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Susceptibility of some Enterobacteriaceae isolated from 4 different aquatic environments in DR Congo (Central Africa), to Amoxicillin/Clavulanic acid and some 3rd generation Cephalosporins

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Abstract

Little is known about the variation of the antibiotic susceptibility of different bacterial strains of the same cells species, isolated from different aquatic environments. The present study aims to evaluate the susceptibility towards some 3rd generation cephalosporins (Ceftriaxon, Ceftazidim and Cefotaxim) and Amoxicillin/Clavulanic acid, of Enterobacteriaceae strains isolated from groundwater, stream, hospital wastewater and slaughterhouse wastewater. Enterobacteria species were isolated on Mac Conkey agar, then identified using enzymatic and MALDI-TOF MS system. The antimicrobial susceptibility was carried out using the disk diffusion method. The antibiotic minimum inhibitory concentrations (MICs) were carried using the VITEK®2 system. Bacterial species mostly identified were *Klebsiella pneumoniae, Escherichia coli, Salmonella typhi* and *Ewingelia americana*. The antibiotic inhibition diameters and the MICs varied depending on the antibiotics, bacterial species and type of aquatic environment hosting the microorganism. Relatively lower MICs were recorded with Cefotaxim against different bacteria in slaughterhouse wastewater, in river water and in well water. Antibiotic resistance was noted with all strains from hospital wastewater. Significant differences (P<0.05) amongst antibiotic inhibition diameter was noted for *K. pneumoniae* and *S. typhi* in most cases. The relative variations in the action mechanisms amongst antibiotics and the intrinsic defense potential of each bacterial strain, as well as the potential influence of the physicochemical properties of each water medium, could partly be at the origin of the relative differences observed at the phenotypic level. It seems necessary to explore the diversity, similarities and differences amongst antibiotic resistance genes in these different types of aquatic biotopes.

Keywords: Aquatic environments; Enterobacteriaceae; 3rd generation Cephalosporins and Amoxicillin/Clavulanic acid; Antibiotic inhibition diameters and MICs; Antibiotic susceptibility variation

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1. Introduction

Cephalosporins are a large group of antibiotics derived from the mold *Acremonium* (previously called *Cephalosporium*). Because of their effectiveness against a wide range of Gram negative bacteria, they are often called broad-spectrum antibiotics [1, 2]. They bind to and block the activity of enzymes responsible for making peptidoglycan, an important component of the bacterial cell wall. Drugs known as cephalosporins included ceftriaxon, cefdinir, cefixim, cefpodoxim, cefditoren, ceftibuten, cefdinir, ceftazidim, cefotaxim, cefoperazon ceftizoxim, among others [2]. 3rd generation Cephalosporins included Cefixim, Cefpodoxim proxetil, Cefotaxim, Ceftazidim and Ceftriaxon. They diffuse well in tissues. Cefotaxime, ceftazidime and ceftriaxone have the particularity of crossing the blood-brain barrier, and are therefore effective in the treatment of meningitis, unlike other 3rd generation Cephalosporins [3].

Amoxicillin works by binding to penicillin-binding proteins and inhibiting peptidoglycan synthesis, which interrupts the construction of the cell wall and ultimately leads to the destruction, or lysis, of the bacteria. With the addition of clavulanic acid, the spectrum Amoxicillin-clavulanate is increased to include all beta-lactamase-producing strains and are often enterobacteria [4].

Enterobacteriaceae is a family of Gram-negative rod-shaped (bacilli) bacteria in the order Enterobacterales of the class Gammaproteobacteria in the phylum Pseudomonadota, domain of Bacteria **[5]**. Recently based on combined results of phenotypic properties, DNA sequences and DNA homology, phylogenetic findings, and 16S rRNA gene sequence study results, 68 genera with a total of 355 species have been proposed [5, 6]. They are cosmopolitan bacteria. They are found in soil, decaying vegetation and organic matters, water sources, sewage, and other environmental aspects. Most of them are mesophilic, hence they are not found in extremely cold and extremely hot environments. They are found scarcely in high salinity, xerophilic (extremely dry), and extreme pH areas [5].

They are commonly known as Gram-negative, rod-shaped (usually short bacilli, about 1-5 μm), facultatively anaerobic, non-sporing, mostly motile with peritrichous flagella (most species in the genera *Klebsiella* and *Shigella* are non-motile), catalase positive, oxidase negative, both lactose fermenter and non-fermenter, usually acid-producing and nitrate-reducing. In addition, the outer membrane (O), flagella (H), and capsule (K) are antigens in most species [5, 7]. In humans, many new species have been described, some associated with specific disease processes. Some established species are now observed in new infectious disease settings and syndromes [6].

Many bacterial species have been reported in groundwater with different antibiotic susceptibilites [8]. During bacterial infiltration through soil layers, different microbiogeochemical reactions and interactions can occur [9, 10]. In groundwater as in river water, different interactions can lead to potential genetic mutations of microorganisms [11]. This could influence the relevant bacterial susceptibility against antibiotics such as Cephalosporins or Amoxicillin/clavulanic acid, and sometimes make it difficult to fight against an infection whose germs are of different aquatic origins. It therefore seems necessary to evaluate, both in rural or in urbanized areas, the variation in susceptibility to Amoxicillin/Clavulanic acid and 3rd generation Cephalosporins (which are the most used to fight infections) of enterobacteria present at the same time in several aquatic environments of different properties.

Waters encountered on continents are often groundwater, streams and wastewater which may be hospital or come from slaughterhouses. In general, they are of different biotic and abiotic properties. Little is known about the susceptibility to the same antibiotic, of different bacterial strains of the same cells species, isolated from different aquatic environments. The present study aims to evaluate the susceptibility towards some 3rd generation Cephalosporins and Amoxicillin/Clavulanic acid, cells strains of some enterobacteria species isolated simultaneously from groundwater, stream, hospital wastewater and slaughterhouse wastewater in an urban area in the Democratic Republic of Congo (DR Congo) (Central Africa).

2. Materials and methods

2.1. Study area and sampling sites

The study was carried out in the city of Butembo, in the east of the DR Congo. Four different types of aquatic environments were choose. They included hospital wastewater (HW), slaughterhouse wastewater (SW), river water (RW) and wells (W). Sampling sites are of different geographic coordinates. The latitudes were 0°7'17.346'' for SW, 0°7'24.8016'' for HW, 0°5'38.06988'' for RW and 0°6'35.3366'' for W. The longitudes were 29°17'10.39812'' for SW, 29°15'48.76776'' for HW, 29°19'32.7576'' for RW and 29°18'42.93288'' for W. Altitudes were 1724m for SW, 1761m for HW, 1807m for RW and 1734m for W. The total depth of this well point was 6m.

