Studies on the Microbial and Parasitological Contamination of Drinking Water Sources in Rural Communities of Southeast Nigeria-Implications for Public Health

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ABSTRACT
Studies on the parasitological and microbial contamination of local water sources in parts of southeast Nigeria was conducted in 40 local villages devoid of good water sources using standard microbiological and parasitological techniques. The studies revealed that cysts and larvae of *Giardia lamblia* (44.4%),*Balantidium coli*(33.3%), *Entamoeba histolytica* (11.1%) and *Strongyloides stercoralis* (11.1%) were parasites implicated in the water samples while the result of the microbial analysis revealed that *Echerichia coli* (28..5%), *Streptoccocci spp* (7.14%), *Staphylococci saprophytic* (21.42%) *Bacillus subtilis* (28.58%), *Bacillus polymyxa* (14.28), *Salmonella typhi* (7.14%), *Vibrio spp* (7.14%), *Pseudomonas putida* (14.28%), and *Pseudomonas aeroginosa* (14.28%) were all isolated from same water samples. The results showed that these local water sources were either contaminated with coliform bacteria, parasites or both at the same time but with different loads. However, some of the diseases which are predominant in the area tend to suggest the presence of the parasites as the responsible factors. Open defecation is still a common practice. Therefore, good and hygienic water sources should be provided to these areas in order to reduce the level of water-related infections to the inhabitants of these villages. Point of source and simple household water treatment techniques should be encouraged. These should deliver measurable health benefits in the interim.

1. INTRODUCTION

In Nigeria, local water sources like dams, ponds, rivers, streams etc are often contaminated by human and animals faeces, sewage effluents, surface runoffs, or industrial pollutants from sources far upstream. In most parts of the developing world, water may be contaminated by parasitic worms, bacteria, protozoa from human and animal wastes or pathogens which use other organisms as an intermediate host. Pathogenic strains of *Echerichia coli* survive briefly outside the body to infect new hosts. *Giardia lamblia* and *Cryptosporidium* spp, which cause diarrhea are common pathogens associated with local water sources, Boulware, *et al*., (2003). Less commonly seen in developed countries are organism such as *Vibro cholerae* which cause cholera and various strains of *Salmonella* which cause typhoid and paratyphoid diseases. Pathogenic *viruses* may also be found in water. The larvae of *flukes* are particularly dangerous in areas frequented by sheep, deer, and cattle. If such larvae are ingested, they can form potentially life-threatening *cysts* in the brain or liver of their hosts if ingested in water (Ashbolt, 2004). According to World Health Organization, diarrhea accounts for an estimated 4.1% of the total daily global burden of disease and is responsible for the death of 1.8 million people every year. It was estimated that 88% of the burden is attributable to unsafe water supply, sanitation and hygiene, and is mostly concentrated in children in developing countries (Nwachukwu, *et al.,* 2004). Water sources contaminated with parasite or microbial agents have significant impact on the economy, locally as well as internationally. People who are infected by waterborne disease are usually confronted with related costs and seldom with a huge financial burden. This is specially the case in less developed countries. The financial losses are mostly caused by cost

for medical treatment and cost for transport, special food and loss of manpower. Many families even sell their land to pay for treatment in good hospitals. On average, a family spends 10% or more of the monthly household income per person infected with waterborne diseases.(Simpson *et al,* 2002)

1.2 RELEVANCE OF THE RESEARCH

Parasitic helminthes and microbial water contamination play a critical role in health problems especially in the rural areas. Lack of information on the health implication of fecal water pollution to the rural dwellers has hindered living a hygienic life. Thus, the availability of knowledge about parasitic helminthes and microbial contamination of local water sources in the study areas. will constitute a set of important tools to minimize the issues of gastro – intestinal health problems and also awaken individuals to preventive measures against the infections caused by water contamination or water related diseases. Therefore, this study will seek to provide information on the parasitological and microbial load in different water sources in the study areas, the sources of contamination, possible control and preventive measure.The study was also done to determine the species composition of parasitic and microbial organisms in the local water sources in the study areas and their implication for public health.

2. METHODOLOGY

The study was carried out in rural communities of Ebonyi State, southeast Nigeria. The Climate is characterized by hot dry period, which stretches from November–April, while the rainy season is from May to October. The maximum temperature during the dry season is 37.7^0C while the minimum temperature is 28^0C. The period of rainy season has a maximum temperature of 25.2^0C with an average rainfall of 34.7 mm to 35.6 mm. The communities are densely populated and lack potable water, good sanitation and other social amenities. Outbreak of enteric diseases is a common occurrence. Agriculture is the major occupation and more than 80% of the population are engaged in subsistence farming.

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2.1 Media Preparation

The respective media used were prepared according to manufacturers' specifications; 28g of nutrient agar and 5.5g of macconkey agar were measured and dissolved in $1000CM^2$ of distilled water, it was then autoclaved at 121^OC for 16 minutes before it was poured and allowed to gel before usage

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2 2 Most Probable Number Analysis of The Water Samples (Presumptive Test)

The macconkey broth was prepared by dissolving to $1dm^3$ of water. It was then dispensed to bijour bottle into single strength broth (5mls) for 5 bottles each, double strength broth (10mls) for 5 bottles each and another double strength broth (50mls), for one bottle respectively with insertion of durham tubes. It was then autoclaved at 121^oC for 15 minute after then it respectively inoculated by pipetting equivalent volume of each strength, 5mls of water for 5mls broth, 1 dml water into 10mls broth and 50mls water into 50ml broth, it was then incubated at 37^OC for 24 hours before it was observed for acid and gas production.

