Conventional Microbiological Approaches in Identification of Pathogenic Bacteria and Protozoons Isolated from the Drinking Water of Gambian Province

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Abstract

This study focuses on conventional approaches while considering coliform bacteria for the contamination of drinking water drawn from the jars in one of the villages found in the Gambia, West Africa. The study intends to rule in pathogenic microorganisms when present in water would cause contamination and hence disease to the inhabitants. 20 samples were collected from 20 different compounds, brought to the laboratory within 30 minutes. Chlorine, Nitrate, Nitrite, Alkalinity, Lead, and Hardness, Copper, Iron and Pesticides were analyzed for some samples and all were subjected to Parasite Counting and Culture after centrifuging to enable a concentrated volume. The chemical parameters were not very deviating from Environmental Protecting Agency (EPA) standards. Almost all the samples shown growth on MacConkey plates but rare growths were seen on EMB agar. The pure cultures were sub-cultured on selective media like; Pseudomonas, Salmonella, Thiosulfate Citrate Bile Sucrose (TCBS) and Charcoal Yeast Extract (CYE) and tests such as oxidase, and the analytical profile index (API) were used to confirm the isolates. Giardia like organism were isolated, also Entameoba histolytica and unidentified organisms were seen. 1(5%) Enterobacter aerogenes, 1(5%) Enterobacter cloacae and 1(5%) Citrobacter freundii were the pathogens confirmed by API. The study concluded that, while the tap water was cleaner and safer, the drinking water from jars contains some pathogenic microbes that can cause gastroenteritis or infectious diarrhea. The storage facilities need to be replaced either with a different and clean container or the jars to be frequently disinfected with chlorine-containing compounds.

Keywords

Enterobacteriaceae; Water Contamination; Indicator Organisms; Disinfection; Water-Borne Pathogens; Gastroenteritis

Introduction

The cleanliness and safety of drinking water is very important for life. The microbiological quality of drinking water has always been a matter of concern. Drinking water, despite all the treatment processes, is never sterile; there are resistant microbes that can survive the entire process; which can find their way through water distribution pipes and subsequently form biofilms [1]. For this reason, constant monitoring of indicator
organisms such as non-specific coliforms, *Escherichia* and *Pseudomonas aeruginosa* that are very commonly found in the human or animal gut as normal flora should be carried out [2].

The primary goal is to detect pathogens found in jar water and to identify these as causative agent for gastrointestinal diseases. In Sub-Saharan Africa, the need for quality water has been felt for long and it requires all the measures to ensure a safe drinking water to the inhabitants. It is in this scope of interest that prompted this study to be carried on. The study aims to ascertain as the main course of drinking water contamination; the imbalance chemical composition, the leaking of pipes that enable microbes to live in water or the contamination during either carrying water or the storage facilities. Contaminated water and drinking water is believed to be due to various sources such as sewage, farm or farm products are seriously threatening public health in many countries. The exact point of sources of these contaminants needs to be figure out and trying ways to solve this avoidable of public threat [1, 3].

Most pathogens are common in farm animals, and at some points their feces are in contact with our drinking water sources [3], and although it is known that the detection and quantification of these pathogens is done by microscopic and culture methods, culture-based methods are often used for days prior to discrimination, sometimes even weeks and allows further delay of subcultures to ensure pure isolation. Since the plate culturing for biological pollutants is annoying and time consuming, standard plaque count Heterotrophic Plate Count (HPC), enzyme/substrate methods, immunological methods, genetic methods, mass spectrometry, DNA sequencing, microarrays and physical methods are sought for [2, 4, 5-7].

HPC is a suitable method for quantification of heterotrophic organisms in drinking water. HPC cannot determine the health hazard dimensions of water, but it can give the necessary signals that the number of microbes is high, that pollution is likely, and that an urgent investigation is needed [2]. Natural water resources have not posed any problems for many years; but due to population growth in recent years and orientation towards free farming techniques in animal breeding; water health and hygiene factors caused significant mortality [8]. In 2004, more than 2400 deaths occurred, accounting for 13.5% of deaths in Gambia. Every 2 million people in the world (mainly children), diarrhoea, dengue fever, etc. and diarrhoea, which causes 1.5 million children death each year, is still the third leading cause of death worldwide [World Health Organization, 2013]. Samendra PS et al 2014 [9] has suggested that drinking water contamination is caused by contamination of source water, microbial contamination in municipal distribution or inadequacies in treatment methods. In response to this hypothesis, a comprehensive analysis is needed to identify the causes of contamination of our water resources and to prevent them.

