Diversity and antibioticsusceptibility of extended-spectrumbeta-lactamase-producingEnterobacteriafrom the urine of human population using bacterio-contaminated water in Butembo urban area (DR Congo, Central Africa)

ABSTRACT

Aims : This study aims to investigate theESBL-producingEnterobacteriaceae in the humanurine samples and to understand the potential influence of the contaminated water the urine donors use.

Study design and Methodology : One hundred urine samples from females and the same number from males were collected from donors of different age group and analyseds.Antibioticsusceptibility tests werecarried out with identified ESBL-producing Enterobacteria strains.

Results : In male urine samples,the identifiedESBLs-producing strains belongs to *E. coli*, *E. agglomerans* and *K. pneumoniae*. They were in 11 samples: 3 from donors aged 20 years or less, 3 also from those aged 21 to 40 years as well as in those aged 41 to 60 years, and 2 in samples from donors aged over than 60 years. In female urine samples, the identified ESBLs-producing strains belongs to *E. coli*, *E. agglomerans*, *Y. frederiksenii*, *S. typhi* and *K. pneumoneae*.Theywere in 27 samples: 3 from donors aged 20 years or less, 9 from those aged 21 to 40 years, 12 from those aged 41 to 60 years, and 3 from donors aged over than 60 years. All the strains were sensitive to Imipenem and resistant toCefotaxim,Ceftazidin, Amoxicillin+Clavulanicacid and Nitrofurantoin. The Multi-AntibioticResistance indexvariedfrom 0.416 (*E. coli*strains, youngest male donors) to 0.916 (*E. coli* strains, oldfemaledonors). Most of strains reacts differently from one antibiotic to another ($P<0.05$), with the exception of *E. coli* strains from most samples. Most of the identified ESBLs-producing species have been reported in surface and groundwater the population use. These water resources could play a role in the human urinary tract infections.

Conclusion : There is a serioushealthproblemwithgreat MAR indices for all bacterialspeciesidentified. Treating water before use could reduce the viability and spreading of ESBLs-producing bacteria and genes into the population.

Keywords : Human urine, ESBLs-producingenterobacteria, diversity, antibioticsusceptibilityprofile,bacteriocontaminated water used

1. INTRODUCTION

Beta-lactamases are a class of diverse enzymes produced by bacteria that degrade the beta-lactam ring, inactivating the beta-lactam antibiotic.Beta-lactamase production in Gram-negative pathogenic bacteria is a public health concern due to the possibility of therapeutic failure, serious consequences for infection control and increased risk of morbidity and mortality in animals and humans [1,2]. Some beta-lactamases are encoded by mobile genetic elements (e.g., plasmids); others are coded on by chromosomes. Both mechanisms give the affected bacteria the ability to hydrolyze a wide variety of penicillins and cephalosporins[3,4].There is a diversity of beta-lactamases, sometimes classified by the Ambler classification system on the basis of molecular structure into 4 classes A, B, C and D. Classes A, C and D have a serine residue on the active site, while class B enzymes also called metallo-beta -lactamases (MBL), have zinc in the active site. Extended-spectrum beta-lactamases (ESBLs) belong to class A [4,5].

Beta-lactamases classifications can alsobebased on the proteins structure, on theiractivities oraccording to theirclinicalrole. The criteria of the classification based on the function are among others, the nature of the substrates and the relative affinity for these substrates [6-8].Members of ESBLsfamilycommonly express beta-lactamases which confer resistance to expanded-spectrum (extended-spectrum) cephalosporins. The prevalence of ESBL-producingbacteria varies from one geographicalarea to another[9,10]. They cause the inactivation of broad-spectrumoxyimino-cephalosporin (third- and fourth-generation) and monobactam (aztreonam) but not cephamycin

(cefoxitin) or carbapenems (meropenem, imipenem, ertapenem, and doripenem) [5,11,12]. Generally, these enzymes are neutralized by beta-lactamase inhibitorssuch as clavulanicacid, sulbactam, and tazobactam[5,11,].

Genesthat encode ESBLs are mostlyfound on transposons or insertion sequences of plasmids in association withotherresistancegenes. As a result, they can spread rapidly, causingresistance to multiple antimicrobialssuch as aminoglycosides, trimethoprim, sulphonamides, tetracyclineschloramphenicol, and fluoroquinolone [5,6]. Most ESBL-producingbacteria are of Enterobacteriaceaefamily[13,14]. Several of them are commonly involved in human infections. This can involve the digestive tract, the respiratory system, sepsis, the skin or the urinary tract [15].

Concerningurinay tract, itisknownthaturine isformed by nephronspresentinside the kidneys. In normal healthyindividuals, there are severalmechanismsthatattempt to preventbacteriafrominvading the bladder or progressing up through the upperurinary tracts [16,17]. The ability of the pathogen to produce infection isinfluenced by the virulence of the specificpathogen and individual'sspecific immune response[16,18]. The urinary tract infection (UTI) can becaused by bacteriumthatinvade the urinaryepitheliumcellscausing irritation and inflammation of thesecells[19]. The symptomsinclude a burning feeling whenurinating, urine that looks cloudy, urine thatappearsred, brightpink or cola-colored, or strong-smelling urine [18]**.** Most of the bacterialorganismsconcerned in the UTIs are sometimesfrom the gastrointestinal tract and can be linked to the poor microbiological quality of food or water consumed[20].

