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Isolation of *E. Coli* from Different School's Drinking Water

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Abstract: ***Water is a vital element in each of our lives. Clean water is important to one's good health. Water is a natural resource and is essential to sustain life. Public and environmental health protection requires safe drinking water, which means that it must be free of pathogenic bacteria. Escherichia coli (E. coli) bacteria. Here Out of 50 water samples, in 12 water samples E. coli were isolated. Microbiological water contamination is detected by microbial water quality indicator. These bacteria indicate feacal pollution and possible presence and ingestion of the other pathogens with polluted water. Waterborne diseases caused by the consumption of contaminated water can affect. a large number of people in a short time.***

Keywords: contaminated water, Fecal pollution

1. Introduction

Water is everywhere on our planet and it's the reason we have organic life. It shapes our mountains, carves our oceans, and drives our weather. water is a chemical compound. . Water is often referred to as the "universal solvent" because of its amazing ability to dissolve so many substances. Accessibility and availability of fresh clean water does not only play a crucial role in economic development and social welfare, but also it is an essential element in health, food production and poverty reduction. Water helps maintain the moisture of internal organs of the body.

Water content of a single cell is 45% to 95% and microorganism contains 80% of body weight as water and human contains water is 70% of their body weight. It is thermal regulator of human body and normal human body contains 42 liters of water in them. Whenever 2.7 liters of water loss from body it can leads to headache, dehydration and weakness. Water is equally important and critical for both humans and environment and it is a key issue in form of drinking water. Dams, canals and wells show importance of water and the impact of human beings on water cycle. . Water maintains normal volume and consistency of fluids such as blood and lymph. Regulates body temperature, removes poisons or toxins from the body through urine, sweat and breathing. Some bacteria are beneficial and some are not.

There are two main sources of water: surface water and groundwater. Surface Water is found in lakes, rivers, and reservoirs. Groundwater lies under the surface of the land, where it travels through and fills openings in the rocks. The rocks that store and transmit groundwater are called aquifers. Groundwater must be pumped from an aquifer to the earth's surface for use. Consumers receive their water from one of two sources: a private well, or a city water system. A household well pumps groundwater for household use. The source of a city water system may be either surface water or groundwater. Coliform bacteria are found in the guts of ruminant animals such as sheep and cattle. In the guts of these animals they play a beneficial role in the nutrition of the animal. However, the waste of these animals also contains a high concentration of these bacteria.

A large number of people in a short time. A number of factors can contribute to microbial contamination of drinking water, including low pressure conditions, low residual chlorine concentrations, and pipe breaks, which can result in the intrusion of pathogenic microorganisms.

Nowadays, for the E. coli detection in water, standardized and regulated conventional techniques are used. These techniques are based on the cultivation of bacteria such as fermentation of multiple tubes, membrane filtration, and methods that use defined substrates, among others. The most important is the prolonged incubation time for the final detection of E. coli, requiring at least 24–28 h. It is worrisome that a positive result of this pathogen is detected when the water has already reached the distribution system of the different houses of the population being too late to generate a suitable response. Furthermore, some conventional methods are questioned for their sensitivity to the interference of microorganisms or antagonistic substances and the deficient detection of viable but non - cultivable bacteria. Additionally, these are expensive methods that require complex equipment, specific reagents, and lack the standardization process to obtain protocols for the sample's analysis. These limitations can place public health at risk. This is why many research studies have focused on the development of fast and accurate methods for E. coli detection in drinking water.

Water - borne diseases are one of the major public health problems in developing countries. It is estimated that contaminated water has caused more than 20 million deaths of which more than 80% were among children under age five. Identification of water born diseases like "diarrhea" caused by the bacterium entero toxigenic Escherichia coli (ETEC) contamination in drinking water, often depends on conventional methods includes culturing, biochemical and more preferable by most probable number (MPN).

