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The Prevalence of Bacteria in Packaged Sachets Water Sold in Nnewi, South East, Nigeria

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Abstract: This study was designed to investigate the potability of sachet water sold in Nnewi metropolis. Ninety (90) sachet water samples obtained from 6 different manufacturers (J to O) were analyzed microbiologically by the multiple tube technique. The mean most probable number (MPN)/100 ml of faecal coliforms in the samples were as follows; J =7.1, K =1.1, L=1.3, M=1.5, N=4.3 and O=5.7. The mean coliform counts at 37°C/100 ml in the samples were: J=2, K=1.0, L=0.3, M=0.3, N=1.3 and O=3.0. Samples L and M were observed to belong to the excellent category while samples J, K, N and O were in the satisfactory category. The mean count at 44°C/100 ml in the water samples were: J=3.3, K=0, L=0, M=0, N=1.3 and O=0.9 thus samples K, L and M belong to excellent category (A) while samples O, N and J belong to the satisfactory category (B). The prevalence of other bacteria isolated from the analyzed samples was *Esch. coli (*36%*), Streptococcus faecalis* (19.4%), *Klebsiella pneumoniae* (19.4%) and *Staphyloccocus aureus* (25%). In conclusion, some of the water samples were found to be in excellent condition and others in satisfactory conditions, efforts however should be made by the manufacturers to still improve.

Key words: Water · Potability · Coliforms · Bacteria

INTRODUCTION

Good quality water is odourless, colourless, tasteless, and free from faecal pollution [1]. Coliform bacteria are indicator organisms mostly used in bacterial water analysis [1].They are easily found in animal faeces especially of human origin; soil and raw surface water[1]. Coliforms are rod-shaped Gram negative organisms which ferments lactose with the production of acid and gas when incubated at 37°C [2]. Faecal coliform is a smaller group within the total coliform family; it inhabits the intestine of mammal and has a relatively short life span. This serves as an indication of contamination by sewage[2]. *Escherichia coli* is the most preferred faecal coliform used in assaying water analysis because it gives indication of faecal contamination. It also does not grow and reproduce in the environment consequently, it is considered to be the species of coliform bacteria that is the best indicator of faecal pollution and the presence of pathogens [1,2]. Recently in this country, the trend of producing sachet water is now on the increase with Nnewi town, South Eastern Nigeria, having lots of these sachet water manufacturers. The need for having potable water is of great public health significance because of water-borne infections. Thus this investigation was undertaken to analyze some of these sachet water samples in order to ascertain their potability.

MATERIALS AND METHODS

A total of (90) sachets water samples randomly selected (15 each) from six (6) different sachet water producers (J to O) in Nnewi were used. They were analyzed as follows;

Multiple Tube Test Presumptive Coliform Count [3]: Fifty (50) ml of each of the water samples was added to 50 ml double strength MacConkey broth, and another 10 ml of each water sample was added to 10 ml double strength MacConkey broth with Durham tubes. The medium contains bromocresol purple and any tube that changes to yellow with gas production is noted while tubes not showing any change in 48 h were considered negative. Reference to McCrady's table showed the most probable number (MPN) of presumptive coliform counts in 100 ml of the sample water analysed.

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Confirming *E. coli* Count by Eijkman's Test [3]: A positive multiple tube test was incubated into 5ml Brilliant green lactose bile broth at different temperatures of 37°C for 48 h and 44°C for 24 h confirmed *Escherichia coli* count and coliform count respectively. *Esch. coli* and *Klebsiella aeruginosa* serve as positive and negative controls respectively. Any tube that changed to yellow with gas production was recorded as confirmed coliform count while tubes not showing any change in 48 h were considered negative. Few drops of Kovac's reagent were added to the tubes incubated at 44°C for 24 h to check for indole production. The tubes with positive indole result as well as positive acid and gas production from lactose were counted and recorded as confirmed *Esch. coli* count. A zero *Esch. coli* count per 100 ml of water sample is classified as "excellent" and belong to category A; Counts of 1-10 belong to category B and are classified as "acceptable"; Counts of 10-50 belong to category C and is unacceptable and finally; Counts greater than 50 belong to category D and is grossly polluted [4, 5].

Viable Count: The number of living bacteria in a liquid culture of the original MacConkey broth was subjected to viable count [6].

Other Bacteria: Identification of other organisms was done using standard methods [3].

Statistical Analysis: The student t-test and ANOVA were used to determine the significance level at $P<0.05$.

RESULTS

Table 1 shows the MPN of the 15 different sachet water samples within 95% confidence limits. Samples K and L with SD 1.1±0.36 and 1.3±0.47 respectively fell within the satisfactory grade while samples M, N, O and J fell within the intermediate grade [4].

The percentage isolation of organisms in the sachets water samples showed that *Esch. coli* had the highest prevalence rate (36.1%). This was followed by *S. aureus* (25.0%), *S. faecalis* (19.4%) and *K. pneumoniae* (11.4%). The viable counts for sachets water J, N and O are 1.0×10^4-1.6×10^4, 2.0×10^3-2.6×10^3 and 2.0×10^3-2.6×10^3 respectively (Table 2).

Table 3 shows the comparison of mean±standard deviation of the different organisms isolated in the sachets water samples. There was no significant difference between sachets water J versus O ($F= 2.73$; $P>0.05$) but there was a significant difference between sachets water J versus N ($F= 1.76$; $P<0.05$) and between sachets water N versus O ($F= 1.90$; $P<0.05$).