In most African cities in general and in Butembo (DR Congo, Central Africa) in particular, the majority of wastewater is discharged into nature without any treatment, due to the non-existence of wastewater treatment plants. Fecal and non-fecal contents of this wastewater can be found in seepage and then contaminate groundwater. Rainwater, which often feeds groundwater following infiltration, can sometimes be polluted [21,22]. The bacterio-contaminants contained in seeping water are rarely all retained before reaching the water table because this process depends on several factors [23-25].

In Butembo as in most African cities, a large part of the population uses stream and groundwater to meet their water needs, due to the insufficiency of public water supply or channeling difficulties linked to the relief[26,27]. In the natural environment, ESBLs-producingbacteriacan be found in various aquatic biotopes including surface water and ground water [14]. In urbanized area ofButembo, various surface and ground water points are contaminated by ESBLs-producingbacteria. Theirabundances and diversity in theseaquaticenvironmentsundergoessignificantspatio-temporal variation [14]. The strains antibiotics susceptibility varieddepending on the antibiotics, bacterialspecies and type of

aquaticenvironmenthosting the microorganism[28]. Several urinary infections cases among others are often reported in hospitals and populations of Butembo. Little is known about the potential relationships between the reported urinay infections in populations and the poor water microbiological quality they use. It is sometimes indicated that several pathogenic enterobacteria can migrate from the gastrointestinal tract after oral ingestion to other functional systems of the human body [13,15,18].This study aims to investigate the diversity and antibioticsusceptibility of ESBL-producingEnterobacteriaceae in the human UTI in Butembo and understand the potential role of the contaminated water the population used in the transmission of this infection.

2. MATERIAL AND METHODS

2.1. Urine samples collection

The study was carried out in the city of Butembo (DR Congo) from May to July 2023. Urine samplesweretakenfrom people who came to the UCG Butembo Central ResearchLaboratorywith a suspectedurinary tract infection. Theywerecollected in sterilebottles. Morning urine collection wasrecommended, or at least 3 hoursafter the last micturition. The study considered a total of 200 urine samples collected from donors of both sexes, namely 100 samples from females and 100 others from males. Due to the abundance of complaints of relatively high infections for certain age groups compared to others within the populations, the study considered for each sex, 6 samples for donors whose age was younger or equal to 20 years old, 54 samples for donors aged from 21 to 40 years, 34 samples for those aged from 41 to 60 years, and 6 for donors aged over than 60 years.

2.2. Isolation and identification of Enterobacteriaceae

The Enterobacteriaceae isolation was done after cytologicalexamination of the urine samples. This cytological examination looks for elements such as inflammatory cells (white blood cells, bacteria, among others) and crystals, allowing us to have an idea respectively of a potential infection and of renal functioning.

After homogenization of each of the urine samples, the isolation of the Enterobacteriaceae was done by spreading on the surface on Cystine Lactose Electrolyte Deficient (CLED) agar medium (W/ANDRADE INDICATOR) poured into petri dishes and then incubated at 37°C for 18 to 24 hours [29,30]. 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 [29,30].

After purification of the strains, their identification was carried out first by the enzymatic method, at Central Research Laboratory, Faculty of Pharmaceutical Sciences, Catholic University of Graben(DR Congo), then using the Matrix assisted laser desorptionionization–time of flight mass spectrometry(MALDI–TOF MS), at the Pasteur Center of Yaounde (Cameroon) [31-33]. The mass-to-charge (*m/z*) ratios are electrodynamicmeasurements of how quicklycharged ions from the clinicalsamplematerial move through the TOF tube and reach a detector.

From 18 to 24 hours culture, selectedcolonywasapplied onto MALDI test plant. Sampleswerethenoverlaidwith matrix and dried. The plate wassubsequentlyloadedinto the MALDI-TOF MS instrument : the samplewasbombarded by the laser. This bombardmentresulted in the sublimation and ionization of both the sample and matrix. Thesegenerated ions wereseparatedbased on their mass-to-charge ratio via a TOF tube, and a spectral representation of these ions wasgenerated and analyzed by the MS software, generating an MS profile. This profile wassubsequentlycompared to a database of reference MS spectra and matched to eitheridentical or the mostrelatedspectracontained in the database, generating an identification for bacteria and analyzed by sofwareassociatedwith the respective system, allowingrapid identification of the microorganism[32-34].

2.3. Detection of ESBL-producing enterobacteria

ESBL-producing enterobacteria have been highlighted by the search for synergy between clavulanic acid and 3rd generation cephalosporins. Enzymes are highlighted by the disc method which consists of looking for an image of champagne cork synergy between an antibiotic disc which contains a β-lactamase inhibitor and the 3rd generation cephalosporin discs. The antibiotic discs used were ceftazidim (30 µg), cefotaxim (30µg), ceftriaxon (30µg), Amoxicillin/Clavulanic Acid (20/10µg) and aztreonam (30µg). The production of ESBLs results in the appearance of an image of "*Champagne cork*" synergy between the discs of Amoxicillin/Clavulanic Acid and 3rd generation cephalosporins [35].

2.4. Antibioticsusceptibilitytests

The antimicrobialsusceptibility tests werecarried out using the disk diffusion methodaccording to the recommendations of the FMS-EUCAST[36]. The medium usedwasMüeller Hinton agar (Biorad) pouredinto Petri dishes. The thickness of the agar wasapproximately 4 mm. The surface of the agar medium wasdriedbefore use. 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

turbidityequivalent to that of the standard 0.5 of the range of McFarlandwascarried out, which corresponds to a bacterialdensity of approximately $1x10^8$ CFU/100 mL. The inoculum wasthendiluted 1/10 ($1x10^7$ CFU/100 mL) before inoculation.

