THE STANDARD ANTIBIOTICS SHOWS THEIR ANTIMICROBIAL EFFECT AGAINST PATHOGENIC BACTERIA ISOLATED FROM DRINKING WATER

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ABSTRACT: Aim of this study is bacterial isolation (pathogenic) from drinking water and makes some awareness about waterborne disease. Infectious diseases caused by pathogenic bacteria, viruses and parasites are the most common and widespread health risk associated with drinking water. Water contamination not only affects the developing countries but is also a major problem in developed countries. Thus, contamination of water resources with pathogenic bacteria leading to illness is a major concern throughout the world. The isolated bacteria were confirmed using DNA isolation method and BLAST sequence editing. The isolated bacteria were antagonistic against the standard antibiotics called Cephalexin, Erythromycin, and Amoxicillin. Amoxicillin is Penicillin antibiotic and treats the stomach ulcer which was caused by *Helicobacter pylori*. Erythromycin and Cephalexin both are bacteriostatic.

Keywords: antibiotics, bacteriostatic, Water contamination, waterborne disease.

INTRODUCTION

India has been gifted with many rivers and Himalaya Mountains to meet majority of water requirements of the country. Water resources mainly accomplish the deeds such as drinking, domestic along with agriculture, aquaculture and in power generation. Therefore, water plays an important role in the lives of human beings. Out of many water resources, the resource that is used for drinking and domestic purpose should be taken into consideration seriously because of contamination problem. Now a day, the water is getting contaminated with several pathogenic microorganisms. Water, as a solvent plays an important role in the metabolism of human beings. It is essential for digestion, absorption of food, transportation of nutrients and excretion of waste products. Hence, Physico-chemical and microbiological quality of drinking water has become an indispensable tool to check before its use. Water acts as a medium for various microorganisms and therefore plays a key role in spreading transferable diseases[1].

Infectious diseases caused by pathogenic bacteria, viruses and parasites are the most common and widespread health risk associated with drinking water. Nearly one-tenth of the global disease could be prevented by improving the water supply, sanitation, hygiene and the management of water resources [2]. Water quality is affected by faecal matter, domestic and industrial sewage and agricultural, in addition to a lack of aware-ness and education among the users [3]. The detection of bacterial indicators in drinking water suggests the presence of pathogenic organisms that are sources of waterborne disease [4]. Indicator microorganisms survive better and longer than pathogens, with uniform and stable properties, and may be easily detected using standard laboratory techniques [5]. These indicator organisms include *Escherichia coli*, thermo tolerant coliforms, total coliforms, streptococci and Clostridium. The two methods commonly used to detect coliforms in water include the multiple fermentation tube technique and the membrane filter technique. Well water is an important source of drinking and household water in both rural and urban areas. Studies [3], [6] assessing the microbiological quality of drinking water have found varying rates of contamination with coliforms and other heterotrophic bacteria. The pathogenic bacteria that usually appear in water contamination include Bacillus sp., *Clostridium botulinum*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Shigella* sp., *Salmonella* sp., *Staphylococcus aureus* and *Vibrio cholera*. Recent reports have shown the presence of pathogenic bacteria in drinking water which are responsible to water borne diseases. Several review papers have also explained the presence of pathogenic bacteria in various water resources. Contaminating pathogenic bacteria will cause several diseases such as diarrhoea and gastric disorders. Mostly the children get affected with these pathogens. As per WHO, nearly 3.4 million people die every year due to the diseases related to water contamination[7]. As per UNICEF, approximately 4000 children die every day due to water contamination [8]. Improving water quality is the major parameter to reduce the water related diseases[4].

The present study investigated water quality at sources and consumption of communities. Most people of such areas use water directly from available sources, without any treatment and therefore are exposed to a variety of water- related diseases. Aim of these study is detecting the pathogenic bacteria and to create awareness about water borne disease.

ISOLATION TECHNIQUES

Sample Collection

The water samples were collected from Puducherry house whom are using different water sources (Puducherry Municipality water, RO purified water, Packaged Can water, Puducherry Regional packaged water bottle). All the water samples pH (7.1, 5.9, 8.2, and 8.5 respectively) were tested and marked. The samples were serially diluted and maintain the diluted sample through pour and spread plates. The dilution range is 10^{-2}, 10^{-3}, 10^{-6}, and 10^{-8} and the cultures were sub cultured periodically.

Morphological identification

The colonies were observed and Gram's staining was done. The results were carefully marked for further bacterial isolation techniques.