Materials and Methods

Source of Water

The Gambia is one of the smallest countries in Africa. The study area is one of the remote villages, 200km away from the coast. Drinking water samples were collected from 20 different jars. All the samples were brought to the laboratory in less than an hour after collection.

Sample Collection Procedures

Sample collection procedure for bacteriological analysis of drinking water was done according to EPA standard, 2nd Edition 2016.

Chemical Tests

Due to the limited number of chemical kits, only the piloting samples and the first two samples were analyzed using chemical parameters. Pure Test Home Water Analysis Kit was used as a guide to the study as to which source of sample should be used for bacterial pathogen isolation and identification.

Bacteria Culture

All of the samples were spun at 1200 rpm at 4°C for 10 minutes (This does not kill bacteria and it was used in the place of membrane filtration). The supernatants were discarded and the deposits were collected for inoculation on MacConkey and Eosin Methylene Blue (EMB) agar plates. MacConkey that is selective for both the coliforms and intestinal pathogens (gram negative) in water were inoculated and incubated for overnight and 48 hours at 37º and 44ºC to enable the growth of *fecal coliform (E.coli)*. EMB agar that can differentiate between *E.coli*, *Enterobacter aerogenes* and some *Enterobacteriaceae (non-coliform)*, were also cultured from the above samples and incubated for overnight and 48 hours at 37ºC.

After establishing all the slides from pure colony as Gram-negative rods, the following sub-cultures were done from the pure colonies into the respective selective media.
**Table 1: Culture and Sub-Culture**

<table>
<thead>
<tr>
<th>Sample Number</th>
<th>Culture Media</th>
<th>Remarks</th>
</tr>
</thead>
<tbody>
<tr>
<td>WA008</td>
<td>CYE agar</td>
<td>Inoculated into MacConkey and Blood agar for determination</td>
</tr>
<tr>
<td></td>
<td>EMB agar</td>
<td>E.coli and Klebsiella spp. suspected</td>
</tr>
<tr>
<td>WA005</td>
<td>CYE agar</td>
<td>Inoculated into MacConkey and Blood agar for determination</td>
</tr>
<tr>
<td></td>
<td>Brilliant Salmonella</td>
<td>Incubated in Triple Sugar Iodine (TSI) and Urea</td>
</tr>
<tr>
<td></td>
<td>Pseudomonas agar</td>
<td>PP on MacConkey</td>
</tr>
<tr>
<td>WA004</td>
<td>MacConkey</td>
<td>PP on new MacConkey plate</td>
</tr>
<tr>
<td>WA012</td>
<td>EMB</td>
<td>Serratia spp. suspected and PP on MacConkey</td>
</tr>
<tr>
<td></td>
<td>TCBS agar</td>
<td>Vibrio spp. suspected and PP on Muller Hinton Agar (MHA)</td>
</tr>
<tr>
<td>WA002</td>
<td>MacConkey</td>
<td>E.coli and Klebsiella suspected and PP on New MacConkey Plates</td>
</tr>
</tbody>
</table>
Brilliant Salmonella that is selective for Salmonella spp. were cultured for at 37ºC for overnight (ISO standard), Pseudomonas agar plates selective for Pseudomonas spp. were also inoculated from the positive MacConkey and EMB agar plates at 30ºC for 48 hours.

TCBS and CYE agar that are selective for Vibrio spp. and Legionella spp. respectively were incubated aerobically at 35ºC for 18-24 hours.

Upon isolating uniform growth colony plates, the culture plates were transported to a Good Clinical Laboratory Practices (GCLP), Fajara MRC Gambia and an ISO 15189 laboratory wherein pure cultures were inoculated into selective media prior to biochemical test. The clearer growth colonies were carried for biochemical test like catalase, oxidase, and Triple Sugar Iodine (TSI), Urea and Analytical Profile Index (API) tests.

