



BACTERIOLOGICAL ANALYSIS OF CHILDREN'S BOTTLE WATER AND DISH WASHING WATER SAMPLES IN WEST BENGAL STATE, INDIA

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ABSTRACT

The present study dealt with the detection of coliforms along with other potential pathogenic bacteria from water bottles of children in the Tarakeswar locality of West Bengal state, India, and exploring their antibiotic susceptibility patterns. A total 6 bacterial isolates were screened from the collected water samples of Hooghly district (West Bengal state, India). Among the 6 isolates, GUW2 and GUW4 and GUW6 were found to be predominant in the feeding bottle water of children and dishwashing water samples. The isolates were either resistant to a single antibiotic: ampicillin or penicillin G, or to both ampicillin and penicillin G. From morphological, biochemical and molecular characterization, GUW2, GUW4 and GUW6 were identified as *Alcaligenes* sp., *Bacillus licheniformis* and *Escherichia coli* respectively. In order to prevent water borne bacterial infections among the people in the study area, regular surveillance for antibiogram of bacteria from drinking water samples is mandatory.

KEYWORDS: Water bacteria, Coliform, Antibiotic, *Alcaligenes* sp., *Bacillus licheniformis*, *Escherichia coli*.

INTRODUCTION

Coliforms are known to be the common resident of all warm-blooded animals including humans. Though the coliforms are not directly related in diseases spread, their presence in drinking water indicates the contamination of water with different pathogens. As pathogenic inspection of water is a complex and lengthy process, it is more convenient to identify the presence of coliforms as an indicator of water pollution. Detection of coliform bacteria in a water sample leads to search for the source of contamination in that water system and to do preventive measures for restoring safe drinking water. Indicator microbes survive better and longer than the pathogens bearing uniform and stable characteristics and thus can easily be detected by standard laboratory techniques. The coliform is defined as the aerobic or facultative anaerobic, non-spore forming, gram-negative rods that ferment lactose with gas production within 48 h, at 35°C. Coliforms are present in the intestinal tracts of animals and are released through fecal matter in the environment. Among these coliforms, *Escherichia coli* have been used for indicator organisms for decades (Rhodes and Kator, 1994). One of the key characteristics of the indicator organism is its presence at a higher concentration than the pathogens, so the methods discriminating the source of coliforms may have greater

predictive and useful value, compared to developing multiple tests that must target specific pathogens. *E. coli* is regarded as the most sensitive indicator of faecal pollution (Rhodes and Kator, 1994). The presence of *E. coli* in large numbers present in the gut of humans and other warm-blooded animals and the fact that they are not generally present in other environments support their continued use as the most sensitive indicator of faecal pollution available (Edberg *et al.* 2000). Mostly different waterborne bacterial pathogens were isolated and characterized using the most probable number (MPN) test, an extremely useful technique in monitoring drinking water quality. The MPN technique is used to test large volumes of samples to get numerical results rapidly. Faecal Coliform bacteria in the water specifies faecal contamination of water containing other harmful or pathogenic organisms, including bacteria, viruses, or parasites. Drinking water contaminated with these organisms can affect the stomach and cause intestinal infection including diarrhoea that can even lead to death. These effects are more rigorous and sometimes acute for infants, children, adults or immune-deficient people. Earlier the bacteriological profiles of Mahananda river water and eye cosmetic samples from Malda, West Bengal state have been reported (Nandi and Mandal, 2016; Das *et al.*, 2016), however, no report has been

made on the antibiotic resistance of bacteria isolated from local niches of drinking water bottles and dishwashing water samples. Therefore, the present study has been designed to detect the coliforms along with other water pathogenic bacteria from water bottles of children in the Tarakeswar locality of West Bengal state (India) and to explore the antibiotic susceptibility patterns of the isolated bacteria.

