

# Microbiological Quality of Drinking Water in Sachets Sold in the City of Yaoundé in Cameroon: Tests of Sensitivity to the Antibiotics of Bacteria Isolated

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#### Abstract

Water is the basic drink for human beings and drinking water in sachets is very popular because of its relatively low cost and availability. The aim of this study is to determine the bacteriological profile of sachet drinking water sold in the city of Yaounde. It was a descriptive cross-sectional study covering the period from March to June 2019, carried out in the application laboratory of ETMS-Yaounde. A total of 230 samples of drinking water in sachets purchased in different markets in the city of Yaounde were analyzed using Mac Conkey's flooding method. The identification was done on the API 20 E Gallery and the susceptibility test on Mueller Hinton media. The size of the sample was 230 packaged sachet drinking water and 213 of the 230 revealed 92% of positive culture of germs, and only 17 samples gave a negative culture, at a percentage of 8% of isolated germs. The isolated bacterial species and their respective abundances in samples were Enterobacter gergoviae (3%), Klebsiella pneumoniae (5%), Proteus mirabilis (5%), Serratia fonticola (5%), Salmonella choler arizonae cloacae (8%), Salmonella spp. (8%), Enterobacter cloacae (10%), Staphylococcus aureus (10%), Pseudomonas aeruginosa (18%) and Staphylococcus epidermidis (28%). All these tested germs were resistant to Amoxicillin and Erythromycin and 70% of tested germs were sensitive to Gentamycin. Overall, the results revealed poor microbiological quality of these waters. This exposes consumers to health risks, and it is important to inform and sensitize consumers about the risks involved, to educate producers and to control their activities by the health services.

Keywords: Drinking water in sachet, Consumers, Bacteriological profile, Cameroon

#### 1. Introduction

Water is the most shared natural resource in the world. Indeed, a man can remain several days without eating but cannot stay for more than three days without drinking water (Akiyo, 2017). Therefore water is the basic drink for human beings, offered to men by nature. Man must be able to drink and to hydrate correctly and avoid body dehydration. Unfortunately, this water which is so precious to life is not always so clean for consumption. By its quality, water can harm health and even human's life. The consumption of water soiled by pathogenic microorganisms is the origin of numerous illnesses. It constitutes a real public health problem (N'diaye, 2008). Among these pathogenic microorganisms, we can mention the bacteria that are unicellular microorganisms, without cores nor individualized organs and of stretched out shape (bacilli) or spherical (cocci). For the pathogenic stumps, they are the causative agent of diarrheas in children, can also cause urinary infections in elderly people, are implicated more and more in infections like dysenteries, fevers generalized, typhoid fevers, gastro-enteritis and cholera (Debabza, 2005). 80% of the illnesses present on the surface of the earth are of water origin and the drinking water is very often contaminated (WHO, 2014). The access to a healthy water constitutes, an indispensable condition to health, an elementary right and a key component of the protective sanitary policies. Thus, facing this phenomenon, several studies have been achieved to appreciate the sanitary risk, the quality and the potability of drinking water in general and the conditions in particular waters (N'diave, 2008; Hadson, 2017). In Cameroon, infections due to the consumption of drinking water have been signaled, and the problem of the sanitary quality of the sold drinking water in plastic sachets on the market

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became the government's priority. The goal of this survey is to evaluate the possible presence of microorganisms capable to have some consequences on the public health and to give an idea of the hygienic quality of sachet drinking water sold in the city of Yaounde.

## 2. Material and Methods

## 2.1 Material

We conducted a transversal, descriptive and analytical study from March to June 2019 at the Medico-Sanitary Technicians application laboratory at the mobile Hygiene in Yaounde (ETMS of Yaounde). Our study population consisted of drinking water in plastic bags sold in the urban areas of the city of Yaounde. The collection of these 230 bags of drinking water was carried out by acquiring in the different sale points of the city (markets and universities). Water bags that were damaged, exposed to the open air or kept in containers of questionable hygienic conditions were excluded from this study.