2.2. Water samplings

The study was carried out during the period from August 2022 to February 2023. This period covers the two main seasons of the locality, which are the rainy season (August-December) and the dry season (January and February). Each water point was sampled once a month. Water samples were taken in sterile 500 mL glass bottles for microbiological analyses, and clean 1000 mL polyethylene bottles for physicochemical analyses. All the samples were then placed in an isothermal container and immediately brought back to the laboratory for analysis.

2.3. Bacteriological analyses

2.3.1. Isolation and identification of Enterobacteriaceae

After homogenization of the water samples, followed or not by decimal dilutions, the isolation of the Enterobacteriaceae was done by spreading on the surface on Mac Conkey agar (Titan Biotech LTD: W/CV, NaCl, 0,15% Bile salts and 1% Lactose) poured into petri dishes and then incubated at 37 °C for 18 to 24 hours [12].

The macroscopic observation consisted of examining the cultural characters of the bacteria expressed in colonies. These include the color, size, contours and surface configuration of the colony. After purification of the strains, their identification was carried out first by the enzymatic method [12], at Central Research Laboratory, Faculty of Pharmaceutical Sciences, Catholic University of Graben, DR Congo, then using the Matrix assisted laser desorption ionization–time of flight mass spectrometry (MALDI–TOF MS) [13, 14], at the Pasteur Center of Yaounde (Cameroon). The mass-to-charge (m/z) ratios are electrodynamic measurements of how quickly charged ions from the clinical sample material move through the TOF tube and reach a detector.

From of 18 to 24 hours culture, selected colony was applied onto MALDI test plant. Samples were then overlaid with matrix and dried. The plate was subsequently loaded into the MALDI-TOF MS instrument: the sample was bombarded by the laser. This bombardment resulted in the sublimation and ionization of both the sample and matrix. These generated ions were separated based on their mass-to-charge ratio via a TOF tube, and a spectral representation of these ions was generated and analyzed by the MS software, generating an MS profile. This profile was subsequently compared to a database of reference MS spectra and matched to either identical or the most related spectra contained in the database, generating an identification for bacteria and analyzed by sofware associated with the respective system, allowing rapid identification of the microorganism [13-15].

2.3.2. 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 [16]. 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 [16].

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 [16].

2.3.3. Antibiotic susceptibility assay

The antimicrobial susceptibility tests were carried out using the disk diffusion method according to the recommendations of the FMS-EUCAST [16]. The antibiotic molecules were chosen in depending on the uses of the population but also on their availability in the laboratory. Those considered were Ceftriaxon, Ceftazidim, Cefotaxim, and Amoxicillin+Clavulanic acid

The inhibition diameters (ID) were measured using the caliper and the results were scored as resistant, sensitive or intermediate according to CA-SFM recommendations [16, 17]. Ceftriaxon, Ceftazidim and Cefotaxim were scored sensitive when ID≥21mm, intermediate when 20mm≤ID≥15mm, and resistant when ID<15mm. Amoxicillin+Clavulanic acid was scored sensitive when ID≥21mm, intermediate when 20mm≤ID≥14mm, and resistant when ID<14mm.

The antibiotic minimum inhibitory concentrations (MICs) against each of the bacteria considered were carried out at the Pasteur Center of Yaounde (Cameroon) using the VITEK®2 technic [18]. VITEK®2 Compact Systems utilize automated growth-based detection using attenuation of light measured by an optical scanner. The optics used in the

systems use visible light to directly measure organism growth. Transmittance optics are based on an initial light reading of a well before significant growth has begun. Periodic light transmittance samplings of the same well measure organism growth by how much light is prevented from going through the well. The VITEK®2 System monitors the growth of each well in the card over a defined period of time. An interpretive call is made between 4 and 16 hours for a "rapid" read but may be extended to 18 hours in some instances. At the completion of the incubation cycle, a report is generated that contains the MIC value along with the interpretive category result for each antibiotic on the card.

From 18h-24h grown colonies a calibrated bacterial suspension in sodium chloride solution, was adjusted to an optical density of 0.5-0.55 McF. A selection of drugs relevant to sepsis management in AST-N233+XN05. Results were automatically reported by Automate Expert System software version 8.01 running with 2018 EUCAST/CA-SFM breakpoints. MICs and interpreted categories expertise by VITEK®2 AES (Advanced Expert System) from D0 workflow were compared to those from D1 workflow [18].

2.4. Physicochemical analyses

The parameters considered included water pH, electrical conductivity, turbidity, nitrite and nitrate concentrations. Some of them (water pH and electrical conductivity) were measured in the field using a multi-parameter HACH/POCKET PRO+ and the others were measured in the laboratory using a spectrophotometer CECIL CE1011. This was done according to the recommended techniques [19].

2.5. Data analysis

The values of the antibiotic inhibition diameters were illustrated by histograms plotted using Excel 2010 software. The values of the antibiotic inhibition diameters were illustrated by histograms plotted using Excel 2010 software. Using the Kruskal-Wallis test, the comparison amongst antibiotic inhibition diameters has been carried out using SPSS version 20.0 software (a value of $P<0.05$ was assumed to be significant).