The agar wasinoculatedwith the bacterial inoculum usingswabmethod. The entire surface of the agar medium wasswabbed in three directions. The antibiotic discs wereplaced on the surface of the inoculated and dried agar. The gap between the discs was 3 cm in order to avoidoverlapping of the inhibition diameters. The Petri disheswerethenincubatedwithin 15 min following the depositing of the discs, at 37 °C aerobically for 24 h.

The antibioticmoleculesconsidereddepend on the uses of the population qndalso on theiravailability in the laboratory. ThoseconsideredwereCeftriaxon, Ceftazidim, AztreonamCefotaxim, and Amoxicillin+Clavulanicacid, Gentamycin, Ciprofloxacin, Chloramphenicol, Doxyciclin, Imipenem, Nalidixicacid and Nitrofiradentoin. The inhibition diameters (ID) weremeasuredusing the caliper and the resultswerescored as resistant, sensitive or intermediateaccording to CA-SFM recommendations[36,37].

2.5. Data analysis

The ESBLs-producing strains were grouped per species. After the antibiotic susceptibility tests, the percentages of sensitive, resistant or intermediate strains were calculated for each species. The comparison using the Kruskal-Wallis test has been carried out amongst the inhibition diameters values for all ESBLs-producing strains for the same antibiotic, then from one antibiotic to another for the same strains per sex and age of urine donor. The average values of the inhibition diameters (and standard deviations) have been calculated for each ESBLs-producing species, considering the sex and age of urine donor patients, then illustrated by histograms. The isolateswhichdisplayedresistance to three or more thanthree classes of antibioticsweredesignated as multidrugresistant (MDR) bacteria. The Multi-Antibiotic Resistance (MAR) Index wascalculatedby using the following formula[38]:

$$\text{MAR Index} = \frac{\text{Number of antibiotic to which an isolate showed resistance}}{\text{Total number of antibiotics used}}$$

3. RESULTS

3.1. ESBLs-producingenterobacteriaisolates

Amongst the 100 urine samples given by male donors, 19 samples were infected with bacterial strains belonging to the Enterobacteriaceae family. They included 3 samples from donors aged 20 years or younger, 7 from those aged 21 to 40 years, 7 from those aged 41 to 60 years and 2 from those aged over 60 years. Amongst the 19 Enterobacteriaceae strains, 11 produced ESBLs and were distributed as follows: 3 strains in the urine samples of donors aged 20 years or less, 3 also in those of donors aged 21 to 40 years old as well in those of donors aged 41 to 60 years, and 2 in urine samples of donors aged over 60 years.

Amongst the 100 urine samples given by female, 48 samples were infected with bacterial strains belonging to the Enterobacteriaceae family. They included 3 samples from donors aged 20 years or younger, 18 from those aged 21 to 40 years, 24 from those aged 41 to 60 years, and 3 from those aged over 60 years. Amongst the 48 Enterobacteriaceae strains, 27 produced ESBLs and were distributed as follows: 3 strains in the urine samples of donors aged 20 years or less, 9 in those of donors aged 21 to 40 years, 12 in those of donors aged 41 to 60 years, and 3 in urine samples of donors aged over 60 years. Abundance of ESBLs-producing strains as well as the number of strains with respect to bacterial species is presented in Table 1. It can be noted that in the urine of male donors, the majority of ESBLs-producing strains are *E. coli*, followed by *K. pneumoniae*. In the urine of female donors, most of ESBLs-producing strains are also *E. coli*, followed by *E. agglomerans*, *Y. frederiksenii*, *S. typhi* and *K. pneumoneae*. The number of ESBLs-producing strains seems to vary first with the sex of the patients, then with their ages (Table 1).

3.2. Antibiotics inhibition diametersagainstESBLs-producingenterobacteriaisolates

The identified ESBLs-producing bacterial strains belong to *E. coli*, *K. pneumoneae*, *E. agglomerans*, *S. typhi* and *Y. frederiksenii*. For each bacterial species, the average value of the inhibition diameter for all the strains identified in the presence of each antibiotic was calculated with respect to donor sex (male or female) and age group. These inhibition diameters are presented in Figure 1. It is observed for all the strains of each of the 5 species that the highest inhibition diameters were recorded in the presence of Imipenem.

With *Y. frederiksenii* strains isolated from the urine of only female donors aged from 41 to 60 years, the Smallest Inhibition Diameter (SID) was 8mm recorded in the presence of Ceftazidin. In the presence of *S. typhi* strains isolated only from urine samples of female donors aged from 41 to 60 years too, the SID was 6mm, recorded in the presence of Aztreonam and Nalidixic acid. With *K. pneumineae* strains isolated from the urine samples from females aged from 20 years or less, it was 6mm, recorded in the presence of Ceftazidin, Ciprofloxacin and chloramphenicol. For other strains

of the same species isolated from male aged over 60 years, it was also 6mm, recorded in the presence of Ceftazidin and Amoxicillin+Clavulanic acid. These SID with *E. agglomerans* strains isolated in the urine of only female aged from 21 to 40 years was 8mm, recorded in the presence of Ceftriaxon.