MATERIALS AND METHODS

Water samples

The study was conducted in five study areas, viz, Tarakeswar, Champadanga, Pursurah, Haripal and Singur of Hooghly district, West Bengal selecting one Anganwadi school in each study areas. A total of 50 water samples (5 from water bottles and 5 from dish and utensil washing water from each Anganwadi School in each of the five study areas) were collected in 500 ml of sterilized non-reactive borosilicate glass bottles.

Bacteriological analysis of water samples

The water samples collected were processed in the laboratory for microbiological analysis (Lacey, 1997). The water samples were diluted upto 10^{-3} dilution. A volume of 0.1 ml of each dilution was pour plated on nutrient agar plate and incubated at 30°C for 72hrs (Azmi and Chatterjee, 2016; Chatterjee *et al.*, 2014). The colonies were isolated and preserved in nutrient agar slants for further characterization. Primary growth of all kinds of bacteria was performed in nutrient broth. The isolates were streaked into MacConkey agar, eosin-methylene blue agar, *Salmonella Shigella* agar and brilliant green agar separately to observe their growth pattern. Gram staining and different biochemical tests were performed for the identification of the isolates (Azmi *et al.*, 2014; Nandi and Mandal, 2016; Holt, 1984; Forbes *et al.*, 2007). The carbohydrate fermentation test of the bacterial isolates using different carbohydrate sources was performed following standard methodologies (Holt, 1984; Forbes *et al.*, 2007).

Molecular characterization of bacteria

Genomic DNA was extracted from the bacteria isolated from test water samples. Quality of each of the extracted DNA samples were evaluated on 0.8% agarose gel, a single band of high-molecular weight DNA has been observed. Fragment of 16S rDNA was amplified by PCR using 8F and 1492R from the above isolated DNA. A single discrete PCR amplicon band of 1500 bp was examined. The PCR amplicon of each of the given sample was purified and further processed for the sequencing. The forward as well as the reverse DNA sequencing of PCR amplicon was carried out with primers: 704F and 907R, with BDT v3.1 cycle (Saitou and Nei, 1987; Tamura *et al.*, 2007).

Antibiotic susceptibility test

The antibiotic susceptibility test for the bacterial isolates was done following disc diffusion method (Bauer *et al.*,

1966), against antibiotics (Hi-Media, India), such as amoxycillin (10 µg/disc), bacitracin (10 units/disc), chloramphenicol (30 µg/disc), ciprofloxacin (30 µg/disc), erythromycin (15 µg/disc), kanamycin (30 µg/disc), polymyxin B (300 units/disc), streptomycin (10 µg/disc), tetracycline (30 µg/disc), Penicillin G (10 units/disc) and vancomycin (30 µg/disc), as described earlier (Nandi and Mandal, 2016). The results were interpreted according to the Clinical and Laboratory Standards Institute's criteria (CLSI, 2011).

RESULTS

A total of 6 bacterial isolates were screened from the collected water samples of Hooghly District. Among these six isolates, the 3 that were designated with GUW2 and GUW4 and GUW6 were found to be predominant in the feeding bottle water of children and dishwashing water samples. All of the isolates showed circular and white to off white colonies on test agar media. The GUW6 produced convex colonies whereas GUW2 and GUW4 produced flat colonies showing no elevation. Phenotypic and bio-chemical properties of these three isolates have been recorded in Table 1. All the three bacterial isolates showed rod shaped vegetative bodies and showed catalase positive and gelatin hydrolysis negative reaction. GUW4 and GUW6 could ferment glucose, lactose, sucrose and mannose while GUW2 could not ferment any of these carbohydrates present in the medium. The GUW6 isolate fermented lactose and produced gas and acid from glucose in TSI agar, but none of the bacterial isolates produced H₂S.

The partial sequences of 16S rRNA genes of GUW2, GUW4 and GUW6 isolates were submitted to GenBank and the accession numbers were provided them were MG893092, MG893093 and MG893094 respectively. The phylogenetic analysis revealed that GUW2 (MG893092) branched with the cluster containing *Alcaligenes* sp. with 67% bootstrap value (Figure 1). The GUW4 (MG893093) was found to be branched with *Bacillus licheniformis* (KX235179) with 64% bootstrap value exhibiting high similarity with that strain (Figure 2). In the neighbor joining tree, GUW6 (MG893094) branched with the cluster containing different strains of *Escherichia coli* characterizing GUW6 as *E. coli* strain (Figure 3).

