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(Research Article)

Antibiotic resistance profile of *Enterococcus spp*. strains isolated from the intestinal tract at the Yaounde Military Hospital Laboratory

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Abstract

Multidrug-resistant *Enterococci* colonizing the intestinal tract of patients are the major sources of infection as well as the spread of resistance. The present study aims to determine the antimicrobial resistance profile of *Enterococcus species* from the intestinal tract of patients admitted to the laboratory of the Yaounde Military Hospital.

The study was conducted in patients from July to November 2020. Stool samples were collected and processed for bacterial isolation and antimicrobial susceptibility testing. Stool samples were inoculated onto selective *Enterococci* media (Bile Esculin azide agar). The isolates obtained were identified to the level of genus and species level: characteristics, Gram staining and use of API 20 *Streptococcus* system. Susceptibility testing was performed using the Kirby-Bauer solid state disk diffusion method.

Of the 35 samples analyzed 19 strains were isolated, 9 *Enterococci* and 10 non-*Enterococci*. *Enterococci* were isolated from 9 (25.7%) of the study subjects. The isolates were *Enterococcus faecium* (36.9%) followed by *Enterococcus faecalis* (10.5%), *Aerococcus viridans* (31.5%), *Streptococcus mitis 1* (10.5%) and finally *Streptococcus mitis 2* (10.5%). Of the 9 *Enterococci* isolates tested, 6 (66.6%) were resistant to Ampicillin, 9 (100%) to Gentamycin and 4 (44.4%) to Amikacin. Other alternative antibiotics to treat infection that may be caused by *Enterococci* have also shown a high rate of resistance in vitro: levofloxacin 6 (66.6%), erythromycin 8 (88.8%), tetracycline 7 (77.7%), chloramphenicol 4 (44.4%). 7 (77.7%) of the *Enterococci* were resistant to vancomycin. Multidrug resistance was predominant in *E. faecium* up to R7 (10.5%), however multi resistance was more pronounced at R3 (94.73%).

This study reveals a high rate of faecal colonization by multidrug-resistant *Enterococci* and an increasing prevalence of strains resistant to vancomycin. Thus, periodic monitoring of antibacterial susceptibilities is recommended to detect emerging resistance and prevent the spread of multi-resistant bacterial strains.

Keywords: *Enterococci*; Resistance; Antibiotics; Intestinal tract; Military hospital

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1. Introduction

For over 80 years, antibiotics have been used as a method of controlling bacteria [1]. However, with repeated use, an antibiotic loses its effectiveness and another mechanism ensues: resistance [2]. Resistance occurs when a bacterium evolves in response to antibiotic use [3]. This results in increased medical costs, a significant increase in hospitalization time and an increase in mortality rates [2]. This being the case, hospitals constitute an environment characterized by high levels of antibiotic use. They can thus constitute real reservoirs of resistant bacteria, from which the latter cause important nosocomial infections [4], but also livestock farms or people in community areas who consume a lot of antibiotics [5]. We can thus observe a worrying evolution of bacteria that are initially resistant to only a few antibiotics, then to more than three classes of antibiotics (MDR, multi-resistant bacteria), then to almost all of them (HRB, highly resistant bacteria), or even to all the antibiotics available (total-resistant bacteria).

In response to this rise in bacterial resistance, the WHO has published its first list of antibiotic-resistant 'priority pathogens', listing the 12 families of bacteria most threatening to human health and to be considered for research [6]. This list was established in an attempt to guide and promote research and development of new antibiotics and is part of the WHO's efforts to address the growing antibiotic resistance worldwide [6]. However, there are multidrug resistant and more virulent bacteria in this list that should be better analyzed, the ESKAPEs [6]. Our interest was thus focused on the study of *Enterococcus spp*. Indeed, *Enterococcus* is a highly resistant bacterium, capable of withstanding harsh living conditions and commensal to the intestinal flora [7]. Clinically, it remains a very neglected bacterium as it is generally considered commensal to the body. However, studies have shown that when exposed to high concentrations of antibiotics, *Enterococcus* has developed resistance mechanisms against these antibiotics over time [8].