DNA Isoaltion [9]

- The cells were grown overnight in Nutrient broth.
- 1.5ml of culture was transferred to a tube and centrifuged at 10,000 rpm for 2 minutes.
- The pellets were collected and repeated the centrifugation with another 1.5ml of culture containing cells. Drained the tubes on a paper towel briefly.
- 10µl of 10% SDS were added. 5µl of proteinase K (20mg/ml) was added. Incubate at 55˚c for 2 hours. After incubation it was chilled on ice for 10 minutes.
- 250µl of 6M Nacl was added. Again it was kept on freezer for 5 minutes.
- After freezing the sample was spinned at 8000 rpm for 15minutes.
- 500µl of supernatant was taken and transferred into a new 1.5ml tube.
- 1ml of 100% ice cold ethanol was added and inverted several times. Again the sample was spinned at 10,000 rpm for 15 minutes.
- The supernatant was removed and rinse with 500µl of 70% ethanol.
- The sample was spinned at 10,000rpm for 5 minutes.
- The supernatant was removed and dry the pellet at room temperature.
- 100µl of 1X TE buffer was added to the pellet.
- 5µl of DNA sample was added to the 0.8% agarose gel and Visualized under the UV Transilluminator.

Agarose Gel Electrophoresis

0.24g of Agarose in 30ml of TAE buffer was mixed. The Agarose solution was boiled till get a clear solution. 1.5µl of EtBr was added the solution gets completely cooled. The clear solution was poured in a gel casting plate with already adjusted gel comb. 5. The casting tray was cooled at room temperature for 30 minutes for solidification. After solidified, 5µl of DNA sample with 2µl of loading buffer were mixed and load in the well. Run the gel 50V for about 20 minutes.

Master Mix Components [10]

- Distelled water   -   16µl
- 10X Assay buffer   -   2.5µl
- Primer mix   -   0.5µl
- dNTPs mix   -   2µl
- Mgcl (30mM)   -   3.0µl
- Taq polymerase   -   0.5µl
- Template DNA   -   1µl

Primer Mix

- Eubac 5'-AGAGTTTGATCCTGGCTC-3'
- 1492RA   5'-GGTTACCTTGTTACGACTT-3'

Pcr Programme For 16s rRNA [11]

Polymerase chain reactions for EUBAC gene can be performed by following the temperature and timing condition programmed in a thermal cycler.

- Initial denaturation at 95˚c for 5 minutes.
- Number of cycles 30.
- Denaturation at 94˚c for 1 minute.
- Annealing at 45˚c for 45 seconds.
- Extension at 72˚c for 1 minute.
- Final extension at 72˚c for 10 minutes.

Check the amplified products in 1.5% Agarose gel electrophoresis and the molecular weight was assessed using molecular weight marker (100bp ladder).

Antimicrobial Activity ([11]

- The broth culture was prepared for test samples.The broth was incubated at overnight.
- The nutrient agar plates were prepared and named properly. The standard antibiotic plates were prepared and named as duplicate plates. The plated were allowed to solidify.
- After solidification the test samples were inoculated by using cotton swab method.
- Prepare standard antibiotic disc in 80µl concentration.
- Place the plates in incubator for 24 hours. Observed the plates and note the zone formation.

Sequencing editing

The obtained sequences were edited based on the electropherogram peak clarities. Sequences with noisy peaks were excluded from the analysis. The sequences were assessed to check the insertion or deletions and codons in MEGA 5.0 software. Sequencing Characterization Multiple sequence alignment and pairwise sequence alignment were performed using Clustal W program implemented in MEGA 5.0 in all the sequences. Nucleotide differences were carefully monitored and the differences were observed and edit manually. Sequences were translated into amino acid sequences using vertebrate mitochondrial codon pattern in the MEGA 5.0 for checking the pseudo-gene status. All the sequences were correctly translated into amino acid sequences with their respective starting primers without any internal stop codon.

Blast Search

The amplified sequences of EUBAC were confirmed by similarity index built in the NCBI's BLAST program. Based on the percentage similarity and query coverage against the reference species, the species were confirmed.

RESULTS

ORGANISMS	GRAM STAINING
Campylobacter coli	Positive
Helicobacter pylori	Positive
Microcystis aeruginosa	Negative
Shigella dysenteriae	Positive

Table 1: Gram's staining

Organisms	Cephalexin	Erythromycin	amoxicillin
Campylobacter coli	Positive	Positive	Positive
Helicobacter pylori	Comparatively positive[1]	Positive	Negative
Microcystis aeruginosa	Positive	Positive	Positive
Shigella dysenteriae	Positive	Positive	Positive

