



Water-related Diseases in the Adamawa Region, Cameroon: A Prospective and Retrospective Case Study and the Susceptibility of Isolated Bacteria to Common Antibiotics

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Water related disease is defined as any significant or widespread adverse effects on human health, such as death, disability, illness or disorders, caused directly or indirectly by the condition, or changes in the quantity or quality of any water. This cross-sectional and analytical study, carried out from July to December 2019 at the Ngaoundere Regional Hospital and Protestant Hospital aimed to determine the prevalence of water related diseases in the Vina Division. Retrospective data of patients records covered the period of five years (2014 to 2018) were analyzed in order to identify

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common water related diseases in the study area. Document review technique was adopted in this study; outpatients/inpatients registers were reviewed and information on socio-demographic characteristics of each individual patient were collected. Also, the bacteriological characterization of stool samples collected from patients was done to identify the most frequent bacteria responsible for water related disease in the area, followed by an antibacterial susceptibility testing of some isolated bacteria using standard methods. Results show that, out of 153073 patients recorded from 2014 to 2019, 63750 were clinically diagnosed for water related diseases, giving a prevalence of 41%. Overall, typhoid fever and malaria were the most prevailing water related diseases confirmed clinically with a prevalence of 43%. Parasitic infections were the least prevailing (3.7%). The study revealed that, females was more prone to water related cases than males. Bacteria were more sensitive to streptomycin and resistant to amoxicillin. This study shows that water related diseases are a major health problem in the Vina Division. Routinely monitoring of drinking water sources is recommended to the different users in order to limit the spread of water related diseases.

Keywords: Water; diseases; prevalence; malaria; typhoid; parasites.

1. INTRODUCTION

Water related diseases are defined as any significant or widespread adverse effects on human health, such as death, disability, illness or disorders, caused directly or indirectly by any changes in the quantity or quality of any water [1]. The World Health Organization defines safe drinking water as, "water that does not represent any significant risk to human health over a lifetime of consumption [2].

Every year more than 3.4 million people die as a result of water related diseases, making it the leading cause of morbidity and mortality around the world [3]. In some countries, statistics are not generally demonstrative and the problem remains complaining. Scientists are also unanimous in all sectors, specifically in the area of housework that, water quality is more important than its quantity [4,5]. Because of the restriction and saturation of the network performed by the public organizations in charge of the matter (production and distribution), many individuals have turned to the use of groundwater [6]. However, population growth and the expansion of human activities (agriculture, poultry, cattle and pigs farming as well as the use of uncontrolled septic tanks) alternates groundwater resources, which is already in limited quantities [7]. The development of these human activities coupled with the climate change and the nature of geological formations make the groundwater increasingly infected and therefore, very dangerous to health when used [8]. The causes of water related diseases include microorganisms, parasites, toxins and chemical contamination of water [2,9].

Water related diseases can be classified as follows: waterborne-vector related diseases that

are spread by insects that depend on water for survival and procreation; water-washed diseases which are caused primarily by water scarcity; water-based diseases in which germs reside in hosts that live in the water; water-borne diseases which are spread through the ingestion of polluted water [1].

Water being incriminated as a path way for the transmission of diseases has led to the establishment of the following research questions:

- What are the various microbes involved in water related diseases?
- What are the micro bacterial susceptibility profiles of the various agents found in water?

In order to answer the above questions, this work has as general objective to determine the prevalence of water related diseases in the vina division and to assess the susceptibility of the germs involved in these infections to commonly used drugs. More specifically, this research aimed to: evaluate the most prevailing water related diseases from 2014 to 2018; carry out a bacteriological stool analysis from the patients suspected of water related diseases; evaluate the susceptibility of isolated bacteria from the stool to commonly used antibiotics.