The *Enterococcus*, as the commensal par excellence of the intestinal tract, is a very resistant germ [5]. Physiologically, speaking, the *Enterococcus* is a germ that is highly resistant to natural environmental conditions. It also has the ability to resist extreme temperature conditions and the acidity of the intestine [5]. For several years *Enterococci* have also acquired the ability to resist antibiotics when exposed to them [7]. Recently tested hypotheses have made it possible to understand that it is through gene exchanges via mobile genetic elements that this resistance could be achieved [5]. This is very dangerous when we know that plasmids are mobile genetic elements. They can therefore be transmitted to bacteria of the same genus as well as to bacteria of other genera. On the other hand, they can be responsible for sepsis or urinary tract infections and endocarditis due to the transmission of the germ in the urethra. However, having developed various resistance mechanisms in the gut as a result of antibiotic therapy, therapeutic overdose due to multiple antibiotic doses and selection pressure mechanisms [5], this germ is becoming highly pathogenic for humans in view of its acquired resistance in the intestine. With this in mind, we decided that it would be judicious to determine the resistance profiles of *Enterococcus* strains isolated from the intestinal tract of patients admitted to the Yaounde Military Hospital.

2. Methodology

This was a cross-sectional study with a descriptive aim over a period of 5 months from 01 July 2020 to 01 November 2020 at the laboratory of the military hospital in Yaoundé. The sampling was non-probability, based on consecutive recruitment of participants who consented to the study. The target population was all patients with a stool culture examination during this period.

2.1. Sampling transport and storage

2.1.1. Levy

The composition of the saprophytic flora depends on the diet, but also on the use of antibiotics. For those who complied with the sampling conditions and who were willing, a sample was taken by the patient in a sterile stool jar. In infants, the sample was taken directly from the nappy. A rectal swab was also taken. Finally, the consistency of the stool was also noted.

2.1.2. Transport and storage

The collected samples were then transported without delay to the laboratory, accompanied by a card bearing all the necessary information (date, patient code and time of collection) and then analyzed immediately. If the samples could not be analyzed directly, they could be kept for a maximum of 1 hour at 4 °C.

2.2. Bacteriological analysis

2.2.1. Plating and Isolation of Enterococci

A Gram stain was applied beforehand to the stool smears on object slides, in order to assess the presence of gram-positive cocci in diplococci. Subsequently, the samples were systematically seeded on Bile-Esculin medium by the quadrant method. The purpose of this medium is to differentiate *Enterococci* from streptococci. Bile tolerance and esculin hydrolysis as presumptive means of identification of *Enterococci* are well recognized. The reaction is positive for bile tolerance and esculin hydrolysis if there is darkening of the medium. Colonies identified as positive underwent a control gram and purification on fresh blood agar for identification using ApiSTREP.

2.2.2. Identification of Enterococcus spp

We produced Mac Farland 4 inocula from the pure colonies. The inoculum was distributed in the gallery. The Api20 STREP gallery contains 20 micro-tubes with dehydrated substrates for the detection of enzymatic activities or sugar fermentations. The enzyme tests are inoculated with a dense suspension that rehydrates the substrates. The reactions produced are reflected by spontaneous color changes or are revealed by the addition of reagents. Fermentation tests are inoculated with an enriched medium (containing a pH indicator) that rehydrates the sugars. Fermentation of the carbohydrates leads to acidification, which results in a spontaneous change in the color indicator. The reading was made using the web API following the results of the biochemical reactions.

2.2.3. Study of resistance to antibiotics

The technique used was the method of diffusion of discs on agar medium. Strains susceptibility testing was done on Mueller Hinton media following the technique recommended by CA-SFM 2020. The agars were inoculated using a sterile loop from a standardized bacterial suspension on a scale of 0,5 Mac Farland (an optical density equal to 0.2 at 650nm) in recommendations of the CA-SFM 2020. Discs of antibiotics were deposited on the surface of the agar using sterile metal forceps. The antibiogram was read by the zones of inhibition using a caliper. The results were compared to the critical values and the bacteria were classified as sensitive, intermediate or resistant according to CA-SFM 2020 standards.

2.3. Data processing and analysis

The data was collected, processed by Excel software (2010 version) and statistical analyzes by SPSS. The results were presented in the form of tables and graphs.

3. Results

35 men and women took part in this study. The results obtained were distributed as follows

3.1. Distribution of the study population by gender and age

The female sex 54.28% (19/35) was the most representative in our study population, with age groups of 1-10 years and 20-30 years respectively of 28.6% and 25.7%.

