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MICROBIAL BIOLOAD OF SOME TAP WATER SAMPLES FROM ENUGU, ENUGU STATE, NIGERIA

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Abstract
Contaminated water has been a source from which infectious diseases are spread in urban areas. Sterile sample bottles were used to collect tap water samples from eleven different locations in Enugu, capital city of Enugu State. Viable bacterial count using surface agar diffusion method was used to estimate and isolate viable organisms. Gram character, coliform count using multiple tube/ most probable number technique and colony characteristics of organisms were used to arrive at our result. Bacteriological screening on all sample groups indicated the presence of microorganism in tap water available in all eleven locations. Further characterization using biochemical methods revealed the absence of *E. coli*, and the presence of *Pseudomonas aeruginosa. Shigella species* and *Salmonella species* in sample groups collected. Suggestions were proffered as to the methods of avoiding possible epidemic as a result of operating water supply unit which fall below WHO standards.

Keywords: microbial bioload, tap water, Enugu, bacteriological screening, coliform

INTRODUCTION

Water is the most abundant of all chemical compounds. It is both an essential ingredient of all living organisms and a major component of the environment in which they live (Brickell, 1996). Water treatment is the most important and direct means of controlling the microbial quality of drinking water (Cooper and Johnson, 1994). Every water supply system is constantly in danger of contamination. John Snow in 1885 connected polluted water with human disease (Purushotham *et al.*, 1986). Therefore, one means of establishing and assuring the purity and safety of water is to set a standard for various contaminants (FAO, 2008). The Safe Drinking Water Act (SDWA) set minimum Standards to be met by all public water systems and are called Maximum Contaminant Levels (MCLs). Two primary mechanisms by which the microorganisms appear in the distribution system are contamination of the treatment plant and growth within the distributions system (Knight, 1979). Indicator organisms as given by European Commission directive on quality of drinking water include coliforms, faecal streptococci, *Pseudomonas aeruginosa* and sulphate reducing anaerobes. WHO has defined indicators to be absent in unpolluted water and present when the source of pathogenic microorganisms of concern is present (Okoye *et al.*, 1987). In spite of the availability of information on portability of drinking water all over the world with the exception of

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inaccessible and impoverished communities, death rate from water-related diseases remains high and is inadequately monitored and reported. A wide range of estimates are available in public literature, ranging from 2 million to 12 million deaths yearly (Table 1). Most of those dying from water related diseases are small children struck by virulent but preventable diarrhoeal diseases (Iwu, 1993).

Table 1: Estimates of water-related mortality

Source	Deaths per year
World Health Organisation	2.2 Million (Diarrhoeal diseases only
Water Dome, 2002	More than 3 Million
Hunter *et al.*,2000	More than 5 Million
UNDP, 2002	More than 5 Million
Hinrichsen *et al.*, 1997	12 Million

Viable microorganisms play major roles in many aspects of water quality control and thus, the assessment of the bacteriological characteristics of water is often of great significance (Nwinuka *et al.,* 2005). Assessment of the bacteriological characteristics of our drinking water has become very important in this age of disturbing antibiotic resistance the Nigerian people. The cleanliness of our drinking water will to an extent ensure that (a) Re-infection by microbes does not occur in patients receiving treatment (b) Resistant strains of pathogens which are water-borne do not occur (c) Expenses accrued as a result of expensive drug therapy required by resistant cases are minimized.

Ridgeway and Oson showed that majority of viable bacteria in chlorinated drinking water are attached to particles. Hence, filtration of drinking water is of paramount importance in homes, hospitals and industries, while boiling of drinking water will ensure that most organisms are killed. These are simple processes and can easily be adhered to for better health.

Although microbial standards vary from place to place, the objective any where is to reduce the possibility of spreading water borne diseases to the barest minimum in addition to being pleasant to drink (Wedlin *et al.,* 1986).

The purpose of this present study is to evaluate the microbial quality of tap water samples in a typical Nigerian city.

MATERIALS AND METHODS

Sources of water samples used in the study and code used to designate each sample group are presented in Table 2.

