Journal of Advances in Microbiology Research

E-ISSN: 2709-944X
P-ISSN: 2709-9431
JRM 2023; 4(1): 86-95

www.microbiojournal.com
Received: 01-11-2022
Accepted: 11-12-2022

Author's details are given below the reference section

Antibiotics susceptibility of some *Bacillus* strains isolated in the ground and rainwater in urbanized area in Cameroon (Central Africa), and potential impact of the season change

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DOI: https://doi.org/10.22271/micro.2023.v4.i1b.72

Abstract
This study assessed the antibiotics susceptibility of *Bacillus cereus*, *B. thuringiensis* and *B. subtilis* strains isolated in wells and rainwater samples in Yaounde (Cameroon). Water samples in wells were collected monthly during a long dry season (LDS), a short dry season (SDS), a long rainy season (LRS) and a short rainy season (SRS), and during the LRS and the SRS for rainwater. The antibiotics considered included Imipenem, Amikacin, Gentamycin, Ciprofloxacin, Ofloxacin, Sulfamethazol, and Tetracycline. With strains from the groundwater, the antibiotic inhibition diameters varied from 9.13 mm (Sulfametazole during SDS) to 32.78 mm (Imipenem during LDS) with *B. thuringiensis*, from 8.2 mm (Sulfametazole during SDS) to 35.25 mm (Imipenem during LDS) with *B. cereus*, and from 5.05 mm (Ofloxacin during LRS) to 29.25 mm (Imipenem during LDS) with *B. subtilis.* With those from the rainwater, they varied from 4.55 mm (Sulfametazole during LRS) to 25.65mm (Imipenem during LRS) with *B. thuringiensis*, from 2.13 mm (Imipenem during LRS) to 20.05mm (Imipenem during SRS) with *B. cereus*, and from 5.03 mm (Gentamicin during SRS) to 25.15mm (Tetracycline during SRS) with *B. subtilis*. *Bacillus* strains isolated during LRS were multiresistant to the majority of antibiotics. The inhibition diameters of the most antibiotics varied significantly from one season to another ($p<0.05$).

Keywords: Antibiotic susceptibility, *Bacillus* strains, ground and rainwater, inhibition diameters variation

1. Introduction
Water consumption varies greatly from one country to another. It depends on their development, population and the resource itself. When it is polluted, water can be one of the main vectors for the transmission of many diseases which are the cause of major human or animal epidemics. It can concern rivers, water bodies, brackish water as well as rainwater, dew, snow and polar ice.
Water found in each type of environment can be polluted by chemicals and microorganisms including protozoa, virus and bacteria [1]. Various bacteria families can be found in aquatic environments. These microorganisms are of various properties. Some properties commonly used to identify bacterial microorganisms are the Gram staining cell wall and spore-forming character. *Bacillus* genera bacteria are known as Gram positive and spore-forming bacteria. They are found in the air, in water or in the soil [2].
For humans, some *Bacillus* species are pathogens or opportunistic pathogens while others are simply commensal. The commensal character of a germ, however, depends on several factors in its environment [3]. In addition to food poisoning, these bacteria cause local and systemic infections, sometimes resulting in the death of the patient [4, 5].
The potential relevance of biological particles for atmospheric processes has also been recognized for many years [6, 7]. Airborne biological particles as a whole are also denoted as bioaerosols. They can include bacterial cells and cellular fragments, fungal spores and fung

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hyphae, pollen grains, viruses, by-products of microbial metabolism and other biological material [8] After emission from the biosphere, primary biological aerosol particles can serve as nuclei for water droplets or ice crystals, leading to the formation of clouds and precipitation [7]. The spores contained in the atmosphere are likely to be transported to the soil surface by precipitation. The recharge of the water table occurs with a delay of one month compared to precipitation, the time necessary to saturate the soil layers above the water table [9].

On the other hand, the growing challenge of the emergence and spread of antibiotic resistance has been the subject of several investigations [10], and has been flagged by world health organizations as one of the major health challenges of the 21st century [11, 12]. The bacteria involved are increasingly varied and above all have become more resistant to antibiotics and its public health consequences have resulted in increased bacterial resistance to various antibiotics [13].

Some work carried out in urban areas in Central Africa has indicated that rainwater contains bacteria of the *Bacillus* genus. Their abundance dynamics undergoes a spatio-temporal variation, and differs from that of groundwater [14]. Few studies have been carried out on the susceptibility to antibiotics of the *Bacillus* species isolated at the same periods in rainwater and groundwater from the same geographical region. Less information's also available regarding the role of the season changing on the bacterial susceptibility to antibiotics. This study aims to assess the seasonal variation in the antibiotics susceptibility to strains of 3 *Bacillus* species (*Bacillus cereus*, *B. thuringiensis* and *B. subtilis*) isolated in underground and rainwater in an urbanized area in Cameroon (Central Africa).

2. Material and Methods

2.1 Study Area and Sampling Sites

Yaounde is the capital of Cameroon. It is located 300 km from the Atlantic coast, between 3°5' North latitude and 11°31' East longitude. The climate is equatorial, characterized by the alternation of two dry seasons and two rainy seasons: a long dry season (LDS) from December to mid-March, a short rainy season (SRS) from mid-March to June, a short dry season (SDS) from July to August and a long rainy season (LRS) from September to November. The annual average temperature is 23.5°C, varying between 16 and 31°C depending on the season, and that of rainfalls is 1650 mm [15].

