human sources were low, while human isolates have higher indices (Fig. 1). Several workers reported higher degree of sensitivity of *E. coli* against ciprofloxacin (Koenraad *et al*., 1995), norfloxacin (Borah, 1994), gentamicin and trimethoprim (Pandey and Mussarrat, 1993).

The variation occurred in antibiotic sensitivity trend of *E. coli* isolated from drinking water confirmed the emergence of antibiotics and antibiotics resistance of *E. coli* species in drinking water. Due to this indiscriminate use of antibiotics the resistant in bacteria increased and the infections. Therefore, the precautions should be taken not to abuse or treat infection indiscriminately with antibiotics. Thus these findings recommended that amikacin and azithromycin, gentamicin, chloramphenicol and tetracyclin are the best choice of drugs, while the ofloxacin, cefdinir, ciprofloxacin and novobiocin antibiotics should be avoided against *E. coli* diarrhoeal infections in this region.

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