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DETECTION OF *ESCHERICHIA COLI* , *STAPHYLOCOCCUS AUREUS* AND *SALMONELLA TYPHI* IN DRINKING WATER OF GOVERNMENT INSTITUTIONS AND ORGANIZATIONS OF GWALIOR CITY

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ABSTRACT

Drinking water should be pure and free of contaminants to ensure proper health and wellness. Poor quality of drinking water is a serious threat for mankind .The contamination of drinking water sources and with microbial pathogens is a hazardous on-going problem. Water samples were collected especially into sterile containers from six designated government institutions and organizations in Gwalior city, India. These government institutions and organizations experience a very large amount of human traffic throughout the day. The water samples were immediately subjected to microbiological analysis in order to evaluate the quality of drinking water. Majority of the samples were found to contaminated with Escherichia coli and half of the samples were contaminated with Salmonella typhi, whereas one sample showed the presence of Staphylococcus aureus bacteria .This explains the high probability of water-borne diseases such as Dysentry, Diarrhea and Typhoid fever, etc. within the people drinking water from these organizations.

KEYWORDS: Drinking water ,Government organizations , Escherichia coli, Staphylococcus aureus, Salmonella typhi.

INTRODUCTION

Worldwide, over one billion people lack access to an adequate water supply; more than twice as many lack basic sanitation[1] .Unsafe water, inadequate sanitation, and insufficient hygiene account for an estimated 9.1 percent of the global burden of disease and 6.3 percent of all deaths, according to the World Health Organization [2]. This burden is disproportionately borne by children in developing countries, with water-related factors causing more than 20 percent of deaths of people under age 14. Nearly half of all people in developing countries have infections or diseases associated with inadequate water supply and sanitation [3].The presence of bacteria like *Escherichia coli* , *Staphylococcus aureus* and *Salmonella etc.* in water is one of the root cause of various diseases and infections .

Escherichia coli is a gram-negative, facultatively anaerobic, rod-shaped bacterium of the genus *Escherichia* that is commonly found in the lower intestine of warm-blooded organisms (endotherms).[4] Most E. coli strains are harmless, but some serotypes can cause serious food poisoning in their hosts, and are occasionally responsible for product recalls due to food contamination.[5][6] 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.[7]E.coli strains can cause gastroenteritis, urinary tract infections, and neonatal meningitis. It can also be characterized by severe abdominal cramps, diarrhea that typically turns bloody within 24 hours, and sometimes fever. In rarer cases, virulent strains are also responsible for bowel necrosis (tissue death) and perforation without progressing to hemolytic-uremic syndrome, peritonitis, mastitis, septicemia, and gram-negative pneumonia.[8]

The presence of *E.coli* in water is a strong indication of recent sewage or faecal contamination. Sewage may contain many types of disease causing organisms. E. coli comes from human and animal waste. During rainfalls, snow melts, or other types of precipitation, *E.coli* may be washed into creeks, rivers, streams, lakes, or groundwater. When these waters are used as sources of drinking water and the water is not treated or inadequately treated, *E.coli*

may end up in the drinking water [9]. Faecalcoliforms and *E.coli* are bacteria whose presence indicates that the water may be contaminated with human or animal wastes. Microbes in these waters can cause short-term effects, such as diarrhea, cramps, nausea, headaches, or other symptoms. They may pose a special health risk for infants, young children, some of the elderly, and people with severely compromised immune systems [10].

Staphylococcus aureus is a gram-positive coccal bacterium that is a member of the Firmicutes, and is frequently found in the nose, respiratory tract, and on the skin. It is often positive for catalase and nitrate reduction. Although *S. aureus* is not always pathogenic, it is a common cause of skin infections such as abscesses, respiratory infections such as sinusitis, and food poisoning. Pathogenic strains often promote infections by producing potent protein toxins, and expressing cell-surface proteins that bind and inactivate antibodies.