Table 1: The most probable number (MPN/100 ml) of the 15 different sachets water and the grades of quality water

Sachets water	ξx±SD 95%	Confidence limits	Grades
J	7.1±3.88	6.10-8.10	Intermediate
K	1.1±0.36	1.0-1.20	Satisfactory
L	1.3±0.47	1.20-1.40	Satisfactory
M	1.5±0.50	1.40-1.60	Intermediate
N	4.3±1.19	4.0-4.60	Intermediate
O	5.7±0.70	5.5-5.90	Intermediate

Table 2: Results on isolation and identification of organisms in the sachets water

Sachets water	% of bacteria	J	N	O
Esch. coli	(36.1%)	6	4	3
K. pneumoniae	(11.4%)	3	3	1
S. aureus	(25.0%)	5	2	2
S. faecalis	(19.4%)	3	2	2
ξ(x±SD)		4.25±1.5	2.75±0.8	2.0±0.7
Viable counts		1.0-1.6×10^4	2.0-2.6×10^3	2.0-2.6×10^3

Table 3: Comparison of mean (±SD) of the different organisms isolated in the water samples

Sachets water Mean (±SD)	Mean comparison	F-value	P-value
J	4.25±1.50	J vs. N	1.76<0.05
N	2.75±0.80	N vs. O	1.90<0.05
O	2.00±0.70	J vs. O	2.73>0.05
ANOVA			11.9>0.05

DISCUSSION

The mean most probable number of faecal coliforms (MPN)/100 ml of water sample in the different sachet water samples were found to fall within the 95% confidence limits. It provided an interval which is likely to contain true values [3]. Based on WHO guidelines for water classification [4], sachets water samples K, L, M having less than three coliforms count per 100 ml belong to the satisfactory category while Sachets water samples J, N, O with less than 9 coliforms count per 100 ml of sample, belong to the intermediate category. Ten (10) coliforms count per 100 ml of sample is classified unsatisfactory and unfit for use [4, 5].

The mean coliforms count per 100 ml of water sample at 37°C was determined and based on WHO guidelines for water classification, Sachets water L and M belong to the excellent grade while K, N, J, O belong to the satisfactory grade [1, 2]. It has been reported that the groups of coliform bacteria that ferment lactose at 30-37°C are mostly found on vegetation and are not considered to be of any particularly epidemiologic importance in the examination of water [1-3]. The ability of coliforms to ferment lactose is the possession of the enzyme α-galactosidase in their system [1].

There were no *Esch. coli* count in samples K, L and M. The presence of *Esch. coli* is an indicator pathogen that determines water pollution. Its presence in water serves as an indication of contamination by sewage [1, 2]. Sachet water samples K, L and M fell within the maximum acceptable concentration (MAC) of total coliforms in water. MAC is none detectable coliforms per 100 ml according to the guidelines for Canadian Drinking Water Quality [7, 8]. However, slight presence of *Esch. coli* was observed in the sachet water samples J, N and O thus signifying that these were relatively not pure for drinking. The presence of *Esch. coli* is most often accompanied by the presence of dangerous enteric pathogens like *Shigella, Salmonella* and *Campylobacter* species [1, 2]. The WHO recognizes the fact that in many villages and small towns, it is very difficult to supply regular water with an *Esch. coli* of zero per 100 ml. So, for chlorinated water, 90% of samples analyses within a year should have a zero *Esch. coli* count per 100 ml but in case of contamination, it should not exceed 5 *Esch. coli* count per 100 ml otherwise investigation should be made on equipment and the water system and the cause of contamination rectified. For unchlorinated samples; most of them contain faecal bacteria but in the case of protected ground water e.g. sealed wells, it is possible to achieve very low levels of contamination.

Apart from *Esch. coli* isolated in sachet water samples of J, N and O, organisms like *Klebsiella pneumoniae, Streptococcus faecalis* and *Staphyloccocus aureus* were also isolated and this finding is supported by those of some researchers who reported that accompanying presence of *Klebsiella pneumoniae, Streptococcus faecalis* and *Staphyloccocus aureus* with *Esch. coli* confirmed the pollution to be of faecal origin though, their presence when compared with *Esch. coli* were of smaller densities than *Esch. coli* [1,8]. The presence of *Staphyloccocus aureus*, a non-coliform organism in the sachet water sample might be as a result of unhygienic handling of equipment since it is an ubiquitous organism.

Statistically, the total number of organisms isolated in J was significantly higher than the values in samples N and there was no significant difference in O. It has been recommended that the routine analysis of *Esch coli* be complemented by a colony count when using Lactose based media [7, 8]. The mean viable count of bacteria/ml of sachets water J, N and O fall within the range of WHO standard (3.0×10^4/ml-3.0×10^5/ml) [5]. There were also no growth in sachet water samples K, L and M.

It has been reported that the drinking of unhygienic water in poor countries had resulted in the death of 2.2 million people per annum, 99% of these are children under the age of five [4]. It has also been found that many countries lack safe drinking water especially in Africa, where only 46% of its population has access to safe drinking water while in Asia, only 8% of its population has access to safe drinking water [9].

In conclusion, sachet water samples K, L and M were found to be in excellent conditions, free from faecal pollution specifically of *Esch. coli* but regular checks should be made on equipment and water system to avoid any contamination. Sachet water samples J, N and O were found to contain faecal pollution and specifically of *Esch. coli*. The public health importance of safe drinking water cannot be overemphasized hence the need for these water manufacturers to adequately make necessary investigation to identify the point of entry and get it rectified.

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