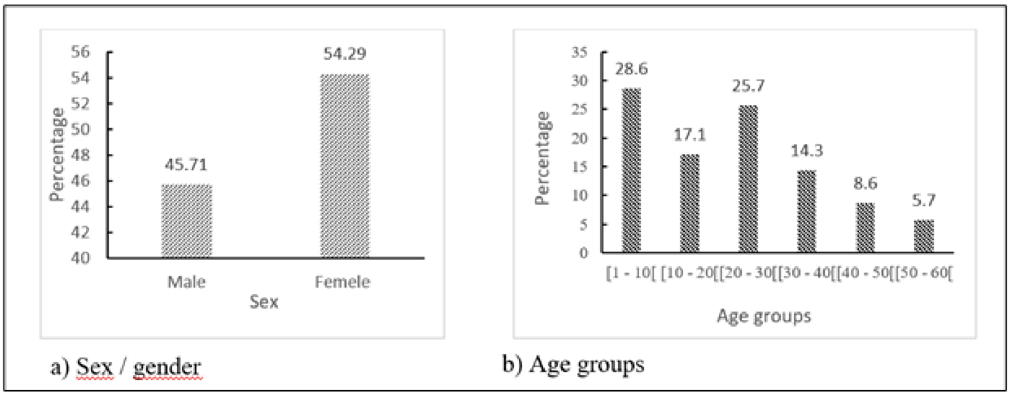

Figure 1 Distribution of the population by gender (a) and age (b)

3.2. Distribution of germs isolated in the study population

We isolated 19 bacterial strains (Table 1), among which 9 strains were *Enterococci* that is a frequency of 47.3% (9/19) and 10 strains were non-*Enterocococci* (*Aerococcus viridans* with 31.5%, *Streptococcus mitis 1* with 10.5%, *Streptococcus mitis 2* with 10.5%.) with a frequency of 52.6% (10/19) The overall frequency of isolated *Enterococci* was 25.71% (9/35), with 77.7% (7/9) *E. faecium* and 22.3% (2/9) *E. faecalis* figure 2.

Table 1 Germs isolated

Germs (n=19)		Effective	Percentage
Enterococci (n=9)	*Enterococcus faecium*	7	36.9
	Enterococcus faecalis	2	10.5
non-*Enterocococci* (n=10)	*Aerococcus viridans*	6	31.6
	Streptococcus mittis 1	2	10.5
	Streptococcus mittis 2	2	10.5

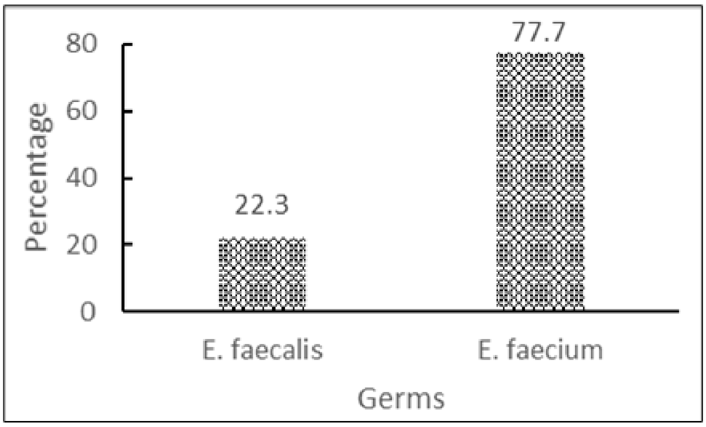

Figure 2 Frequency of *Enterococcus species*

3.3. Frequency of positive cultures by gender and age groups

The age groups of 1-10 years with a frequency of 31.5%; those of 20-30 years with a frequency of 26.31% and the female sex (52.63%) are the most exposed (Figure 3a and 3b). nevertheless, there is no significant difference in the isolation of *Enterococci* with age (*P= 0.353*) and sex (*P = 0.836*).