2. MATERIALS AND METHODS

2.1 Type, Duration and Study Area

This cross-sectional and analytic study was carried out from June 2019 to December 2019 in the vina division, Adamawa region of Cameroon. This region is located in the heart of Central Africa, between 6-8° North latitude and 11-16° East longitude (Fig. 1). It extends over a length

of about 410 km from West to East between Nigeria and the Central African Republic, for a total area of 67827 Km². The region receives an average of 1540 mm of rainfall per year, from March to October. The temperature is moderate with an annual average around 25°C. On hydrological level, the Adamawa region is called “the water tower of Cameroon” because it feeds three of the four major watersheds of this country, namely the lake Chad Basin, the Niger basin in the North and the Sanaga Atlantic basin [7].

2.2 Study Population

The study population consists of all patients who were referred to the laboratory for gastroenteritis or abdominal problem; and patient’s registers

with water related diseases from 2014 to 2018. All diseases not related to water were not included in this study, Moreover, patients with incomplete information in the registers were excluded.

2.3 Retrospective Scheme of Work

In order to determine the prevalence of water related diseases, retrospective data of patients records covered the period of five years, from 2014 to 2018 were exploited. Document review technique was adopted to review patient’s registers and information on the frequency and type of water related diseases as well as socio-demographic information.

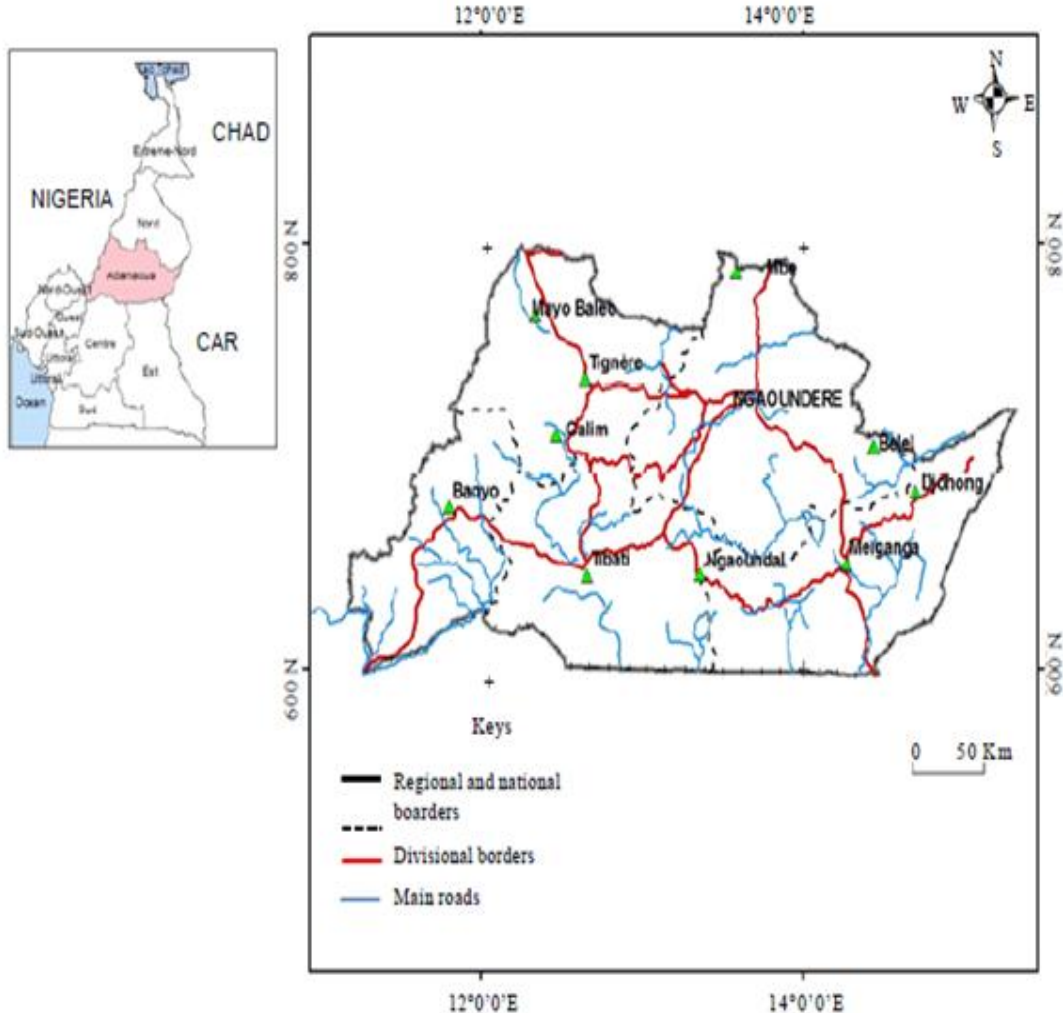


Fig. 1. Geographic representation of Adamawa Region [7]