Six wells points encoded W1-D5, W2-D5, W3-D3, W4-D3, W5-D6 and W6-D6 were chosen in 3 different administrative districts of Yaounde. Wells W1-D5 and W2-D5 were choose in N°5 district area, wells W3-D3 and W4-D3 in N°3 district area, and W5-D6 and W6-D6 in N°6 district area. At the same time, one station was installed in each of the 3 districts to collect rainwater. It was named R1-D5 in N°5 district area, R2-D3 in N°3 district area and R3-D6 in N°6 district area. The location of each sampling wells and each station for rainwater collection is indicated in Figure 1. The geographic coordinates of each sampling site are indicated in Table 1.

The meteorological data during the sampling period have been downloaded from the NASA web site (https://power.larc.nasa.gov/data.access.viewer/). During this sampling period, the rainfall varied from 0.34 mm/day (January 2022) to 11.8 mm/day (July 2021). The air temperature and the air relative humidity varied from 20.02°C (July 2021) to 24.28°C (March 2021), and from 75.26% (January 2022) to 91.25% (August 2021) respectively. The insolation varied from 2.59 Kw-h/m^2/day (July 2021) to 5.54 Kw-h/m2/day (September 2021). The temporal variation of these data is presented in Figure 2.

2.2 Water Sampling

Rainwater and wells were analyzed once a month, from March 2021 to March 2022. This study period therefore includes 4 different seasons: a LDS, a SRS, a SDS and a LRS. Samples were collected once a month. During the short rainy season, it rains at least once a week, while during the short dry season it rains 1 to 2 every 15 days. During the long rainy season, it rains 3 to 5 times a week.

Each rainwater sample was collected using an autoclaved glass container and placed in the yard of a specific dwelling. The said container was opened just when the rain started to fall. The collected water was poured into a sterile 500 mL glass vial. After the rain, it was then immediately transported in a refrigerated conditions to the laboratory.

For each well chosen, the water sample was taken using a sterile bucket attached to a rope. The collected water was poured into a sterile 500 mL glass vial. After taking this sample, the hydrological parameters of the well were measured using a graduated metal rope, also sterilized in an autoclave and connected to ballast at its end.

2.3 Bacterial isolation and identification

Bacteriological analyzes consisted of the isolation of *Bacillus cereus*, *B. thuringiensis* and *B. subtilis*. *Bacillus cereus* was isolated on Mossel agar medium (Humeau laboratories) containing Polymyxin and the egg yolk added after autoclaving. *Bacillus thuringiensis and B. subtilis* was isolated on were isolated on Luria Bertani agar culture medium (Sigma-Aldrich) [16, 17]. For that, 100 µl or a diluted water sample was analyzed, by the surface plating method. Petri dishes were then incubated at 36 ± 1°C for 24 hours. The morphological identification of the colonies considered the size and the outlines the color. *B. cereus* on Mossel agar medium formes colonies of around 5mm diameter, pink colour (mannitol negative) and typically sorrounded by an opaque halo due to egg precipitation (lecethinase positive) [18-20]. On Luria Bertani agar, the colonies of *B. thuringiensis* are white, with a diameter varying from 0.5 to 1 mm [18, 19], and colonies of *B. subtilis* are yellow [16]. Biochemical identification of strains isolated was further performed using API 20E system [18, 19, 21].

2.4 Antibiotics susceptibility tests

2.4.1 Nutrient media and culture conditions

The medium used was Müeller Hinton agar (Biorad) poured into Petri dishes. The thickness of the agar was approximately 4 mm. The surface of the agar was dried before use [22]. From an 18-24 h culture on non-selective agar medium (Plate Count Agar), a bacterial suspension in saline solution (0.9% NaCl) with a turbidity equivalent to that of the standard 0.5 of the range of McFarland was carried out, which corresponds to a bacterial density of approximately $1x10^8$ CFU/100 mL The inoculum was then diluted 1/10 ($1x10^7$ CFU/100 mL) before inoculation [22].

The agar was inoculated with the bacterial inoculum by the swab method. The entire surface of the agar was swabbed in three directions. The antibiotic discs were placed on the

surface of the inoculated and dried agar. The gap between the discs was 3 cm in order to avoid overlapping of the inhibition diameters. The Petri dishes were then incubated within 15 min following the depositing of the discs, at 37°C aerobically for 24 h [22].

2.4.2 Antibiotic susceptibility assay

The antimicrobial susceptibility tests were carried out using the disk diffusion method according to the recommendations of the FMS-EUCAST [22]. The antibiotic molecules were chosen in depending on the uses of the population but also on their availability in the laboratory.

A total of 7 antibiotics belonging to five major families were used. Antibiotic of the β-lactam family was Imipenem. Those of the Aminoglycoside family included Amikacin and Gentamycin. Antibiotics of the Quinolone family were Ciprofloxacin and Ofloxacin. That of the Sulfonamide family was Sulfamethazol, and that of the Cyclin family was Tetracycline. The inhibition diameters were measured using the caliper and the results were scored as resistant, sensitive or intermediate according to CA-SFM recommendations and others [22, 23].