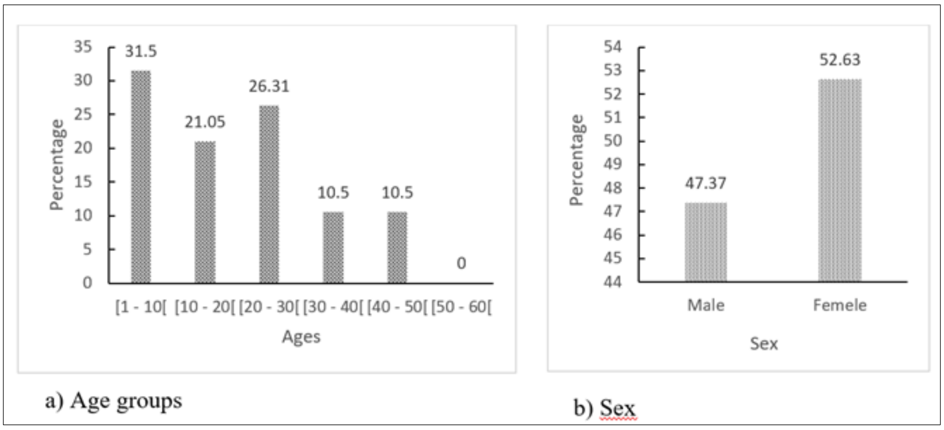

Figure 3 Positive culture by age (a) and sex (b)

3.4. Antibiotic resistance of *Enterococcus species* isolated from the intestinal tract in patients admitted to the Yaounde Military Hospital

The resistance was pronounced on the strains of *Enterococci* isolated. The resistance was strictly above 40% on all families of antibiotics tested with a resistance of 100% in aminoglycosides.

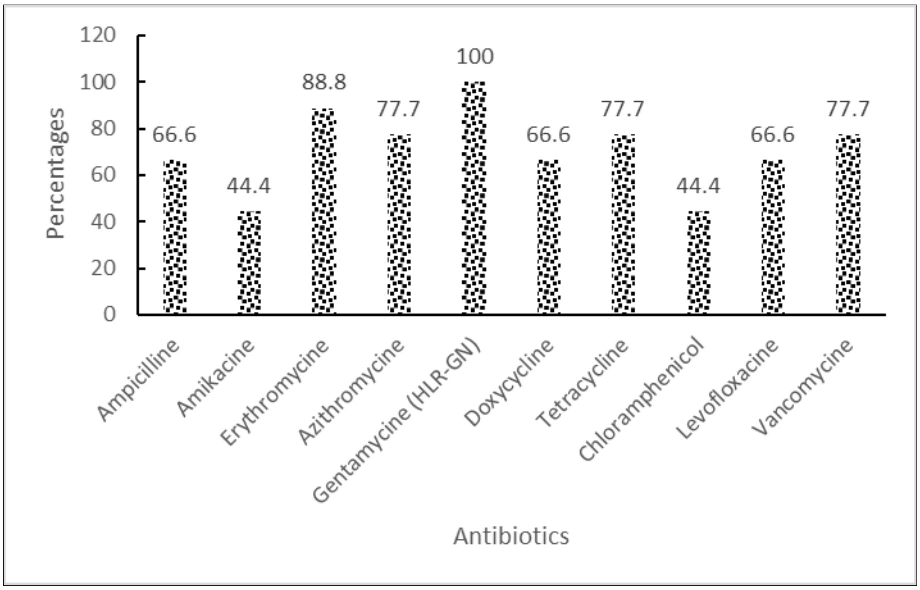

Figure 4 Resistance profile of isolated *Enterococci*

3.4.1. Resistance profile of Enterococcus faecalis

Resistance of *E. faecalis* was observed at 50% for Ampicillin, 100% for Gentamicin and 50% for Vancomycin (Figure 5).