In particular, S. aureus is one of the most common causes of bacteremia and infective endocarditis. Additionally, it can cause various skin and soft tissue infections,[11] particularly when skin or mucosal barriers have been breached

Salmonella is a genus of rod-shaped (bacillus) gram-negative bacterium of the Enterobacteriaceae family. The two species of Salmonella are Salmonella enterica and Salmonella bongori. *Salmonella enterica* is the type species and is further divided into six subspecies[12] that include over 2500 serovars. Infection with non-typhoidal serovars of *Salmonella* generally results in food poisoning. Infection usually occurs when a person ingests foods that contain a high concentration of the bacteria. Infants and young children are much more susceptible to infection, easily achieved by ingesting a small number of bacteria. In infants, infection through inhalation of bacteria-laden dust is possible. *Salmonella typhi* is responsible for typhoid fevers.

The organisms enter through the digestive tract and must be ingested in large numbers to cause disease in healthy adults. An infection can only begin after living salmonellae (not merely Salmonella-produced toxins) reach the gastrointestinal tract. Some of the microorganisms are killed in the stomach, while the surviving ones enter the small intestine and multiply in tissues. Gastric acidity is responsible for the destruction of the majority of ingested bacteria, but *Salmonella* has evolved a degree of tolerance to acidic environments that allows a subset of ingested bacteria to survive.[13]

The present study was carried out to investigate drinking water from government run institutions and organizations (Colleges, Public Places, Hospitals) and to discover any microbial pathogens in these water samples as a source of biological environmental health hazard.

MATERIALS AND METHOD

Sampling

Six water samples were collected from different water sources run by government organizations within Gwalior city, India were named as A, B, C, D, E, and F and were analyzed for bacterial and fungal contamination. Each sample was collected in sterile container sealed with screw cap after disinfection of dispensing point with flame. Then, samples were kept on ice till analysis take place in the laboratory within three hours.

Methods

A. Multiple tube fermentation technique for coliform bacteria (MPN test):

In the multiple-tube method, a series of tubes containing a suitable selective broth culture medium (lactose-containing broth, such as MacConkey broth) was inoculated with test portions of water samples. After a specified incubation time at a given temperature, each tube showing gas formation was regarded as “presumptive positive” since the gas indicates the possible presence of coliforms. However, gas may also be produced by other organisms, and so a subsequent confirmatory test was essential. The two tests are known respectively as the presumptive test and the confirmatory test. For the confirmatory test, a more selective culture medium (brilliant green bile broth) was inoculated with material taken from the positive tubes. After an appropriate incubation time, the tubes are examined for gas formation as before. The most probable number (MPN) of bacteria present was then estimated from the number of tubes inoculated and the number of positive tubes obtained in the confirmatory test. The analysis of water samples was done using procedure of standard methods.[14]

B. EC-MUG Test for confirmation of *E. coli* :

This test was done for confirmation of presence of E. coli in water samples and may be knitted into the Multiple Tube Fermentation (MTF) procedure, as a confirmatory test. For the confirmation of E.coli we choose to use EC-MUG test, by using BGLB and tryptone broth (indole test) at 44.5ºC.Here, MUG stands for 4-methlumbelliferyl-β-D-glucoronide. It is the substrate for the enzyme β-glucoronidase. This enzyme is primarily found only in E. coli. The analysis of water samples was done using procedure of standard methods.[14]

C. Test for *S. aureus*

Staphylococci have the unique ability of growing on a high salt containing media [15]. Isolation of coagulase-positive staphylococci on Phenol Red Mannitol Agar supplemented with 7.5% NaCl was studied by Chapman [16]. Mannitol Salt Agar (MSA) is recommended for use as a selective and differential medium for the isolation of pathogenic staphylococcus aureus .Therefore, resulting Mannitol Salt Agar Base was used for the isolation of coagulase positive staphylococci from water samples. The media(500ml) was prepared accordingly.

S.aureus ferment MSA and produce yellow colored colonies surrounded by yellow zones confirming presence of S.aureus in the specimen samples.

The petri plates were allowed to warm to room temperature and the agar surface to dry before inoculating. Thereafter, these plates were heavily inoculated and then the specimen were streaked .The specimen to be cultured was on a swab, which was further rolled over the agar surface. Plates were incubated at 35-37ºC for 24-48 hours and then results were analyzed.