E. coli numbers in freshwater are determined by counting the number of yellow and yellow brown colonies growing on a 0.45 micron filter placed on m - TEC media and

incubated at 35.0º C for 22 - 24 hours. The addition of urea substrate confirms that colonies are E. coli. This bacteria is a preferred indicator for freshwater recreation and its presence provides direct evidence of fecal contamination from warm - blooded animals. Although usually harmless, E. coli can cause illnesses such as meningitis, septicemia, urinary tract, and intestinal infections. . Bacteriophages or phages are viruses that infect prokaryotes and are capable of killing them specially the Bacteria. Bacteriophage means bacteria eater and this name is because when they first discovered, they appeared to eat bacterial cells. Among the coliform bacteria, Escherichia coli are the most abundant and best indicator of water quality and presence of pathogens. It comprises n seven (97%) of feacal coliform bacteria in human faces and available indicator of feacal contamination (Nold, 2005).

E. coli was first discovered by T. Escherichia in 1885 for feacal of health individuals and in 1891, frank land stated it as organisms with sewage characteristics that provide evidence for potentially dangerous pollution and so must be identified (Hutchinson, 1994). Event though, *E. coli* is indicator of contamination and pathogen presence, some of its strain such as 0157: H7, enter hemorrhagic and entro invasive are pathogenic and causes illness in mammals including humans, can cause severe disease and may be fatal in small children and the elderly. More than 80% of diseases in the world are attributed to unsafe drinking water or in adequate sanitation practices (WHO, 2003). This work was carried out to find out whether *E. coli* is present or absent in drinking water sources.

Scientific Classification of Escherichia coli;
Domain: Bacteria
Phylum: Proteobacteria
Class: Gammaproteobacteria
Order: Enterobacterales
Family: Enterobacteriaceae
Genus: Escherichia
Species: E. coli
Binomial Name: Escherichia coli

Escherichia coli also known as E. coli is a Gram - negative, facultative anaerobic, rod - shaped, coliform bacterium of the genus Escherichia that is commonly found in the lower intestine of warm - blooded organisms (endotherms). Most E. coli strains are harmless, but some serotypes can cause serious food poisoning in their hosts, and are occasionally responsible for food contamination incidents that prompt product recalls. Among the coli form bacteria, Escherichia coli is the most abundant and best indicator of water quality and presence of pathogens. It comprises n seven (97%) of feacal coliform bacteria in human faces and available indicator of feacal contamination (Nold, 2005). Global E. coli - related morbidities and mortalities are high.

The harmless strains are part of the normal microbiota of the gut, and can benefit their hosts by producing vitamin K2, (which helps blood to clot) and preventing colonization of the intestine with pathogenic bacteria, having a symbiotic relationship. *E. coli* is expelled into the environment within fecal matter. The bacterium grows massively in fresh fecal matter under aerobic conditions for 3 days, but its numbers decline slowly afterwards.

Mekonen Wolditsadik, & Jida Leta *et al*. (2014) found that the average indicator bacteria (*E. coli*) obtained from all sites were different. The average counts of *E. coli* were detected in shower sample (203.57cfu/100ml), cafeteria (138.57cfu/ 100ml) and the least count were obtained from spring (80.7cfu/100ml). The highest average *E. coli* colony counts were observed at shower (203.57cfu/ 100ml) and the lowest mean counts, 80.7cfu/ 100 ml. E. coli colony were found in spring sample.

Frederick Adzitey *et al*. (2015) found that a total 56 water samples *Escherichia coli* isolates were screened against nine different antibiotics. Susceptiblity to gentamicin (91.07%), ciproflaxin (94.64%), ceftrioxine (89.29%). Resistance to erythromycin (85.71%), vancomycin (94.64%) was high.

C. A. Adinortey, D. H. A. K. Amewowor, E. P. Otwe, I. K. A. Galyuon and D. K. A. Asante *et al*. (June 2010 - May 2012) found that in all, 389 E. coli isolates were obtained comprising 261 and 128 from clinical and environmental samples respectively. All *E. coli* isolates were 100% sensitive to Imipenem. The percentage sensitivities of clinical E. coli isolates to ampicillin (0 - 24.1%), tetracycline (16.0 - 28.4%), cotrimoxazole (16.8 - 22.0%), cefuroxime (27.6 - 43.2%) and nalidixic acid (22.1 - 47.8%) were found to be relatively low. The sensitivity of environmental isolates to the 16 antibiotics was higher than that of clinical isolates, except for nalidixic acid, aztreonam and amikacin to which isolates from environmental samples were less sensitive.