Materials

Table 2: Sources of water samples used in the study

S/N	Sample code	Area of collection in Enugu
1	SA	New Haven
2	SB	Abakpa
3	SC	Trans-Ekulu
4	SD	GRA
5	SE	Iva valley
6	SF	Coal camp
7	SG	P and T
8	SH	Asata
9	SI	Obiagu
10	SJ	Presidential Rd
11	SK	Secretariat Qtrs

Microbiological culture media
Nutrient agar (Biotec, England), Mckonkey broth (purple) (Lab. M. England) cetrimide agar (Biolife, England), deoxycholate citrate agar (Biolife, UK). McConkey agar (Lab M., England) were used as procured from their respective manufacturers.

Equipment
The following instruments and equipment were used in the study: autoclave, hot air oven, incubator (Gallenkamp, England) and oil immersion microscope (Vickers, England).

Sample collection
Eleven batches of tap water samples were collected from eleven different locations using sterile sample bottles and following strictly accepted steps for collecting drinking water samples for bacteriological analysis as published by Lake County Health Department and Community Health Centre (Robertus, 1991) and Homeowner Bacteriological Collection Instruction (Vitetta and Thorpe, 1991).

Determinations
Viable bacteria count was performed on the samples collected using surface agar diffusion method described by APHA AWNA (1995), Cheesbrough (2005) and Jawetz *et al.,* 1976). A 1:10 dilution was aseptically prepared and serial dilution carried out to yield 10^{-2} dilution of each sample collected and labelled. Underside of over dried agar plate was marked into five divisions and drops containing 0.015 ml of each diluted sample deposited on the surface of prepared nutrient agar. Extra plates were prepared, over dried and incubated under same laboratory conditions to act as control. The plates were incubated for 24 h at 37^{O}C. Colonies developed were counted. Bioload of water samples were determined by counting the colonies and calculating the cfu/ml of the original suspension as described by Okore (2004). Presumptive coliform count using the method described in Talaro and Talaro (1996) was used to obtain the most probable number (MPN). Sterile McConkey broth (purple) was prepared and inoculated into each water sample and incubated for 24 h at 37^{O}C to check for the presence of *E. coli* in all water samples. Controls were prepared without inoculates and also incubated under similar condition. Isolates were collected from colonies observed on the nutrient agar plates. Isolated organisms were stored in appropriate condition throughout the period of the experiment. Cultural/colonial characteristics were studied and the Gram characteristics of typical colonies determined with references to official standards (Cheesbrough, 2005). Biochemical characterisation was done using glucose, lactose, sucrose and mannitol broths containing methyl orange indicator. Sterile test tubes containing the sugars were sterilized with upturned Durham tubes at 121^{O}C for 15 min. Surface growths of typical colonies were scrapped off using a sterile platinum wire loop and introduced into the test tubes. The test tubes were then incubated at 37^{O}C for 24 h. Sterility of the broths was ensured by incubating tubes which were not inoculated with organisms as controls. The indicator incorporated into the broths indicated colour change for acid production. Results were recorded as for samples which contained organisms in them.

RESULTS AND DISCUSSSION
The results of the viable bacterial count of the respective water samples are shown in Table 3.

Table 3 = Viable bacterial count of water samples

Sample code	Viable bacterial count (cfu/ml)
SA	9.17×10^3
SB	3.50×10^4
SC	1.63×10^5
SD	5.92×10^4
SE	3.97×10^5
SF	5.08×10^5
SG	4.00×10^5
SH	1.04×10^6
SI	1.37×10^5
SJ	6.78×10^5
SK	5.79×10^5