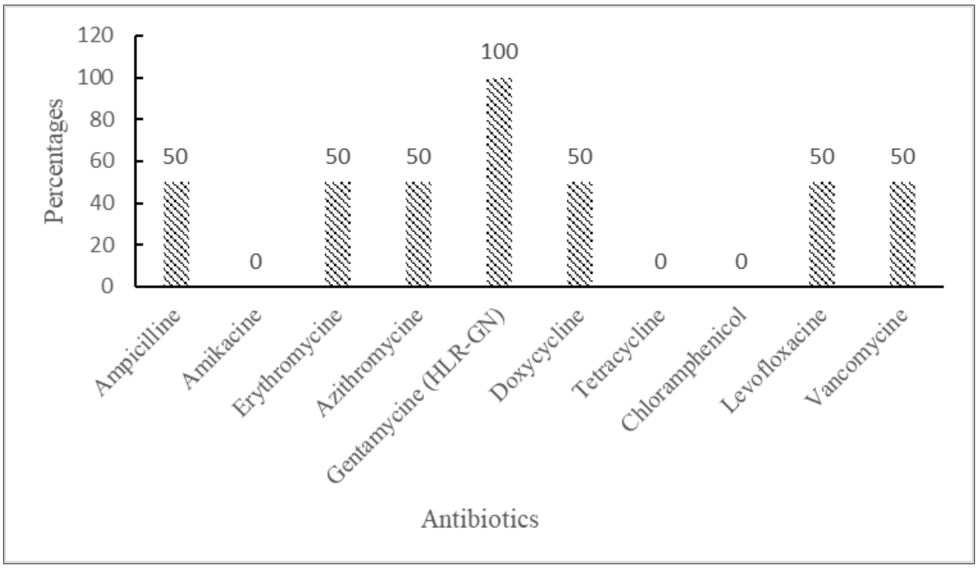

Figure 5 Resistance of *Enterococcus faecalis*

3.4.2. Resistance profile of Enterococcus faecium

E. faecium showed resistance strictly above 50% for all antibiotics tested. It is 71.4% for Ampicillin, 100% for Gentamicin and 85.7% for Vancomycin (figure 6).

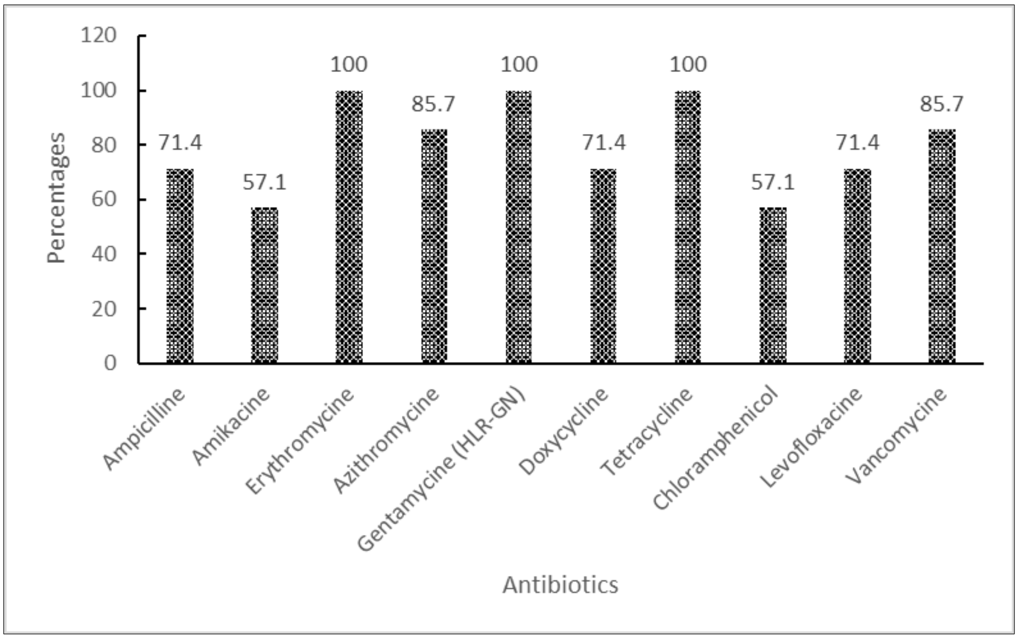

Figure 6 Resistance of *Enterococcus faecium*

3.5. Frequency of exposure to antibiotics

Of the 35 subjects, the history of exposure to one or more antibiotics was up to 68.5% (figure 7).