All of the 3 bacterial isolates (GUW2, GUW4 and GUW6) were sensitive to amoxycillin (10 µg), bacitracin (10 units), chloramphenicol (30 µg), ciprofloxacin (30 µg), erythromycin (15 µg), kanamycin (30 µg), polymyxin B (300 units), streptomycin (10 µg), tetracyclin (30 µg) and vancomycin (30 µg). *Alcaligenes* sp. GUW2 was resistant to ampicillin (10 µg), *Bacillus licheniformis* GUW4 showed resistance to penicillin G (10 units) and *Escherichia coli* GUW6 was resistant to both ampicillin (10µg) and penicillin G (10 units).

Table 1: Phenotypic features of bottled water and dishwashing water bacteria.

Isolate	Shape	Gram stain	Ca	In	MR	VP	Cit	Oxi	Ure	Nitr	H ₂ S	Star	Gel	Lip
G UW2	Rod	-	+	-	-	-	+	+	-	-	-	-	-	-
G UW4	Rod, Spore	+	+	-	+	-	+	+	-	-	-	-	-	+
G UW6	Short Rod	-	+	+	+	-	-	-	-	+	-	-	-	-
Carbohydrate utilization test														
Isolate	Glucose		Lactose		Sucrose		Mannose							
G UW2	-		-		-		-							
G UW4	+		+		+		+							
G UW6	+		+		+		+							

Ca: Catalase; In: Indole; MR: Methyl Red; VP: Voges Proskauer; Cit: Citrate; Oxi: oxidase; Ure: Urease; Nitr: Nitrate; Star: Starch; Gel: Gelatinase; Lip: Lipid; '+': positive; '-': negative.

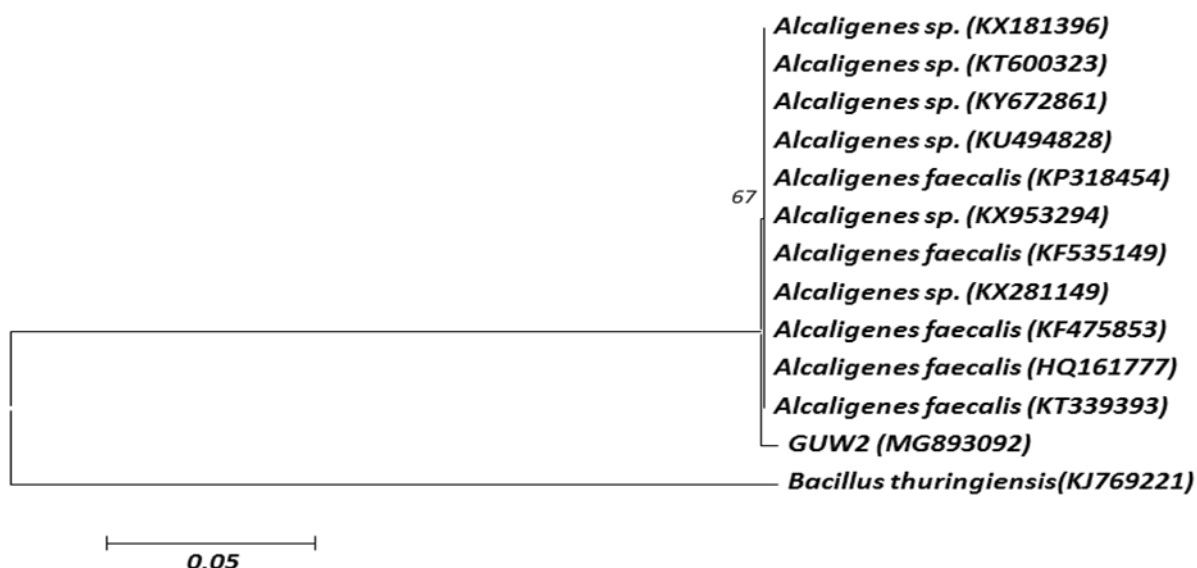


Figure 1: Neighbour joining tree constructed using partial 16S rDNA sequence of G UW2 with other related bacterial partial 16S rDNA sequence.