2.4 Collection of Stool Samples and their Bacteriological Analysis

One hundred and three (103) stool samples (5 grams per sample) were collected in sterile test tubes previously contained pepton solution, from patients who came in the hospital suffering of gastroenteritis or abdominal pain.

After macroscopic observation of these stool samples (its consistency, the presence of mucus, the presence of blood), they were immediately taken into the microbiology laboratory for analyses and culture. The stool samples were then inoculated on *Salmonella Shigella* agar and Endo agar culture media initially prepared as describe by the manufacturer. The cultures were incubated for 24 hours at 37°C and the bacteria growth noted.

2.5 Isolation and Identification of Some Pathogenic Bacteria from the Stool Culture

Based on macroscopic characteristics of bacteria on different plates, isolates were selected for further characterization.

The identification of colonies was done in pure culture on Muller Hinton basis on cultural characteristics described by Fodouop et al., [8]. Sub culturing was performed and the identity of strains isolates was confirmed by using API 20E Strips as describes by the manufacturer of the kit.

2.6 Antimicrobial susceptibility testing of some pathogenic bacteria from the stool culture

Antimicrobial sensibility was determined by the disk diffusion test (Britania), described by Fodouop et al., [8]. Three to five identical colonies from each agar plate were transferred with a sterile wire loop into test tube containing 5 mL of distilled water. The turbidity of each bacterial suspension was adjusted to give a turbidity value of 0.5 McFarland standard, resulting in a suspension containing approximately 1 to 2×10^8 CFU/mL. Mueller-Hinton agar plates were inoculated by streaking the swab over the entire sterile agar surface. After allowing the inoculum

to dry at room temperature (25°C), 6 mm diameter disks each with fixed concentration of the antibiotics was tested for antibacterial activity by introducing duplicate disks per plate.

The plates were allowed to stand at 25°C for 1 hour to allow the antibiotics diffuse and then incubated at 37°C for 18 hours, subsequently, the plates were examined for bacterial growth inhibition and the inhibition zone diameter (IZD) measured to the nearest millimeter using a transparent ruler. A standard table of antibiotic susceptibilities was used to determine whether the strain was resistant (R), intermediate (I) or susceptible (S) to the specific treatment tested Antibiotics employed and their concentrations.

Antibiotic discs (Oxoid, England) used were: Ampicillin (amp, 10µg), Tetracycline (t, 30µg), Trimethoprim/sulphamethazole (te, 25µg), Amoxicillin (am, 30 µg), Chloramphenicol (c, 30µg), Amoxicillin/clavulanic (ax, 30 µg), Streptomycin (str.), Erythromycins (e, 15 µg), and Penicillin (sxt,10 µg).

2.7 Data Analysis

Data were collected and the graph was plotted using Microsoft office and excels software. These data were analyzed using XL Stat. Chi-square test of independence was performed to explore the relationship between different parameters.

3. RESULTS

3.1 Socio-demographic Characteristic of Patients

3.1.1 Distribution of patients with water related diseases

Out of the 153073 patients recorded from 2014 to 2019 in the various hospitals, 63750 were clinically diagnosed for water related diseases, giving a prevalence of water diseases of 41% (Fig. 2). Among these biologically diagnosed cases, 27421(43.01%) were suffering from typhoid fever, 27641 (43.35%) from malaria, 6349 (6.96%) from other gram negative bacteria diseases and 2339 (3.67%) from other parasitic infections (Fig. 3).