2.5 Data analysis

The values of the antibiotic inhibition diameters were illustrated by histograms plotted using Excel 2010 software. The Kruskal-Wallis and Mann-Whitney tests were performed to compare the antibiotic inhibition diameters tested between seasons (a value of $P<0.05$ was assumed to be significant). Statistical analyzes were performed using SPSS version 25.0 software.

3. Results

3.1 Antibiotics inhibition diameters with *Bacillus* species isolated

With *Bacillus* strains isolated in the groundwater, it was noted that the inhibition diameters ranged from 5.05 to35.25 mm for the whole antibiotics used. With *B. thuringiensis*, the inhibition diameters varied from 9.13 mm to 32.78 mm. The greatest value was obtained with Imipenem (family of beta lactams) during the long dry season while the smallest was obtained with Sulfametazole (family of sulfonamides) during the short dry season.With *B. cereus* the inhibition diameters varied from 8.2 to 35.25 mm. The highest value was also obtained with Imipenem during the long dry season while the lowest value was recorded also with sulfametazole during the short dry season. With *B. subtilis* inhibition diameters varied from 5.05 to 29.25 mm. The lowest value was recorded with Ofloxacin (Quinolone family) during the long rainy season while the highest value was noted with Imipenem during the long dry season. The temporal variation with respect to the antibiotic used, of the inhibition diameters of each *Bacillus* species isolated from wells samples is presented in Figure 3. The antibiotic inhibition diameters relatively varied with the season changing.

With *Bacillus* strains isolated in the rainwater samples, it was noted that inhibition diameters varied from 2.13 to 25.65 mm for the whole antibioticas used. With *B. thuringiensis*, the inhibition diameters varied from 4.55 mm to 25.65 mm. The greatest value was obtained with Imipenem during the long rainy season while the smallest value was obtained with sulfametazole during the same season. With *B. cereus* the inhibition diameters varied from 2.13 to 20.05 mm. These values were obtained with Imipenem during the long and the short rainy season respectively. Inhibition diameters in *B. subtilis* varied from 5.03 to 25.15 mm. The smallest value was recorded with Gentamicin (family of Aminoglycosides) and the largest value was noted with Tetracycline (family of Cyclins) during the short rainy season. The temporal variation with respect to the antibiotic used, of these inhibition diameters is showed in Figure 3. The antibiotic inhibition diameters also relatively varied with the season changing.

3.2 Antibiotic susceptibility of *Bacillus* species

In groundwater, *B. thuringiensis* was sensitive to Imipenem (Beta-lactam family) during the long and short dry season and was resistant against others of the same family during the short and long rainy season. It has a high resistance against Gentamicin and Akamicin (family of Aminoglycosides) and Sulfamethazole (Sulfamides family), and moderate resistance against Ciprofloxacin and Ofloxacin (family of quinolones) and Tetracycline (Cyclins). *B. cereus* showed a high sensitivity to Imipenem, a medium sensitivity to ciprofloxacin and ofloxacin, a high resistance and a medium resistance against the sulfonamides and aminoglycosides family respectively. *B. subtilis* showed a high resistance against antibiotics of the Aminoside and Sulfonamide family in all seasons and an average sensitivity to Imipenem of the beta-lactam family. In rainwater, a strong resistance of all species of *Bacillus* against all the families of antibiotics tested was recorded, with the exception of *B. subtilis* which was sensitive to antibiotics of the cyclin family during the short rainy season. The susceptibility of the 3 *Bacillus* species against all the antibiotics used is presented in Table 2 for groundwater and Table 3 rainwater. The results of the sensitivity to the antibiotics tested showed that the *Bacillus* species isolated during the long rainy season were mostly multiresistant to the majority of antibiotics.

3.3 Comparison of antibiotic inhibition diameters

Using the Kruskal-Wallis test, a comparison of the inhibition diameters of bacteria amongst the isolation seasons of the cell strains (4 different seasons for groundwater and 2 different seasons for rainwater), was made for each type of water and each antibiotic tested. The results are presented in Table 4. When cell strains were isolated from groundwater, it was noted for *B. thuringiensis* that the inhibition diameter varied significantly ($P<0.05$) from one of the 4 seasons to another at the presence of Imipenem, Ofloxacin and Ciprofloxacin. When the strains were instead isolated from rainwater, the inhibition diameters varied significantly from one rainy season to another ($P<0.05$) only when the cells were at the presence of Gentamycin or Tetracyclin (Table 4).

With *B. cereus* when the bacterial strains were isolated from the groundwater, the inhibition diameters varied significantly from one of the 4 seasons to another ($P<0.05$), at the presence of Imipenem, Gentamycin, Ciprofloxacin, Sulfamethazole/trimethoprim and Tetracyclin. With the strains isolated from rainwater, the inhibition diameters varied significantly from one rainy season to another ($P<0.05$), only at the presence of Imipenem, Ofloxacin and Sulfamethazole/Trimethoprim (Table 4).

For *B. subtilis* cells, the inhibition diameters varied significantly from one of the rainy season to another at the presence of all the antibiotics tested ($P<0.05$) with the

strains isolated from rainwater. The same observation is made with the strains isolated during the 4 different seasons of the year from groundwater, except when the cells were at the presence of Sulfamethazole/Trimethoprim (Table 4). It is noted from Table 3 that the profiles susceptibility of strains isolated from a water type varied relatively depending on the species. This susceptibility profiles for each cell species to the antibiotics seems to vary from season to season. However, this depends on the bacterial species, its original aquatic biotope and the antibiotic tested.