Several ESBLs-producing *E. coli* strains have been identified in the urine of patients of both sexes and of various age groups. In the urine of male aged from 20 years or less, the SID was 6.33 mm recorded in the presence of Doxyciclin. Still in the urine of male aged from 21 to 40 as well as those aged from 41 to 60, it was 7mm all recorded in the presence of Nitrofurantoin. For strains isolated from the urine of female aged from 20 years or less, it was 6.5 mm, recorded in the presence of Cefotaxim and Ceftazidin. In the urine of female aged from 21 to 40 and from 41 to 60 years, it was 9.12 mm and 6.66 mm, respectively, recorded in the presence of Doxyciclin and Ceftazidin. For strains isolated from the urine of female aged over 60 years, the SID was 8.33 mm recorded in the presence of chloramphenicol (Fig. 1).

Table 1. ESBL-producingEnterobacteriaceaespeciesidentified in urine infectedwith respect to the urine donorsages and sex

Urine donorsages	Male			Female		
	Urine infec-ted	Urine infected and Enterobacteriaceaestrainsidentified	Urine infected and ESBL-producingstrainsidentified	Urine infec-ted	Urine infected and Enterobacteriaceaestrainsidentified	Urine infected and ESBL-producingstrainsidentified
≤ 20 yearsold **(6** samples**)**	**(6)**	**(3)** *E. coli*-1 *E. coli*-2 *E. coli*-3	**(3)** *E. coli*-1 *E. coli*-2 *E. coli*-3	**(4)**	**(3)** *E. coli*-1 *E. coli*-2 *K. pneumoneae*	**(3)** *E. coli*-1 *E. coli*-2 *K. pneumoneae*
21 to 40 years old **(54** samples**)**	**(17)**	**(7)** *E. agglomerans*-1 *E. agglomerans*-2 *E. agglomerans*-3 *K. pneumoniae* *E. coli*-1 *E. coli*-2	**(3)** *E. coli*-1 *E. coli*-2 *E. coli*-3	**(37)**	**(18)** *E. coli*-1 *E. agglomerans*-2 *E.coli*-2 *E. agglomerans*-3 *E.coli*-3 *E. agglomerans*-4 *E.coli*-4 *E. agglomerans*-5 *E.coli*-5 *E. agglomerans*-6 *E. coli*-6 *Y. frederiksenii*-1 *E. coli*-7 *Y. frederiksenii*-2 *E. coli*-8 *Y. frederiksenii*-3 *E. agglomerans*-1 *K. pneumoneae*	**(9)** *E. coli*-1 *E. coli*-6 *E.coli*-2 *E. agglomerans*-1 *E.coli*-3 *E. agglomerans*-2 *E.coli*-4 *E. agglomerans*-3 *E.coli*-5
41 to 60 years old**(34** samples**)**	**(15)**	**(7)** *E. coli*-1 *E. coli*-2 *E. coli*-3 *E. coli*-4 *E. coli*-5 *E. coli*-6 *E. agglomerans*	**(3)** *E. coli*-1 *E. coli*-2 *E. coli*-3	**(31)**	**(24)** *E. coli*-1 *E. agglomerans*-6 *E. coli*-2 *E.agglomerans*-7 *E. coli*-3 *E. agglomerans*-8 *E. coli*-4 *Y. frederiksenii*-1 *E. coli*-5 *Y. frederiksenii*-2 *E. coli*-6 *Y. frederiksenii*-3 *E. coli*-7 *S. typhi*-1 *E. agglomerans*-1 *S. typhi*-2 *E. agglomerans*-2 *S. typhi*-3 *E. agglomerans*-3 *Y. pseudotuberculosis*-1 *E.agglomerans*-4 *Y. pseudotuberculosis*-2 *E. agglomerans*-5 *Y. pseudotuberculosis*-3	**(12)** *E. coli*-1 *Y. frederiksenii*-1 *E. coli*-2 *Y. frederiksenii*-2 *E. coli*-3 *Y. frederiksenii*-3 *E. coli*-4 *S. typhi*-1 *E. coli*-5 *S. typhi*-2 *E. coli*-6 *S. typhi*-3
> 60 years old**(6** samples**)**	**(3)**	**(2)** *K. pneumoneae*-1 *K. pneumoneae*-2	**(2)** *K. pneumoneae*-1 *K. pneumoneae*-2	**(3)**	**(3)** *E. coli*-1 *E. coli*-2 *E. coli*-3	**(3)** *E. coli*-1 *E. coli*-2 *E. coli*-3
Total	**41**	**19**	**11**	**75**	**48**	**27**

