"Studies on Prevalence and Multiple Antibiotic Resistance Patterns of E. coli Isolated from Drinking Water"

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ABSTRACT

E. coli is the primary cause of human urinary tract infections, as well as pneumonia, and traveler's diarrhea. 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. Antibiotic resistance is a type of drug resistance where a microorganism is able to survive exposure to an antibiotic. In the present study a total of 27 water sample collected randomly had poor hygiene area and a total of 18 E. coli isolates were identified. The overall spectrum of antibiotic resistance was showed that 2 isolates, Oxacillin and penicillin was highly resistance. 10 isolate resistance to Ampicillin, Cephalothin, Cephoxitin and Metronidazole. The spectrum showed variation in resistance. When study data analyzed for Over All Antibiotic Sensitivity it is found that out of total antibiotics more than 70% isolates showed sensitivity towards Piperacillin, Tetracycline, Vancomycim and Chloremphenicol and this drug can be recommended for elimination of E.coli. Thus these findings recommended that Piperacillin and Vancomycin 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.

Key Words: Multiple drug resistance, fecal coliform, diarrhea.

INTRODUCTION

Escherichia coli inhabit the intestinal tract of humans and other warm-blooded mammals. It constitutes approximately 0.1% of the total bacteria in the adult intestinal tract. However, it is now known that certain types of E. coli exist that are more capable of causing disease than other types. If these types are present in water or food that is ingested, then an infection can result. E. coli is the primary cause of human urinary tract infections, as well as pneumonia, and traveler's diarrhea.

E. coli is an indicator of fecal pollution of water. The presence of large numbers of E. coli in water is a strong indicator of recent fecal pollution, and so the possible presence of other intestinal bacteria that cause serious disease (i.e., Vibrio, Salmonella, Shigella). 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, Y.S. Patil 2004, Charan 2004).

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 Escherichia 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).

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The widespread use of antibiotics both inside and outside of medicine is playing a significant role in the emergence of resistant bacteria. Antibiotics are often used in rearing animals for food and this use among others leads to the creation of resistant strains of bacteria. In some countries antibiotics are sold over the counter without a prescription which also leads to the creation of resistant strains

Resistance to antimicrobial drugs is increasing day by day worldwide in almost all bacterial genera and to almost all drug classes. Studies with E. coli are of particular relevance because this species can occupy multiple niches, including human and animal hosts. The emergence, propagation, accumulation, and maintenance of strains of antimicrobial-resistant (AR) pathogenic bacteria have become a worldwide health concern in human and veterinary medicine. Alhussain et al. (2005) recorded the excellent susceptibility to meropenem and variable susceptibility to aminoglycoside and fluoroquinolones but greatly reduced susceptibility to beta lactam beta lactamase inhibitors combination, trimethoprim and sulphamethoxazole in Extended Spectrum Beta Lactamase (ESBL) producing E. coli. During the work on in vitro susceptibilities of E. coli ampicillin-sulbactam and amoxicillin-clavulanic acid. Birgul and Nedim (2007) found that more organism were susceptible to amoxicillin-clavulanic acid than ampicillin-sulbactam.

The use, misuse and abuse of antibiotics are held to be responsible for this antibiotic resistance development (Austin et al., 1999; Bronzwaer et al., 2002). Keeping in view the public health effects of waterborne pathogens i.e., E. coli, since it has been used as an indicator of water quality and to assist the control of water borne diseases (Ejaz and Ahmad, 2001; Kjerschener et al., 2004).

Efforts are being taken by all technological advancements including antibiotic usage to control transmission of waterborne diseases, but multi-drug resistance by these Escherichia coli warrant the beginning of steps to prevent the public health hazards (Tambekar et al., 2006; Pandey and Musrat, 1993; Parveen et al., 1997). 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 Escherichia coli in drinking Water available in various sources. The data collected from this study would allow us to control the spread and develop strategies for treatment of the enterococcal infections.

MATERIALS AND METHODS
In present study a total of 27 drinking water samples were collected in sterile container from hotels, restaurants and hospitals of Neemuch City. All the drinking water samples were analyzed for its potability and presence of E.coli. Out of that 18 samples were found to be contaminated with E.coli.

All samples were subjected to perform for lactose fermentation in Mac Conkey Double strength broth (MPN). After 24 Hr of incubation period and at 37° C incubation temperature confirm the presence of coliforms. Positive Drinking water samples were inoculated on Mac Conkey and EMB agar medium (Hi-Media) and place them for 24 Hr incubation period at 37°C temperature. For confirmation of E. coli the positive samples were inoculate on EMB medium and Incubated at 37° C for 24 Hr. Green metallic sheen were observed and confirm by using Biochemical Tests and gram staining.

All the isolates were subjected to perform Antibiotic Sensitivity Test by Kirby and Buyer Method. Study Data were subjected for analysis. In present study 17 antibiotics were used against 18 Isolates of e coli. Plates were observed for growth at 12, 24, and 36 hrs and the diameter of circular zone of inhibition were measured using zone measurement scale (Table 1).