In addition, with regard to strains isolated from groundwater, the Mann-whitney U test was made to compare the inhibition diameters between the seasons taken two by two, in order to know precisely between which seasons the susceptibility to antibiotics varied. It appeared a significant difference ($P<0.05$) for the *B. thuringiensis* strains inhibition diameters at the presence of Imipenem, Ofloxacin, Ciprofloxacin between the long dry season and the long rainy season and between the short dry season and the short rainy season. Concerning the *B. cereus* strains, a significant difference in the susceptibility to antibiotics ($P<0.05$) was recorded between the long and short dry season at the présence of Imipenem, Gentamycin, Akamicin, Ofloxacin, Ciprofloxacin and Tetracycline. A significant difference in the cells susceptibility ($P<0.05$) was also recorded between the short dry and rainy season at the presence of Imipenem, Gentamycin, Ciprofloxacin, Sulfamehtazol and Tetracycline. For the *B. subtilis* strains, a significant difference ($p<0.05$) was observed between the long dry season and the short dry rainy season, then between the short dry season and short rainy season at the presence of Imipenem, Gentamycin, Akamicin, Ofloxacin, Ciprofloxacin and Sulfamethazol.

4. Discussion

4.1 Antibiotic resistant *Bacillus* strains in wells and rainwater

Bacillus species isolated from different water types were highly resistant to several antibiotics used (Tables 2-3). This would reflect the multidrug resistance. This multi-resistance was observed for different families of antibiotics tested during the long and short rainy season for rainwater and during almost all seasons for groundwater. Egorov *et al* [24] indicated during all the time that synthesis of antibiotics has existed in nature (more than 2 billion years), bacteria have developed resistance mechanisms to their toxic action. Antibiotic resistance can occur via three general mechanisms: prevention of interaction of the drug with target, efflux of the antibiotic from the cell, and direct destruction or modification of the compound [25].

All the *Bacillus* strains isolated were resistant to Sufamethazol. Sulfonamides are the antibiotics to which bacteria are generally sensitive; however, in this study there was a high level of resistance. The target of sulfonamides in bacteria is the enzyme dihydropteroate synthase [26].

The role of bacterial enzymes in resistance development is rather versatile and involves several key mechanisms [24, 25]. The enzymes involved in cell wall biosynthesis, as well as the synthesis of nucleic acids and metabolites, serve as a direct target for antibiotics. These enzymes in most cases included a)-those as the targets of antimicrobial drugs, b)-those modifying the cell targets of antimicrobial drugs, c)-those modifying antimicrobial drugs, and d)-bifunctional enzymes [24, 27, 28].

Intrinsic antibiotic resistance and acquired antibiotic resistance are also known [29]. The acquired resistance mechanisms also include modification of antibiotic targets by genetic mutation or post-translational modification of the target [29-31]. Various antibiotic resistance mechanisms and may vary with respect to the considered cells strains and antibiotic. They include (i)modifications to the cell wall or outer membrane, reducing permeability for the antibiotic substance, (ii) expression of degradation enzymes that can render the substance harmless, (iii) protection of the molecular target of the antibiotic by preventing the antibiotic from entering the cell or pumping it out faster than it can enter, or (iv) alterations to the site of primary action and production an alternative target (usually an enzyme) that resists inhibition by the antibiotic [32-34].

Rainwater comes from the atmosphere. Many strains isolated from rainwater were also resistant against many antibiotics. Some studies carried out on the antibiotics susceptibility of culturable microorganisms in bioaerosols noted that this susceptibility varied with respect to antibiotic tests on one hand and the bacterial species considered, and most of germs have different antibiotic resistance genes distribution patterns [35]. It has been indicated that air represents an important active reservoir of diverse antibiotic resistance genes (ARGs) [35], and their origins may be extensive, including human activity such as wastewater treatment plants, gray water, municipal solid waste landfill, livestock farm, and medical industry and natural phenomena such as volcanic eruptions [36, 37].

4.2 Relative variation of antibacterial susceptibility with the change of the aquatic biotope

A relative variation of the bacterial resistance to an antibiotic has sometimes been noted with the change of the type of aquatic biotope (Tables 2-4). This would reflect the impact of the biotope properties on the genotypic properties of the microorganism. Working on the abundance dynamics of the strains of an aquatic species of Aeromonadaceae their antibiotic susceptibility, Signe *et al* [38] noted that there was a positive and significant relationship between the *A. hydrophila* susceptibility against Oxacillin and pH ($P<0.01$) and between susceptibility against Amoxicillin-clavulanic acid and temperature. They noted that the increase in water temperature affect the cells strains antibiotic susceptibility. On the contrary, a negative and significant relationship ($P<0.05$) between cell species susceptibility against Imipenem and pH was noted. The water physicochemical properties would influence the genetic makeup of aquatic microorganisms. This would then have repercussions on the enzymes activity in the presence of antibiotics.

It has thus been suggested that the regulation of some bacterial genes would be regulated by complex mechanisms including interactions of some abiotic characteristics of water [38, 39]. In addition, Dutta and Ramamurthy [40] indicated that the ecosystem is continuously exposed to a wide variety of chemicals and antimicrobials, which act as a source for the spread of antimicrobial resistance genes. It has also been suggested that water chemicals could potentially stimulate the conjugative transfer of plasmid-encoded multidrug resistance genes within and across the bacterial species [40].