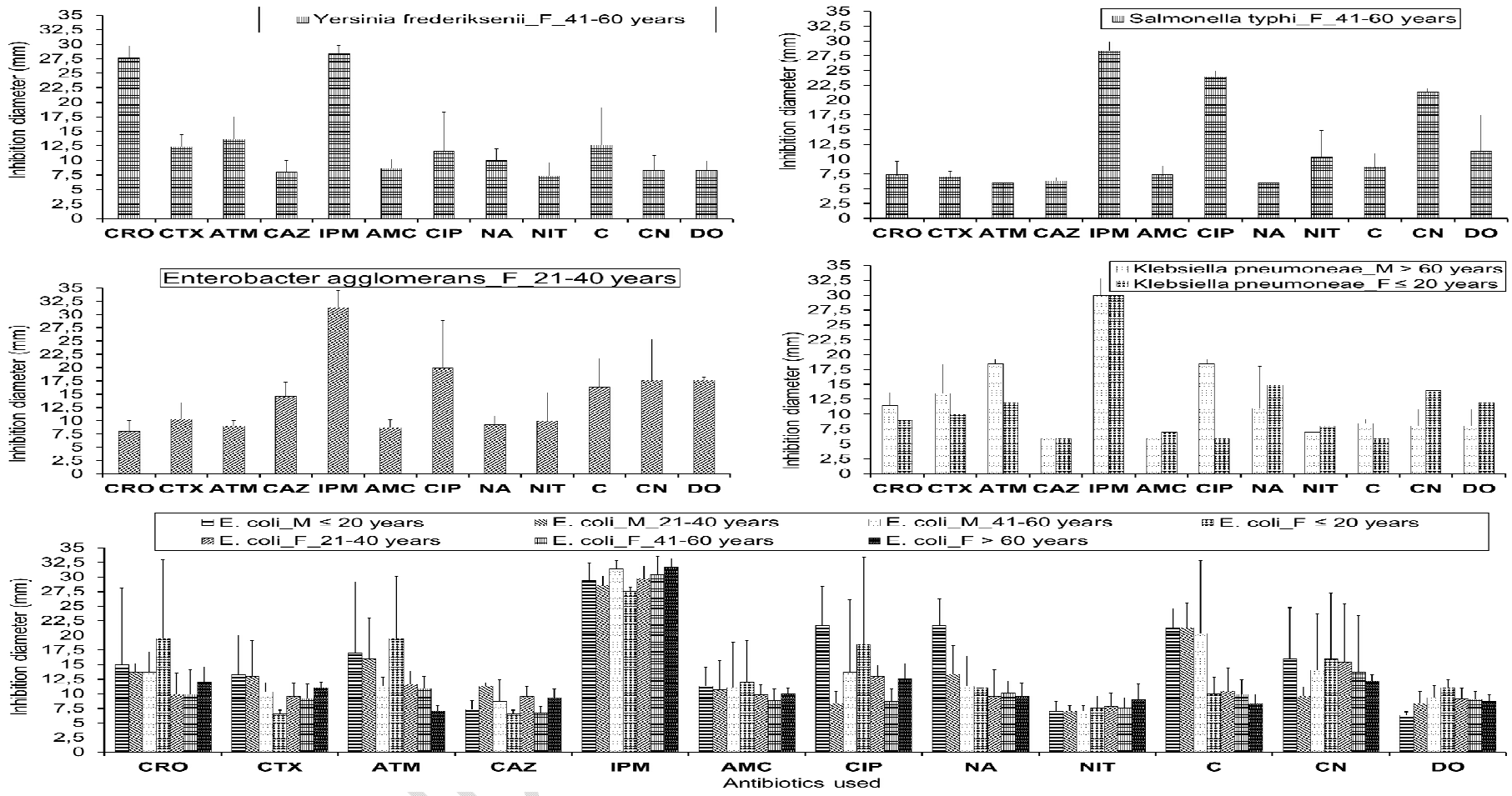

Fig. 1.Mean values of the antibiotics inhibition diametersagainstESBLs-producingbacterialstrains ((CRO : Ceftriaxon; CTX : Cefotaxim ; ATM : Aztreonam ; CAZ : Ceftazidin ; IPM : Imipenem ; AMC : Amoxicillin+Clavulanicacid ; CIP : Ciprofloxacin ; NA : Nalidixicacid ; NIT : Nitrofurantoin ; C : chloramphenicol ; CN : Gentamicin ; DO : Doxyciclin)

3.3. Antibioticssusceptibility of ESBLs-producingenterobacteriaisolates

Antibiotic susceptibility tests showed that the percentages of sensitive, resistant or intermediate strains vary relatively from one bacterial species to another for all the antibiotics tested, and from one antibiotic to another for the same bacterial species. The percentage of sensitive strains for all antibiotics varied from 0 to 100 (Fig. 2).

All strains of the 5 bacterial species were sensitive to Imipenem. Additionally, all strains of *K. pneumoneae* were sensitive to Ciprofloxacin and Gentamicin. All strains of *Y. frederiksenii* were also sensitive to Ceftriaxon. The same observation was noted for *E. agglomerans* against Doxyciclin. Amongst all the antibiotics used, all strains of *E. coli* were resistant to 3 (Ceftazidin, Nitrofurantoin, Doxyciclin), those of *K. pneumoneae* resistant to 6 (Ceftriaxon, Ceftazidin, Cefotaxim, Nitrofurantoin, chloramphenicol, Amoxicillin+Clavulanic acid), those of *E. agglomerans* resistant to 5 (Ceftriaxon, Cefotaxim, Aztreonam, Amoxicillin+Clavulanic acid, Nalidixic acid), those of *S. typhi* resistant to 7 (Ceftriaxon, Cefotaxim, Aztreonam, Ceftazidin, Amoxicillin+Clavulanic acid , Nalidixic acid, chloramphenicol), and those of *Y. frederiksenii* resistant to 8 (Cefotaxim, Ceftazidin, Amoxicillin+Clavulanic acid, Ciprofloxacin, Nalidixic acid, Nitrofurantoin, Gentamicin and Doxyciclin) (Fig. 2).