3. Results

3.1. Spatio-temporal variation of the presence of bacterial species in the aquatic environments considered

A total of 4 bacteria species identified were most often isolated. These are *Klebsiella pneumoniae, Escherichia coli, Salmonella typhi* and *Ewingelia americana* (Figure 1).
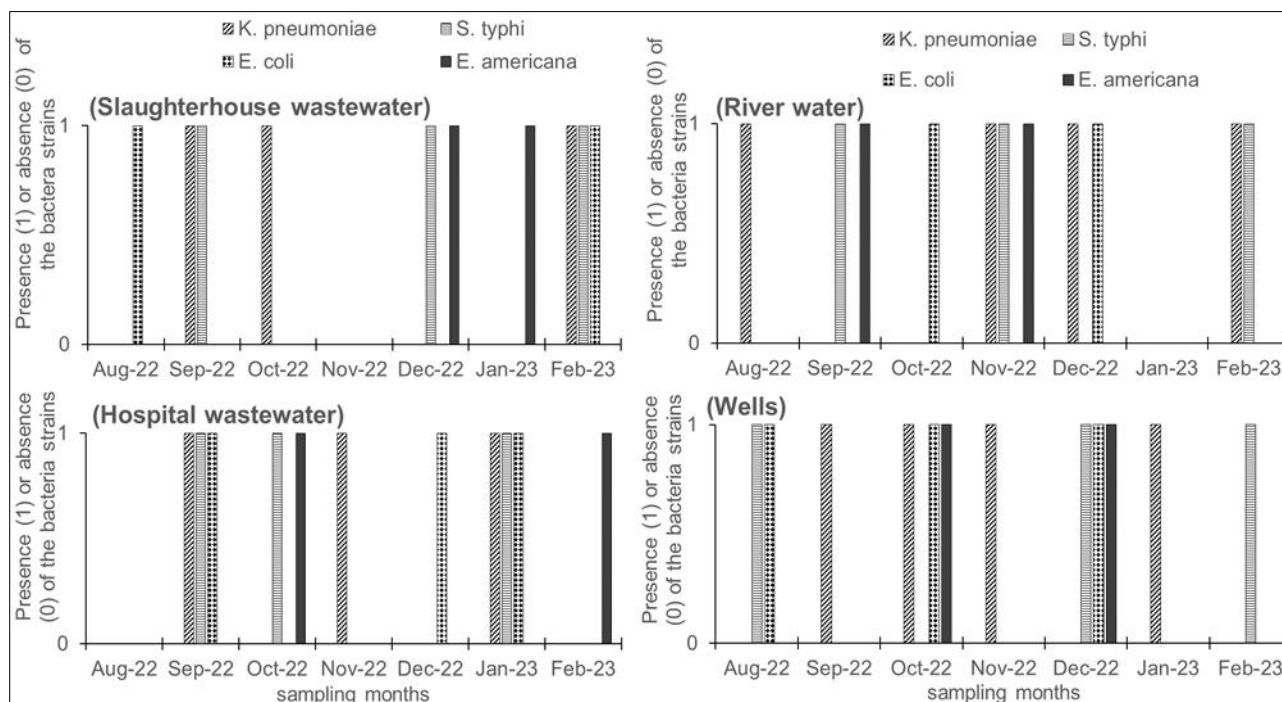

Figure 1 Presence or absence of each bacterial strains considered in each of the aquatic environment of water during the sampling period

The frequency of presence or absence varied from one biotope to another. For example, in slaughterhouse wastewater, none of these species was identified during the month of November 2022. The same is true of hospital wastewater during the month of August 2022, as well as in January 2023 in river waters. In the well water, at least one of the bacterial species considered was identified in each month of the study period (Figure 1). In the 4 different aquatic biotopes sampled, *S. typhi* and *K. pneumoniae* were most often isolated. Additionally, *E. coli* has been isolated several times from well water and hospital wastewater (Figure 1).

3.2. Spatial variation of the antibiotics inhibition diameters with respect to each bacteria species and aquatic environment considered

The inhibition diameters varied from one antibiotic to another, from one biotope to another and from one bacterium to another. The inhibition diameters recorded with Amoxicillin+Clavulanic acid were relatively low (6-12mm) in the most of cases, with the exception for *K. pneumoniae* (20mm) isolated from slaughterhouse wastewater and for *S. typhi* (24mm) isolated from river waters. For strains isolated from hospital wastewater, the inhibition diameters recorded varied from 6-12mm; an exception was observed for *K. pneumoniae* (Ceftriaxone: 18mm and Ceftazime: 16mm) (Figure 2).
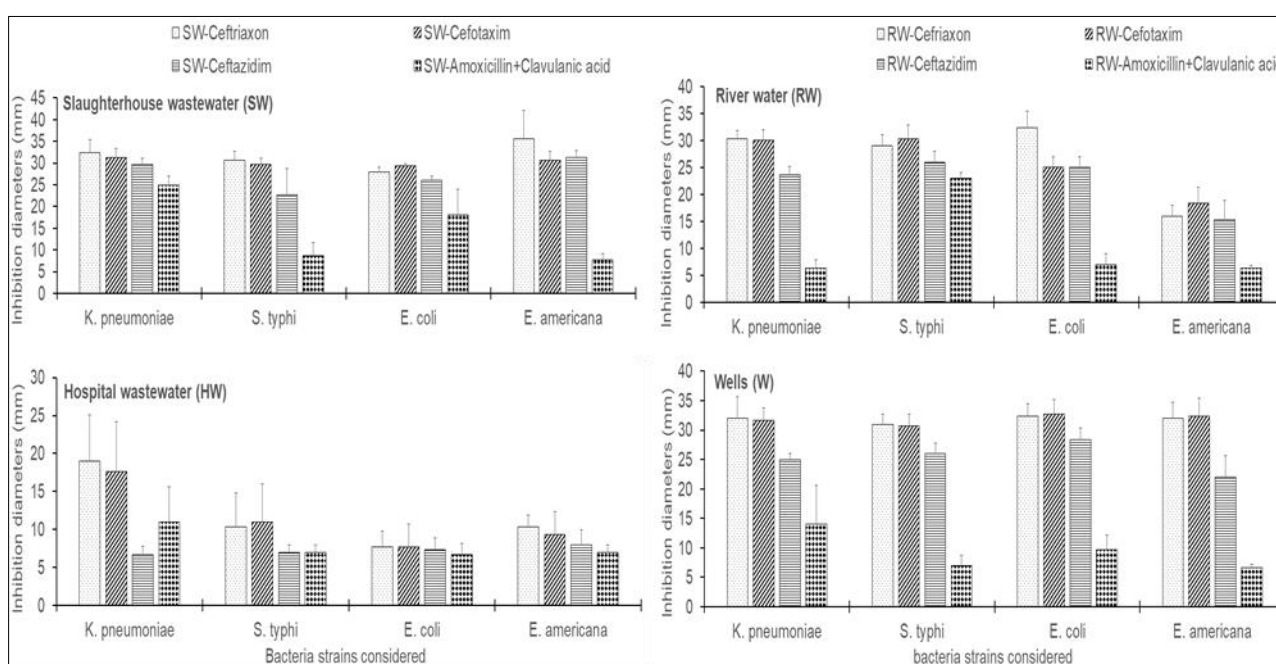

Figure 2 Mean values of the antibiotics inhibition diameters during the sampling period with respect to each bacteria strains and aquatic biotope considered (hospital wastewater (HW), slaughterhouse wastewater (SW), river water (RW) and wells (W))

3.3. Spatial variation of the antibiotics MIC with respect to each bacteria species and aquatic environment considered

The MICs varied depending on the antibiotics, from one bacterium to another on the one hand, and depending on the aquatic biotope hosting the germ on the other hand. Most of the high MICs (>16 and >32 µg/ml) were recorded in hospital wastewater for the four drugs tested. The MICs of Amoxicillin+clavulanic acid were relatively higher (>16 µg/ml) against the different bacteria in all the biotopes explored. Relatively low MICs (≤ 1 µg/ml) were recorded with Cefotaxime against different bacteria in slaughterhouse wastewater, in river water and in well water. The same is true for Ceftazidim in wastewater from the slaughterhouse and wells (Table 1).