Table 2: Antibacterial Activity

Comparatively positive[1] is low level of zone of inhibition.
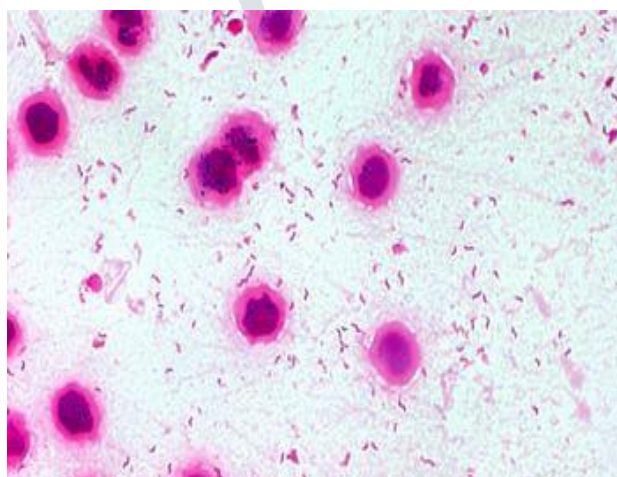
Figure 1a: *Campylobacter coli*   **Figure 1b:** *Campylobacter coli* **plate**
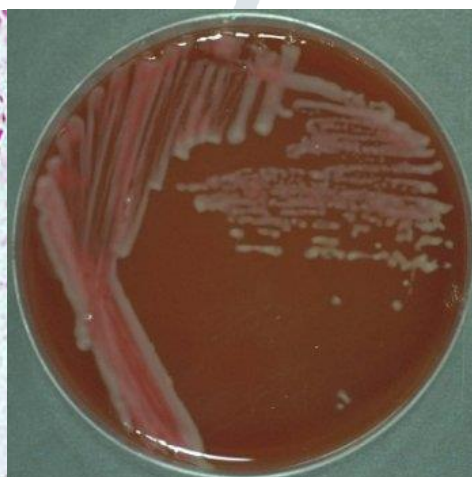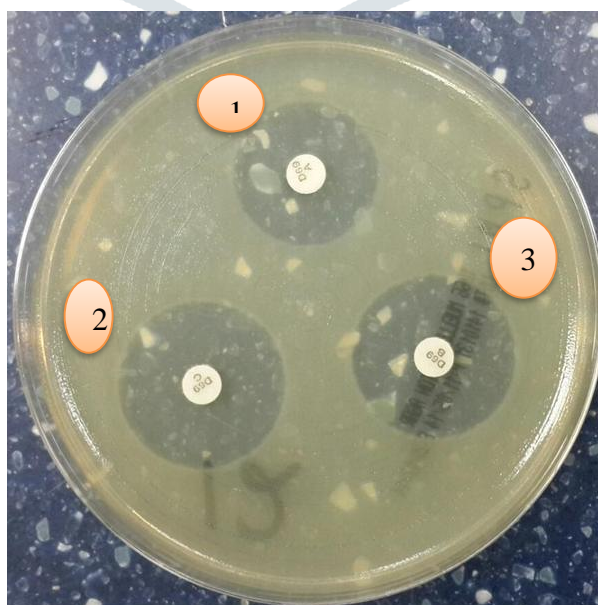
Figure 1c: *Campylobacter coli* **Antibacterial Activity**
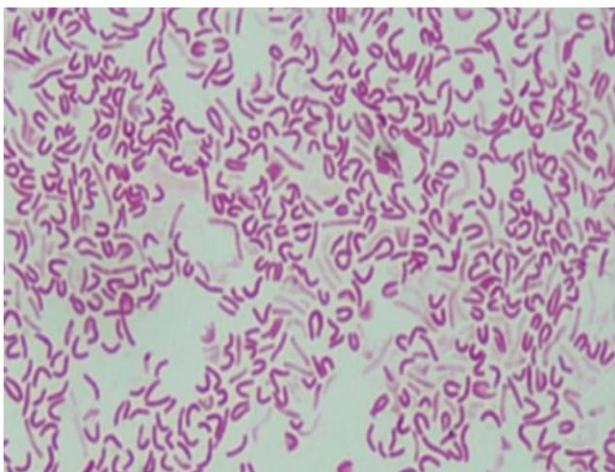
Figure 2a: *Helicobacter pylori*