The resistant, sensitive or intermediate character of the groups of ESBLs-producing bacterial strains identified with respect to sex and age group of urine donors is presented in Table 2. It can be noted that all the bacterial strains identified are sensitive to Imipenem. In addition, *E. coli* strains isolated from the urine of male and female donors aged 20 years or less, *E. agglomerans* isolated from the urine of female donors aged from 21 to 40 years, as well as *S. typhi* strains isolated from the urine of female donors aged from 41 to 60 years are sensitive to Gentamicin (Table 2). Several other *E. coli* strains from the urine of male and female donors aged 20 years or less, as well as from male donors aged from 41 to 60 years, and *K. pneumoneae* isolated from the urine of male donors aged over 60 years, have an intermediate status for more than one antibiotic tested (Table 2).

It isnotedthat all ESBLs-producingisolatesidentified are multidrugresistantbacteria. The Multi-Antibiotic Resistance (MAR) Index has been calculated. Its values variedfrom 0.416 to 0.916. The highestwasrecordedwith*E. coli* strainsisolatedfromfemale urine donorsagedfrom 41 to 60 yearsold and over, and the lowestwasregisteredfrom*E. coli*strainsisolatedfrom male urine donorsaged of 20 yearsold or less (Table 2).

The comparison using the Kruskal-Wallis test has been carried out amongst the inhibition diameters values for all ESBLs-producing strains for the same antibiotic, then from one antibiotic to another for the same strains with respect to sex and age of urine donor. It appears that each

identified ESBLs-producing Enterobacteriaceae strains reacts differently from one antibiotic to another (P<0.05), except the *E. coli* strains isolated from the urine of male donors aged less than or equal to 20 years and those aged from 41 to 60 years, as well as the *E. coli* strains isolated from the urine of female donors aged from 21 to 60 years. This test also showed that Aztreonam, Ceftazidin, chloramphenicol and Doxyciclinreact differently from one ESBLs-producing Enterobacteriaceae strain to another (P<0.05), while the activities of the other antibiotics do not differ significantly from one strain to another (P>0.05).

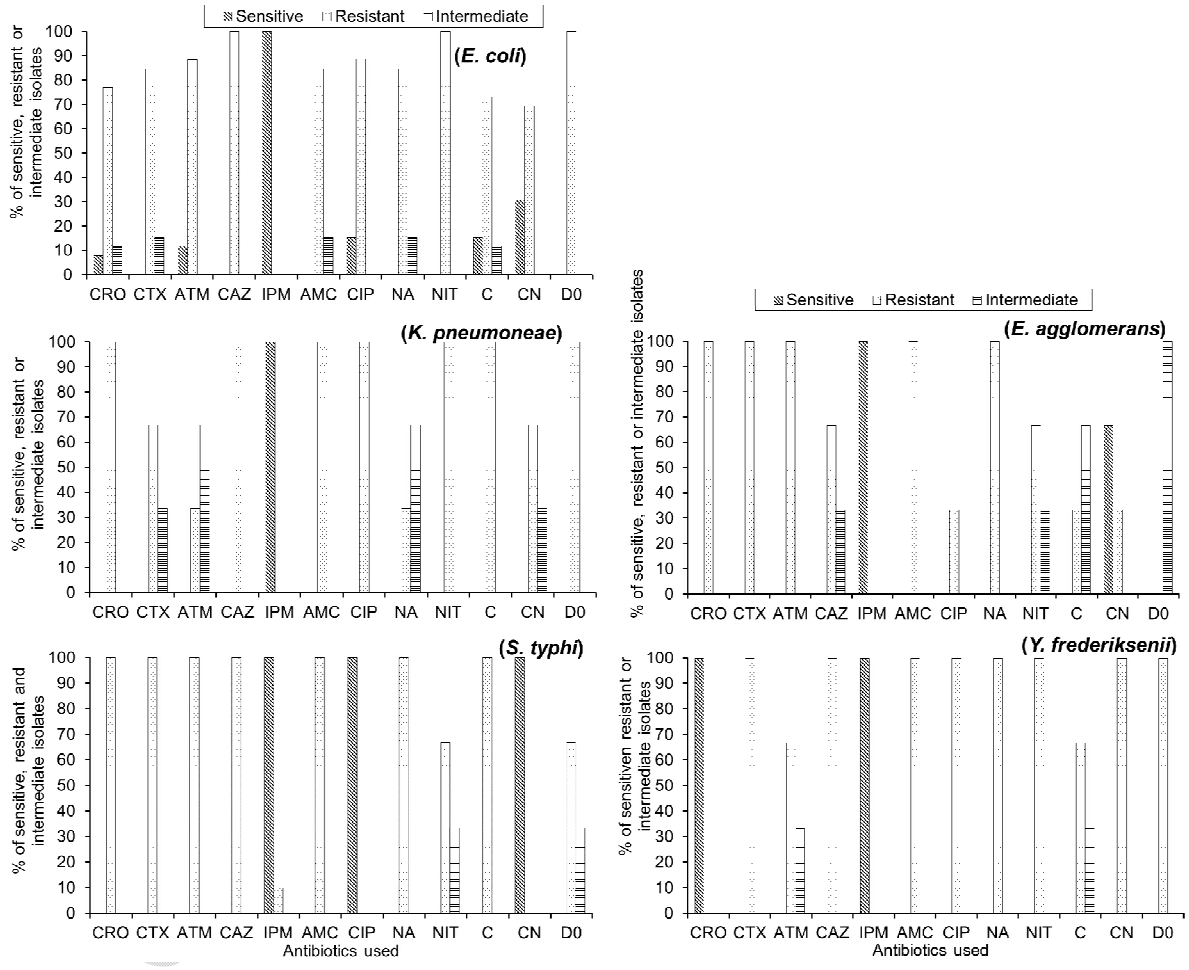

Fig. 2.Percentage of sensitive, resistant or intermediate*E. coli, K. pneumoneae, E. agglomerans, S. typhi* and *Y. frederikseniii*solateswith respect to antibioticsused (CRO : Ceftriaxon; CTX : Cefotaxim ; ATM : Aztreonam ; CAZ : Ceftazidin ; IPM : Imipenem ; AMC : Amoxicillin+Clavulanicacid ; CIP : Ciprofloxacin ; NA : Nalidixicacid ; NIT : Nitrofurantoin ; C : chloramphenicol ; CN : Gentamicin ; DO : Doxyciclin).