Table 1 Ranges of MIC values depending on the bacteria and the aquatic biotope sampled

Antibiotic used and aquatic biotope sampled		MIC value			
Antibiotic	Aquatic environment	*Klebsiella pneumoniaie*	*Salmonella typhi*	*Escherichia coli*	*Ewingelia americana*
Ceftriaxon	SW	≤ 4	≤ 4	≤ 4	≤ 4
	HW	8	>32	>32	>32
	RW	≤ 4	≤ 4	≤ 4	8
	W	≤ 4	≤ 4	≤ 4	≤ 4
Cefotaxim	SW	≤ 1	≤ 1	≤ 1	≤ 1
	HW	8	>32	>32	>32
	RW	≤ 1	≤ 1	≤ 1	≤ 1
	W	≤ 1	≤ 1	≤ 1	≤ 1
Ceftazidim	SW	≤ 1	≤ 1	≤ 1	≤ 1
	HW	>32	>32	>32	>32
	RW	16	16	16	16
	W	≤ 1	≤ 1	≤ 1	≤ 1
Amoxicillin+Clavulanic acid	SW	2	>16	10	>16
	HW	>16	>16	>16	>16
	RW	>16	2	>16	>16
	W	>16	>16	>16	>16

3.4. Antibiotic scored susceptibility

From the mean antibiotic inhibition diameters indicated above, antibiotic susceptibility has been assessed with respect to bacteria considered and aquatic biotope sampled. Results are shown in Table 2. Resistance to the four antibiotic molecules is noted in all bacterial species in hospital wastewater. The four bacterial species are resistant to Amoxicillin+Clavulanic acid in the different biotopes explored. An exception was noted for *K. pneumoniae* in slaughterhouse wastewater and *S. typhi* in river water. Susceptibility of all four bacterial species to Ceftriaxone, Cefotaxime and Ceftazime was observed in river water, well water and slaughterhouse wastewater (Table 2).

Table 2 Antibiotic susceptibility with respect to bacteria considered and aquatic biotope sampled

Antibiotics used and aquatic biotope sampled		Bacteria considered and the antibiotic susceptibility			
Antibiotics	Aquatic biotope	*Klebsiella pneumoniaie*	*Salmonella typhi*	*Escherichia coli*	*Ewingelia americana*
Ceftriaxon	SW	S	S	S	S
	HW	I	R	R	R
	RW	S	S	S	I
	W	S	S	S	S
Cefotaxim	SW	S	S	S	S
	HW	I	R	R	R

	RW	S	S	S	I
	W	S	S	S	S
Ceftazidim	SW	S	S	S	S
	HW	R	R	R	R
	RW	S	S	S	I
	W	S	S	S	S
Amoxicillin+Clavulanic acid	SW	S	R	I	R
	HW	R	R	R	R
	RW	R	S	R	R
	W	R	R	R	R

3.5. Comparison amongst some considered parameters

Several comparisons of the antibiotics inhibition diameters have been carried out between the parameters considered. The first was made amongst antibiotics used for each bacteria and each aquatic environment sampled. Results are presented in Table 3. A significant difference in antibiotic inhibition diameter (P<0.05) is noted for *K. pneumoniae* in river water and in well water, for *S. typhi* in slaughterhouse wastewater, river and well water. A significant difference in antibiotic inhibition diameter (P<0.05) is also noted for *E. coli* in slaughterhouse wastewater, river water and well water. Finally, for *E. americana* a significant difference (P<0.05) is observed only in well water (Table 3).

Table 3 "P" value of the comparison of inhibition diameters amongst antibiotics used for each bacteria and each aquatic biotope sampled

Bacteria strains considered	Aquatic biotopes sampled			
	Slaughterhouse wastewater	Hospital wastewater	River water	Wells
Klebsiella pneumoniae	P=0.061	P=0.091	P=0.024*	P=0.025*
Salmonella typhi	P=0.027*	P=0.622	P=0.039*	P= 0.024*
Escherichia coli	P=0.019*	P=0.971	P=0.024*	P=0.031*
Ewingelia americana	P= 0.052	P=0.275	P=0.063	P=0.024*

* : Significant difference (P<0.05)

The second comparison of inhibition diameters was carried out amongst bacteria considered for each antibiotics and each aquatic environment sampled. Results are presented in Table 4. A significant difference in inhibition diameter (P<0.05) is observed for cefotaxim in river water, for ceftazidim in slaughterhouse wastewater, and for amoxicillin+Clavulanic acid in water waste from the slaughterhouse and river water (Table 4).

Table 4 "P" value of the comparison of inhibition diameters amongst bacteria considered for each antibiotics and each aquatic biotope sampled

Antibiotic used	Aquatic biotope sampled			
	Slaughterhouse wastewater	Hospital wastewater	River water	Wells
Ceftriaxon	P=0.062	P=0.110	P=0.074	P=0.315
Cefotaxim	P=0.085	P=0.205	P=0.026*	P=0.099
Ceftazidim	P=0.036*	P=0.754	P=0.099	P=0.147
Amoxicillin+Clavulanic acid	P=0.019*	P=0.664	P=0.034*	P=0.189

* : Significant difference (P<0.05)

The last comparison of the inhibition diameters carried out was amongst aquatic environment sampled for each bacteria and antibiotics considered. Results are presented in Table 5. A significant difference (P<0.05) is observed in *K. pneumoniae* for Ceftazidim and Amoxicillin+Clavulanic acid; in *E. coli* and *E. americana*, a significant difference is noted for Ceftriaxone, Cefotaxim and Ceftazidim. No significant difference was reported in *S. typhi* for the different antibiotics (Table 5).