Water related diseases Other diseases

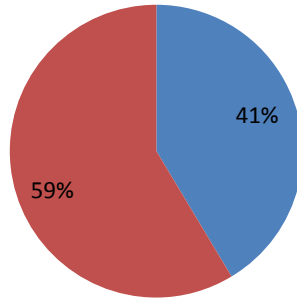


Fig. 2. Distribution of patients according to their types of diseases encountered during the retrospective study

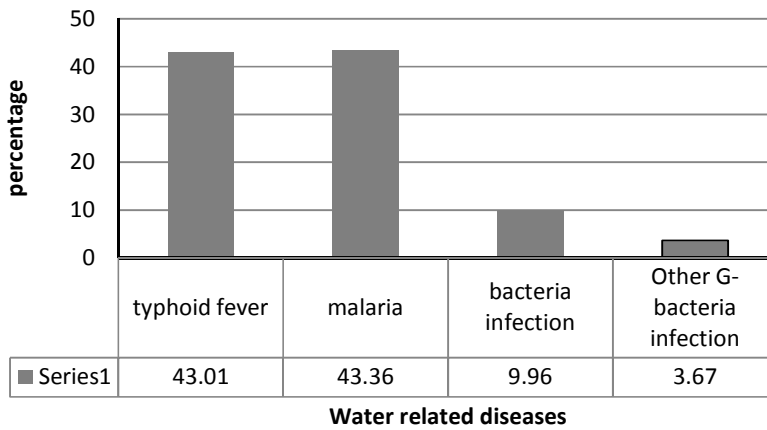


Fig. 3. Distribution of different type of water related diseases from 2014 to 2019 in Vina division

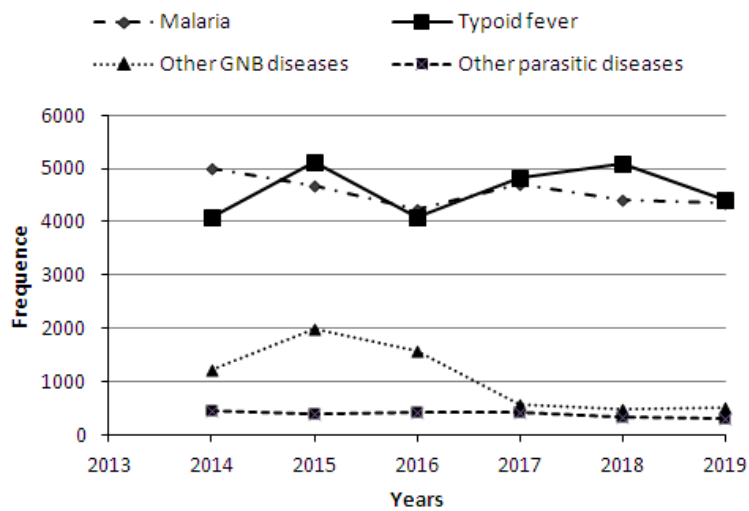


Fig. 4. Yearly distribution of patients infected by water related diseases from 2014 to 2019.

3.1.2 Yearly distribution of water related diseases

Fig. 4 shows the yearly distribution of the different water related diseases from 2014 to 2019. As shown on this figure, typhoid malaria and other parasitic infections had a stable trend throughout the years concerned. the frequency of the two above mention diseases were the highest amount all. Other gram negative bacteria diseases had an unstable trend throughout the interested period.

3.1.3 Socio-demographic characteristics of patients infected by *Salmonella typhi* from 2014 to 2019 years

Fig. 5 shows that out of a total of 63750 cases of water-borne pathologies recorded during these

six years 31212 were men and 32,538 women; or a sex ratio of 1.04 in favor of women.

Out of the 27641 and 27421 patients respectively positive for typhoid and malaria, 15183 and 13363 patients were female and 12458 and 14058 were male, giving a sex ratio of 1.21 and 1.05.

The evolution of malaria and typhoid fever in respect to age group shows that adults are more infected followed by children and adolescents. The least to experience the diseases was the elder (Fig. 6). *Trichomonas intestinalis* was the most prevailing parasite followed by *Gardialambia*. The least parasite encountered was *Shistosomia mansoni* (Fig. 7).

