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ORIGINAL RESEARCH ARTICLE

"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 thesefindings recommended that Piperacillin and Vancomycim 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 fluroquinolones but greatly reduced susceptibility to beta lactam beta lactamase inhibitors combination, trimethoprime and sulphamethoxazole in Extended Spectrum Bbeta 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; Kjrschner *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 *Escherchia 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 37^0 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 37^0C temperature. For confirmation of *E coli* the positive samples were inoculate on EMB medium and Incubated at 37^0C 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 1: Antibiotics Used in Study

SN	Antibiotics	Quantity Used	SN	Antibiotics	Quantity Used
1	Chloremphenicol	30 mcg	10	Cephoxitin	30 mcg
2	Clindamycin	2 mcg	11	Metronidazole	5 mcg
3	Erythomycin	15 mcg	12	Tetracycline	30 mcg
4	Gentamycin	30 mcg	13	Penicillin	10 mcg
5	Oxacillin	10 mcg	14	Co-trimoxazole	25 mcg
6	Vancomycin	15 mcg	15	Ceftraxone	30 mcg
7	Ampicillin	30 mcg	16	Piperacillin	15 mcg
8	Cephalothin	30 mcg	17	Amikacin	2 mcg
9	Carbenicilline	100 mcg			

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 Vancomycim, 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 Vancomycim (**Table 2**).

Table 2: Antibiotic resistance frequency

	Chloremphenicol	Clindamycin	Erythomycin	Gentamycin	Oxacillin	Vancomycim	Ampicillin	Cephalothin	Carbenicilline
1	E1	E6	E1	E1	E1	E1	E1	E1	E2
2	E6	E8	E3	E5	E6	E2	E2	E2	E8
3	E8	E10	E9	E8	E7	E5	E3	E3	E9
4	E9	E11	E13	E18	E9	E6	E7	E4	E10
5	E13	E12	E15	E11	E10	E7	E8	E5	E11
6	E15	E13	E16	E3	E11	E15	E12	E7	E12
7	E17	E14	E17	E17	E12		E14	E9	E13
8		E15	E18	E9	E14		E15	E10	E14
9				E4	E15		E16	E15	E15
10					E16		E17	E17	E16
11					E17				
12					E18				
Total	7	8	8	9	12	6	10	10	10

	Cephoxitin	Metronidazole	Tetracycline	Penicillin	Co-trimoxazole	Ceftraxone	Piperacillin	Amikacin	
1	E1	E1	E7	E1	E1	E2	E1	E3	
2	E2	E6	E8	E5	E2	E3	E5	E4	
3	E4	E7	E9	E6	E5	E6	E10	E5	
4	E5	E8	E10	E7	E6	E8	E17	E8	
5	E7	E9	E16	E8	E10	E9	E18	E12	
6	E11	E11	E18	E9	E11	E12		E13	
7	E12	E13		E10	E17	E13		E14	
8	E14	E15		E12		E14		E15	
9	E15	E17		E13		E15		E16	
10	E17	E18		E14		E16		E18	
11				E15		E18			
12				E18					
Total	10	10	6	12	7	11	5	10	

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**).

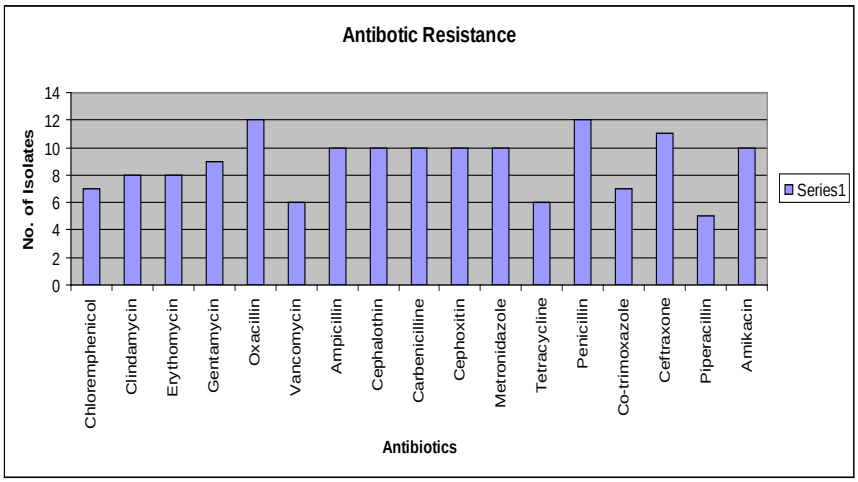

Fig 1: Overall spectrum of antibiotic Resistance

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* infection (**Fig 2**).