Figure 2b: *Helicobactor pylori* **plates**

Figure 2c: *Helicobactor pylori* **Antibacterial Activity**
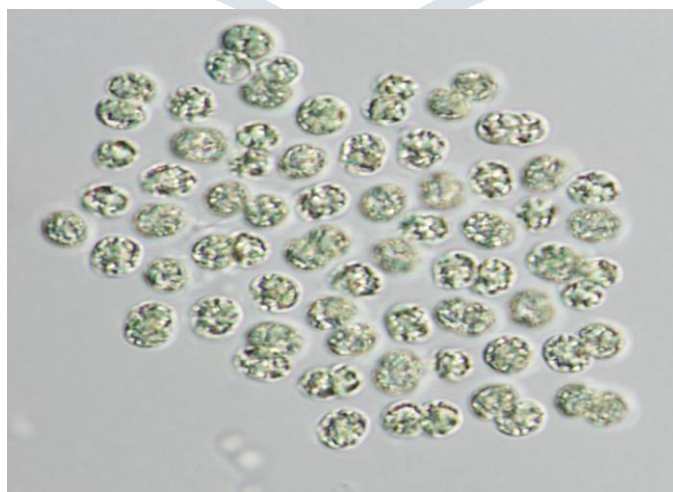
Figure 3a: *Microcystis aeruginosa*
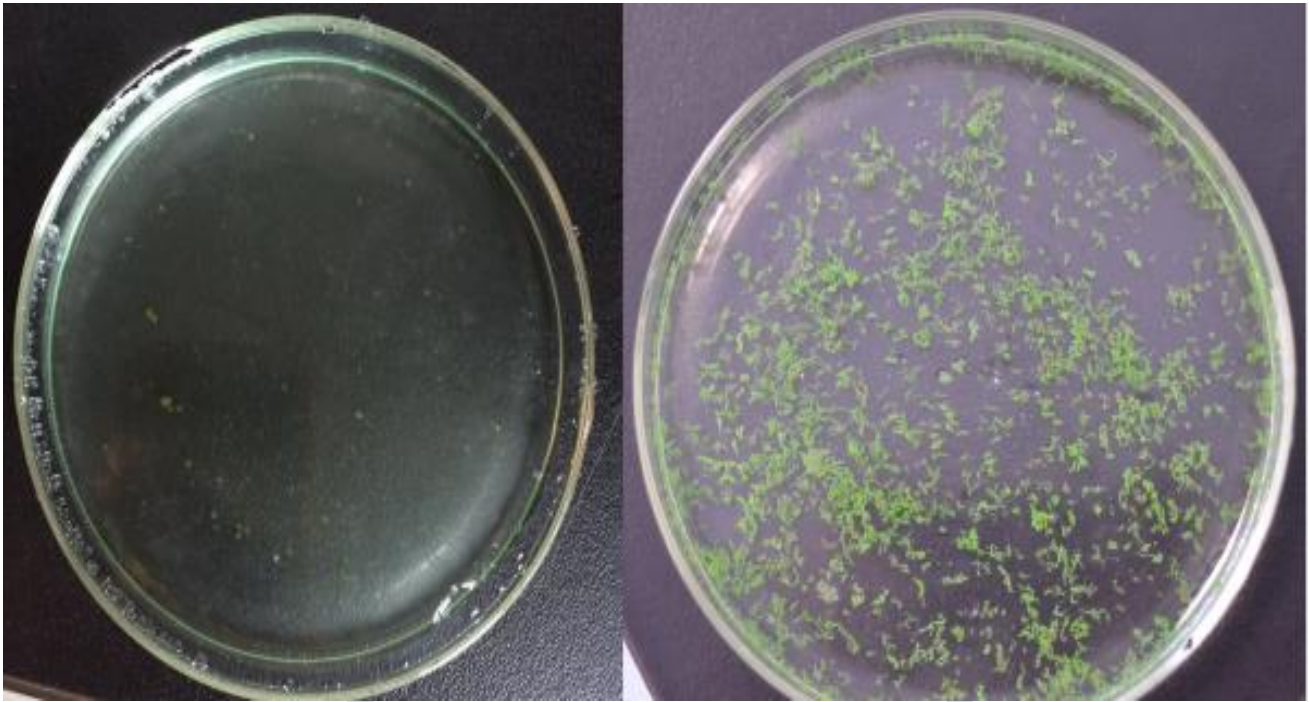
Figure 3b: *Microcystis aeruginosa* **plates**