#### 2.2 Method

The plastic water bags were collected in the markets: Acacia, Central, Mendong, Mokolo and at the University of Yaounde I campus. Two sachets per brand were used for the study; then labelled and transported to the ETMS-Yaoundé laboratory where we kept them refrigerated at 4 °C in a refrigerated enclosure. The labels bore three-letter codes corresponding to the names of the 13 brands found, namely: "BON, SUP, AQU, ROS, OME, SER, ALP, OSM, GOL, PER, NAT, MIN and EAM".

Bacteriological analyses were carried out using solid medium cultures. Petri dishes were placed and the different culture media were prepared and poured into them (Mac Conkey, Chapmann, DNA agar, Mueller Hinton) according to the manufacturer's procedure (SCHARlab). On the first day of analysis, after shaking the water bag vigorously, the Mac Conkey medium was inoculated by flooding and incubated at 37 °C for 18 to 24 hours (Hadson, 2017; Nébout et al., 2010). The results were expressed in colony-forming unit per milliliter (CFU/ml). The second day of analysis consisted of the interpretation of the culture media. Indeed, the Petri dishes were taken out of the oven, colonies counted and a smear was made, which was to be stained with Gram and read with an optical microscope using the 100 X objective. For this, we used the API 20 E gallery for the identification of the cultivated germs. On the third day of analysis we performed sensitivity tests of pathogens to antimicrobial agents.

The inoculum was prepared from a bacterial strain and inoculated by flooding on Mueller Hinton. After drying the medium, the antibiotic discs were deposited 30 mm apart using the flaming tongs. The plates were incubated at room temperature for 30 minutes to allow pre-diffusion of the antibiotic into the agar. Petri dishes were incubated in an oven at 37 °C for 18-24 hrs in an inverted position. Using a ruler, graduated in millimeters (mm) measurement of the inhibition diameters of the different antibiotics tested to determine the MIC. The results were recorded on the antibiotic susceptibility chart by diameter and the antibiotics were categorized into: Sensitive (S); Resistant (R) and Intermediate (I).

Spearman rank correlations were used here to assess the robustness of the relationships between the bacteriological species isolated and the nature of the sachets. This correlation is denoted r and two variables are more or less strongly related depending on whether r is more



or less close to 1. The dependency relationships between the biological variables (abundance of bacteria) and the different water brands were investigated using Principal Component Analysis (PCA). This analysis made it possible to distinguish the bacterial germs that characterized each brand (Nébout et al., 2010). Ward's aggregation method allowed the grouping between nodes (Hammer et al., 2014). The analyses were performed using SPSS version 16.0 software and the results were evaluated at 5 % safety threshold.

#### 3. Results

3.1 Characteristic of Nature of Isolated Germs



Figure 1. Distribution of samples according to the presence of germs (A) and the nature of germs (B)

The results show that there were more positive cultures (213 water sachets with a frequency of 92%) than negative cultures (17 sachets with a frequency of 8% (Figure 1A). Figure 1B shows that, there was a majority of Gram-negative bacilli (BG-) with a frequency of 80% and less Gram-positive cocci (CG+) with a frequency of 20%.







Figure 2. Distribution of isolated germs in the different samples ALP, AQU, BON, GOL, MIN (A) and NAT, OME, OSM, PER, ROS, SER, SUP (B)

On Figure 2A, in the ALP sample, *Enterobacter cloacae* constituted 25% of the identified germs, *Enterobacter gergoviae* 25%, *Klebsiella pneumoniae* 25%, and *Salmonella choler arizonae* 25%. In the AQU sample, *Enterobacter cloacae* comprised 33.33%, *Salmonella* spp. 33.33%, and *Staphylococcus epidermidis* 33.33%. In the BON sample, *Staphylococcus aureus* accounted for 33.33%, and *Staphylococcus epidermidis* accounted for 63.67%. In the GOL sample, *Enterobacter cloacae* accounted for 33.33%, *Klebsiella pneumoniae* 16.67%, *Salmonella* spp. 16.67%, and *Pseudomonas aeruginosa* 16.67%. In the MIN sample *Staphylococcus aureus* constituted 50% and *Pseudomonas aeruginosa* 50%.