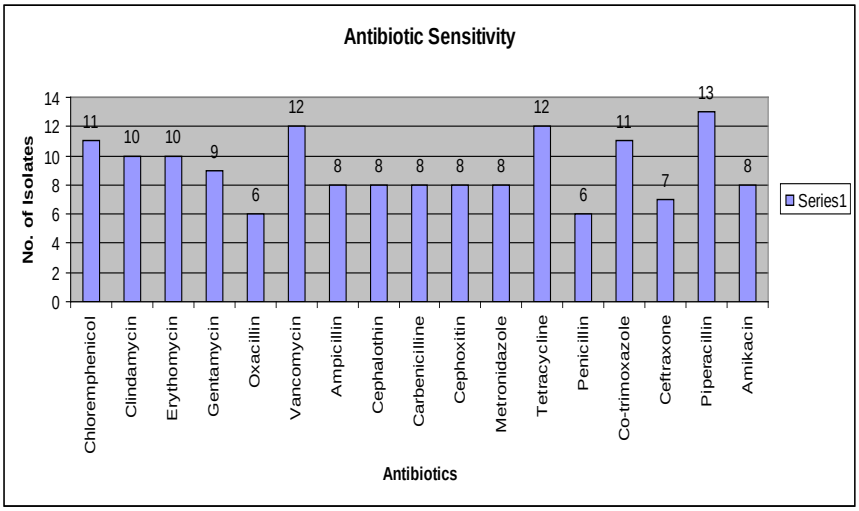

Fig 2: Overall spectrum of antibiotic Resistance

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 Vancomycim (o mm zone of inhibition), ampicillin, Cephalothin, Carbenicilline and Cephoxitin. Data showed E 1 isolate showed 100 % resistance against Vancomycim, ampicillin, Cephalothin, Carbenicilline and Cephoxitin (**Fig 3**).

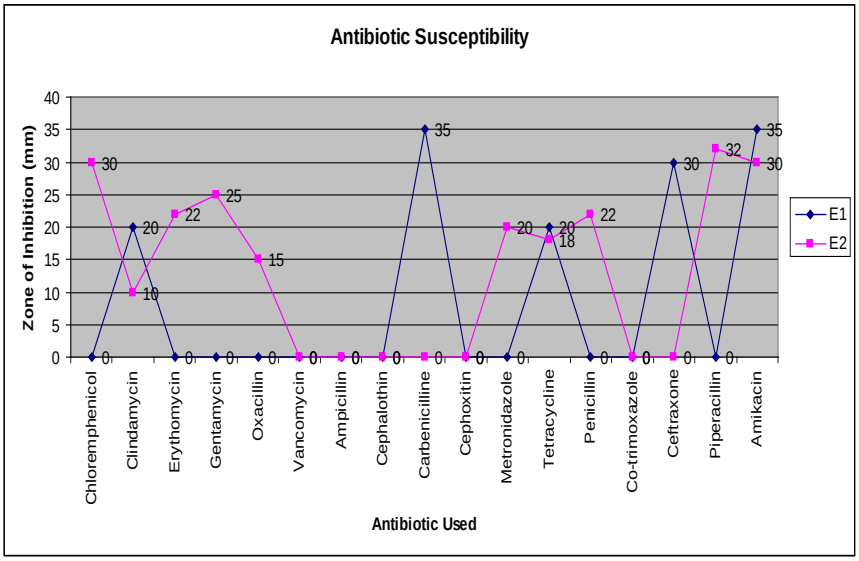

Fig 3: Antibiotic Susceptibility of Isolate E 1 and E 2

Whereas E2 showed 100 % resistance against Vancomycim (o 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%), furfuroxime one (58%) and nitrofurotoin (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**).