4.3 Variation of the antibacterial susceptibility with the season changing

Significant variations of the antibiotics susceptibility and inhibition diameters from one season to another have been recorded for a given antibiotic against the same bacterial species isolated from the same aquatic biotope (Tables 2-4). This would suggest the evolutionary and ecological aspects of the chemical and biological reactions in bacteria. It has been indicated that the accumulation of antibiotics in the environment, including wastewaters and drinking water, can contributed to the development of antibiotic resistant bacteria and the dissemination of ARGs. It has been indicated that ARGs can temporary be transferred horizontally to other microorganisms within the atmosphere or aquatic environment, thus promoting the dissemination of antibiotic resistance [29].

For rainwater, the temporal variation of the antibiotic susceptibility of the *Bacillus* isolated strains could be related to meteorological factors such as wind and lightning. It is indicated that aeration in wastewater treatment processes can increase microbial activity, intensify aerosolization, and increase bioaerosol emissions. High aeration shear can affect the size distribution and morphology of activated sludge flocs and further affect the size of bioaerosol particles discharged into the air. And these particles in the air can be transported at different speeds from one region of the world to another [37, 41, 42].

In addition, the presence and concentration of dust particles in the atmosphere would vary according to the seasons. The concentration of these particles in the atmosphere should be relatively high in the dry season, and relatively low in the rainy season. Their wind transport from one part of the world to another could be influenced by wind speeds, the degree of humidity in the atmosphere, as well as by sunshine duration and the sun intensity. Kumar and Attri [43] indicated that the dominant bacterial species in the air are different in different seasons. Some authors have suggested that the influence of season on the concentration of microorganisms in the atmosphere is intertwined with various factors, and the distribution of dust-associated microorganisms may change vertically in space [37, 44].

Accoding to Kataki *et al* [45], many factors may affect the microbial emission load in bioaerosol. They include: a)-the seasonal impact on bioaerosol formation although bioaerosol concentration may vary with respect to type of microbial contaminant [46], b)-the wind speed and direction which directly influence microbial emission around a wastewater treatment plants [47, 48], c)-the sampling height which incease with the decreasing of the concentrations of the microorganisms and total suspended particles [49], d)-the mode and aeration and bioaerosol formation which have the aeration basin in wastewater treatment process trains [50], e)-the chemical components in bioaerosol, the proportion depending upon the source of generation [51, 52], and f)-the meteorological factors (including temperature, humidity, wind speed), the seasonal variation (the dominant bacteria in the air vary with respect to the seasons), the geographic conditions and location, as well as the particle size and atmospheric pollutants [37].