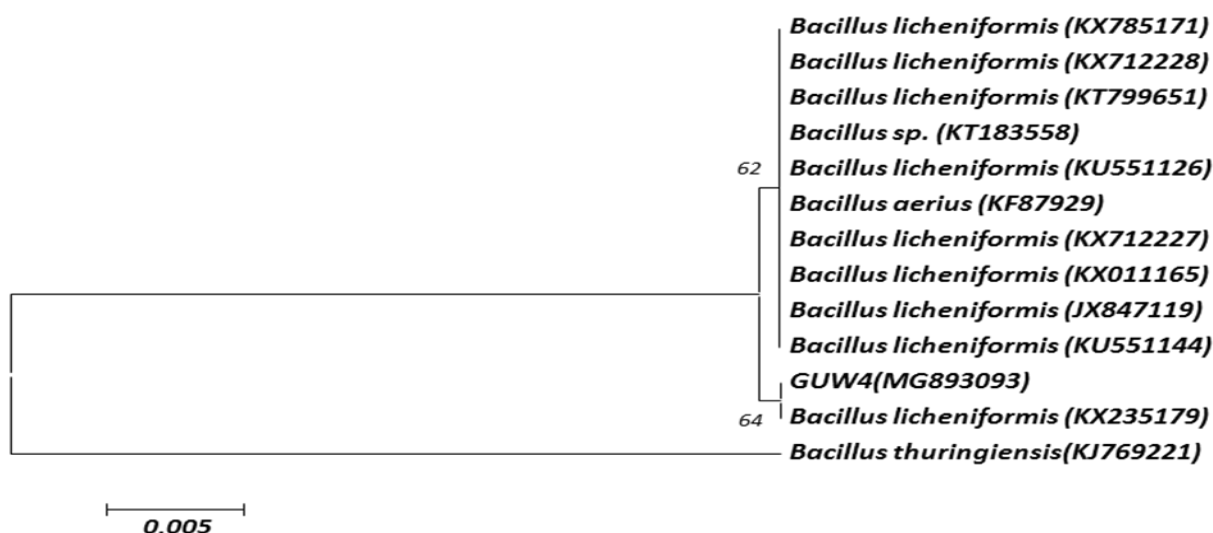


Figure 2: Neighbour joining tree constructed using partial 16S rDNA sequence of G UW4 with other related bacterial partial 16S rDNA sequence.