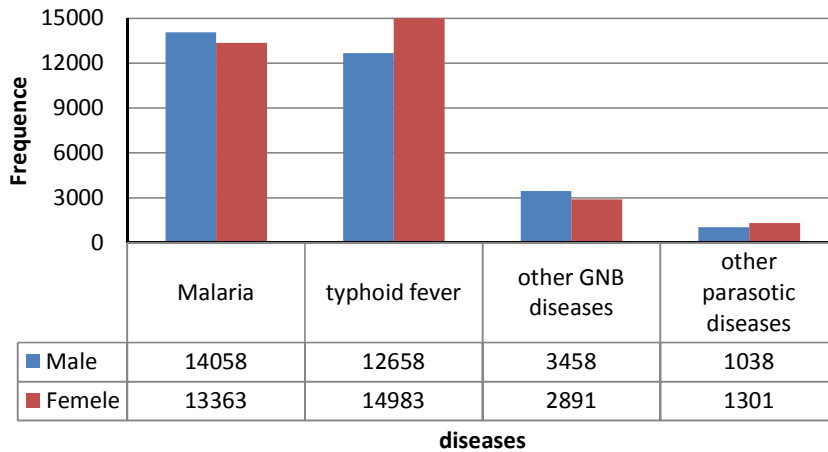


Fig. 5. Sex distribution of patients infected from water related disease in Vina Division

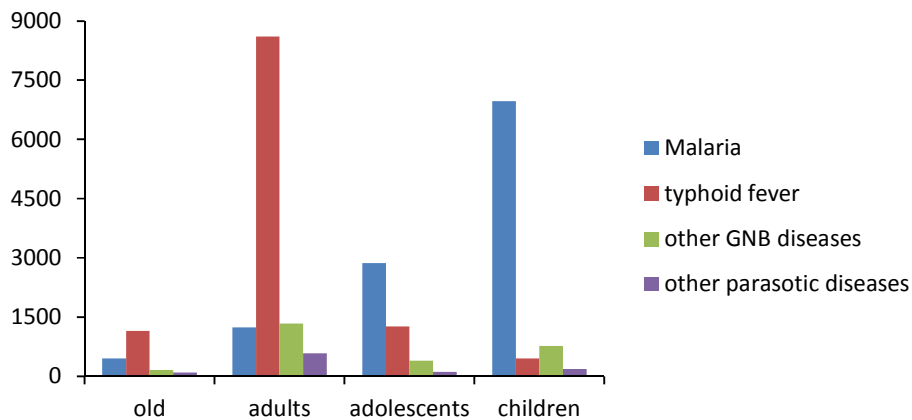


Fig. 6. Age group distribution of patients infected water related diseases from 2014 to 2019 in Vina division

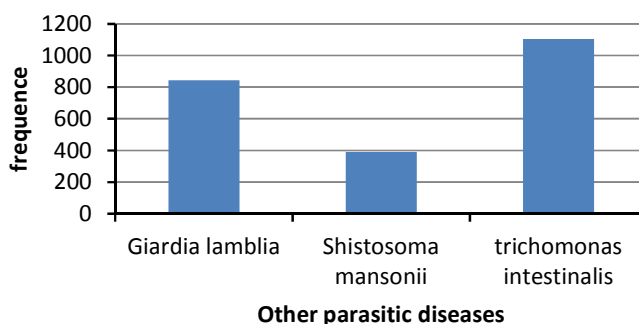


Fig. 7. Frequency of parasitic infections according to different parasites from 2014 to 2019 in vina divion

3.2 Bacteriological Characteristics of Stool

After 24 hours incubation of culture at 37°C, bacteria growth has led to the changes of the golden yellow color of Endo media to a metallic pink color (photo 1 A). The growth on S-S media gave a black color after incubating for 24hrs (Photo 1B). The following 8 bacteria strains were identified based on their biochemical characteristics on API 20E gallery (Table 2): *Escherishia coli*, *Salmonella* spp, *Klebsiella*

pneumoneae, *Klebsiella oxitoca*, *serratia faciria*, *Serratia fonticola*, *Enterobactera erogenes* and *Citrobacterfreundii*.