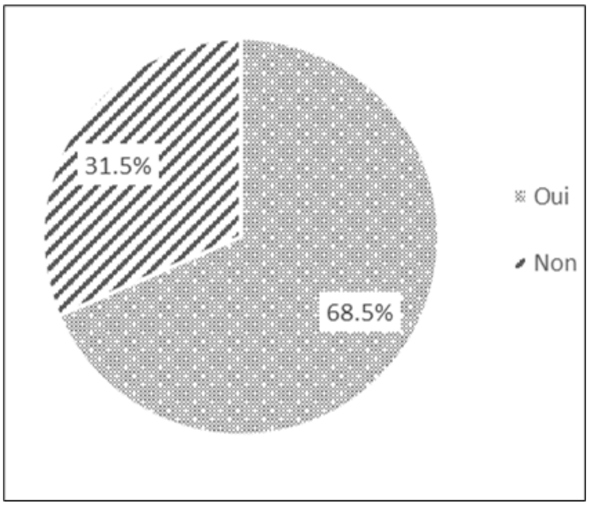

Figure 7 Antibiotic exposure

3.6. Multidrug resistance in Enterococci

Multidrug resistance to the families of antibiotics presented in Table 2, ranged from R3 (94.73%) to R7 (10.5%). It was more observed in *E. faecium* up to R7 (10.5%).

Table 2 Multiresistance model of *E. faecalis* and *E. faecium*

Level of MDR	Resistance characteristics	Eff of *E. faecium* with resistance pattern	Eff of *E. faecalis* with resistance pattern	Total MDR (%)
R3	AMP, HLR-GN, VAN	4		18 (94.73%)
	AMP, HLR-GN, DOX	3	1	
	AMP, HLR GN, LEV	3		
	AMP, HLR GN, AMK	4		
	AMP, HLR-GN, CHL	3		
R4	AMP, HLR-GN, VAN, LEV	3		16 (84. 21%)
	AMP, HLR-GN, VAN, DOX	3		
	LEV, VAN, DOX, HLR-GN	4		
	LEV, VAN, DOX, AMP	2		
	AMP, HLR-GN, AMK, DOX	2		
	AMP, HLR-GN, AMK, CHL	2		
	AMP, HLR-GN, AMK, LEV	2		
	AMP, HLR-GN, AMK, VAN	2		
	CHL, LEV, VAN, AMK	2		
R5	AMP, HLR-GN, VAN, LEV, DOX	2		12 (63.15%)
	AMP, HLR-GN, VAN, LEV, AMK	2		
	LEV, VAN, DOX, AMP, AMK	2		
	LEV, VAN, DOX, HLR-GN, AMP	2		
	LEV, VAN, DOX, HLR-GN, CHL	2		
	AMP, HLR-GN, AMK, DOX, VAN	2		
R6	AMP, HLR-GN, VAN, LEV, DOX, AMK	2		6 (31.57%)
	AMP, HLR-GN, VAN, LEV, AMK, CHL	2		
	AMP, HLR-GN, VAN, LEV, CHL, DOX	2		
R7	AMP, HLR-GN, VAN, LEV, DOX, AMK, CHL	2		2 (10.5%)

Legend: MDR: Multidrug resistance, R3: resistance to three antibiotics, R4: resistance to four antibiotics, R5: resistance to five antibiotics, R6 resistance to six antibiotics, R7: resistance to seven antibiotics, AMP: Ampicillin, HLR-GN: Highly resistant to gentamycin, TE: Tetracycline, AMK: Amikacin, CIP: Ciprofloxacin, CHL: Chloramphenicol, ERY: Erythromycin, VA: Vancomycin

The combined resistance to gentamycin and Ampicillin in *E. faecium* isolates was higher than that of *E. faecalis* (71.4 vs. 50%), Table 3.

Table 3 Combined aminoglycoside and Ampicillin resistance in *E. faecium* and *E. faecalis*

	Number (%) of combined antibiotics found with phenotype		
Isolated germs	HLR-GN	AMP	HLR-GN, AMP
E. faecium (7)	7 (100 %)	5 (71.4 %)	5 (71.4 %)
E. faecalis (2)	2 (100 %)	1 (50 %)	1 (50 %)