Table 5 "P" value of the comparison of inhibition diameters amongst aquatic biotope sampled for each bacteria and antibiotics considered

Bacteria strains considered	Antibiotic used			
	Ceftrixon	Cefotaxim	Ceftazidim	Amoxicillin+Clavulanic acid
Klebsiella pneumoniae	P=0.078	P= 0.075	P=0.021*	P= 0.043*
Salmonella typhi	P=0.062	P= 0.090	P= 0.077	P=0.077
Escherichia coli	P=0.028*	P=0.017*	P=0.044*	P=0.058
Ewingelia americana	P=0.024*	P= 0.023*	P= 0.019*	P=0.532

* : Significant difference (P<0.05)

3.6. Some physico-chemical parameters of the aquatic biotopes sampled

During the study period, electrical conductivity varied from 480 to 750 µS/cm in hospital wastewater, from 320 to 920 µS/cm in river water, from 340 to 1060 µS/cm in water waste from the slaughterhouse and 180 to 250 µS/cm in well water (Figure 3).
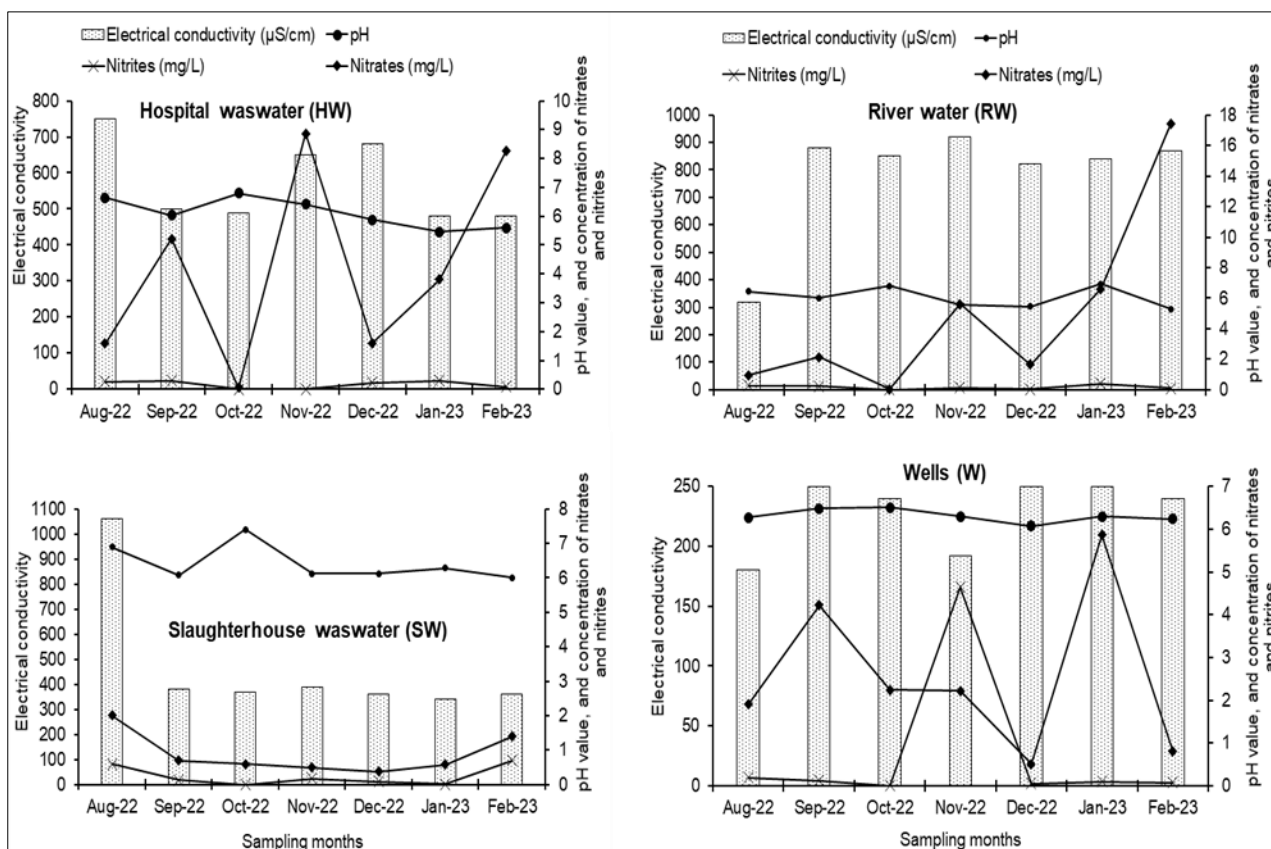

Figure 3 Temporal variation of the considered physicochemical parameters in each of the sampled aquatic biotope

The pH oscillated from 5.46 to 6.64 for the hospital wastewater, from 5.28 to 6.8 for the river water, from 6 to 7.4 for the slaughterhouse wastewater and from 6.07 to 6.5 for well water. The nitrite content varied from 0 to 0.3 mg/L in

hospital wastewater, from 0 to 0.38 mg/L in river water, from 0 to 0.6 mg/L in slaughterhouse wastewater and 0 to 4.6 mg/L in river water. The concentration of nitrates varied from 0.05 to 8.86 mg/L in hospital wastewater, from 0.05 to 17.39 mg/L in river water, from 0.58 to 2, 01 mg/L in slaughterhouse wastewater and 0.5 to 5.86 mg/L in well water (Figure 3).

4. Discussion

Variations in the frequency of presence or absence of bacteria in the different aquatic biotopes sampled were noted. This frequency would depend on the one hand on the intrinsic properties of the bacteria allowing it to resist the different constraints of the environment. It would also depend on the chemical and nutritional properties of each biotope, allowing the survival of each bacteria to be maintained.

The present study showed that inhibition diameters of different antibiotics against germs from hospital wastewater are relatively lower in most cases. In addition, these strains are also resistant to the considered antibiotics in most cases. It is stated that antibiotics are used worldwide to treat and prevent bacterial infections in humans, animals and even plants. They are also widely used to stimulate the growth of animals to increase meat production [20]. It is also indicated that wastewater can contain many contaminants such as drugs, antibiotics, disinfectants, metals and other antimicrobial compounds. These compounds can be found in surface water, in soil and groundwater at different levels of concentration depending on the source and their behavior (degradation rate and adsorption) [21]. Their release into the natural environment is likely to trigger resistance in bacteria [22].

Antimicrobial resistance can be intrinsic or acquired. In the latter case, resistance can arise from mutation of bacterial DNA or from the acquisition of resistance genes by means of horizontal gene transfer, with DNA passing from one bacteria to another [23-25]. It is also indicated that the release of hospital wastewater in the natural environment is a source of both antibiotics and antibiotic resistance genes in groundwater, based on their spatial spreading [26]. In addition, it has been indicated anthropogenically impacted surface waters are an important reservoir for multidrug-resistant bacteria including extended spectrum beta lactamase-producing microorganisms, and antibiotic-resistant genes [27].