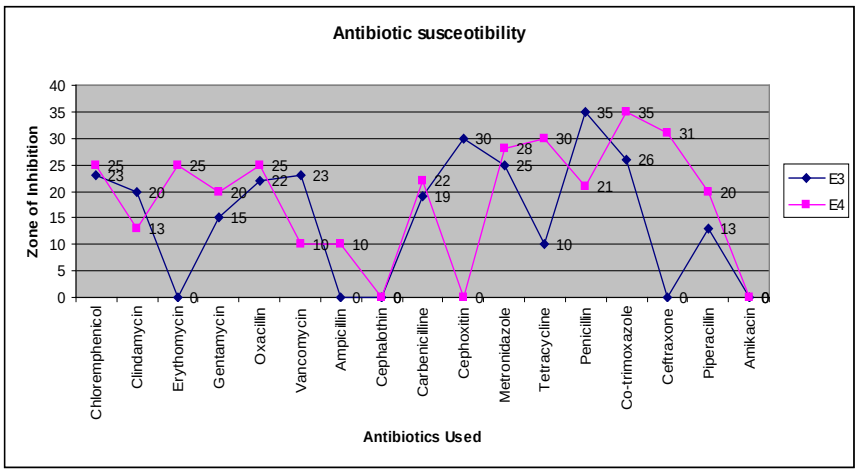

Fig 4: Antibiotic Susceptibility of Isolate E 3 and E 4

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

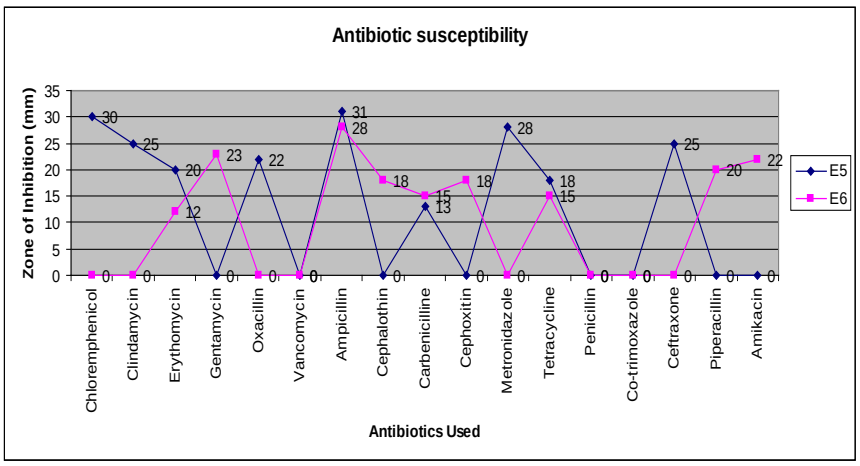

Fig 5:- Antibiotic Susceptibility of Isolate ES 5 and ES 6

Several workers reported higher degree of sensitivity of *Escherichia coli* against ciprofloxacin (Koenraad *et al*., 1995), norfloxacin (Borah, 1994), gentarnicin and trirnethoprirn (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, Cephoxitin and Penicillin. whereas E8 showed 100 % resistance against Ampicillin, Gentamycin, Penicillin, Amikacin Metronidaxazole, Tetracycline etc antibiotics (**Fig 6**).

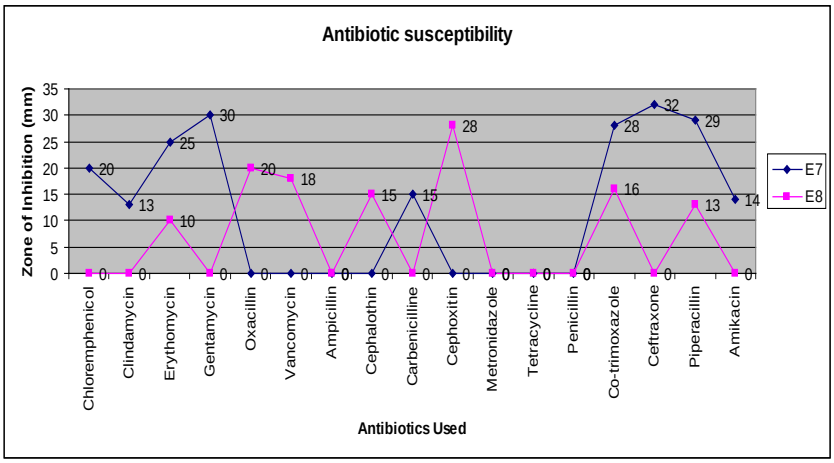

Fig 6: Antibiotic Susceptibility of Isolate ES 7 and ES 8

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, cefipime, enoxobid, noroxin, tarivid, ciproxin, avelox, amikacin, kanamycin, rocifin, 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, Vancomycim, Cephalothin Cephoxitin, Penicillin, Co-trimaxazole and Amikacin (**Fig 7**).