In the OME sample (Figure 2B), *Pseudomonas aeruginosa* constituted 50%, *Klebsiella pneumoniae* 25%, and *Serratia fonticola* 25%. In the WHO sample, *Staphylococcus epidermidis* constituted 50%, and *Staphylococcus aureus* 50%. In the ROS sample, *Proteus mirabilis* constituted 40%, *Pseudomonas aeruginosa* 20%, and *Staphylococcus epidermidis* 40%. In the SER sample, *Pseudomonas aeruginosa* constituted 33.33% and *Salmonella choler arizonae* 66.67%. In NAT, PER and SUP samples *Staphylococcus epidermidis* constituted 100% of the isolated germs.





Figure 3. Level of contamination by abundance of bacteria

A distribution of isolated germs by level of water contamination gave the following respective percentages per species on Figure 3 above: *Enterobacter gergovia* (3%), *Klebsiella pneumonae* (5%), *Proteus mirabilis* (5%), *Serratia fonticola* (5%), *Salmonella* spp. (8%), *Salmonella choler aezonae* (8%), *Enterobacter cloacae* (10%), *Staphylococcus aureus* (10%), *Pseudomonas aeruginosa* (18%), and *Staphylococcus epidermidis* (28%).

3.2 Antimicrobial Susceptibility Testing of Pathogens

The antibacterial specificities of each of these species are summarized in Table 1.

	Amoxicillin	Azithromycin	Gentamicin	Ceftriaxone	Cefotaxim	Cefuroxim	Erythromycin	Ofloxacim
Enterobacter cloacae	R	S	S	R	R	R	R	S
Enterobacter gergoviae	R	R	R	S	R	R	R	R
Klebsiella pneumoniae	R	R	R	Ι	R	Ι	R	S
Proteus mirabilis	R	R	S	R	R	Ι	R	S
Pseudomonas aerugenosa	R	S	S	R	R	R	R	S
Serratia fonticola	R	R	S	I	Ι	R	R	S
Staphylococcus aureus	R	S	S	R	Ι	Ι	R	S
Staphylococcus epidermidis	R	R	S	R	Ι	R	R	R
Salmonella choler aezonae	R	Ι	S	S	S	Ι	R	R
Salmonella spp	R	R	S	S	S	Ι	R	S

Table 1. Table of sensitivity / resistance of isolated bacteria to some antibiotics (red colour= resistant antibiotics; blue= Sensitive; white = intermediate resistance)

Amoxicillin is the first antibiotic used for sensitivity testing. There has been 100% resistance of isolated germs to Amoxicillin. The germs tested with Azythromycin showed that there was 100% resistance for *Staphylococcus epidermidis*, *Salmonella* spp, 100% intermediate for *Salmonella choler arizonae* and 100% sensitivity for *Staphylococcus aureus*, *Pseudomonas aerugenosa* and *Enterobacter Cloacae*, (Table 1).



The germs tested with Gentamicin showed 100% resistance to *Enterobacter gergovia*, and *Klebsiella pneumonae*, 100% sensitivity for *Enterobacter cloa*cae, *Proteus mirabilis*, *Pseudomonas aeruginosa, Salmonella choler aezonea, Salmonella* spp., *Serratia fonticola, Staphylococcus aureus* and *Staphylococcus epidermidis*.

The germs tested with Ceftriaxon were 100% resistant to *Enterobacter cloacae*, *Proteus mirabilis*, *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Staphylococcus epidermidis*. *Serratia fonticola* and *Klebsiella pneumonae* were 100% intermediate tested to Ceftriaxon. 100% of sensitivity with *Salmonella choler aezonea*, *Salmonella* spp. and *Enterobacter gergovia*.