Carnot, J. S. Guerra, T. S. Souza and L. C. Carneiro*et al*. (2014) Isolated and identified E. coli from in nature water samples and verified the presence of plasmids that caused bacteria resistance. Collected 24 water samples from two different stations (a water treatment station and a water captation station). From the contaminated samples, they evaluated antibiotic resistance to amoxicillin (5 µg) cephalexin (5 µg), azitromicin (5 µg), ampicilin (5 µg), tetracycline (5 µg) and ciprofloxacin (10 µg). The results showed a frequent occurrence of multiple resistance to the main antimicrobials utilized, including cephalexin (67.44%), amoxicilin (62.79%), ampicilin (58.13%), tetracycline (37.20%), azithromycin (32.55%), ciproflaxacin (18.60%). Observed the presence of different plasmodia l profiles, including occurrences of amoxicillin (30%), ampicillin (30%), tetracycline (30%) and ciprofloxacin (10%). The study showed that the samples presented plasmids with genes resistant to important antibiotics used for public health.

I Garba, MB Tijjani, MS Aliyu, SE Yakubu, A Wada - Kura, OS Olonitola *et al*. (2009) biochemical analysis of the samples showed that out of 63 confirmed *Escherichia coli* isolated, 41 (45.5%) were from well water, tap water had 14 (23.3%) while packaged water had 8 (13.3%). The susceptibility profile of the isolates to nine antimicrobial agents indicated that majority of the isolates were highly susceptible to Chloramphenicol, Gentamycin, Perrfloxacin, Tarivid, Augmentin, Streptomycin, Sparfloxacin, and

Ciprofloxacin, moderate susceptibility to Septrin and resistant to Amoxacilin were observed. None of the water samples met the WHO standards for drinking water and thus pose a serious health risk to its consumers and users if not properly treated.

Łuczkiewicz, K. Jankowska, S. Fudala - Ksiazek, and K. Olańczuk – Neyman *et al*. (2010) antimicrobial resistance of fecal coliforms (n = 153) and enterococci (n = 199) isolates was investigated in municipal wastewater treatment plant based on activated sludge system. Susceptibility of selected strains was tested against 19 (aminoglycosides, aztreonam, carbapenems, cephalosporins, β - lactam/β - lactamase inhibitors, fluoroquinolones, penicillines, tetracycline and trimethoprim/ sulfamethoxazole) and 17 (high - level aminoglycosides, ampicillin, chloramphenicol, erythromycin, fluoroquinolones, glycopeptides, linezolid, lincosamides, nitrofuration, streptogramins, tetracycline) antimicrobial agents respectively. Resistance to nitrofuration and erythromycin was common among enterococci (53% and 44%, respectively), and followed by resistance to ciprofloxacin (29%) and tetracycline (20%). The resistance phenotypes related to glycopeptides (up to 3.2%) and high - level aminoglycosides (up to 5.4%) were also observed. Most frequently, among Escherichia coli isolates the resistance patterns were found for ampicillin (34%), piperacillin (24%) and tetracycline (23%).

A Larson *et al*. (2019) examined total 314 *E. coli*samples. The E. coli antibiotic resistance profile showed highest resistance against tetracycline (37.6%), ampicillin (34.2%), sulfamethoxazole - trimethoprim (21.4%), and nalidixic acid (13%). Some 19.7% (95% CI [12.9, 28.0], n = 23) of the E. coli isolates displayed multidrug resistance, defined as resistance to at least three classes of antibiotics.