The antibiotic inhibition diameters against the wells strains varied from 6.66mm (Amoxicillin+Clavulanic acid against *E. americana*) to 32.66mm (Cefotaxim against *E. coli*). This suggests bacterial resistance in some cases. Groundwater contamination can sometimes result from human impact. In most countries in the world, domestic wastewater, hospital wastewater and slaughterhouse wastewater are sometimes discharged into the environment without any prior treatment. They often contains a variety of antibiotic resistance genes [28]. These wastewaters, with all their bacterial and non-bacterial contents, added to rainwater which contains a diversity of airborne biological particles (also denoted as bioaerosols) and resistance genes, can then contaminate surface water and groundwater [25, 29, 30]. Contamination of surface water could induce interactions firstly between the chemical molecules of spilled wastewater and those of rivers and streams, which could thus lead to the formation of new chemical complexes. Secondly, there could be interactions between these newly formed chemical complexes or the arriving chemical molecules and the bacterial flora. Regarding the contamination of groundwater, water during its infiltration could carry various molecules and bacteria into the groundwater. The degree of this groundwater pollution depends on the self-purifying power of the soil surrounding the water table [31, 32].

Relative differences amongst antibiotics activities have been noted. Those antibiotics mainly act at the cell wall level. The bacterial cell wall, which is located at the periphery of Gram-positive bacteria and within the periplasm of Gram-negative bacteria, comprises a glycopeptide polymer synthesized through cross-linking of glycans to peptide stems on alternating saccharides, which is known commonly as peptidoglycan. Cell wall formation, recycling, and remodelling require numerous enzymes, including a family of enzymes with similar active site character despite distinct and sometimes overlapping roles as carboxypeptidases, endopeptidases, transpeptidases, and transglycosylases, known as "penicillin-binding proteins" (PBPs). The number of PBPs differs between bacteria, in which some are considered essential and others redundant. In general, inhibition of one or more essential PBPs results in impaired cell wall homeostasis, loss of cell integrity, and is ultimately bactericidal [33, 34].

Ceftriaxon acts by inhibiting the mucopeptide synthesis in the bacterial cell wall [35]. The beta-lactam moiety of Ceftriaxon binds to carboxypeptidases, endopeptidases, and transpeptidases in the bacterial cytoplasmic membrane. These enzymes are involved in cell-wall synthesis and cell division. Binding of Ceftriaxon to these enzymes causes the enzyme to lose activity; therefore, the bacteria produce defective cell walls, causing cell death [35].

Cefotaxim inhibits bacterial cell wall synthesis by binding to one or more of the PBPs which in turn inhibits the final transpeptidation step of peptidoglycan synthesis in bacterial cell walls, thus inhibiting cell wall biosynthesis. Bacteria eventually lyse due to ongoing activity of cell wall autolytic enzymes (autolysins and murein hydrolases) while cell wall assembly is arrested [36]. Cefotaxim has activity in the presence of some beta-lactamases, both penicillinases and cephalosporinases, of gram-negative and gram-positive bacteria [36].

Ceftazidim exhibits its bactericidal effect primarily through direct inhibition of specific PBPs in susceptible bacteria [37, 38]. Clavulanic acid binds and inhibits beta-lactamases that inactivate Amoxicillin resulting in Amoxicillin having an expanded spectrum of activity. Amoxicillin inhibits bacterial cell wall synthesis by binding to one or more of the PBPs which in turn inhibits the final transpeptidation step of peptidoglycan synthesis in bacterial cell walls, thus inhibiting cell wall biosynthesis. Bacteria eventually lyse due to ongoing activity of cell wall autolytic enzymes (autolysins and murein hydrolases) while cell wall assembly is arrested [4]. The relative variations in the action mechanisms of antibiotics on the one hand and the intrinsic defense potential of each bacterial strain on the other hand could partly be at the origin of the relative differences observed amongst antibiotics activities at the phenotypic level.

Concerning the physicochemicals of water samples, the pH of the 4 biotopes sampled overall is between 5.28 and 7.4, and the electrical conductivity varied from 180 to 1060 µS/cm. Although the relationship between the abiotic properties of the water sampled and the phenotypic properties of the isolated bacteria has not been assessed, it is known that some water chemical characteristics can significantly impact some bacterial susceptibility against some drugs. For example, significant relationship has been indicated between the pH of the stream water and the *Aeromonas hydrophyla* susceptibility against Oxacillin [39]. According to Chaturvedi *et al* [27], some abiotic parameters of water such as Biochemical Oxygen Demand, Chemical Oxygen Demand, pH and the dissolved oxygen content can be significantly correlated to some resistence genes. An *et al* [40] also noted that the nutrients concentration in an aquatic environment impacts the selection of antibiotic resistance bacteria and exchange of antibiotic resistance genes.

5. Conclusion

Wells, stream, hospital wastewater and slaughterhouse wastewater in Butembo (DR Congo, Central Africa) contains enterobacteriaceae including *Klebsiella pneumoniae, Escherichia coli, Salmonella typhi* and *Ewingelia americana*. Their presence or absence frequency varied with respect to the aquatic biotope and bacterial species considered. The antibiotic inhibition diameters and the MICs values of Ceftriaxon, Ceftazidim, Cefotaxim and Amoxicillin/Clavulanic acid varied depending on the antibiotics, the bacterial species and the type of aquatic environment hosting the microorganism. Most of the high MICs and microbial resistance against the four antibiotic were recorded with strains from hospital wastewater. Significant differences in antibiotic inhibition diameter has been noted in most cases amongst bacteria for each antibiotic and each aquatic biotope. The relative variations in the action mechanisms amongst antibiotics on the one hand and the intrinsic defense potential of each bacterial strain on the other hand, as well as the potential influence of the physicochemical properties of each water medium, could partly be at the origin of the relative differences observed at the phenotypic level. It seems necessary to explore the diversity, similarities and differences amongst antibiotic resistance genes in these 4 types of aquatic biotopes.

Compliance with ethical standards

Disclosure of conflict of interest

No conflict of interest to be disclosed.

References

[1] Werth BJ. 2022. Cephalosporins : Pharmacokinetics, indications, contraindications, use during pregnency and breasfeeding, adverse effects. https://www.merckmanuals.com/professional/infectious-diseases/bacteria-and-antibacterial-drugs/cephalosporins (accessed 20th October 2023)

[2] Pope C. 2023. Third generation cephalosporins. https://www.drugs.com/drug-class/third-generation-cephalosporins.html (accessed 20th October 2023)

[3] Buxeraud J, Faure S. 2021. Les céphalosporines. Actualités Pharmaceutiques 60 (607) : S24-S27. https://doi.org/10.1016/j.actpha.2021.04.008.