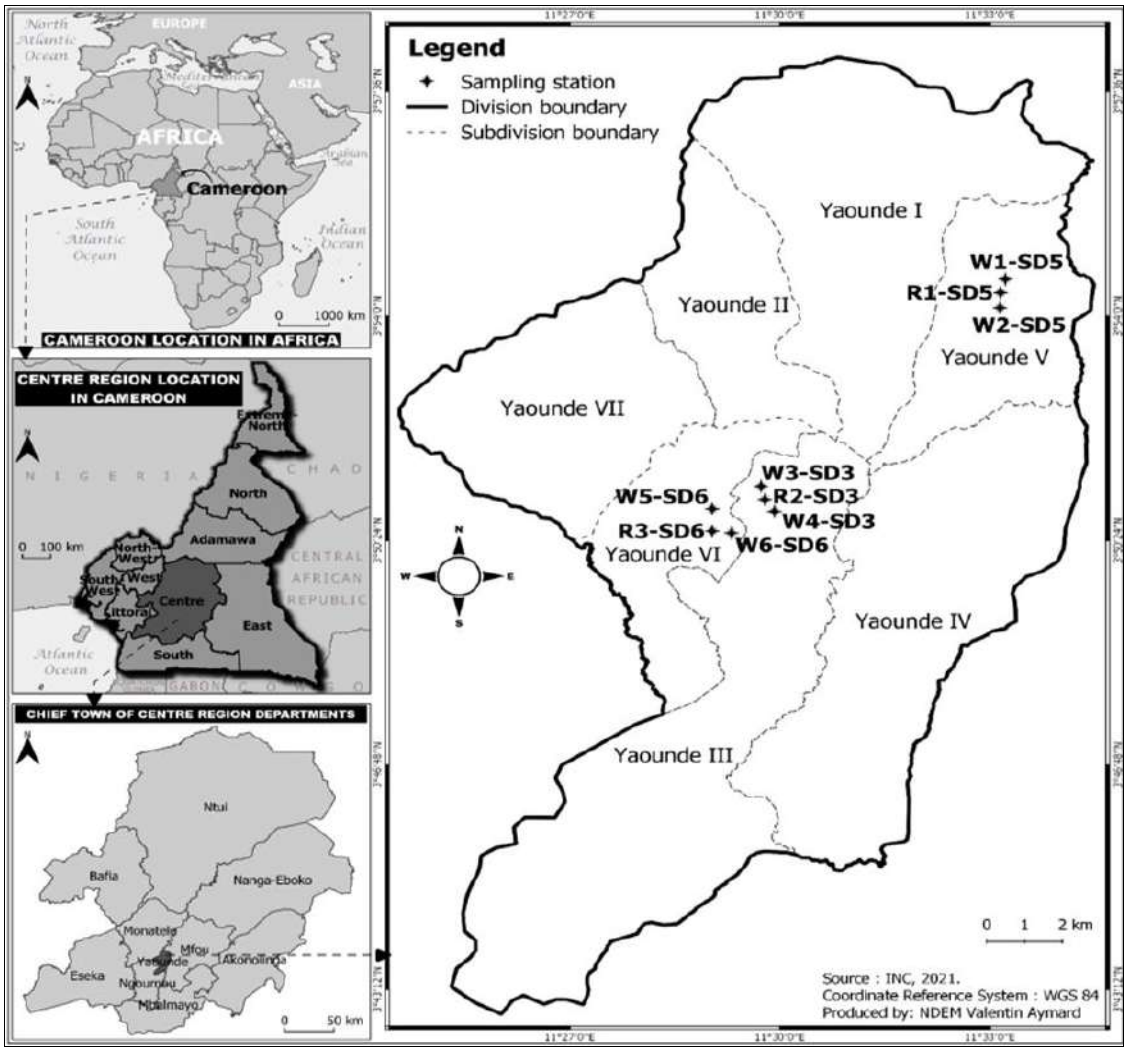

Fig 1: Sampling sites of the water samplings (National Institute of Cartography, Cameroon, 2020, modified)

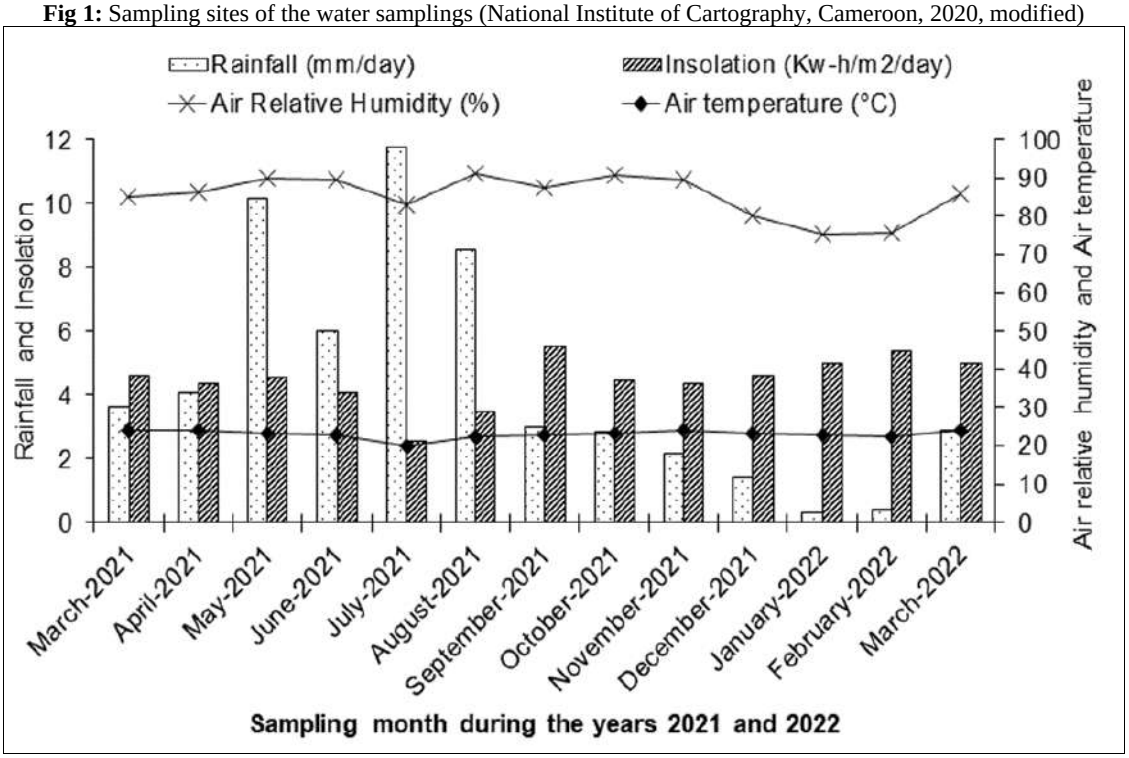

Fig 2: Temporal variation of rainfall, insolation, air relative humidity and air temperature during the sampling period