<table>
<thead>
<tr>
<th>SN</th>
<th>Antibiotics</th>
<th>Quantity Used</th>
<th>SN</th>
<th>Antibiotics</th>
<th>Quantity Used</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Chloramphenicol</td>
<td>30 mcg</td>
<td>10</td>
<td>Cephalothin</td>
<td>30 mcg</td>
</tr>
<tr>
<td>2</td>
<td>Clindamycin</td>
<td>2 mcg</td>
<td>11</td>
<td>Metronidazole</td>
<td>5 mcg</td>
</tr>
<tr>
<td>3</td>
<td>Erythomycin</td>
<td>15 mcg</td>
<td>12</td>
<td>Tetracycline</td>
<td>30 mcg</td>
</tr>
<tr>
<td>4</td>
<td>Gentamycin</td>
<td>30 mcg</td>
<td>13</td>
<td>Penicillin</td>
<td>10 mcg</td>
</tr>
<tr>
<td>5</td>
<td>Oxacillin</td>
<td>10 mcg</td>
<td>14</td>
<td>Co-trimoxazole</td>
<td>25 mcg</td>
</tr>
<tr>
<td>6</td>
<td>Vancomycin</td>
<td>15 mcg</td>
<td>15</td>
<td>Ceftraxone</td>
<td>30 mcg</td>
</tr>
<tr>
<td>7</td>
<td>Ampicillin</td>
<td>30 mcg</td>
<td>16</td>
<td>Piperacillin</td>
<td>15 mcg</td>
</tr>
<tr>
<td>8</td>
<td>Cephalothin</td>
<td>30 mcg</td>
<td>17</td>
<td>Amikacin</td>
<td>2 mcg</td>
</tr>
<tr>
<td>9</td>
<td>Carbenicilline</td>
<td>100 mcg</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 1: Antibiotics Used in Study
RESULT AND DISCUSSION
In the present study a total of 27 water sample collected randomly had poor hygiene area and a total of 18 *E. coli* isolates were identified. Study data indicated that all the strains of *E. coli* showed sensitivity for Vancomycin, Piperacillin and Tetracycline as compare to other antibiotics and showed highest resistance for Oxacillin and penicillin. The result analysis showed that for both antibiotics 12 isolates showed resistance. And the very high sensitivity showed by and Vancomycin (Table 2).

Table 2: Antibiotic resistance frequency

<table>
<thead>
<tr>
<th>Antibiotics</th>
<th>Series 1</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chloramphenicol</td>
<td>1</td>
</tr>
<tr>
<td>Chloramycin</td>
<td>2</td>
</tr>
<tr>
<td>Erythromycin</td>
<td>3</td>
</tr>
<tr>
<td>Gentamycin</td>
<td>4</td>
</tr>
<tr>
<td>Oxacillin</td>
<td>5</td>
</tr>
<tr>
<td>Vancomycin</td>
<td>6</td>
</tr>
<tr>
<td>Ampicillin</td>
<td>7</td>
</tr>
<tr>
<td>Cephalothin</td>
<td>8</td>
</tr>
<tr>
<td>Carbenicillin</td>
<td>9</td>
</tr>
</tbody>
</table>

The overall spectrum of antibiotic resistance was showed that 2 isolates, Oxacillin and penicillin was highly resistance. 10 isolate resistance to Ampicillin, Cephalothin, Cephoxitin and Metronidazole. The spectrum showed variation in resistance (Fig 1).
When study data analyzed for Over All Antibiotic Sensitivity it is found that out of total antibiotics more than 70% isolates showed sensitivity towards Piperacillin, Tetracycline, Vancomycin and Chloremphenicol and this drug can be recommended for elimination of E.coli infection (Fig 2).

![Fig 2: Overall spectrum of antibiotic Resistance](image)

When study data analyzed for antibiotic susceptibility to each isolates, E1 showed 30 mm zone of inhibition for Chloremphenicol, 10 mm for Clindamycin, 22 mm for Erythromycin, 25 mm for Gentamycin, 15 mm for Oxacillin as compare to Vancomycin (0 mm zone of inhibition), ampicillin, Cephalothin, Carbenicilline and Cephoxitin. Data showed E1 isolate showed 100 % resistance against Vancomycin, ampicillin, Cephalothin, Carbenicilline and Cephoxitin (Fig 3).

![Fig 3: Antibiotic Susceptibility of Isolate E 1 and E 2](image)

Whereas E2 showed 100 % resistance against Vancomycin (0 mm zone of inhibition), ampicillin, Cephalothin, Carbenicilline and Metronidazole and Piperacillin. Tambekar and Patil in 2006 found maximum resistance to ofloxacin (92%) followed by novobiocin (86%) and cefdinir (82%) and ciprofloxacin (79%). The antibiotics such as cefazolin (64%) floxacin one (58%) and nitrofurtoin (51%) Were moderately effective against the isolates.