[4] Evans J, Hanoodi M and Wittler M. 2023. Amoxicillin Clavulanate. https://www.ncbi.nlm.nih.gov/books/NBK538164/ (accessed 20th October 2023)

[5] Dahal P. 2022. Enterobacteriaceae- Definition, Characteristics, Identification. https://microbenotes.com/enterobacteriaceae/#enterobacteriaceae-habitatecology (accessed 20th October 2023)

[6] Janda JM, Abbott SL. 2021. The changing face of the family Enterobacteriaceae (Order: "Enterobacterales"): New members, taxonomic issues, geographic expansion, and new diseases and disease syndromes. Clinical Mocrobiology Reviews 34 (2): e00174-20. https://doi.org/10.1128/CMR.00174-20.

[7] Tankeshwar A. 2022. Enterobacteriaceae family. https://microbeonline.com/enterobacteriaceae/#google_vignette (accessed 20th October 2023)

[8] Singh AK, Das S, Kumar S, Gajamer VR, Najar IN, Lepcha YD, Tiwari HK, Singh S. 2020. Distribution of antibiotic-resistant Enterobacteriaceae pathogens in potable spring water of Eastern Indian Himalayas: Emphasis on virulence gene and antibiotic resistance genes in Escherichia coli. Frontiers in Microbiology 11, 581072. https://doi.org/10.3389/fmicb.2020.581072.

[9] Xia X, Cheng L, Zhu Y, Liu Y, Wang K, Ding A, Cai Z, Shi H, Zuo L. 2020. Response of soil bacterial community and geochemical parametersto cyclic groundwater-level oscillations in laboratory columns. Vadose Zone Journal 19:e20011. https://doi.org/10.1002/vzj2.20011.

[10] Zhang X, Gao X, Li C, Luo W, Wang Y, Luo X. 2023. Seasonal dynamics and interaction of shallow groundwater geochemical properties and microbial community patterns. Chemical Geology 638, 121703. https://doi.org/10.1016/j.chemgeo.2023.121703.

[11] Yin H, Cai Y, Li G, Wang W, Wong PK, An T. 2022. Persistence and environmental geochemistry transformation of antibiotic-resistance bacteria/genes in water at the interface of natural minerals with light irradiation. Crit Rev Environ Sci Technol 52 (13) : 2270-2301. https://doi.org/10.1080/10643389.2021.1877062.

[12] Denis F, Ploy Mc, Martin C, Cattoir V. 2016. Bactériologie médicale, Techniques usuelles, 3ème édition. Paris, Elsevier Masson, 2016.

[13] Roux-Dalvai F, Gotti C, Leclercq M, Helie MC, Boissinot M, Arrey TN, Dauly C, Fournier F, Kelly I, Marcoux J, Bestman-Smith J, Bergeron MG, Droit A. 2019. Fast and accurate bacterial speciesidentification in urine specimens using LC-MS/MS mass spectrometry and machine learning. Molecular Cellular Proteomics 18 : 2492-2505. https://doi.org/10.1074/mcp.TIR119.001559.

[14] Mortier T, Wieme AD, Vandamme P, Waegeman W. 2021. Bacterial species identification using MALDI-TOF mass spectrometry and machine learning techniques: A large-scale benchmarking study. Computational and Structural Biotechnology Journal 19 : 6157-6168. https://doi.org/10.1016/j.csbj.2021.11.004.

[15] Clark A, Kaleta E, Arora A, Wolk D. 2013. Matrix-assisted laser desorption ionization–time of flight mass spectrometry: a fundamental shift in the routine practice of clinical microbiology. Clinical Microbiology 26 (3):547-603. https://doi.org/10.1128/CMR.00072-12.

[16] FMS-EUCAST. 2022. French Microbiology Society-European Committee on Antimicrobial Susceptibility Testing/Comité de l'Antibiogramme de la Société Française de Microbiologie. Recommandations 2022. https://www.sfm-microbiologie.org/boutique/comite-de-lantibiograme-de-la-sfm-casfm/

[17] Shakir ZM, Alhatami AO, Ismail Khudhair Y, Muhsen Abdulwahab H. 2021. Antibiotic Resistance Profile and Multiple Antibiotic Resistance Index of Campylobacter Species Isolated from Poultry. Archives of Razi Institute 76 (6) : 1677-1686. https://doi.org/10.22092/ari.2021.356400.1837.

[18] Paluch M, Lleres-Vadeboin M, Poupet H, Chanard E, Wilhelm N, Nadji S, Prots L, Bala Y, Zambardi G, Cattoen C. 2023. Multicenter evaluation of rapid antimicrobial susceptibility testing by VITEK®2 directly from positive blood culture. Diagnostic Microbiol Infect Disease 106, 115950. https://doi.org/10.1016/j.diagmicrobio.2023.115950.

[19] Rodier, J, Legube B, Merlet N. 2016. L'analyse de l'eau. 10ème édition, Dunod, Paris, pp.1600.

[20] Manyi-Loh C, Mamphweli S, Meyer E, Okoh A. 2018. Antibiotic Use in agriculture and its consequential resistance in environmental sources: Potential public health implications. Molecules 23 (4):795. https://doi.org/10.3390/molecules23040795.

[21] Subbiah M, Mitchell SM, Ullman JL and Call DR. 2011. β-Lactams and florfenicol antibiotics remain bioactive in soils while Ciprofloxacin, Neomycin, and Tetracycline are neutralized. Appl Environ Microbiol 77 (20) : 7255-7260. https://doi.org/10.1128/AEM.05352-11.

[22] Pruden A, Larsson DG, Amezquita A, Collignon P, Brandt KK, Graham DW, Lazorchak JM, Suzuki S, Silley P, Snape JR, Topp E, Zhang T, Zhu YG. 2013. Management options for reducing the release of antibiotics and antibiotic resistance genes to the environment. Environ Health Perspectives 12(8) : 878-885. https://doi.org/10.1289/ehp.1206446.

[23] Berendonk TU, Manaia, CM, Merlin C, Fatta-Kassinos D, Cytryn E, Walsh F, Burgmann Sorum H, Norstrom M, Pons M, Kreuzinge N, Huovinen P, Stefani S, Schwartz T, Kisand V, Baquero F, Martinez JL. 2015. Tackling antibiotic resistance: the environmental framework. Nature Reviews Microbiology 13: 310–317. https://doi.org/10.1038/nrmicro3439.