Table 4: Cultural and Gram characteristics of isolated organisms

Isolates	Gram stain	Colony colour	Chromogenesis	Arrangement	Shape	Gram Character
SA	Red	Creamy	Non-diffusible	Singly dispersed	Rod	G-ve
SBi	Red	Yellow	Non-diffusible	Singly dispersed	Rod	G-ve
SBii	Red	Creamy	Non-diffusible	Singly dispersed	Rod	G-ve
SCi	Red	Creamy	Non-diffusible	Singly dispersed	Rod	G-ve
SCii	Red	Yellow	Non-diffusible	Singly dispersed	Rod	G-ve
SDi	Red	Yellow	Non-diffusible	Singly dispersed	Rod	G-ve
SDii	Red	Bluish green	Non-diffusible	Singly dispersed	Rod	G-ve
SEi	Red	Creamy	Non-diffusible	In chains	Rod	G-ve
SEii	Red	Creamy	Non-diffusible	Singly dispersed	Rod	G-ve
SFi	Red	Creamy	Non-diffusible	Irregular cluster	Coccus	G-ve
SFii	Red	Creamy	Non-diffusible	Singly dispersed	Rod	G-ve
SGi	Red	Creamy	Non-diffusible	Singly dispersed	Rod	G-ve
SGii	Purple	Creamy	Non-diffusible	Singly dispersed	Rod	G+ve
SGiii	Red	Creamy	Non-diffusible	Singly dispersed	Rod	G-ve
Shi	Red	Creamy	Non-diffusible	Singly dispersed	Rod	G-ve
SIi	Purple	Yellow	Non-diffusible	In chains	Coccus	G+ve
SIii	Purple	Creamy	Non-diffusible	In chains	Coccus	G+ve
SIiii	Purple	Creamy	Non-diffusible	Irregular cluster	Coccus	G+ve
SIiv	Red	Yellow	Non-diffusible	Singly dispersed	Rod	G-ve
SJi	Red	Bluish green	Diffusible	Singly dispersed	Rod	G-ve
SJii	Red	Bluish green	Diffusible	Singly dispersed	Rod	G-ve
Ski	Red	Bluish green	Diffusible	Singly dispersed	Rod	G-ve
SKii	Red	Creamy	Non-diffusible	Singly dispersed	Rod	G-ve
SKiii	Red	Creamy	Non-diffusible	Singly dispersed	Rod	G-ve

Coliform test
Only sample SG showed evidence of gas production indicating a positive presumptive test. However, the most probable number test executed using the presumptive test positive sample, gave negative result. This implied that the faecal coliform test showed absence of faecal coliform bacteria within confidence limits.

Fermentation test
By observing the fermentation of the following sugars: lactose, sucrose, glucose, and mannitol and matching the result of reported tests, the following organisms were identified (Table 5).

Table 5 – Sugar fermentation characters of the bacterial isolates

Sample No	Glucose	Lactose	Sucrose	Mannitol	Organisms
SGi	AG	AG	AG		*Enterobacter aerogenes*
SGiii	A	-	-		*Salmonella specie*
SFi	A	-	-		*Shigella dysentariae*
SBi	A	-	-		*Shigella dysentariae*
SDiii	A	-	-		*Shigella dysentariae*
SBii	A	-	A	A	*Serratia*
SEi	A	-	-	A	*Shigella flexneri*
SEii	A	-	-	A	*Shigella flexneri*
SDii	-	-	-	-	*Pseudomonas aeruginosa*
SJi	-	-	-	-	*Pseudomonas aeruginosa*
SJii	-	-	-	-	*Pseudomonas aeruginosa*

Sample SA had the lowest microbial load while SH had the highest, above the acceptable range. Sample SG contained organism producing both acid and gas. Confirmation tests did not confirm *E. coli* as present. *Salmonella* species in SG were red, singly dispersed rod, Gram negative glucose fermenter. Colonial morphology showed entire, creamy coloured and non-diffusible colonial growth. Deoxycholate citrate agar did not inhibit its growth. *Shigella flexneri* was seen in SE, identified as in the case of *Salmonella* species.
P. aeruginosa in SJ and SD was confirmed using cetrimide agar medium. *Serratia* was seen in SB and *Enterobacter aerogenes* in SG.

Other organisms which were non-sugar fermenters were not confirmed and thus not reported. A few coliform organism were detected, an indication that pollution took place in the distribution system.

It is therefore recommended that tap water samples should be filtered and boiled to eliminate microbes before use in hospitals, homes and in industries producing foods, cosmetics and drugs.

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