Figure 3c: *Microcystis aeruginosa* **Antibacterial Activity**
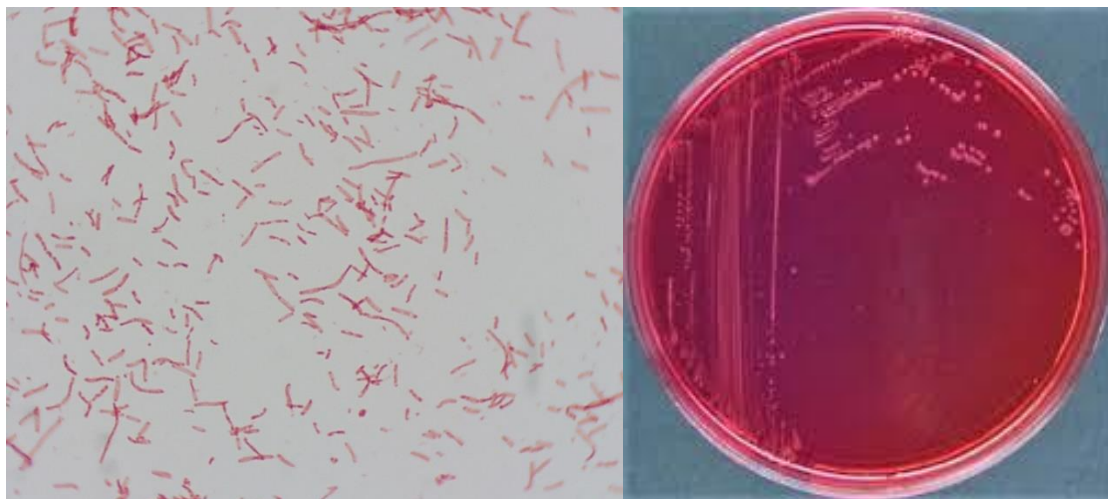
Figure 4a: *Shigella dysenteriae*   **Figure 4b:** *Shigella dysenteriae* **plates**
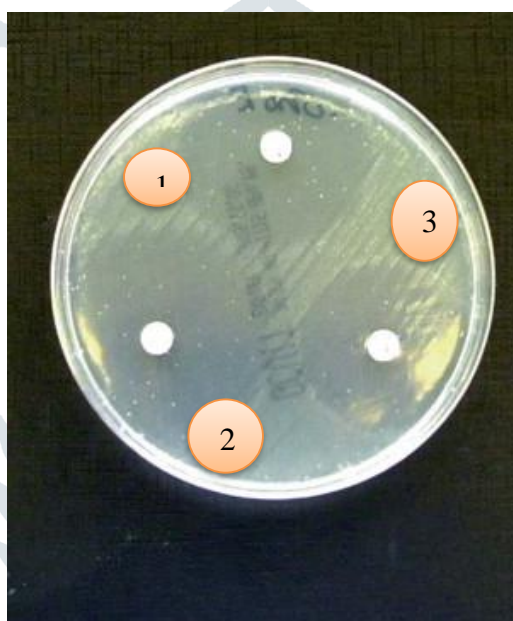
Figure 4c: *Shigella dysenteriae* **Antibacterial Activity**

1- Cephalexin

2- Erythromycin

3- **A**moxicillin
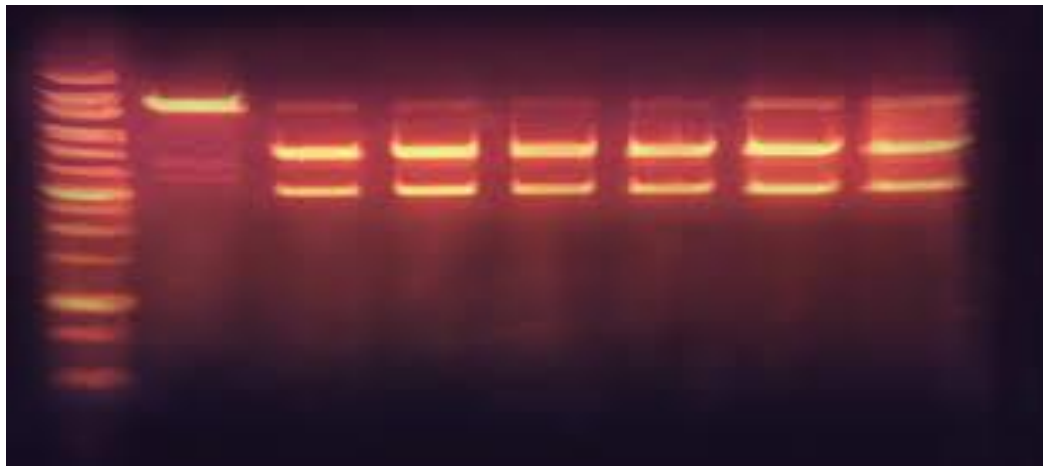
Figure 5: DNA isolation lane 2,3,4,5
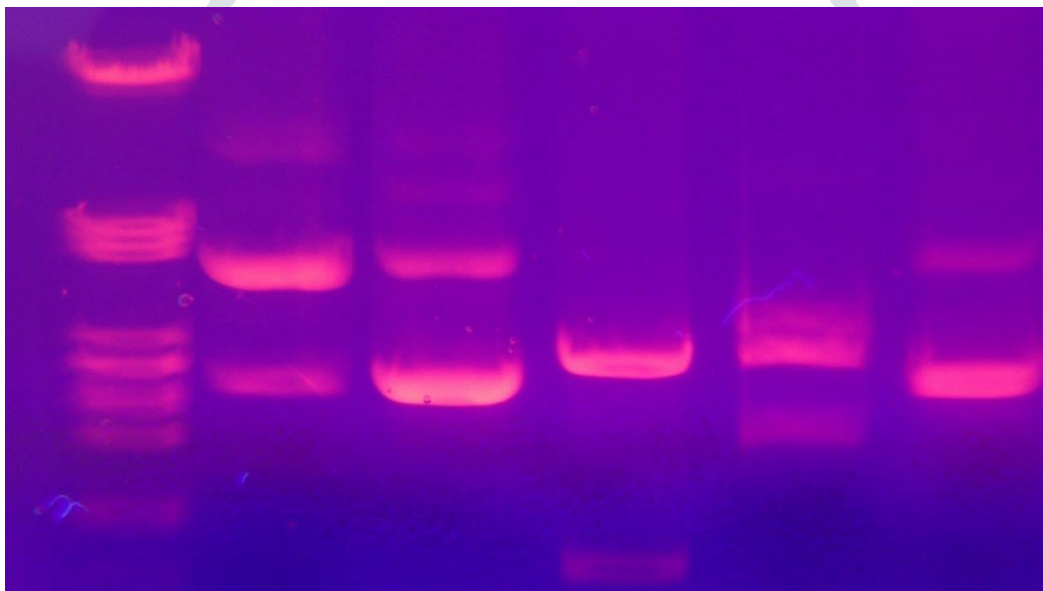
Figure 6: DNA isolation lane 1,2,3,4