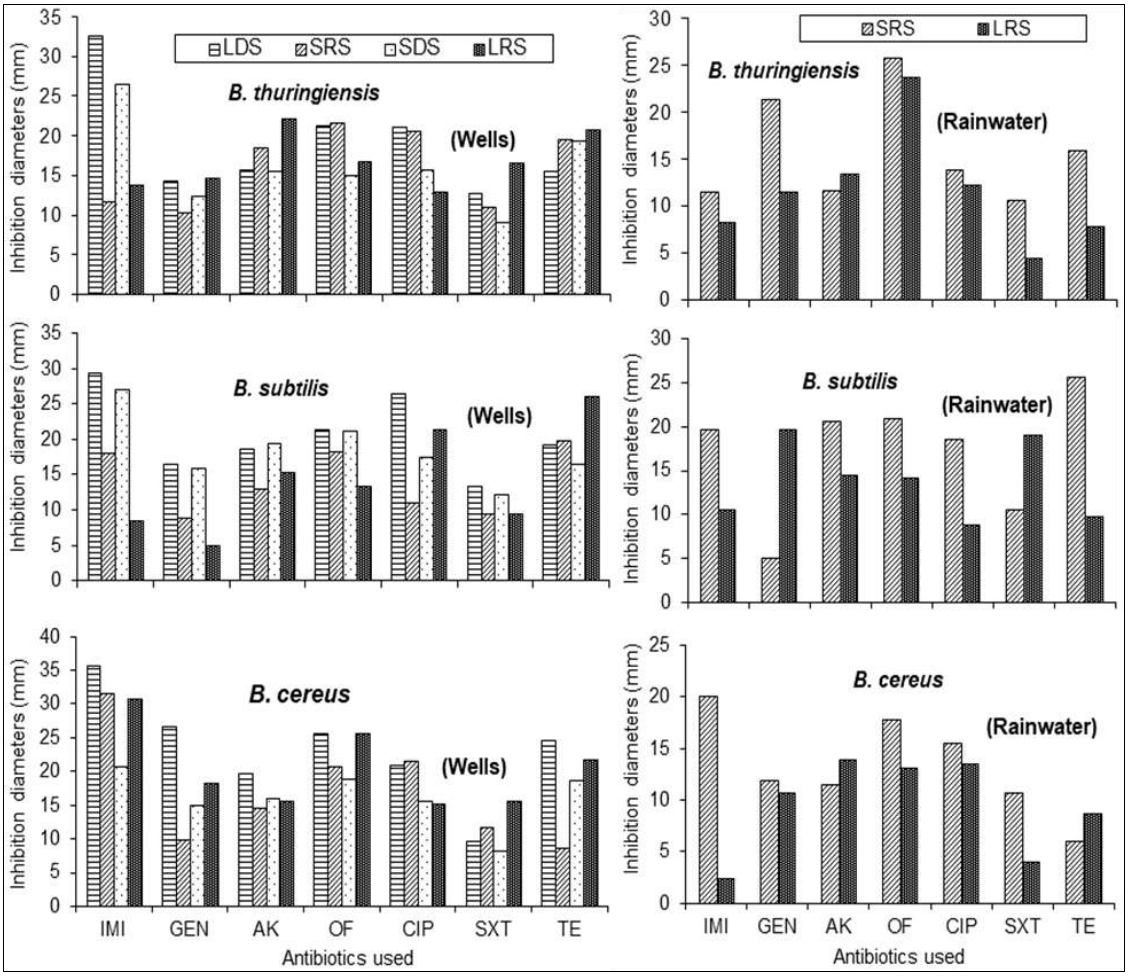

Fig 3: Seasonal variation with respect to the antibiotic used, of the inhibition diameters of each *Bacillus* species isolated from wells and from rainwater (LDS: long dry season; SRS: short rainy season; SDS: short dry season; LRS: long rainy season)

Table 1: Geographic coordinates of sampling sites

Location of the sampling site and nature of the water sampled		Geographic coordinates of the sampling site and total depth of well			
Location of the sampling site	**Nature of the water sampled**	**Latitude**	**Longitude**	**Altitude (m)**	**Total well depth (m)**
W1-SD5	Wells	03°54'19.9"N	11°33'12.9"E	692	9.5
W2-SD5	Wells	03°54'18.0"N	11°33'11.3"E	707	17.3
W3-SD3	Wells	03°51'14.4"N	11°29'43.9"E	728	11.5
W4-SD3	Wells	03°51'04.2"N	11°29'48.5"E	725	5
W5-SD6	Wells	03°50'31.0"N	11°29'07.1"E	711	20
W6-SD6	Wells	03°50'31.5"N	11°29'10.2"E	720	17
R1-SD5	Rainwater	03°50'31.5"N	11°33'06.3"E	729	/
R2-SD3	Rainwater	03°51'04.2"N	11°29'48.5"E	725	/
R3-SD6	Rainwater	03°50'31.0"N	11°29'07.1"E	711	/

Table 2: Antibiotic inhibition diameter (mm) and antibiotic susceptibility (R: resistant, I: intermédiaire, S: sensitive) of strains of the bacterial species isolated in groundwater during the long dry season (LDS), the short rainy season (SRS), the short dry season (SDS) and the long rainy season (LRS)