3.3 Sensibility Profile of Identified Bacteria to Antibiotics

As shown in Table 1, bacteria strains were either resistant or possess intermediate activity to other antibiotics. Streptomycin was the most effective antibiotic on the different bacteria strains.

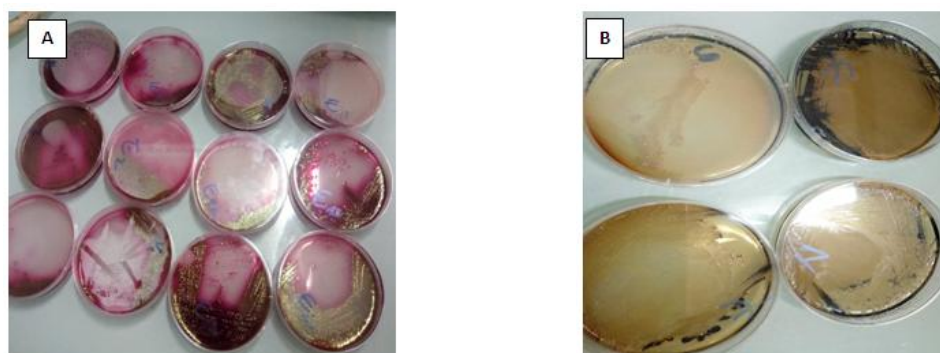


Photo 1. Colonies of *Escherichia coli* and *salmonella* Sppon Endo SS culture media

Table 1. Susceptibility of different strains to Antimicrobials

Strains	Amc	Ax	Am	Sxt	C	STR	T	E	TE
S-1	R	I	I	R	R	S	I	I	I
S-2	I	S	I	I	R	S	I	I	I
S-3	R	R	R	R	I	S	I	R	I
S-4	I	S	R	S	S	S	I	I	I
S-5	R	R	R	R	S	S	I	R	I
S-6	R	R	R	R	R	S	I	R	R
S-7	R	R	R	R	R	S	R	I	I
S-8	R	R	R	R	R	S	R	I	R

S-1: *Escherishia coli*, S-2: *Salmonella* spp, S-3: *Klebsiella pneumoneae*, S-4: *Klebsiella oxitoca*, S-5: *Serrate afaciria*, S-6: *Serratia fonticola*, S-7: *Enterobacter aerogenes*, S 8: *Citrobacter freundii*. Resistance: 7.8-5, Intermediate: 8.5-13, Sensibile: >13, S: strain

3.4 Sociodemographic Distribution of Patients Involved in the Bacteriological Stool Characteristic

During the prospective phase of study, we recorded 68 males and 38 females suffering from gastroenteritis (Fig. 7). The age group that were mostly consulted for abdominal pains or diarrhea were mostly children from 0-6 years old, followed by 6-16 and 15-25 years as seen in Fig.9. Most of the stool samples were soft, followed by liquid, mushy, the least were liquid mucus (Fig. 10). Fig. 11 gives data on patient's location. It can be noted from this figure that, most patients were from Baladji followed by Haoussa quarter, Onaref quarter, Burkina quarter.

explained by the fact that there is an inadequate water supply in the Vina Division. Typhoid and malaria were the most prevailing water related disease, this result is in line with a report on drinking water quality guidelines by [10]. According to the UN, third World Water Development Report, more than 600 million people in most parts globally are forced to live without safe water and sanitation services.