Campylobacter coli **16S ribosomal RNA (16S rRNA), partial sequence**

GAGGACAACAGTTGGAAACGACTGCTNATACTCTATACTCCTGCTTAACACAAGTTGAGTAGGSAAAGTT
TTTCGGTGTAGGATGAGACTATATAGTATCAGCTAGTTGGTAAGGTAATGGCTTACCAAGGCTATGACGC
TTAACTGGTCTGAGAGGATGATCAGTCRCRCTGGAACTGAGACGCGGTCCAGACTCNTACGGGAGGCNGC
NGTAGGGAATATTGCGCAATGGGGGGAAACCCTNAGCAGCNACGCCGCGTGGAGGATGACNCTTTTAGGAG
CGTAAACTNNNTTTCTTAGGGAAGAATTCTGANGGTACCTNAGGAATAAGCNCCGGCNNNNNNNNNNNNNN
NNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNN
NNNNNNNNNNNNNNNNNNCCATNNAACTGCTTGAGAAACTGATAATCTAGAGTGAGGGAGAGGCNGATGGA
ATTGGTGGTGTAGGGGTNAAATCCGTAGAGATCACCAAGAATACCCATTGCGAAGGCGATCTGCTAGAAC
TCAACTGAGCTAATGCGTNAAAGCGTGGGGAGCNAACAGGATTAGATACCCTNGTAGTCCACGCCCTNAA
CGATGTATACTAGTTGTTGCTCTGCTAGTNAGGGCNGTAATGCTCTAACGGATTAAGTATACCGCCTNGG

GAGTACGGTCGCTAGANNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNN
NNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNCGGCTTGTCTAGCTCGTGTCGTGA
GATGTTGGGTTAAGTCCCCGAACGAGCGCAACCCACGTATTTAGTTGCTAACGGTTCGGCCGAGCACTCT
AAATAGACTGCCTTCGTAAGGAGGAGGAAGGTGTGGACGACGTCAAGTCATCATGGCCCTTATGCCCAGG
GCGACACACGTGCTACAATGGCATATACAATGAGACGCAATACCGCGAGGTGGAGCAAATCTATAAAATA
TGTCCCAGTTCGGATTGTTCTCTGCNACTCGAGAGCATGAAGCCGGAATCGCTAGTAATCGTAGATCAGC
CATGCTACGGTNAATACGTTCC