Table 2.Profile of antibioticsactivity (R : resistent ; S : sensitive ; I : intermediate) against ESBL-producingEnterobacteriaceaeidentified in urine sampleswith respect to the urine donorsex and age and Multi-Antibiotic Resistance (MAR) Index

Groups of cellsstrainsbased on the urine donorage and sex	Antibioticsused and MAR Index												
	CRO	**CTX**	**ATM**	**CAZ**	**IPM**	**AMC**	**CIP**	**NA**	**NIT**	**C**	**CN**	**DO**	**MAR Index**
*E. coli*_M ≤ 20 yearsold	**I***	R	**I***	R	**S***	R	**I***	**S***	R	**I***	**S***	R	0.416
*E. coli*_M_21-40 yearsold	R	R	R	R	**S***	R	R	R	R	**I***	R	R	0.833
*E. coli*_M_41-60 yearsold	R	R	R	R	**S***	R	R	R	R	**I***	**I***	R	0.75
*E. coli*_F ≤ 20 yearsold	**I***	R	**I***	R	**S***	R	**I***	R	R	R	**S***	R	0.583
E. coli_F_21-40 yearsold	R	R	R	R	**S***	R	R	R	R	R	**I***	R	0.833
*E. coli*_F_41-60 yearsold	R	R	R	R	**S***	R	R	R	R	R	R	R	0.916
*E. coli*_F> 60 yearsold	R	R	R	R	**S***	R	R	R	R	R	R	R	0.916
*E. agglomerans*_F_21-40 yearsold	R	R	R	R	**S***	R	**I***	R	R	R	**S***	**I***	0.666
*K. pneumoneae*_M> 60 yearsold	R	R	**I***	R	**S***	R	**I***	R	R	R	R	R	0.75
*K. pneumoneae*_F ≤ 20 yearsold	R	R	R	R	**S***	R	R	**I***	R	R	R	R	0.833
*S. typhi*_F_41-60 yearsold	R	R	R	R	**S***	R	**S***	R	R	R	**S***	R	0.75
*Y. frederiksenii*_F_41-60 yearsold	**S***	R	R	R	**S***	R	R	R	R	R	R	R	0.833

M : male urine donor; F : female urine donor; CRO : Ceftriaxon; CTX : Cefotaxim ; ATM : Aztreonam ; CAZ : Ceftazidin ; IPM : Imipenem ; AMC : Amoxicillin+Clavulanicacid ; CIP : Ciprofloxacin ; NA : Nalidixicacid ; NIT : Nitrofurantoin ; C : chloramphenicol ; CN : Gentamicin ; DO : Doxyciclin.

4. Discussion

4.1. Antibioticssusceptibility of ESBLs-producingenterobacteriaisolates

The identifiedESBLs-producingenterobacterialisolateswere*E. coli, K. pneumoneae, E. agglomerans, S. typhi* and *Y. frederiksenii* species. Some authors have isolated many strains of the same species or the genus from human and animals, and also from food-producing animals and animal-derived foods, with some studies indicating genetic similarities between these isolates and those found in human infections [39-41].

This studyshowedthat all the identified ESBLS-producingisolates in urine samples are multipledrugresistant. This has also been noted by Mwanzo*et al*[14] about isolatesfrom surface and ground water samplesin the urbanized area ofButembowhereESBLs-producingbacterialabundances and diversityundergoessignificantspatio-temporal variation. It has also been noted that the antibioticESBLs-producingbacteriavarieddepending on the antibiotics, bacterialspecies and type of aquaticenvironmenthosting the microorganism[28]. It issometimesindicatedthatsuchresistancedevelops in bacteria due to genetic mutations and/or acquiredgenomes[42].

Somemechanisms of ESBLs- producingbacterialresistance have been suggested. Antibiotics spread out in the cellthrough the occurrence of mutations in the genewhichspecifically encodes the outer membrane porinprotein[43,44]. Throughstrong efflux pumping, the number of antimicrobials are launched out of the cell. Theiroverexpressionallowsresistance to beformerly effective [44,45]. By changing the arrangement of the targets, the binding affinity of antibiotics can bereduced[44,46].

The MAR index variedfrom 0.416 to 0.916. It isknownthatbacteriahaving MAR index ≥ 0.2 originatefrom a high-risk source of contamination whereseveralantibiotics are used[47]. Development of resistance to certain drugssuch as macrolids, lincosamines, and streptograminscan can beattained by doingmethylation of their binding site and the 16 s rRNA by the action of enzyme callederythromycin ribosomal methylase[44,48]. The development of the concernedantibioticresistancetakes place due to horizontal distribution of ESBL plasmidsthroughbacteriophages or through horizontal genetransferwhich engage the genes for the concernedantibiotic binding proteins[49]. Attainment of the van gene cluster ismainlyresponsible for the development of resistancethatultimately causes less binding affinity to the target[50]. This can lead to enzymes modification like phophotransferases, nucleotidyltransferases, acetyltransferases, or through mutation and efflux mechanisms[50]. Resistance couldalsobe due to the mutations occurring in the subunits of the DNA gyrase and topoisomerase[44,50].