Hassan Momtaz, Farhad Safarpoor Dehkordi, Ebrahim Rahimi & Amin Asgarifar *et al.* (2013) studied total of 448 water samples from tap water and mineral water. The culture method showed that 34 (7.58%), 4 (0.89%) and 3 (0.66%) of all 448 water samples were positive for Escherichia coli, Salmonella species, and Vibrio cholera, respectively. The culture technique showed that 34 (23.61%), 4 (2.77%) and 3 (2.08%) out of 144 tap water and only 7 (2.3%) out of 304 bottled drinking water were positive for presence of E. coli, Salmonella species, and Vibriocholerae, respectively. Among the tap water samples, 27 (18.75%), 14 (9.72%), and 5 (3.47%) were positive for these bacteria in the three seasons, respectively. Totally, 46 (10.26%), 5 (1.11%), and 3 (0.66%) samples out of the 448 tap water and bottled drinking waters, were positive for E. coli, Salmonella species, and Vibrio cholerae, respectively.

Shar *et al*. (2007) isolated total and faecal coliform bacteria from all samples of drinking water of Khairpur city having a surface reservoir as the primary source of water. The total coliform counts (log10 3.0 - 3.94 CFU/100 mL) and faecal coliform (E. coli) counts (log10 1.46 - 2.47 CFU/100 mL) were found to be higher than the maximum microbial contaminant level (MMCL) established by WHO. Liu et al. (2008) determined presence of viable and viable but non - culturable (VBNC) cells of E. coli O157: H7 in drinking water and river water samples. Viable E. coli O157: H7 as few as 3 to 4 CFU/L in tap water, 7 CFU/L in river water and 50 VBNC cells in 1 Litre of river water were detected in the study. Nagvenkar and Ramaiah (2009) studied Mandovi and Zuari River in central west coast of India to estimate different human pathogenic bacteria and showed a mean abundance of 123 CFU/mL of E. coli in the waters.

Ibekwe *et al*. (2011) investigated the antimicrobial resistance pattern of *E. coli* isolated from small channels arising from middle Santa Ana River in Southern California and identified the source of contamination to be that of humans and animals.24% of the 600 isolates exhibited resistance to more than one antimicrobial agent. Most multiple resistances were associated with inputs from urban runoff and involved the antibiotics rifampicin, tetracycline, and erythromycin.

2. Material and Method

Study Area:
The study was conducted at Nabira Mahavidyalaya, Katol in Nagpur district of Maharashtra State. It is the administrative headquarters of Katol taluka, one of the 14 talukas of this district. Water samples were collected from water taps of various schools and colleges of Katol and nearby cities. Katol is a city and municipal council in Nagpur District of Maharashtra. Katol is located at 21.27°N 78.58° E

Media: EMB, of Lactose broth, of Mueller Hinton Agar, Nutrient Broth

Collection of sample
A total of 50 samples were collected from various school's drinking water. Study was conducted between December 2019 to January 2020. Water sample are collected to estimate the presence and number or population of *E. coli.* . The water samples were collected from sources and collected in sterile bottles. The sampling bottles were labeled and sealed with paper tape. After collection of sample test tubes were tightly closed bottle to avoid any contamination and to protect from environmental pathogen contamination. Then transported to the laboratory for microbiological analysis. Out of 50 samples, 12 samples are found to be contaminated by *E. coli.*

- **Gram staining**
- **Biochemical Test was done**

Biochemical test includes following tests
- Indole test
- Methyl red test
- Voges - Proskauer test
- Citrate test

Bacteriological analysis
1) Presumptive Coliform Test
2) Confirmed Coliform Test
3) Completed Coliform Test

Antibiotics susceptibility testing:
Antibiotic sensitivity profiles of E. coli isolates were studied against different antibiotics Briefly, bacteria were grown in nutrient broth. Mueller - Hinton Agar (Hi - media) was used as a medium to study the susceptibility to antibiotics. Zones

of inhibition were recorded. antimicrobials are used for the control and treatment of bacterial associated infectious diseases and for growth promotion purposes as well. Various studies have reported resistance of E. coli strains to antibiotics.

The Modified Kirby - Bauer disk diffusion method was used to evaluate the susceptibility or resistance of E. coli isolates to 11 selected antibiotics, i. e. erythromycin (15 µg), chloramphenicol (30 µg), gentamicin (10 µg), ciprofloxacin (5 µg), cephalotin (30 µg), penicilin - G (10 units), cotrimoxazole (25 µg), ceftriaxone (30µg), Vancomycin (30 µg), amoxicillin (10µg), ampicillin (10 µg).