Lactic Acid Bacteria Group Hindering Growth of *Helicobactor pylori*
NNNNNNAAGGNTTTCGGATCGTAAAGCTCTGTTGTAAGTCAAGAACGGGTGTGAGAGTGGAAAGTTCACA
CTGTGACGGTAGCTTACCAGAAAGGGACGGCTAACTACGTGCCAGCAGCCGCGGTAATACGTAGGTCCCG
AGCGTTGTCCGGATTTATTGGGCGTAAAGCGAGCGCAGGCGGTTTGATAAGTCTGAAGTTAAAGGCTGTG
GCTCAACCATAGTTCGCTTTGGAAACTGTCAAACTTGAGTGCAGAAGGGGAGAGTGGAATTCCATGTGTA
GCGGTGAAATGCGTAGATATATGGAGGAACACCGGTGGCGAAAGCGGCTCTCTGGTCTGTAACTGACGCT
GAGGCTCGAAAGCGTGGGGAGCGAACAGGATTAGTCCTTGGTGGTAGTCAGCGATTTCATTGCGGCTGCC
CCCCGGTAGGGGAAGCCCGGATTAAAAAGGTTTTTCGAATCGTAAAGCTTTTTGTAGTTCAGAAAGGGG
TTTGAGAATTGGAAAGTTTCCCCTGTGACGGTGCTTACCAAAAGGGGAAGGCAAATACGTGCCGCACCCG
GGTAATCCCTAGGGTCGGGCTGTTCCCGGATTTTTGGGGGAATAAAGGAGGGCGGGGGGNNTAGTATTAT
TTAAAATTTTAAGGGGGTGGGACAACACAGATTTTCTTGTGGAACCGGTCAATATTGGGTGCAAAAGGGG
GAAAAGGGGAAAGTCCGTGGTTAGCCTTAAAAGTGGCGTATATTTTNGGGAGAGAACCCGGTGGGGAAGG
CGCTCTTTTGGATTATTTACTTGATCTTAAGGGTGAAAGGCGGGGGGGCACAAAAGAAATGATTCCCCGG
GTATAAAAA

Microcystis aeruginosa **16S ribosomal RNA, partial sequence**
ATCATGGAGAGTTTGATCCTGGCTCAGGATGAACGCTGGCGGCGTGCCTAACACATGCAAGTCGAACGGG
AATCTTCGGATTCTAGTGGCGGACGGGTGAGTAACGCGTAAGAATCTAACTTCAGGACGGGGACAACAGT
TGGAAACGACTGCTAATACCCGATATGCCGCGAGGTGAAACCTAATTGGCCTGAAGAAGAGCTTGCGTCT
GATTAGCTAGTTGGTGGGGTAAGAGCCTACCAAGGCGACGATCAGTAGCTGGTCTGAGAGGATGAGCAGC
CACACTGGGACTGAGACACGGCCCAGACTCCTACGGGAGGCAGCAGTGGGGAATTTTCCGCAATGGGCGA
AAGCCTGACGGAGCAACGCCGCGTGAGGGAGGAAGGTCTTTGGATTGTAAACCTCTTTTCTCAAGGAAGA
AGTTCTGACGGTACTTGAGGAATCAGCCTCGGCTAACTCCGTGCCAGCAGCCGCGGTAATACGGGGGAGG
CAAGCGTTATCCGGAATTATTGGGCGTAAAGCGTCCGCAGGTGGTCAGCCAAGTCTGCTGTCAAATCAGG
TTGCTTAACGACCTAAAGGCGGTGGAAACTGGCAGACTAGAGAGCAGTAGGGGTAGCAGGAATTCCCAGT
GTAGCGGTGAAATGCGTAGAGATTGGGAAGAACATCGGTGGCGAAAGCGTGCTACTGGGCTGTATCTGAC
ACTCAGGGACGAAAGCTAGGGGAGCGAAAGGGATTAGATACCCCTGTAGTCCTAGCCGTAAACGATGGAT
ACTAGGCGTGGCTTGTATCGACCCGAGCCGTGCCGAAGCTAACGCGTTAAGTATCCCGCCTGGGGAGTAC
GCACGCAAGTGTGAAACTCAAAGGAATTGACGGGGGCCCGCACAAGCGGTGGAGTATGTGGTTTAATTCG
ATGCAACGCGAAGAACCTTACCAAGACTTGACATGTCGCGAACCCTGGTGAAAGCTGGGGGTGCCTTCGG
GAGCGCGAACACAGGTGGTGCATGGCTGTCGTCAGCTCGTGTCGTGAGATGTTGGGTTAAGTCCCGCAAC
GAGCGCAACCCTCGTTCTTAGTTGCCAGCATTAAGTTGGGGACTCTAAGGAGACTGCCGGTGACAAACCG
GAGGAAGGTGGGGATGACGTCAAGTCAGCATGCCCCTTACGTCTTGGGCGACACACGTACTACAATGGTC
GGGACAAAGGGCAGCGAACTCGCGAGAGCCAGCGAATCCCAGCAAACCCGGCCTCAGTTCAGATTGCAGG
CTGCAACTCGCCTGCATGAAGGAGGAATCGCTAGTAATCGCCGGTCAGCATACGGCGGTGAATTCGTTCC
CGGGCCTTGTACACACCGCCCGTCACACCATGGAAGCTGGTCACGCCCGAAGTCATTACCTCAACCGCAA
GGAGGGGGATGCCTAAGGCAGGGCTAGTGACTGGGGTGAAGTCGTAACAAGGTAGCCGTACCGGAAGGTG
TGGCTGGATCACCTCCTTA