Results
In this study, 20 out 20 samples showed distinct colonies on MacConkey agar, this gives 100% as the total of isolates from the these plates. There were only 4 out 20 growth on EMB agar and since these were already pure colonies on MacConkey agar, there were neglected as part of the study after confirming them as Gram-negative and no metallic sheen were detected. Out of the sub-cultures from MacConkey, there were 4 out 20 (20%) on Pseudomonas, selective agar media and they were all oxidase positive. Only 5% of the samples have shown growth on TCBS agar and this was Gram-negative Vibrio. 20% of the samples have shown growth on CYE agar however, these isolated has pure growth on MacConkey and Blood agar. Only 1% of the samples were seen as growth on Brilliant Salmonella agar. Sample number WA004 shown API positive for Enterobacter aerogenes, WA010 shown Enterobacter cloacae and Citrobacter freundii.

Table 1 is showing the sub-culturing of more samples that are positive and Table 2 shows the culture growth on selective media.

Discussion
All of the Nitrates, Nitrites, Arsenic, and lead, the hardness of water, iron, acidity and alkalinity are
within the EPA limit. These are great parameters as far as drinking water is concerned. The chlorine level has been seen as a very interesting parameter in this study. Although the chlorine level in the tap sample is bit higher, the level in the jar has shown a decreased level. This indicates that jar storage of drinking water can help reduce the chlorine content. However, the chemical reactions that lead to dechlorination require an interesting further study. The Copper level is slightly more than 1ppm (1.3ppm) and according to EPA, problems can start developing and its consequences can lead to severe gastrointestinal reactions if reached a level of 3ppm. In addition, Wilson Disease patients accumulate copper in their bodies, thus developing into the deterioration of the brain and liver. The copper level has cause for a concern and this has been seen both in the tap and jar water. This means that, these areas should monitor the copper content of drinking water supplied to the community. Since Chlorine inhibits the growth of bacteria, a bacteria culture growth in tap water was normal.

The prevalence of Giardia-like organism in drinking water is really a setback with regards to environmental sanitation and domestic hygiene practices. Giardia is categorized as a fecal pathogen and should not be seen in any form of drinking water. Giardia can cause abdominal pain, severe diarrhea, flatulence, vomiting, weight loss, mal-absorption with lactose intolerance and impairment of growth. Among children under 3 years and undernourished, the symptoms can be severe and in an adult, the disease has no symptoms. In immune-compromised patients, gastrointestinal disorders or intestinal bacterial infections are more prone to Giardia infections.

This menace can be prevented with improved environmental sanitation practices and personal hygiene in order to prevent our body contacts with foods and water contaminated with feces containing cysts. In addition, since Giardia is somehow resistant to chlorine, a clever chlorine monitoring is sought for the presence of other free-living molecules and amoeba are part of water normal flora are normally found in water and they are healthy to consume. In recent years, the rise of resistancy has been observed and pathogens have been prominent in hospitals and clinics. Among these are; Escherichia, Enterobacter, Klebsiella strains. Although E.cloacae and E.aerogenes have been seen a decreased occurrence in European countries, they have been identified in this study and may be the factors for causing infections in human populations of the area. These can only be controlled via improving the environmental conditions in order to avoide water contamination.

Enterobacter aerogenes (K. aeromobilis) and Enterobacter cloacea are one of those versatile bacterial pathogens that are confronting antibiotic treatments. They are opportunistic and has developed a high multi-resistance for antibiotics in humans. Their resistance is due to their acquiring of numerous genetic mobile elements and the presence of the regulatory cascades that monitors the bacterial membrane permeability and inactivates the antibiotic efficiency. These Gram-negatif, rod-shaped, facultative anaerobic and non-spore-forming bacterial genus are a common nosocomial.