[24] Manouore Njoya A, Ehet JS, Poutoum Yogne Y, Metsopkeng CS, Moldovan C-V, Chinche Belengfe S, Ngando L, Kamdem Simo M, Nana PA, Mouafo Tamnou EB, Sime-Ngando T, Nola M. 2022. Proteus bacteria species from hospital sewage and Mfoundi River in Yaounde (Cameroon, Central Africa): Comparison of the diversity, abundance and susceptibility against some β-lactams, Quinolones and Aminoglycosides antibiotics. J Adv Microbiol Res 3 (2): 34-46. https://doi.org/10.22271/micro.2022.v3.i2a.47.

[25] Djiala Tagne MR, Nougang ME, Mouafo Tamnou EB, Manouore Njoya A, Ngo Bahebeck P, Baleng SD, Nana PA, Yogne Poutoum Y, Bricheux G, Metsopkeng CS, Sime-Ngando T, Nola M. 2023. 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. J Adv Microbiol Res 4 (1): 86-95. https://doi.org/10.22271/micro.2023.v4.i1b.72.

[26] Guan X, Guo Z, Wang X, Xiang S, Sun T, Zhao R, He J, Liu F. 2023. Transfer route and driving forces of antibiotic resistance genes from reclaimed water to groundwater. Environ Pollution, 330, 121800. https://doi.org/10.1016/j.envpol.2023.121800.

[27] Chaturvedi P, Chowdhary P, Singh A, Chaurasia D, Pandey A, Chandra R., Gupta P. 2021. Dissemination of antibiotic resistance genes, mobile genetic elements, and efflux genes in anthropogenically impacted riverine environments. Chemosphere 273, 129693. https://doi.org/10.1016/j.chemosphere.2021.129693.

[28] Uluseker C, Kaster KM, Thorsen K, Basiry D, Shobana S, Jain M, Kumar G, Kommedal R, Pala-Ozkok I. 2021. A Review on occurrence and spread of antibiotic resistance in wastewaters and in wastewater treatment plants: Mechanisms and perspectives. Frontiers in Microbiology 12, 3003. https://doi.org/10.3389/fmicb.2021.717809.

[29] Brillard J, Dupont CMS, Berge O, Dargaignaratz C, Oriol-Gagnier S, Doussan C, Broussolle V, Gillon M, Clavel T, Bérard A. 2015. The water cycle, a potential source of the bacterial pathogen Bacillus cereus. BioMed Research International 356928. http://dx.doi.org/10.1155/2015/356928.

[30] Després VR, Huffman JA, Burrows SM, Hoose C, Safatov AS, Buryak G, Frohlich-Nowoisky J, Elbert W, Andreae MO, Poschul U, Jaenicke R. 2012. Primary biological aerosol particles in the atmosphere: a review. Tellus B: Chemical and Physical Meteorology 64, 15598. https://doi.org/10.3402/tellusb.v64i0.15598.

[31] Nola M, Noah Ewoti OV, Nougang M, Moungang ML, Chihib NE, Krier F, Servais P, Hornez JP, Njiné T. 2010. Involvement of cell shape and flagella in the bacterial retention during percolation of contaminated water through soil columns in tropical region. J Environ Sci and Health Part A 45 (11): 1297-1306. https://doi.org/10.1080/10934529.2010.500877.

[32] Nola M, Noah Ewoti OV, Nougang ME, Moungang LM, Chihib NED, Krier F, Hornez JP, Njiné T. 2012. The growth of Escherichia coli in soil layers separating the soil surface from the underground water table, in Central Africa: The hierarchical influence of the soil chemical characteristics. Res J Environ Earth Sciences 4 (2):196-206.

[33] Fisher JF, Mobashery S. 2020. Constructing and deconstructing the bacterial cell wall. Protein Sci 29 (3): 629-646. https://doi.org/10.1002/pro.3737.

[34] Sayed ARM, Shah NR, Basso KB, Kamat M, Jiao Y, Moya B, Sutaria DS, Lang Y, Tao X, Liu W, Shin E, Zhou J, Werkman C, Louie A, Drusano GL, Bulitta JB. 2020. First Penicillin-Binding protein Occupancy patterns for 15 beta-Lactams and beta-Lactamase inhibitors in Mycobacterium abscessus. Antimicrob Agents Chemother 6(1). pii: AAC.01956-20. https://doi.org/10.1128/AAC.01956-20.

[35] Schleibinger M, Steinbach CL, Topper C, Kratzer A, Liebchen U, Kees F, Salzberger B, Kees MG. 2015. Protein binding characteristics and pharmacokinetics of ceftriaxone in intensive care unit patients. Br J Clin Pharmacol 80 (3): 525-33. https://doi.org/10.1111/bcp.12636.

[36] Imming P, Sinning C, Meyer A. 2006. Drugs, their targets and the nature and number of drug targets. Nat Rev Drug Discov 5 (10): 821-34. https://doi.org/10.1038/nrd2132.

[37] Sutaria DS, Moya B, Green KB, Kim TH, Tao X, Jiao Y, Louie A, Drusano GL, Bulitta JB. 2018. First Penicillin-Binding protein occupancy Patterns of beta-Lactams and beta-Lactamase inhibitors in Klebsiella pneumoniae. Antimicrob Agents Chemother 62(6). pii: AAC.00282-18. https://doi.org/10.1128/AAC.00282-18.

[38] Shirley M. 2018. Ceftazidime-Avibactam: A review in the treatment of serious Gram-negative bacterial infections. Drugs 78 (6): 675-692. https://doi.org/10.1007/s40265-018-0902-x.

[39] Signe Mbiada J, Lontsi Djimeli C, Manouore Njoya A, Eheth JS, Tchakonté S, Tamsa Arfao A., Ateba Bessa H, Bricheux G, Nola M, Sime-Ngando T.2015. Assessment of the potential effect of some streams properties on the isolated Aeromonas hydrophila strains susceptibility against some β-Lactams and sulfamids. Res Biotechnol 6 (5): 33-44.

[40] An XL, Su JQ, Li B, Ouyang WY, ZhaoY, Chen QL, Cui L, Chen H, Gillings MR, Zhang T, ZhuYG. 2018. Tracking antibiotic resistome during wastewater treatment using high throughput quantitative PCR. Environ International 117 : 146-153. https://doi.org/10.1016/j.envint.2018.05.011.