Shigella dysenteriae 16S ribosomal RNA, partial sequence

TGGCTCAGATTGAACGCTGGCGGCAGGCCTAACACATGCAAGTCGAACGGTAACAGAAAGCAGCTTGCTG
TTTGCTGACGAGTGGCGGACGGGTGAGTAATGTCTGGGAAACTGCCTGATGGAGGGGGATAACTACTGGA

AACGGTAGCTAATACCGCATAACGTCGCAAGACCAAAGAGGGGGACCTTCGGGCCTCTTGCCATCGGATG

TGCCCAGATGGGATTAGCTAGTAGGTGGGGTAACGGCTCACCTAGGCGACGATCCCTAGCTGGTCTGAGA

GGATGACCAGCCACACTGGAACTGAGACACGGTCCAGACTCCTACGGGAGGCAGCAGTGGGGAATATTGC

ACAATGGGCGCAAGCCTGATGCAGCCATGCCGCGTGTATGAAGAAGGCCTTCGGGTTGTAAAGTACTTTC

AGCGGGGAGGAAGGGAGTAAAGTTAATACCTTTGCTCATTGACGTTACCCGCAGAAGAAGCACCGGCTAA

CTCCGTGCCAGCAGCCGCGGTAATACGGAGGGTGCAAGCGTTAATCGGAATTACTGGGCGTAAAGCGCAC

GCAGGCGGTTTGTTAAGTCAGATGTGAAATCCCCGGGCTCAACCTGGGAACTGCATCTGATACTGGCAAG

CTTGAGTCTCGTAGAGGGGGGTAGAATTCCAGGTGTAGCGGTGAAATGCGTAGAGATCTGGAGGAATACC

GGTGGCGAAGGCGGCCCCCTGGACGAAAACTGACGCTCAGGTGCGAAAGCGTGGGGAGCAAACAGGATTA

GATACCCTGGTAGTCCACGCCGTAAACGATGTCGACTTGGAGGTTGTGCCCTTGAGGCGTGGCTTCCGGA

GCTAACGCGTTAAGTCGACCGCCTGGGGAGTACGGCCGCAAGGTTAAAACTCAAATGAATTGACGGGGGC

CCGCACAAGCGGTGGAGCATGTGGTTTAATTCGATGCAACGCGAAGAACCTTACCTGGTCTTGACATCCA

CAGAACCTTGTAGAGATACGAGGGTGCCTTCGGGAACTGTGAGACAGGTGCTGCATGGCTGTCGTCAGCT

CGTGTTGTGAAATGTTGGGTTAAGTCCCGCAACGAGCGCAACCCTTATCCTTTGTTGCCAGCGGTCCGGC

CGGGAACTCAAAGGAGACTGCCAGTGATAAACTGGAGGAAGGTGGGGATGACGTCAAGTCATCATGGCCC

TTACGACCAGGGCTACACACGTGCTACAATGGCGCATACAAAGAGAAGCGACCTCGCGAGAGCAAGCGGA

CCTCATAAAGTGCGTCGTAGTCCGGATTGGAGTCTGCAACTCGACTCCATGAAGTCGGAATCGCTAGTAA

TCGTGGATCAGAATGTCACGGTGAATACGTTCCCGGGCCTTGTACACACCGCCCGTCACACCATGGGAGT

GGGTTGCAAAAGAAGTAGGTAGCTTAACCTTCGGGAGGGCGCTTACCACTTTGTGATTCATGACTGGGGT

GAAGTCGTAACAAGGTA

SUMMARY AND CONCLUSION

An adequate, safe and accessible supply must be available to all. Improving access to safe drinking-water can result in significant benefits to health. Every effort should be made to achieve a drinking water quality as safe as possible. Many people struggle to obtain access to safe water. A clean and treated water supply to each house may be the norm in Europe and North America, but in developing countries, access to both clean water and sanitation are not the rule, and waterborne infections are common. Two and a half billion people have no access to improved sanitation, and more than 1.5 million children die each year from diarrheal disease. According to the WHO, the mortality of water associated diseases exceeds 5 million people per year. From these, more that 50% are microbial intestinal infections, with cholera standing out in the first place. In general terms, the greatest microbial risks are associated with ingestion of water that is contaminated with human or animal faeces. Wastewater discharges in fresh waters and costal seawaters are the major source of faecal microorganisms, including pathogens. Acute microbial diarrheal diseases are a major public health problem in developing countries. People affected by diarrheal diseases are those with the lowest financial resources and poorest hygienic facilities. Children under five, primarily in Asian and African countries, are the most affected by microbial diseases transmitted through water.

ABBREVIATION

UNICEF   United Nations Children's Fund

PCR   Polymerase Chain Reaction

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