# Prevalence and Antibiotic Sensitivity Profile of Human Enteric Pathogens from Different Water Sources in Salinity Affected Villages of Vidarbha (India)

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**Abstract:** Waterborne diseases are among the leading causes of morbidity and mortality in developing countries and around 2.2 million people die every year due to basic hygiene related diseases like gastroenteritis, diarrhoea, typhoid, dysentery. A total of 260 samples were analysed from various sources such as surface water (13) shallow ground water (42), deep ground water (129) and public water supply (76) for the presence of thermotolerant coliform (*E.coli*) and faecally associated *Salmonella typhi and Pseudomonas aeruginosa* and recorded 97 to 99% samples contaminated with coliform (by MPN technique) of which 75 content *Escherichia coli*, 50 samples contaminated with faecally associated *S.typhi* and high incidences (36%) of *P. aeruginosa* showing health risks from microbial growth and biofilms in drinking water distribution system. Most of the strains of *E.coli* were resistance to lincomycin and vancomycin and sensitive to levofloxacin, tobramycin and moxifloxacin and the strains of S. *typhi* sensitive to ciprofloxacin, levofloxacin and tobramycin and resistant to ampicillin, cephalothin and almost all strains of *P. aeruginosa* were resistance to cephalothin, clindamycin and lincomycin and sensitive to ofloxacin. Study indicated high incidences of thermotolerant *E.coli* and *S. typhi* in surface water due to surface faecal contamination or open defecation in villages and *P. aeruginosa* in public water supply due to biofilm. The findings recommended that levofloxacin, tobramycin, Moxifloxacin, ciprofloxacin and ofloxacin should be the drug of choice for diarrhoeal diseases caused by these organisms, while the lincomycin, vancomycin, ampicillin, cephalothin antibiotics should avoid in these infections in the region. The study indicated that most of water is contaminated with coliform and faecally associated microorganisms and unsafe for drinking purpose, hence properly treated water should be used for drinking purpose.

**Key words:** Water quality, coliform, faecally associated microorganisms, antibiogram, *E.coli, Salmonella typhi, Pseudomonas aeruginosa*)

## INTRODUCTION

Waterborne infections are most common causes of morbidity and mortality in the underdeveloped and developing countries and 80% of the infectious diseases are waterborne in India. Around 2.2 million people die due to basic hygiene related diseases like gastroenteritis, diarrhoea, typhoid, and dysentery. Interventions in hygiene, sanitation and water supply proved to control these diseases. Universal access to safe drinking water and sanitation are essential steps in reducing these preventable diseases. The normal inhabitant of human intestine, *Escherichia coli, Salmonella typhi and Pseudomonas aeruginosa* has central place in water faecal pollution. About 50%

deaths (4.6 million) in children under 5 years of age occur due to diarrhoea disease caused by polluted water[13, 9]. The use of antibiotics to combat these infections is a common practice but indiscriminate use of antibiotics leads to drug resistance in these microbes, which warrants the initiation of steps to prevent public health hazard[17] .

Gautam *et al.,*[5] in Northern India and Lateef *et al.*[10] in Hungeri revealed the presence of multiple drug resistance enteric bacterial pathogens and recorded association of multiple antibiotic resistance profiles with point and non point sources of *E.coli.* The repeatability of antibiotic resistance indexing of *E. coli* and *S. typhi* were used to identify the source of faecal contamination in drinking water in Purna Valley of

Vidarbha[18, 20] . *S. typhi* are the contaminant of water and causes enteric fever, septicemia and acute gastroenteritis. Multiple drug resistance and continuous increase in the emergence in these organisms has been reported in India[11,14]. *P. aeruginosa* are environmental bacteria frequently present in small number in normal intestinal flora of humans and animals and particularly good at forming biofilm and associated with deterioration of bacteriological quality of drinking water including test, odour and turbidity[16].

In view of its public health implication, we undertook to determine the prevalence and drug resistant *E. coli, S. typhi and P. aeruginosa* 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, S. typhi and P. aeruginosa* in drinking water available in various sources such as tube surface water, shallow ground water, deep ground water and public water supply scheme in salinity affected villages of Vidarbha and determined their multiple antibiotic resistances indexing for proper use of antibiotics.