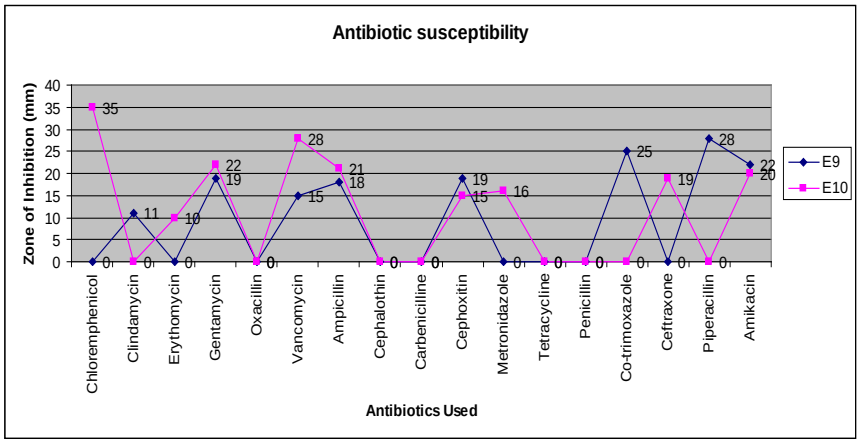

Fig 7:- Antibiotic Susceptibility of Isolate ES 9 and ES 10

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

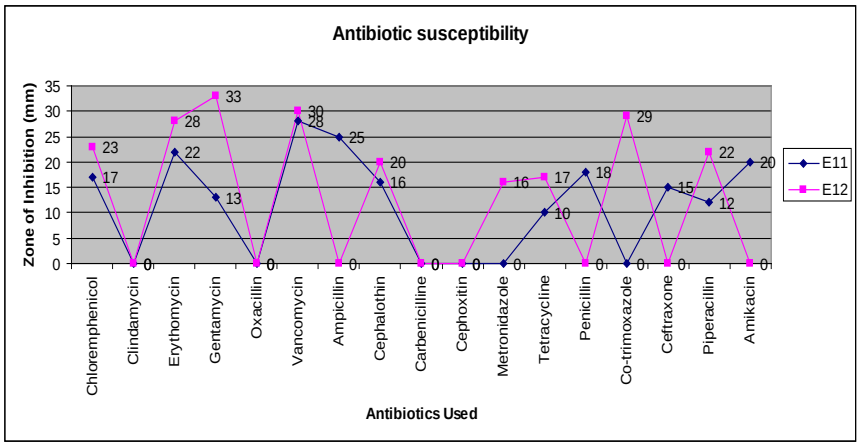

Fig 8: Antibiotic Susceptibility of Isolate ES 11 and ES 12

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**).