Women are more exposed and children are most susceptible to water related diseases. This is due to their roles in water collection, clothes washing and other domestic activities. Fonyuy [11] also reported that females were more prone to waterborne diseases as compared to males in the north west region of Cameroon and women are also responsible for the care of sick family members. Children within 0-6 age groups are more vulnerable to water related diseases. This can be explained by the fact that, children are more exposed to stagnant water than adults. In addition, their immune systems

4. DISCUSSION

Considering 153073cases of consultations reviewed, gives a prevalence of water related diseases of 41%. This shows that, water related diseases are amongst the leading causes of consultation in the Vina Division. This could be

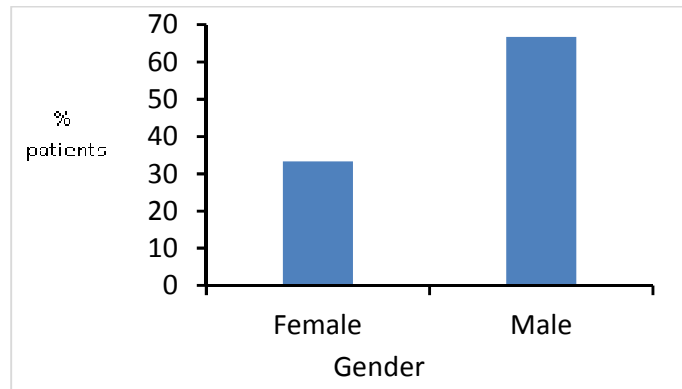


Fig. 8. Sex representation of patients who were involved in the bacteriological study

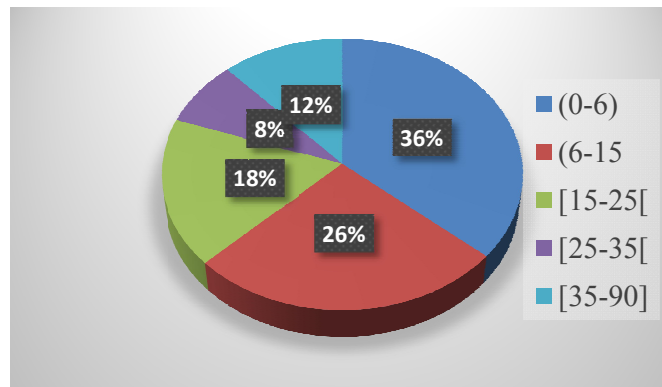


Fig. 9. Age representation of patients involved in the bacteriological study

Table 2. Biochemical characteristics of bacteria from cultured stool

Code	ONPG	ADH	LDC	ODC	CIT	H ₂ S	URE	TDA	IND	VP	GEL	GLU	MAN	INO	SOR	RHA	SAC	MEL	AMY	ARA
S-1	+	-	+	-	+	-	-	-	-	-	-	+	+	-	+	+	+	+	-	+
S-2	+	-	+	-	+	-	-	-	-	-	-	+	+	-	+	+	+	+	-	+
S-3	+	-	+	+	+	-	-	-	-	-	-	+	+	-	+	+	-	+	-	+
S-4	+	-	+	+	-	-	+	-	-	+	-	+	+	+	+	+	+	+	+	+
S-5	+	+	+	+	+	-	-	-	-	-	-	+	+	+	+	+	-	+	-	+
S-6	+	-	+	+	+	-	-	-	-	-	-	+	+	-	+	+	+	-	-	+
S-7	+	-	-	-	-	+	-	-	-	-	-	+	+	-	+	+	+	+	-	+
S-8	+	-	+	+	+	-	-	-	-	-	-	+	+	-	+	+	+	-	-	+

+: positive reaction, - :negative reaction, ONPG : OrthoNitroPhenyl-βDGalactopyranosidase), ADH : Arginine Dihydrolase, LDC: Lysine Decarboxylase, ODC : Ornithine Decarboxylase, CIT: Citrate utilization, H₂S:H₂S production, URE: Urease, TDA:Tryptophane DeAminase, IND: Indole, VP: production,acetoin production (Voges Proskauer, GEL: Gelatinase, GLU : fermentation/oxidation (Glucose), MAN: Manitol, INO; Inositol, SOR: Sorbitol, RHA: Rhamnose, SAC: Saccharose, MEL: Melibiose, AMY: Amygdalin, ARA: Rabinose. S: strain. S-1: Escherishia coli, S-2: Salmonella spp, S-3: Klebsiella pneumoneae, S-4: Klebsiella oxitoca, S-5:serratia faciria, S-6: Serratia fonticola S-7: Enterobacter aerogenes, S-8: Citrobacter freundii