## MATERIALS AND METHODS

To study the water quality and antibiotic sensitivity profile of human enteric pathogens, a total of 260 drinking water samples were collected from surface water (13) shallow ground water (42), deep ground water (129) and public water supply (76) for the isolation and detection of thermotolerant coliform (*E.coli*) and coliform associated *S. typhi and P. aeruginosa* from different localities of salinity affected villages of Akola and Buldhana district of Vidharbha between June 2007 to December 2007 by using sterilized plastic water sample collection bottle.

The bacteriological examination was performed within the 24 h of collection using standard Multiple Tube Fermentation Technique (MTFT) for determination of Most Probable number (MPN) index, nine multiple tube dilution technique using double and single strength Bromo-Cresol Purple MacConkey medium and Membrane filter techniques (MFT) by using M-EC test agar (Hi-media Lab. Mumbai), which detect only *E.coli* with production of yellow colour colonies on membrane filter; Eijekman's test for Thermotolerant coliform (TTC) by using Brilliant Green Bile broth (BGLB) for detection of gas production and Tryptone broth for production of Indole and Manja's Rapid $H_2S$ test for detection of fecal

contaminations in drinking water. The MPN Index was calculated from MPN table and index of water more than 10 coliforms/dl is designated as polluted or unhealthy for drinking purpose or non-potable[1].

The isolation and identification of *E.coli* was made based on MFT incubated at $44.5^0C$ and standard bacteriological tests such as morphological, cultural, biochemical and some special tests by subculturing the MFT positive (yellow color colonies on membrane filter in M-EC test agar) colonies in respective medium. The modified $H_2S$ test medium[12] was used to detect potability of water and coliform associated organisms. Blackening in $H_2S$ medium was recorded after 24 and 48h of incubation at room temperature and at $37^0C$. The $H_2S$ positive test was further processed for the presence of coliform associated microorganisms, *S. typhi* was identified by subculturing on Salmonella Shigella agar medium and Xylose lysine deoxycholate agar (XLD). Identification of *S. typhi* was made on the basis of selective test. *P. aeruginosa* were isolated from MPN positive test by using cetrimide agar and identified by performing oxidase and citrate utilization test.

Antimicrobial agents susceptibilities were determined according to the procedures of CLSI (formerly NCCLS) by various combinations of antibiotics disc (Hi-media Pvt. Ltd., Mumbai) (Table 1). The MAR indices for antibiotics were calculated as per Tambekar and Patil[19]. The statistical analysis was performed with the Statistical Package for Social Sciences 15 for Windows (SPSS Inc.; Chicago, IL, USA) software.

**Table1:** Antibiotics used in the study.

| Antibiotics (µg/disc) | Quantity | Antibiotics (µg/disc) | Quantity |
|---|---|---|---|
| Amikacin | 30 | Lincomycin | 2 |
| Ampicillin | 10 | Meropenem | 10 |
| Ceftazidime | 30 | Moxifloxacin | 5 |
| Ceftriaxone | 30 | Nalidixic acid | 30 |
| Cephalothin | 30 | Netilmicin | 30 |
| Cephotaxime | 30 | Nitrofuront | 300 |
| Ciprofloxacin | 5 | Norfloxaci | 10 |
| Co-trimoxazole | 25 | Ofloxacin | 2 |
| Gentamicin | 10 | Sparfloxaci | 5 |
| Imipenem | 10 | Tobramycin | 10 |
| Levofloxacin | 5 | Vancomycin | 30 |

## RESULTS AND DISCUSSION

Water and sanitation are the primary driver of public health which means that once we can secure access to clean water and to adequate sanitation facilities for all people, irrespective of the difference in their living conditions, a huge battle against all kinds

of diseases will be won. More than half of the world's population lives in villages in rural areas and most of those without access to safe drinking water supply[6]. The majority of diseases in developing countries are infectious in nature caused by bacteria, viruses and other microbes, which are shed in human faeces and pollute water supplies, which people use for drinking and washing purposes. Faecal indicator bacteria have been used to measure water quality and personal hygiene standards in a variety of settings[7]. The aims of the present studies were to determine the prevalence of enteropathogens in water, determine their antibiogram for the assessment and management of microbial risks in drinking water, which will aid in developing practical plans to adopt, and provide proper antibiotic use.