D. Test for *S.typhi :*

Bismuth Sulfite Agar is a highly selective and differential medium. It is recommended for the isolation of *Salmonella* species, especially *S. typhi*, from food and clinical specimens[17]. Bismuth Sulfite Agar(BSA) was first described by Wilson and Blair as a combination of bismuth and medium sulfite for selection of typhoid and paratyphoid groups of bacteria from stool specimens. A modified version of the original formula of Wilson and Blair is recommended by the American Public Health Association for the examination of specimens for evidence of *Salmonella*.[18] The presence of causes *S.typhi* causes a black or green metallic colony and brown or black precipitate .[19]

The BSA media(500ml) was prepared accordingly. The petri plates were inoculated by using BSA and then the specimen were streaked using swab .Plates were incubated for 48 hours at 35 ^{0}C. After 24 hours these plates were re-examined for typical colonies and the results were noted.

RESULTS AND DISCUSSION:

The results of all the tests are given in the form of tables below :

Table 1 : Showing results of MPN Test

Sample	10ml	1ml	0.1ml	Combination of Positives	MPN/100ml
A	+ + + + +	+ - - + +	+ + + - +	5-3-4	210
B	+ - + + +	- - - - -	- - - - -	4-0-0	13
C	+ + + + -	- + - - +	+ - - - -	4-2-1	26
D	- + + + +	+ + - + -	- - - + +	4-3-2	39
E	+ + + + +	- - + - -	- + - - -	5-1-1	46
F	+ + + + +	+ - + + +	- + - - -	5-4-1	170

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Graph 1 : Showing comparison of MPN /100ml of all samples

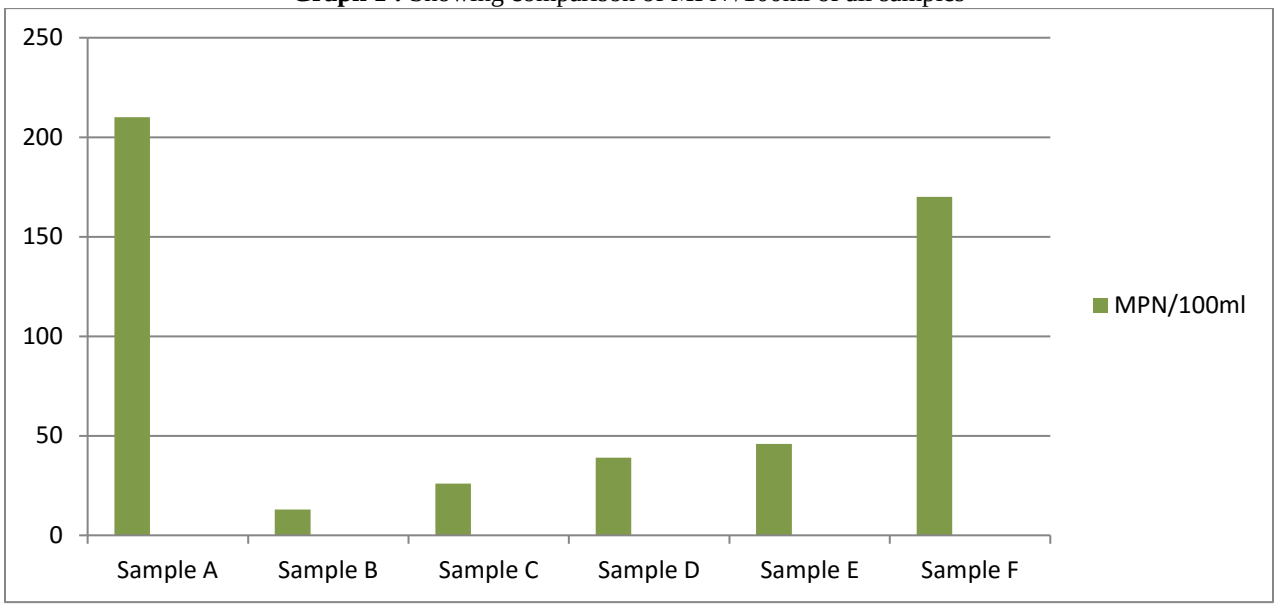

Table 2 : Showing results of EC-MUG Test for confirmation of *E. coli*

Sample	Presence- Absence(P-A) of *E.coli*
A	P
B	A
C	P
D	P
E	A
F	P

The samples showing Presence of E.coli showed a positive reaction i.e. observance of a bright blue fluorescence when these were subjected to long-wave (366 nm) ultraviolet (UV) light.