Bacterial species and isolation period		**Antibiotics used**						
		IMI10	**GEN10**	**AK30**	**OF5**	**CIP5**	**SXT25**	**TE30**
Bacillus thuringiensis	LDS	32.7 (S)	14.3 (R)	15.8 (R)	21.4 (I)	21.1 (I)	12.8 (R)	15.6 (R)
	SRS	11.8 (R)	10.4 (R)	18.5 (I)	21.6 (I)	20.7 (I)	11 (R)	19.5 (I)
	SDS	26.5 (S)	12.4 (R)	15.5 (R)	15 (R)	15.7 (R)	9.2 (R)	19.4 (I)
	LRS	13.9 (R)	14.7 (R)	22.2 (I)	16.8 (R)	13 (R)	16.7 (R)	20.8 (I)
Bacillus cereus	LDS	35.5 (S)	26.5 (S)	19.7 (I)	25.5 (S)	20.8 (I)	9.5 (R)	24.5 (S)
	SRS	31.5 (S)	9.8 (R)	14.5 (R)	20.6 (I)	21.5 (I)	11.7 (R)	8.5 (R)
	SDS	20.7 (I)	15 (R)	16 (R)	18.7 (I)	15.5 (R)	8.2 (R)	18.5 (I)
	LRS	30.7 (S)	18.2 (I)	15.6 (R)	25.5 (S)	15.2 (R)	15.5 (R)	21.7 (I)
Bacillus subtilis	LDS	29.5 (S)	16.5 (R)	18.7 (I)	21.5 (I)	26.5 (S)	13.5 (R)	19.2 (I)
	SRS	18.1 (I)	9 (R)	13 (R)	18.3 (I)	11 (R)	9.5 (R)	19.8 (I)
	SDS	27 (S)	16 (R)	19.5 (I)	21.2 (I)	17.5 (R)	12.3 (R)	16.5 (R)
	LRS	8.5 (R)	5 (R)	15.3 (R)	13.5 (R)	21.5 (I)	9.5 (R)	26.1 (S)

IMI: Imipenem; GEN: Gentamycin; AK: Amikacin; OFX: Ofloxacin CIP: Ciprofloxacin; SXT: Sulfamethazole/Trimethoprim; TE: Tetracyclin

Table 3: Antibiotic inhibition diameter (mm) and antibiotic susceptibility (R: resistant, I: intermédiaire, S: sensitive) of strains of the bacterial species isolated in rainwater during the short rainy season (SRS) and the long rainy season (LRS)

Bacterial species and isolation period		**Antibiotics used**						
		IMI10	**GEN10**	**AK30**	**OF5**	**CIP5**	**SXT25**	**TE30**
Bacillus thuringiensis	SRS	11.5 (R)	21.4 (I)	11.7 (R)	25.8 (S)	13.9 (R)	10.6 (R)	15.9 (R)
	LRS	8.3 (R)	11.6 (R)	13.5 (R)	23.8 (I)	12.2 (R)	4.5 (R)	7.8 (R)
Bacillus cereus	SRS	20.1 (I)	12 (R)	11.5 (R)	17.9 (I)	15.5 (R)	10.8 (R)	6 (R)
	LRS	2.5 (R)	10.7 (R)	14 (R)	13.1 (R)	13.6 (R)	4 (R)	8.7 (R)
Bacillus subtilis	SRS	19.5 (I)	5 (R)	20.5 (I)	20.8 (I)	18.5 (I)	10.5 (R)	25.5 (S)
	LRS	10.5 (R)	19.5 (I)	14.3 (R)	14 (R)	8.7 (R)	18.9 (I)	9.7 (R)

IMI: Imipenem; GEN: Gentamycin; AK: Amikacin; OFX: Ofloxacin CIP: Ciprofloxacin; SXT: Sulfamethazole/Trimethoprim; TE: Tetracyclin

Table 4: P-values of the Kruskal-Wallis test comparing for each of the bacterial species and each of the sampled water type, the antibiotics inhibition diameters among cells strains isolation seasons

Antibio-tics used	**P-values for each bacterial species isolated from each water type**					
	Bacillus thuringiensis		***Bacillus cereus***		***Bacillus subtilis***	
	Groundwater	**Rainwater**	**Groundwater**	**Rainwater**	**Groundwater**	**Rainwater**
IMI10	0.007**	0.149	0.022*	0.019*	0.005**	0.021*
GEN10	0.616	0.021*	0.003**	0.372	0.005**	0.020*
AK30	0.300	0.462	0.086	0.237	0.007**	0.019*
OFX5	0.010*	0.309	0.143	0.028*	0.007**	0.020*
CIP5	0.018*	0.306	0.043*	0.137	0.003**	0.020*
SXT25	0.065	0.146	0.010*	0.027*	0.067	0.019*
TE30	0.291	0.021*	0.004**	0.381	0.021*	0.021*

*: $P<0.05$; ** $P<0.01$; IMI: Imipenem; GEN: Gentamycin; AK: Amikacin; OFX: Ofloxacin; CIP: Ciprofloxacin; SXT: Sulfamethazole/Trimethoprim; TE: Tetracyclin

5. Conclusion

Bacillus strains found in ground and rainwater undergoes various susceptibility profile against Imipenem (β-lactam family), Amikacin and Gentamycin (Aminoglycoside family), Ciprofloxacin and Ofloxacin (Quinolone family), Sulfamethazol (Sulfonamide family), and Tetracycline (Cyclin family). This antibiotics susceptibility and inhibition diameters can significantly vary from one season to another for a given antibiotic against the same bacterial species isolated from the same aquatic biotope. This seasonal

variation in susceptibility to antibiotics would be due to several factors including anthropogenic and meteorological factors, as well as the temporal variation of the abiotic properties of aquatic biotopes.

6. Conflict of Interest
Not available

7. Financial Support
Not available

8. References
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How to Cite This Article
Tagne MRD, Nougang ME, Tamnou EBM, Njoya AM, Bahebeck PN, Baleng SD, et al. Antibiotics susceptibility of some *Bacillus* strains isolated in the ground and rainwater in urbanized area in Cameroon (Central Africa), and potential impact of the season change. Journal of Advances in Microbiology Research. 2023;4(1):86-95.