A total of 260 samples were analysed from various sources such as surface water (13) shallow ground water (42), deep ground water (129) and public water supply (76) for the presence of thermotolerant coliform (*E.coli*) and faecally associated *S. typhi and P. aeruginosa* and recorded all most all (97 to 99%) samples contaminated with coliform (by MPN technique) in which 75 content *E. coli* (table 2). It includes surface water (54%), shallow ground water (24%), deep ground water (29%) and public water supply (28%). Out of total, 50 water samples contaminated with faecally associated *Salmonella spp.* from surface water (43%), shallow ground water (28%), deep ground water (15%) and public water supply (18%). The study reported high incidence (36%) of *P. aeruginosa* in drinking water showing health risks from microbial growth and biofilms in drinking water distribution system (Fig 1). Most of the strains of *E.coli* were resistance to lincomycin and vancomycin and sensitive to levofloxacin, tobramycin, and Moxifloxacin (Fig. 2) and the strains of S. typhi sensitive to ciprofloxacin, levofloxacin, and tobramycin and resistant to ampicillin, cephalothin (Fig. 3) and almost all strains of *P. aeruginosa* were resistance to cephalothin, clindamycin, and lincomycin and sensitive to Ofloxacin (Fig. 4). These finding supported the observations of several previous studies[2,20]. Comparatively high antibacterial sensitivity observed due to rare or occasional use of the drug and could be attributed to the fact that these drugs were seldom used[3].

Study indicated high (13/13=100%) incidences of thermotolerant *E.coli* and *S. typhi* in surface water due to surface faecal contamination or open defecation in villages. Tewari and Agarwal[21]0 showed most of *E. coli* were resistance to one or other drug singly or in combination and maximum resistance was observed in with ampicillin, chloramphenicol, tetracycline,
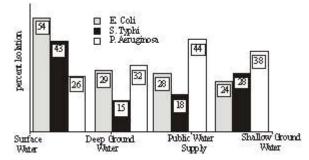
sulphonamide, streptomycin. Most of the isolated strains of *E.coli* were resistance to linomycin (85%) and vancomycin (66%) and sensitive to levofloxacin (91%), tobramycin (91%), and moxifloxacin (90%) (Fig 2) Typhoid fever remains a serious problem in India due to emergence of multidrug resistance in *S. typhi*. The Multiple Antibiotic Resistance (MAR) profile of *S. typhi* strains isolated from Akola and Buldhana district showed highest sensitivity to ciprofloxacin (98%), levofloxacin (96%) tobramycin (92%), ofloxacin (90%), gentamicin (90%), sparfloxacin (90%), and resistant to ampicillin (96%), cephalothin (92%), nalidixic acid (92%) and nitrofurantoin (84%) (Fig 3).
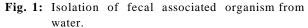
*P. aeruginosa* is a common organism found in water and its growth can cause problems with color, taste, odour, and turbidity to the drinking water. It is a typical biofilm-producing organism that grows on tubing, fittings and showerheads as wells as in sinks and sink drains[8]. The study reported high incidences (36%) of *P. aeruginosa* in drinking water indicating danger to human health due to its biofilm producing ability, which supported the finding of previous researchers[22]. In present study *P. aeruginosa* showed 61% resistance and 39% sensitive to tested antibiotics. All the isolated strains of *P. aeruginosa* were resistance to cephalothin (99%), clindamycin (98%), linomycin (97%) vancomycin (95%), ampicillin (95%), ofloxacin (94%), erythromycin (92%). The higher-level resistance to this antibiotic might be attributed to antibiotic and antibiotics resistance bacterial emergence because of improper and extensive use of these antibiotics. *P. aeruginosa* were highly sensitive to alizarin (94%), ofloxacin (98%), netilmicin (82%), gentamycin (82%), ceftriaxone (67%) (Fig 4).