It isalsoindicatedthatgenesthat encode ESBL are mostlyfound on transposons or insertion sequences of plasmids in association withotherresistancegenes. As a result, they can spread rapidly, causingresistance to multiple antimicrobialssuch as aminoglycosides, trimethoprim, sulphonamides, tetracyclines, chloramphenicol, and fluoroquinolone [5,6].

Otherbetalactamantibiotics inactivation mechanisms by Gram-negativebacteria have been reported. The periplasm of Gram-negativebacteria releases beta lactamase which has a higheraffinity to betalactamantibioticsthanthat of betalactamantibiotics to theirtargets. The genecodingbetalactamasemaybelocated in the immobile genetic chromosomes or extra-chromosoma mobile geneticelementssuch as a plasmid, integrin, or a transposon. The resistantgenesevolveeither by gene-level mutations or acquisition of resistantgenesfromotherbacteria of the same or differentspecies[5]. Bacteriahaving MAR index $\geq$ 0.2 alsoconfirm the presence of multidrug-resistantgenesoriginatingfrom the environmentwherethereis an abuse of thesedrugs and alsothat the plasmidscontain one or more resistancegenes, eachencoding a single antibioticresistancephenotype[47,51].With the exception of Imipenem (carbapenemes class), all identified strains were resistant to the majority of antibiotics used. Imipenem is currently among the latest molecules in clinics[52]. Excessive use of this molecule could lead to the development of resistance.

4.2. Potentialrelationshipbetweenhuman urine infection and poorbacterial water quality in Butembo

Mwanzo*et al* [14] noted in urbanized area in Butembo that various aquatic environments including well, river water and hospital wastewater harbored various species of ESBLs-producingenterobacterialstrains.Theyweremainly*Escherichia coli, Ewingelia americana, Erwina*spp, *Citrobacterfreundii, Edwarsiella*spp, *Klebsiella ozaenae, Shigella*spp, *Enterobacter aerogenes, E. agglomerans, Citrobacterdiviersys, Proteus mirabilis, Serratia fonticola, Serratia ficana, Moellerellawisconsensis, Klebsiella rhinoscleromatis* and *Providenciarettgeri*species.Debabza*et al*[53]analysed more than 250 Enterobacteriaceaestrainsisolatedfrom influent wastewater, effluent wastewater, and sludge in a municipal wastewatertreatment plant and from river water receivingtheir effluent in Algeria. Theynotedthat56.30% weredefined as extended-spectrumbeta-lactamase producerswith 17.50% found in the river water. In the urban area withoutwastewatertreatment plant, the municipal sewagemaybe a reservoir of the dissemination of ESBLs–producingEnterobacteriaceaeinto the environment, therebycontaminatingnatural water resourcesincludinggroundwatersometimesmostlydrinked by the population. This can

alsodissiminate mobile geneticelementswhich are involved in spreadingESBLs-genesamong the bacterial population [5].

The presence of mutiltidrugresistantESBLs-producingbacteria in soil have been reported by someauthors[10,54], and the phylogeneticanalysis of the prevalentESBLs-carryingorganisms in soilindicatedthat the genes can behorizontallytransferredacrossdifferentbacterialorders and classes [55]. It isknownthatthe soil rarely retains all the chemical and biological pollutants contained in the seeping water from the soil surface to the groundwater. This retention depends on several factors including the mineralogical and petrographic properties of the soil particles, the anatomical and physiological properties of the germs contained in these infiltration waters, as well as the chemical characteristics of this seeping water [23-25]. Following the infiltration of microbial pollutants from the soil to the water table during the infiltration of runoff water, for instance, these genes and enzymes could then be found in groundwater which will be consumed by populations.

In addition, the use of antimicrobial agents has precipitated the proliferation of antimicrobialresistantstrains of bacteriawithin the commensal microbiota, engendering a potentialhazard to humanhealth via the consumption of contaminatedwater and foods[41,56]. The unregulated and excessive use of antimicrobials in aquaticenvironments hasalso been identified as a significant driver of antimicrobialresistanceESBLs-producingenterobacteria. For example, it has been indicatedthataquaticanimalssuch as fish and shrimp can serve as reservoirs for antimicrobialresistancegenes[57,58], whichcould lead as a threat to humanhealththrough the consumption of rawseafood, thuscontributing to a public healthcrisis[59].

5. Conclusion

The ESBLs-producing bacteria represent a real danger for human health, because of their multi-resistance to antibiotics. In areas without public drinking water for various reasons, people should treat groundwater before consumption.This can be done by simple and inexpensive means such as boiling water or adding chlorine or ozone. This would reduce the viability of the ESBLs-producing bacteria and genes in drinking water as well as the level of human infections. This could also reduce the spread of these microbial contaminants into the natural environment.

ATTACHED DOCUMENT :

ETHICAL APPROVAL CERTIFICATE

CONSENT

This study was approved by the health ethics committee, Provincial Directorate of North Kivu (authorization ref.: CNES/DP-NK 002054125001-105/2023).

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