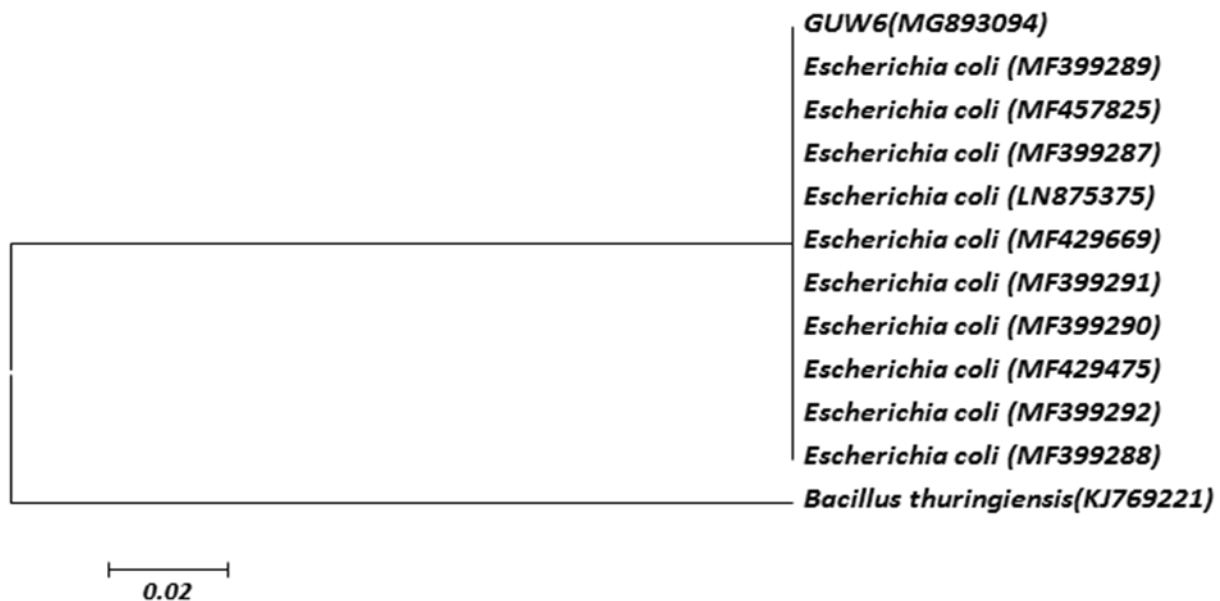


Figure 3: Neighbour joining tree constructed using partial 16S rDNA sequence of G UW6 with other related bacterial partial 16S rDNA sequence.

DISCUSSION

The three water bacterial isolates were identified as three distinct bacteria exhibiting different biochemical, physiological and molecular characters. Presence of these three bacteria in drinking water indicated the presence of organic waste materials in the water. Pujari *et al.* (2007) summarized that improper sanitation system in India might be the reason of the water contamination with these kinds of bacteria. Lack of sanitation and personal hygiene is the main cause of the water contamination leading to several diseases in children and adults of India. Presence of *E. coli* G UW6 indicated the high risk of contamination with other pathogenic bacteria in the collected water samples. *E. coli* is an enteric gram-negative bacillus, and is known as a noninvasive commensal possessing the capacity to cause enteric infections. *Alcaligenes* sp. is one of the main causes of opportunistic infections such as nosocomial septicemia, meningitis, peritonitis, enteric fever, appendicitis, cystitis etc (Madigan and Martinko, 2005). *Bacillus* spp. infection is correlated with the consumption of a variety of foods including raw milk and meat products. Infection by *Bacillus* spp. may directly result from the consumption of the bacteria or toxins produced by the organisms. Waterborne transmission of *Bacillus gastroenteritis* has not been recorded in previous works (Bartram *et al.*, 2003). Earlier the antibiotic resistance patterns of potential pathogenic bacteria isolated from Mahananda river water and eye cosmetic samples from Malda, West Bengal state, India, were reported (Das *et al.*, 2016; Nandi and Mandal, 2016). In the current study, the isolated bacteria had resistance either to ampicillin or penicillin G, or to both the antibiotics (ampicillin or penicillin G).

The contamination of drinking water with *Bacillus* spp. is prevalent and persistent, which might be due to the resistance of their spores towards disinfectant. So proper care and appropriate decontamination methods should be taken up to avoid the water borne pathogenic infection. Hence, the present study is of a severe concern, as contaminated water plays crucial role in the prevalence of waterborne and water related bacterial disease outbreaks in the rural areas of such unstudied niches from West Bengal state, India.

CONCLUSION

From the morphological, biochemical and molecular characterization point of view, the isolated water bacteria, such as G UW2 (MG893092), G UW4 (MG893093) and G UW6 (MG893094) isolates were identified as *Alcaligenes* sp., *Bacillus licheniformis* and *Escherichia coli*, respectively; all the bacterial isolates are clinically relevant having the capacity to cause infections to humans. Regular surveillance of such bacteria and exploring their antibiotic susceptibility status, is therefore, mandatory, to prevent water borne infections. Also, proper hygienic environment is a must for maintaining the good health of the common people and children in the study area.

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