The germs tested with Cefotaxim showed that there was 100% resistance to *Enterobacter cloacae*, *Enterobacter gergovia*, *Klebsiella pneumonae*, *Proteus mirabilis* and *Pseudomonas aeruginosa*; 100% intermediate of *Serratia fonticola*, *Staphylococcus aureus* and *Staphylococcus epidermidis*; 100% sensitivity of *Salmonella choler aezonae* and *Salmonella spp.*.

The germs tested with Cefuroxim showed that there was 100% resistance to *Enterobacter cloacae*, *Enterobacter gergoviae*, *Pseudomonas aeruginosa*, *Serratia fonticola* and *Staphylococcus epidermidis*; 100% intermediate of *Klebsiella pneumonae*, *Proteus mirabilis*, *Salmonella* spp., *Staphylococcus aureus* and *Salmonella choler aezonae*.

The germs tested with Erythromycin showed 100% resistance to *Enterobacter gergovia*, *Klebsiella pneumonae*, *Proteus mirabilis*, *Pseudomonas aeruginosa*, *Serratia fonticola*, *Staphylococcus aureus* and *Staphylococcus epidermidis*; no isolated germs were intermediate or sensitive to this antibiotic.

The germs tested with Ofloxacim showed 100% resistance to *Enterobacter gergovia*, *Staphylococcus epidermidis*, *Salmonella choler aezonae*; 100% sensitivity to *Enterobacter cloacae*, *Klebsiella pneumonae*, *Proteus mirabilis*, *Serratia fonticola*, *Staphylococcus aureus Pseudomonas aeruginosa* and *Salmonella* spp. (Table 1).

3.3 Interactions Between Identified Species in Water

Some correlations between isolated species were consigned in Table 2 according to abundance and characteristics of species.

	Pseudomonas	Serratia	Staphylococcus	Staphylococcus	Salmonella	Salmonella
	aerugenosa	fonticola	aureus	epidermidis	choler aezonae	Spp.
Enterobacter						
cloacae	0.438	0.678*	-0.251	-0.335	-0.128	0.630*
Enterobacter						
gergoviae	-0.198	-0.147	-0.155	-0.278	0.008	0.807**
Klebsiella						
pneumoniae	0.919**	0.487	-0.229	-0.41	-0.135	0.135
Pseudomonas						
aerugenosa	1	0.579*	-0.206	-0.466	0.018	0.064

Table 2. Correlations between the different bacterial germs isolated



Serratia						
fonticola	0.579*	1	-0.273	-0.29	-0.161	0.371
Salmonella						
choler arizonae	0.018	-0.161	-0.17	-0.304	1	-0.074
Salmonella spp	0.064	0.371	-0.273	-0.389	-0.074	1

\* Correlation significant at a threshold of 5 %.

\*\* Correlation significant at a threshold of 1%.

Relationships between the different species isolated from the plastic water bags (table II) gave significant and positive correlations at the 5% threshold between *Enterobacter cloacae* and the species *Serratia fonticola* (r = 0.678; p = 0.11), *Salmonella* spp. (r = 0.630; p = 0.21). At 1% threshold, significant correlations were also observed between *Enterobacter gergoviae* and *Salmonella* spp. (r = 0.807; p = 0.01) and between *Klebsiella pneumoniae* and *Pseudomonas aerugenosa* (r = 0.919; p = 0.000). Overall, the genus Staphylococcus represented by the species *Staphylococcus aureus* and *Staphylococcus epidermidis* was negatively correlated with the other isolated species.