3. Result and Discussion

Out of 50 water samples, in 12 water samples *E. coli* were isolated. All positive tubes from presumptive test were streaked on Eosin methylene blue (EMB) agar for the detection of coliform colonies especially E. coli. Positive confirmed samples that showed typical coliform colonies i. e. metallic green sheen colonies of E. coli. All samples are then subjected for antibiotic susceptibility test. The average indicator bacteria (*E. coli*) obtained from all sites were different.

The isolation of coliform especially *E. coli* from water sources is attributable to contamination by human and animal origin and this is of health significance as these organisms have generally been agent of gastroenteritis in humans. The tap water found positive might have been contaminated by water hoses connected to the tap, which was normally left on the ground after used and reused without cleaning.

Water quality based on most probable number method were confirmation of coliform. Out of total 50 water samples, in 12 samples *E. coli* were isolated. All samples were subjected for antibiotic susceptibility pattern. Following data provides descriptive information about it.

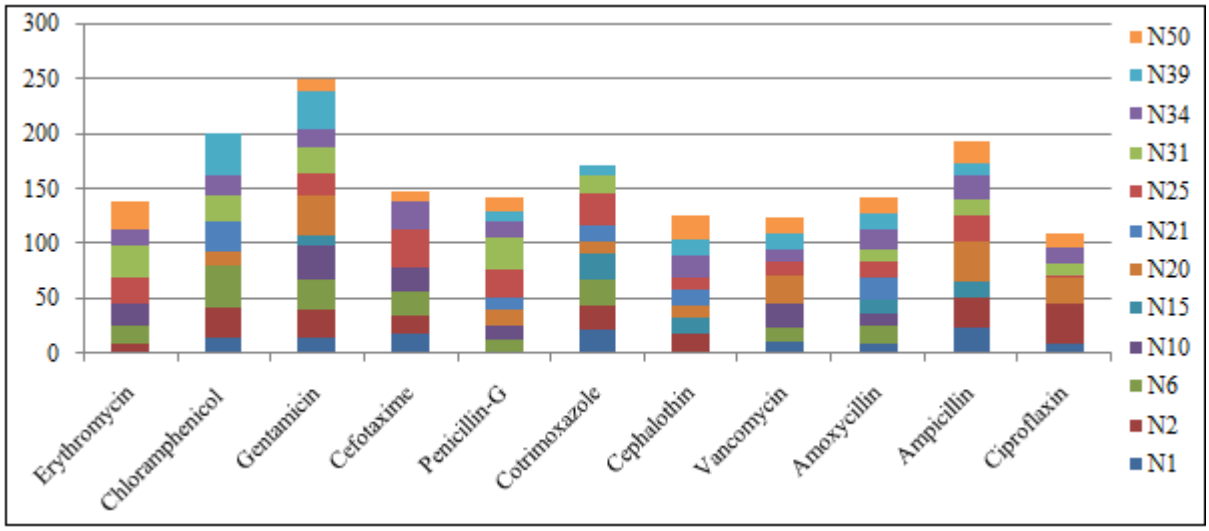

Figure 1

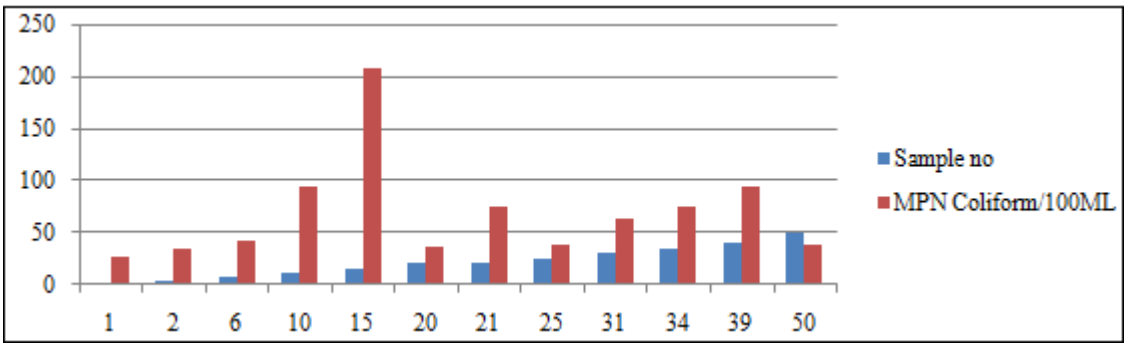

Figure 2

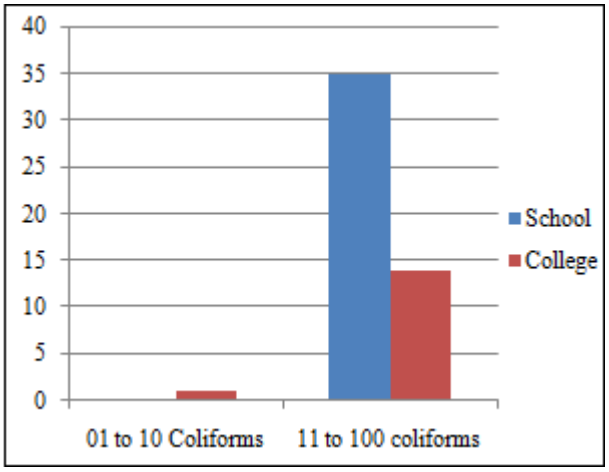

Figure 4
Number of coliforms found in schools and colleges

S= Sensitive
R= Resistant

- In a present study isolate N1 is resistant to Erythromycin, penicillin - G and there is no zone found. Isolate N1 is sensitive to Chloramphenicol and Gentamicin measure 15 mm zone, Cefotaxime measure 18mm zone, Cotrimoxazole measure 22 mm zone, Vancomycin measure 11 mm zone, Amoxicillin measure 10 mm zone, Ampicillin measure 24 mm zone, Ciproflaxin measure 10 mm zone.
- Isolate N2 is resistant to Penicillin - G, Vancomycin, Amoxicillin and there is no zone found. Isolate N2 is sensitive to Erythromycin measure 10 mm zone, Chloramphenicol measure 27 mm zone, Gentamicin measure 25 mm zone, Cefotaxime measure 17 mm zone, Cotrimoxazole measure 21mm zone, Cephalothin measure 18mm zone, Ampicillin measure 28mm zone and also sensitive to Ciproflaxin measures 36 mm zone.
- Isolate N6 is resistant to Cephalothin, Ampicillin, Ciproflaxin and there is no zone found. Isaolate N6 is sensitive to Erythromycin measure 15mm zone, Chloramphenicol measure 38 mm zone, Gentamicin measure28 mm zone, Cefotaxime measure 22 mm zone, Cotrimoxazole measure 24 mm zone also sensitive to Penicillin - G measure 12mm zone.