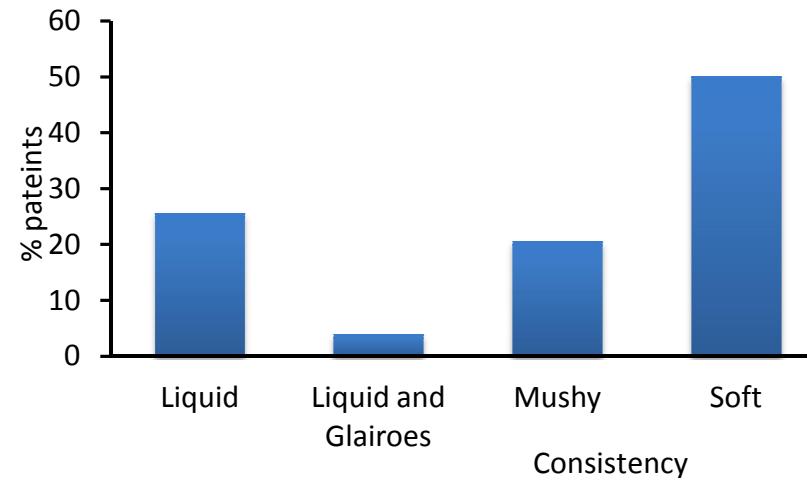


Fig. 10. Percentage of stool consistency collected during the bacteriological study

and detoxification mechanisms are not fully developed, so, they are often less able to respond to a water related infection [12,13].

The stable trend of malaria can be because of the active health campaign on the distribution of treated mosquitoes net since 2014, this goes in affirmation of a work carried out in Nigeria [14]. Typhoid is the first leading water related diseases in the study area. This is due to the contamination of domestic water used by the people and low level of personal hygiene. Similarly, typhoid and diarrhea are water borne diseases which have claimed several million lives globally. Parasitic infection got an unstable trend and least prevailing infection during the period of study, this result could be explained by the fact that current education on hand washing techniques has been going on in Cameroon and that the government has launched a free health campaign on the distribution of anti-parasitic drugs in schools and homes.

We found out that most of the bacteria that grew on the culture media were pathogenic and were mostly bacteria that are water related.

There were the following bacteria in the stool sample based on the biochemical characteristics studied; *Escherishia coli*, *Klebsiella pneumoneae*, *Klebsiella oxitoca*, *Serratia faciria*, *Serratia fonticola*, *Enterobacter aerogenes* and *Citrobacter freundii*. These results can be explained by the facts that the region has few treated water sources and hence the population is forced to consume inappropriate water. Besides, there is little practice of hygiene, especially as fruits and vegetables are not being properly washed before selling or consuming. Most of the bacteria that were identified in our experimental study are mostly the same observed during the retrospective study giving us an affirmation of the water quality of Vina Division. A similar study was carried out by Mercy et al., [15] shows that diarrhea and typhoid are among the most widely known illnesses that are linked to the consumption of faecally contaminated food and water in Nigeria. Poor personal hygiene at the household level and poor household water handling practices could explain the high prevalence of diarrhoeal and typhoid diseases in the study area.

From the antimicrobial susceptibility testing, streptomycin was the most sensitive antibiotic on all the eight isolates bacteria strains; and the prevalence of resistant strains was very high.

The prevalence of antimicrobial resistance in the healthy human population is often higher in developing countries than in developed countries. This resistance can be attributed to the uncritical use of antibiotics [16]. Also, the extensive reservoirs of resistance genes in the bacterial populations of developing countries facilitates the introduction of resistance genes into the bacterial flora [17].

5. CONCLUSION

The sensitivity of streptomycin to all strains could be explained by the fact that this antibiotic is rare to find and manipulation of this antimicrobial agent is rare except in cases of treatment of patients who are resistant to tuberculosis usual therapy. Amoxicillin appeared to be non effective drug against these bacteria. This can be explained by the fact that, it is an over the counter drug which is easily manipulated and frequently prescribed.

DISCLAIMER

The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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