Follwoing Escherichia coli and K. pneumonia, E. cloacea, is believed to be the third broad spectrum Enterobacteriaceae species involved in nosocomial infections. This is due to the diffusion of most frequent extended spectrum β-lactamases (ESBL) and carbapenemases. A proper care should be taken for this almost superbug. Although it lives as commensal in the human intestinal tract, it is also a nosocomial pathogen that can cause bacteremia, septic, endocarditis, arthritis, osteomyelitis etc. It is recently reported as a member of the ESKAPE (Enterococcus faecium, Staphylococcus aureus, Klebsiella pneumoniae, Acinetobacter baumannii, Pseudomonas aeruginosa, Enterobacter spp.) as one of the main contributors to the health human infection problem.

In contrast to its environmental benefits in reducing Nitrate to Nitrite, Citrobacter freundii of Enterobacteriaceae family is a key pathogenic opportunistic bacteria. Although it is a normal flora in the intestinal tract of humans, it causes respiratory tract infections, blood and urinary tract infections. Just as other emerging Enterobacteriaceae, it has been recently reported expressing resistance to broad-spectrum antibiotics like; vancomycin, cephalosporins, piperacillin and piperacillin-tazobactam. This is a threat to human health in the future.

The identification of Bacillus spp. in drinking water does not cause a greater risk since, until today, bacillus infections has not been confirmed as waterborne. In addition, the majority of Bacillus spp. are non-pathogenic to human. A similar study by Subedi M and Aryal M in Public perception about drinking jar water and its bacteriological analysis in Kathmandu Valley, Nepal found E.coli, Klebsiella spp, and Enterobacter aerogens in
the jar water. Of those faecal coliforms, all were sensitive to antibiotic ciprofloxacin and resistant to ampicillin. The finding indicates that jar water is not safe for drinking purpose without treatment. Even in these regions, though most population rated their drinking jar water good but found to be highly concerned with the quality. Among total water samples, 91.2% (n=52) were found contaminated with total coliforms and 59.6% were with faecal coliforms. This definitely signals that the water stored in jars are not clean and safe enough for drinking until and unless proper hygienic measures are put in place. This is obvious for the people of the region and Gambia at large where the study was conducted. The norm of sticking to the traditional and cultural drinking jar is at its peak. And since these people have lived with these water storage facilities for centuries, it will not be easy to convince them that their jars are not very clean and unsafe for drinking. During the consenting, some even interrupted by claiming that their jars are clean and free of pathogens that can infect humans.

Among the limiting factors are insufficient chemical parameters for all the samples. In the provision of these, the study would have a better-shaped chemical analysis and a clear identification would have been seen. Since the project is self-funded, only a small portion of the samples was taken for chemical analysis.

For the bacterial culture, there are insufficient of culture media that are selective for the targeted bacteria. A special example was the absence of BCYE agar for selectively culturing of Legionella spp. In addition, there was no membrane filtration machine and this compelled the use of centrifuging at 1200 rpm on 4 degrees for 10 minutes. Although this does not kill bacteria, membrane filter papers would have produced better results.

The microscope used for parasite counting could not enable more videos or photographs to be taken. Again, the counting was not done by a very qualified parasitology. Above all, lack of funding was the biggest limitation to the study. It was self-funded by researcher.

**Recommendations**

Since it is clearly manifested that some caustic agents (CA) of gastrointestinal diseases are also the same pathogen isolated from the storage of the drinking water, an action is highly needed to be taken. Although it will cost the Ministry of Health a huge of money to halt the public or improvised them from using jars storage facilities for drinking water, an efficient cleaning and disinfection methods and facilities can be circulated in order to improve the standards of drinking water. Drinking water treatment plans that involve; the four method approaches; Filtration, Water Softeners, Distillation and Disinfection should be practiced even at the household level. The problems identified in this study have something to do with either poor water storage facilities or improper sanitation and cleaning practices with regards to water storage. On this ground, highly sanitation level in terms of collecting and storing of drinking water should be given an importance.

This study when published should be given a chance to be presented before the ministry responsible for public health to throw more lights on the underlining effects and consequences that her citizens in remote areas are prone to.

A broad spectrum drinking and domestic water analysis are sought for in Gambia. These must include; chemical, conventional microbiology and molecular approaches as to the strain types and to ascertain that the users are not prone to pandemics or outbreaks in the specific area. The researcher and his team are willing to volunteer for the broad-based nation-wide water analysis if the source of funding is allocated.

**References**