Study analysis for E3 showed 35 mm zone of inhibition for Penicillin, as compare to other antibiotics. E3 showed 30 mm for Cephoxitin, 0 mm for Erythromycin, and 15 mm for Gentamycin. E3 showed, highest resistance against Erythromycin, ampicillin, Cephalothin, Ceftaxone and Amikacin as compare to other antibiotics Used.
E4 showed 35 mm zone of inhibition for Co-trimoxazole and resistance against Cephalothin, Amikacin and Cephoxitin antibiotics (Fig 4).

In present study E 5 isolate showed 100% resistance against Vancomycin, resistance against Gentamycin, Vancomycin, Clindamycin, Cephoxitin, Penicillin, Co-trimazaxole and Amikacin. E 6 isolate showed 100% resistance against Vancomycin, Clindamycin, Cephoxitin, Penicillin, Co-trimazaxole (Fig 5).

Several workers reported higher degree of sensitivity of Escherichia coli against ciprofloxacin (Koenraad et al., 1995), norfloxacin (Borah, 1994), gentarnicin and trimethoprin (Pandey and Mussarrat, 1993), whereas Wimmerstedt and Kahlmets (2008) investigated the trimethoprime resistance in ampicillin resistant than ampicillin susceptible isolates of E. coli.

When E 7 and E8 tested for their susceptibility it was found that E7 showed 100% resistance against Oxacillin, Vencomycin, Ampicillin, Cephalothin and Penicillin, whereas E8 showed 100% resistance against Ampicillin, Gentamycin, Penicillin, Amikacin Metronidaxazole, Tetracycline etc antibiotics (Fig 6).
In 2009 A.H. Shar, Y.F. Kazi and I.H. Soomro worked on Antibiotic Susceptibility of Thermo-Tolerant Escherichia coli 2 Isolated from Drinking Water of Khairpur City, Sindh, Pakistan, observed that levoflaxin, cefpime, enoxobid, noroxin, tarivid, ciproxin, avelox, amikacin, kanamycin, rocin, pipenedic acid. Antibiotic susceptibility of E9 showed highest resistance against Clindamycin Oxacillin Cephalothin Carbenicillin, Tetracycline and Piperacillin as compare to other antibiotics. Whereas E 10, showed 100 % resistance against Gentamycin, Vancomycin, Cephalothin Cephoxitin, Penicillin, Co-trimaxazole and Amikacin (Fig 7).

Isolated strain E11 when tested for their susceptibility it was found that E 11 isolate showed 100 % resistance against Clindamycin, Oxacillin, Ampicillin, Carbenicillin, Penicillin, Gentamycin, Penicillin, Amikacin, Penicillin, and Ceftraxone whereas E 12 isolate showed 100 % resistance against Gentamycin, Oxacillin, Ampicillin, Carbenicillin, Penicillin, Amikacin, Penicillin, and Ceftraxone (Fig 8).

Similarly E13 when tested for their susceptibility it showed 0 mm zone of inhibition for Chloremphenicol, Clindamycin, and Erythromycin, 23 mm for Gentamycin, 22 mm for Oxacillin as compare to other antibiotics (Fig 9).
E 15 isolate showed 100% resistance against Ampicillin, Gentamycin, Penicillin, Amikacin Metronidaxazole, Tetracycline etc antibiotics whereas E 16 showed 100% resistance against Gentamycin, Vancomycin, Cephalothin Cephoxitin, Penicillin, Co-trimaxazole and Amikacin.

Isolated strain E 17 when tested for their susceptibility it was found that E 5 showed 0 mm zone of inhibition for Chloremphenicol, 13 mm for Clindamycin, 0 mm for Erythromycin, 16 mm for Gentamycin, 0 mm for Oxacillin as compare to other antibiotics. E 18 showed 22 mm zone of inhibition for Chloremphenicol, 20 mm for Clindamycin, 0 mm for Erythromycin, 10 mm for Gentamycin, 0 mm for Oxacillin.

The prevalence of strains resistant to tetracycline, ampicillin, chloramphenicol, and streptomycin were 9% to 35% in 1986 and 56% to 100% in 1998. These findings demonstrate that resistance gene reservoirs are increasing in healthy persons.

CONCLUSION

The use of antibiotics to combat these infections is a common practice. The drug resistances displayed by these Escherichia 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).

Study analysis indicated that all the strains of E. coli showed sensitivity for Vancomycin and Tetracycline as compare to other antibiotics and showed highest resistance for Oxacillin and penicillin. The result analysis showed that for both antibiotics 12 isolates showed resistance. And the very high sensitivity showed by Piperacillin and Vancomycin. 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 Piperacillin and Vancomycim are the best choice of drugs, while the flouxacin, cefdinir, ciprofloxacin and novobiocin antibiotics should be avoided against E coli diarrhoeal infections in this region.

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REFERENCES