Figure 4. Characteristics of the water in plastic sachets analyzed according to the isolated species

The principal component analysis gave along two main axes (Figure 4), a grouping of the variables into two groups. Group I, to the left of the figure following Component 1. This group is characterized by bushy variables composed of the species *Salmonella choler aezonae*, *Staphylococcus epidermidis* and *P. mirabilis*, major characteristics for the brands NAT, MIN, EAM, SER and minor for the brands OSM, PER, OME, ROS. Group II on the

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right, has a more dispersed appearance, characterized by the species *Enterobacer gergoviae*, Salmonella spp. and *Enterobacter cloacae* with the marks AQU, SUP and ALP. The minor organisms; *Serratia fonticola, Klebsiella pneumoniae* and *Pseudomonas aeruginosa* were characteristic of the bacteriological profile of the BON brand.

## 4. Discussion

This study has highlighted that the factors contributing to the definition of the production environment of conditioned water are: the hygiene of the building, the method of water supply, the maintenance of the water storage equipment and the place of storage of the finished product. The adverse effects of the above-mentioned factors are the possible elements of contamination ingested in the water bags when the hygienic conditions are insufficient, which lead to diseases. Thus, two hundred and thirty (230) water samples out of the 213 collected were positive for contamination, which corresponded to a frequency of 92% of the total samples (Figure 2). This prevalence is relatively high compared to those observed in previous studies: 29.13% in Abidjan (N'diaye 2008; Cristian, 2008); 41% in Dakar (Senegal) (Hadson, 2017). This difference can be explained by the fact that this work was carried out in 6 communities of Abidjan and in all districts of the Dakar region, whereas we worked in 4 districts of the city of Yaounde.

Most of the germs isolated were Gram-negative bacilli (80%) of which some fermentative and one non-fermentative (*Peudomonas aeruginosa*), which is not an indicator of water contamination and other germs also Gram-positive. Cocci (20%) indicators of environmental contamination showed that these waters are not free of microorganisms (N'diaye 2008; Cristian, 2008; Tourab, 2013). This prevalence is lower than that found in Abidjan (21.64%) but is equally dominant in this population (Sekhri, 2011). This difference could be explained by the fact that Gram-negative bacilli are more resistant in the environment, especially under unfavorable environmental conditions (Degbey et al., 2011, DEPES, 2019). These germs should not be present in plastic sachet water, as they are intended for direct consumption.

As for the sensitivity tests of isolated germs to the different antibiotics used, all isolated germs have shown resistance to Amoxicillin and Erythromycin with a frequency of 100% (Diallo, 2013), these resistances would be due to the abusive and anarchic use of these antibiotics and self-medication (WHO, 2014; Calmet, 2012). The sensitivities observed for Gentamicin and Ofloxacim could indicate the efficiency of these antimicrobials related to their mode of action and their broad spectrum (Degbey et al., 2011). The bacteriological profile of the tested sachet waters could also be influenced by the storage method of the sachets. Faced with this situation we can say that these waters are unfit for human consumption.

#### 5. Conclusion

This survey whose objective was to determine the different bacteriological nature of water in plastic sachets sold in the city of Yaounde permitted to put in evidence the existence of germs which could be pathogenic as well as non-pathogenic that are indicators of fecal and environmental contamination. Drinking water in plastic sachets sold at the scene of the urban city of Yaounde and valued generally by the population, are germ-carriers due to the handling using dirty hands or hands poorly washed and also by airborne carriers due to our



environment. The research of the contamination proved to be positive and more than half of the samples analyzed presented a positive culture with a frequency of 92%, either to the germs targeted during the survey or to other normally absent germs in drinking water. All isolated germs showed resistance to the antibiotics (Amoxicillin and Erythromycin) and a major sensitivity to Gentamicin. In the light of these results, these waters in sachets are therefore unfit for human consumption and it is necessary to warn consumers of the health risks involved. However, these sachet water not only meet a vital need for consumers, but also provide a source of income for producers and sellers. Banning them without adequate replacement measures is not an option. On the other hand, education of producers and monitoring of their activities by communal hygiene services can limit health risks.

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