- Isolate N10 is resistant to Cephalothin, Ampicillin, Ciproflaxin, Chloramphenicol, Cotrimoxazole and there is no zone found. Isolate N10 is sensitive to Erythromycin measure 20mm zone,, Gentamicin measure 30 mm zone, Cefotaxime measure 22 mm zone, Penicillin - G measure 14mm zone, Vancomycin measure 22 mm zone and also sensitive to Amoxicillin measure 12 mm zone.
- Isolate N15 is resistant to Erythromycin, Chloramphenicol, Cefotaxime, Penicillin - G, Vancomycin and ciproflaxin and there is no zone found. Isolate N15 is sensitive to measure 20mm zone,, Gentamicin measure 10mm zone, Cotrimoxazole measure 25mm zone, Cephalothin measure 15mm zone, Ampicillin measure 14 mm zone and also sensitive to Amoxicillin measure 14 mm zone.
- Isolate N20 is resistant to Erythromycin and Amoxicillin and there is no zone found. Isolate N20 is sensitive to Chloramphenicol measure 13mm zone, Gentamicin measure 36 mm zone, Penicillin - G measure 15 mm zone, Cotrimoxazole measures 10 mm zone, Cephalothin measures 11 mm zone, Vancomycin measures 27 mm zone, Ampicillin measures 36 mm zone and also sensitive to Ciproflaxin measure 23 mm zone.
- Isolate N21 is resistant to Erythromycin, Gentamicin, Cefotaxime, Vancomycin, Ampicillin, Ciproflaxin and there is no zone found. Isolate N21 is sensitive to Chloramphenicole 27 measure mm zone, Penicillin - G measures 10 mm zone, Cotrimoxazole measure 15 mm zone, Cephalothin measure 14 mm zone and also sensitive to Amoxicillin measures 20 mm zone.
- Isolate N25 is resistant to Chloramphenicol and there is no zone found. Isolate N25 is sensitive to Erythromycin measures 24mm zone, Gentamicin measure 20 mm zone, Cefotaxime measure 35 mm zone, Penicillin - G measure 25 mm zone, Cotrimoxazole measure 30 mm zone, Cephalothin measure 11 mm zone, Vancomycin measure 13 mm zone, Amoxicillin measure 15mm zone, Ampicillin measure 24 mm zone and also sensitive to Ciproflaxin measures 3 mm zone.
- Isolate N31 is resistant to Cefotaxime, Cephalothin, Vancomycin and there is no zone found. Isolate N31 is sensitive to Erythromycin measure 30 mm zone, Chloramphenicoll measures 25mm zone, Gentamicin measure 25 mm zone, Cotrimoxazole measure 15 mm zone, Amoxicillin measure 10 mm zone, Ampicillin measure 15 mm zone and also sensitive to Ciproflaxin measure 10 mm zone.