Table 3 : Showing results Test for *S. aureus*

Sample	Presence- Absence(P-A) of *S. aureus*
A	A
B	A
C	A
D	A
E	A
F	P

Only Sample F showed presence of *S. aureus* which appeared as yellow colonies with yellow zones in the media.

Table 4 : Showing results Test for *Salmonella*

Sample	Presence- Absence(P-A) of *Salmonella typhi*
A	P
B	A
C	P

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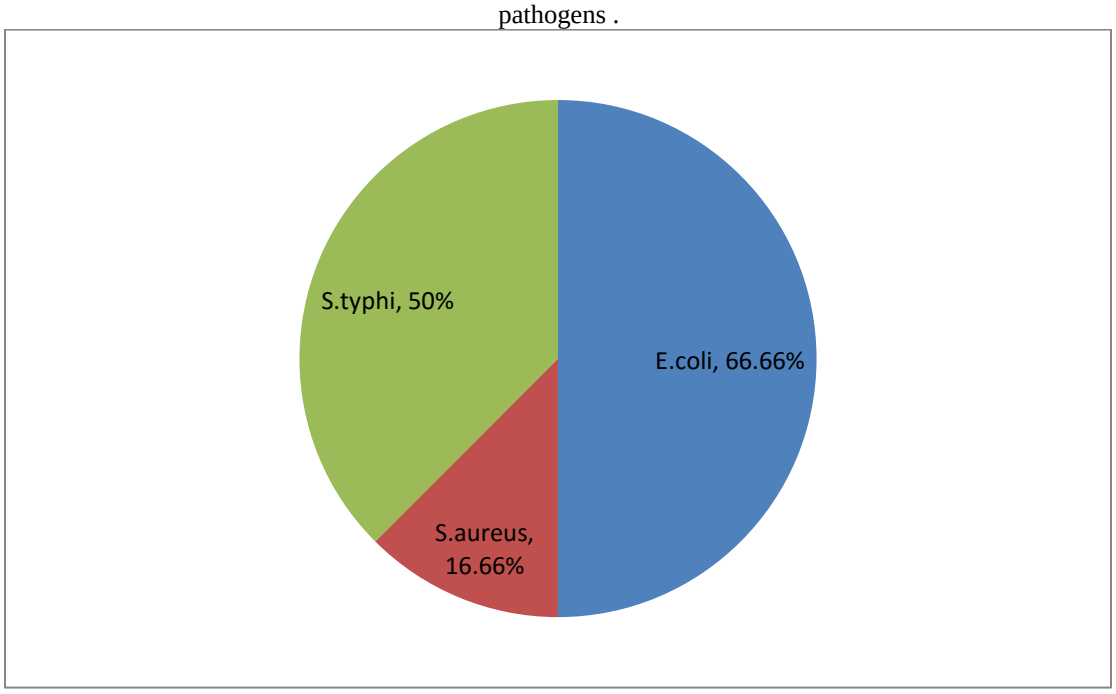
D	P
E	A
F	A

On Bismuth Sulfite Agar, Samples A, C and D formed round black colonies surrounded by a black zone which were be several times the size of tile colony confirming the presence of *Salmonella typhi*. Tile tone had a distinct metallic sheen when viewed by reflected light.

Pie-chart 1 : Showing the % of the samples of Government Institutions and organizations having presence of pathogens .



CONCLUSION

Presence *of E.coli, S.aureus, S.typhi* in drinking water is highly undesirable and can lead to many diseases. Four out of the six samples (A ,C, D, and F) were reported having *E.coli*, whereas sample F was detected with *S.aureus*, and *S.typhi* was found in three samples namely :A ,C and D. From the obtained results in our study we can conclude that: Most of the water coolers, water dispenser systems in Government institutions and organizations in Gwalior city were found to be contaminated with different microbial pathogens, bacteria and fungi.

It is highly recommended to have Periodical monitoring and cleaning of water sources for pollutants should be done (both chemical & microbial) along with Periodical testing of water coolers and dispenser tanks for their microbial contamination. The risk of microbial contamination in tanks can be reduced by several well-known practices.

A preventive approach to pathogen pollution should be taken by developing countries like India in the form of a source water protection program for all major freshwater sources and their supplies to all places .We also propose all government institutions and organizations to encourage infrastructure planning, extensive cleaning of water sources in public places including technological advances, to ensure that improved water treatment measures are taken for public welfare.

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