2.3 Confirmatory Test In the confirmatory, 1ml of positive test that bottles with both acid and gas production were inoculated into brilliant green broth prepared by dissolving 20g into 1dm3, autoclaved at 121^OC for 15 minutes with insertion of durham tubes, it was respectively incubated at 35^OC for 48 hours before observed for gas production.

2.4 Completed Test

Eosine methylene blue agar was prepared by desolving 15g in $1dm^3$ of water autoclaved at 121^0C of 15 minutes, then a loopfull of the Brillant green broths showing gas production was inoculated onto the agar, using sterile wire loop. It was incubated at 35^0C for 24 hours before it was sub cultured on sterile nutrient agar plate for identification and characterization.

2.5 Biochemical Characterization of the Bacterial Isolates

Apart from the macroscopical observation of the colour, consistency and texture, the isolates were further identified using gram staining, voges proskauer (VP), indole, catalase, sugar fermentation test and oxidase test and performed according to. Cheesbrough (2000) and Ochie and Kolhalker (2008)

2.6 Gram Staining Test
A loopful of each isolate was emulsified on a drop of physiological saline made on clean microscopic slide. The smear was allowed to air dry and was heat-fixed by passing over a bunsen flame for three to four consecutive times. The heat fixed smear was flooded with crystal violet, allowed to stay for 60 seconds, washed using clean tap water, then flooded again with Lugol's iodine and allowed for 30 seconds, then washed off before decolourised with acetone for 10 to 15seconds which was washed off immediately. The smears was counter stained with safranin, allowed to stay for 30 minutes and washed off with clean tap water, allowed to air dry, 2 drops of oil immersion was added on the slides and observed microscopically using oil immersion lens to observe for presence of Gram negative rods indicating *Salmonella typhi* (Cheesbrough, 2000).

2.7 Motility Test
The medium used was the indole motility semi solid medium for determination of motility. A little portion of the respective bacterial isolates were collected and inoculated on the semi medium using sterile wire loop. It was incubated at 37^0C for 24 hours before it was observed for diffused turbidity in the medium (Ochie and Kolhaker, 2008).

2.8 Catalase Test
Some colonies of the test organism was collected using sterile glass rod and was immersed in 3ml hydrogen peroxide in clean test tubes. Then it was observed for immediate bubbling / gas production. (Willy *et al.,* 2008).

2.9 Oxidase Test
Some colonies of the organism were emulsified on a fitter paper impregnated with freshly prepared oxidase reagent (Tetramehtyl papraphenylenediamine dihydrochloride).

2.10 Indole Test
A little portion of the isolate was inoculated in 5 ml of sterile peptone water in sterile Bijou bottles prepared according to the manufacturers specification using sterile wire loop and was incubated at 37^OC for 48 hours after which, 3-4 drops of indole reagent (Kovac reagent) was added and observed for production of pink ring colour (Ochie and Kolhaker, 2008).

2.11 Voges Prokauer Test
Each isolate was inoculated into 5mls of sterile VP broth in bijou bottles using sterile wire loop and was incubated at 37^OC for 48 hours. 3 drops of VP reagent were respectively added followed by 5mls of 40% potassium Hydroxide and observed for formation of pink to red colour within 5 minutes (Cheesbrough, 2006).

2.12 Sugar Fermentation
The sugars used are glucose, fructose and lactose a 1% of differential sugar solutions was prepared according to Ochie and Kolhalker (2008) and was dispensed into bijou bottles with durharm tubes inverted 3 drops of phenol red before sterilized at 121^OC for 15p. After sterilization a little portion of the isolate was inoculated into 10mls of the sterile sugar solution using sterile wire loop. This was incubated for 2 days at 37^OC, after which it was observed for change of colour for acid and gas production.

2.13 Centrifugation
The water samples were in triplicates and allowed to sediment overnight for proper sedimentation of parasite ova, egg and cyst, then 10mls of the water samples were measured into test tubes and then centrifuged for 5 minutes at 2500rpm. The supernatant was discarded while the sediments were microscopically observed using x10 and x40 with condenser iris sufficiently closed to create good contrast (Cheesbrough, 2000).

3. RESULTS
The result of the research showed that nine (9) species of bacteria were isolated from the water samples, (4) four out of these isolates are gram positive which include *streptococcus* specie, *Staphylococcus saprophyticus, Bacillus subtilis* and *Bacillus* polymyra, while the gram negative organisms are *E. Coli. Salmoniella typhi, vibro* spp, *Pseudomonas putida* and *Pseudomonas aeroginosa*. These isolates are catalase positive while four are oxidase positive, four (4) are voges

proskauer negative while one (1) isolate showed indole positive. Moreover, all the isolates showed acid or gas production on the fungi fermentation test as in table 1 below.