- Isolate N34 is resistant to Cotrimoxazole and there is no zone found. Isolate N34 is sensitive to Erythromycin measure 14 mm zone, Chloramphenicol measures 17 mm zone, Gentamicin measure 15 mm zone, Amoxicillin measure 18 mm zone, Ampicillin measure 22 mm zone, Ciproflaxin measure 10 mm zone. Cefotaxime measure 25 mm zones, Cephalothin measure 20 mm zone and also sensitive to Vancomycin measure 11 mm zone.
- Isolate N39 is resistant to Erythromycin, Ciproflaxin, Cefotaxime and there is no zone found. Isolate N39 is sensitive to Cotrimoxazole measure 10 mm zone, Chloramphenicol measures 40 mm zone, Gentamicin measure 35 mm zone, Penicillin - G measure 10 mm zone, Amoxicillin measure 15 mm zone, Ampicillin measure 10 mm zone, Cephalothin measure 15 mm zone and also sensitive to Vancomycin measure 13 mm zone.
- Isolate N50 is resistant to Chloramphenicol, Cotrimoxazole and there is no zone found. Isolate N50 is sensitive to Erythromycin measure 26 mm zone, Gentamicin measure 12 mm zone, Amoxicillin measure 15 mm zone, Ampicillin measure 20 mm zone, Ciproflaxin measure 12 mm zone. Cefotaxime measure 10 mm zone, Cephalothin measure 22 mm zone, Penicillin - G measure 13 mm zone and also sensitive to Vancomycin measure 15 mm zone.
- These 12 isolates show maximum resistance to Gentamicin followed by Cefotaxime, Cotrimoxazole, Chloramphenicol, Ampicillin, Erythromycin, Amoxicillin, Cefotaxime, Vancomycin, Penicillin - G, Cephalothin, Ciproflaxin. Thus finding recommended that Gentamicin, chloramphenicol, Cotrimoxazole are the best choice of drugs against *E. coli* infection.

4. Conclusion

It can conclude from the study that antibiotic resistant *E. coli* is common in school's drinking water source in Katol and surrounding area. Ciproflaxin is the least effective antibiotic followed by Cephalothin while Gentamicin is the most effective antibiotic. The E. coli isolates also varied patterns to commonly antibiotics used.

Major source of water was well water for most of the school's. Attention should be given to proper handling of the water. On conclusion it is clear that E. coli appears to be the best indicator of bacteriological quality of water. So water authorities should have steps to control coliforms in drinking water to prevent from water borne disease. Water, especially water from a private water source like a well, should be treated using chlorine, ultra - violet light, or ozone, all of which act to kill or inactivate E. coli. The isolates have a variety of resistant and sensitivity patterns to commonly used antibiotics is concluded from the study.

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