Antibiotic-resistant bacteria and antibiotics discharged in various amounts in the environment, indiscriminate use of antibiotics in medical, veterinary, and agricultural practices lead to multiple antibiotic resistances in bacterial pathogens[4]. The antibiotics and antibiotic resistance bacterial pathogens mixed with ground water through the phenomenon of percolation and contaminated the ground water. The success of antimicrobial therapy in various waterborne diseases depends upon proper selection of the drugs. The findings recommended that levofloxacin, tobramycin, Moxifloxacin, ciprofloxacin, and ofloxacin should be the drug of choice for diarrhoeal diseases caused by these organisms, while the lincomycin, vancomycin, ampicillin, cephalothin antibiotics should avoid against these infections in the region. The study indicated that most of water is contaminated with coliform and faecally associated microorganisms and unsafe for drinking purpose, hence properly treated water should be used for drinking purpose.

**Table 2:** Quality of drinking water by various tests and isolation of *E.coli, S.typhi and P. aeruginosa* from various districts and sources.

| District | Taluka | MPN/24 | | MPN/48 | | H2S/24 | | H2S/48 | | M/E. coli | Salmoella typhi | Pseudomonas aeruqinosa |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Safe | Polluted | Safe | Polluted | Safe | Polluted | Safe | Polluted | | | |
| Akola District | Akot | 5 | 50 | 3 | 52 | 37 | 18 | 21 | 34 | 17 | 10 | 32 |
| | Balapur | 2 | 1 | 2 | 1 | 1 | 2 | 1 | 2 | 3 | 2 | 0 |
| | Mrtizapur | 0 | 4 | 0 | 4 | 2 | 2 | 0 | 4 | 0 | 0 | 0 |
| | Sub-total | 10 | 122 | 8 | 124 | 86 | 46 | 32 | 100 | 42 | 22 | 47 |
| Buldhana District | J.Jamod | 0 | 35 | 0 | 35 | 17 | 18 | 5 | 30 | 11 | 8 | 15 |
| | Malkapur | 1 | 10 | 1 | 10 | 9 | 2 | 2 | 9 | 7 | 0 | 0 |
| | Nandura | 0 | 29 | 0 | 29 | 20 | 9 | 5 | 24 | 4 | 1 | 3 |
| | Sangrampur | 0 | 14 | 0 | 14 | 5 | 9 | 2 | 12 | 0 | 7 | 9 |
| | Shegaon | 6 | 25 | 4 | 27 | 11 | 20 | 3 | 28 | 5 | 7 | 12 |
| | Telhara | 0 | 8 | 0 | 8 | 3 | 5 | 0 | 8 | 6 | 5 | 7 |
| | **Sub -total** | 7 | 121 | 5 | 123 | 65 | 63 | 17 | 111 | 33 | 28 | 46 |
| **Total** | | **17** | **243** | **13** | **247** | **151** | **109** | **49** | **211** | **75** | **50** | **93** |
| Source | Public Water supply | 6 | 70 | 5 | 71 | 49 | 27 | 18 | 58 | 21 | 14 | 33 |
| | Deep Ground water | 8 | 121 | 5 | 124 | 81 | 48 | 28 | 101 | 37 | 19 | 41 |
| | Shallow Ground water | 3 | 39 | 3 | 39 | 17 | 25 | 2 | 40 | 10 | 12 | 16 |
| | Surface Water | 0 | 13 | 0 | 13 | 4 | 9 | 1 | 12 | 7 | 5 | 3 |
| **Total** | | **17** | **243** | **13** | **247** | **151** | **109** | **49** | **211** | **75** | **50** | **93** |



**Fig. 1:** Isolation of fecal associated organism from water.



**Fig. 2:** Antibiogram of *E.coli* isolated from Akola and Buldhana district.



**Fig. 3:** Antibiogram of *S. typhi* isolated from Akola and Buldhana district.



**Fig. 4:** Antibiogram of *P. aeruginosa* isolated from Akola and Buldhana district.

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