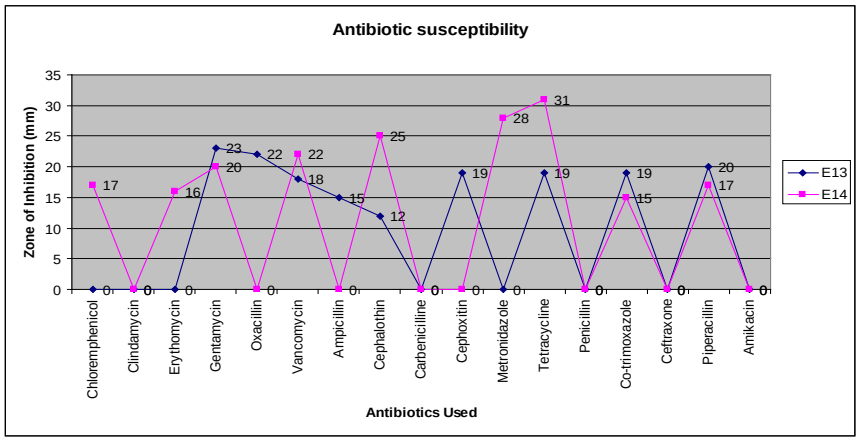

Fig 9: Antibiotic Susceptibility of Isolate E 13 and E 14

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E 15 isolate showed 100 % resistance against Ampicillin, Gentamycin, Penicillin, Amikacin Metronidaxazole, Tetracycline etc antibiotics whereas E 16 showed 100 % resistance against Gentamycin, Vancomycim, Cephalothin Cephoxitin , Penicillin , Co-trimaxazole and Amikacin. .

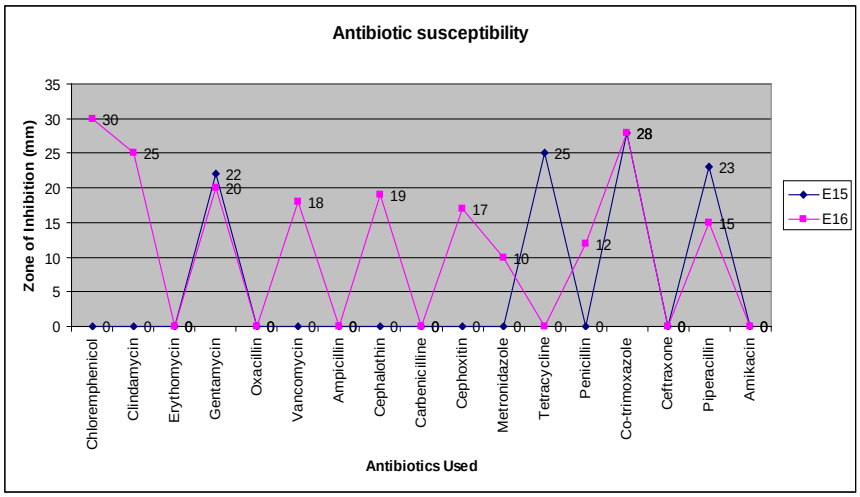

Fig 10: Antibiotic Susceptibility of Isolate E 15 and E 16

Isolated strain E17 when tested for their susceptibility it was found that E5 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. E18 showed 22 mm zone of inhibition for Chloremphenicol, 20 mm for Clindamycin, 0 mm for Erythromycin, 10 mm for Gentamycin, 0 mm for Oxacillin.

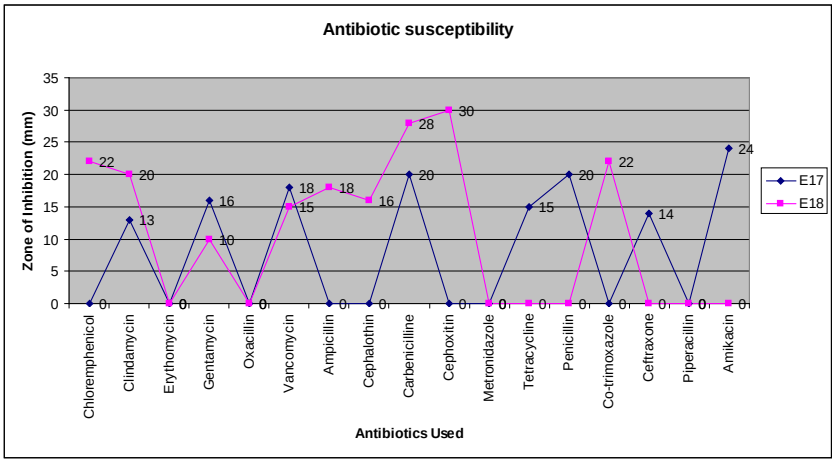

Fig 11: Antibiotic Susceptibility of Isolate E 17 and E 18

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. It has been reported that beta-lactamase producing *E. coli* which had become resistant to ceftriaxome can become sensitive to the same antibiotic when the inhibitor sulbactam is added (Abdul *et al*., 2005).

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 Vancomycim 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 f**o**xacin, cefdinir, ciprofloxacin and novobiocin antibiotics should be avoided against *E coli* diarrhoeal infections in this region.

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