4. Discussion

Following the rapid emergence of resistance to *Enterococci*; the increasing incidence of colonization and infection; vancomycin resistant *Enterococci* (VRE) have become a real health problem which is today a serious concern for doctors and health authorities [9]. The objective of this study was to determine the resistance profile of strains of *Enterococcus spp* isolated from the intestinal tract of patients coming to the laboratory of the Yaounde Military Hospital. Of the 35 participants who constituted our study population, the female sex was in majority (54.28%) with a sex ratio M/F of 0.8. This observation could be linked on the one hand to the frequentation of hospitals by women because for men it is difficult to go to the hospital: and on the other hand, to the predominance of the female sex in the Cameroonian population. The most exposed age group is that of 1-10 years with a frequency of 31.5%. This result can be explained by the fact that their immune system is not yet completely immunocompetent, which makes them more at risk than elderly. These results are similar to those found by *Abamecha and al* (2015) [10]. On the other hand, we can report that a large proportion of invasive infections caused by multidrug resistant *E. faecium* was found. Nosocomial enterococci, but also resulted in the partial replacement of *E. faecalis* by *E. faecium* as the cause of nosocomial infections. [9] Indeed, several studies have shown an increase in the proportion of nosocomial enterococcal infections caused by *E. faecium* [11, 4]. Concerning our study, the isolates obtained were *E. faecium* with 36.9% followed by *E. faecalis* with 10.5%. This species distribution is comparable to the distribution of enterococci species in other studies such as that of *Maschieto and al* (2004) or *Gonsu and al* (2015) [12, 4]. Beside this aspect, we can also note that in this study, enterococcal isolates were predominant (47.4%), which corroborates with the report from India which described 81% of blood isolates as *E. faecium* [13] or the study conducted in Singapore who also reported an increase in *E. faecium* of 78.9 to 91.8% over a 5-year period from 2006 to 2010 from clinical cultures [14].

Regarding the resistance of *Enterococci* to commonly used antibiotics, *Enterococci* isolates have intrinsic resistance relative to penicillin and ampicillin. Furthermore, *E. faecium* is less susceptible to β-lactamins than *E. faecalis* because their penicillin binding proteins (PBPs) have lower affinities for these antibiotics and some strains have a plasmid-encoded β-lactamase. [15] The study, shows an Ampicillin resistance rate of 50% of *E. Faecalis* isolates, which is higher than the resistance rates of 0-8.3% observed respectively in Kuwait, Hong Kong and Brazil [16, 17, 18] and comparable to the rates of 60.7% and 66%, in Gaza [19] which sufficiently shows that the resistance is growing over the years. Ampicillin resistance rates were observed at 71.4% in isolated *E. faecium*, that is more than 31.4% of resistance rates reported in Hong Kong [16] and 66.7% in Gaza [19]. However, a resistance rate comparable to ours of 82% has been reported in Iran [20]. The higher prevalence of resistance to β-lactam antibiotic in patients here could be due to the phenomenon of selection pressure. As for resistance to aminoglycosides, they are frequently used in combination with paro-active antibiotics for severe enterococci infections [9]. Enterococcal resistance to gentamicin occurs by different mechanisms.

It is therefore important to test sensitivity to this agent. Gentamicin resistance is a good predictor of resistance to other aminoglycosides except streptomycin [15]. Although high-level aminoglycosides resistance (HLAR) may be considered important for severe infections, we determined high-level resistance in *enterococci* species *faecalis* and *faecium* in *enterococci* isolates from fecal samples in patients. The study reveals a high-level resistance to gentamicin (100%) of enterococcal isolates. These results are higher than the resistance rate reported from Kuwait and Saudi Arabia [17, 21] and comparable to those conducted by Mahto and Ngandjio (2010) [22] in Cameroon. Of the 35 subjects, 68.5% had a history of exposure to one or more antibiotics. This phenomenon is explained by the fact that before coming to the hospital, patients practice significant self-medication and the hospital is for the most part a last resort. These results are similar to those of *Abamecha et al* (2015) [10] who found a medication rate of 92.7%. Clinically, aminoglycosides and betalactam antibiotics are most often used in invasive enterococcal infections. In our study, *E. faecium* showed resistance to seven antibiotics (Table 2). Concomitant resistance to aminoglycosides (HLAR) and β-lactam (Ampicillin) was high; 50% of *E. faecalis* and 71.4% of *E. faecium* strains (Table 3). This finding is of great concern, because the synergistic activity of the combination of β-lactam antibiotics with HLAR in the treatment of enterococcal infections is proscribed. Also, controlling the spread of these organisms becomes a real challenge. Indeed, due to the high prevalence of concomitant resistance to aminoglycosides and beta-lactams, attempts have been made to search for alternative antibiotics in different studies. Fluoroquinolones have been among the dominant classes of antimicrobial agents in the last decade and are widely used for nosocomial infections empirically [23].

Regarding our study, 66.6% of 1 enterococcal isolates were resistant to levofloxacin. Other alternative antibiotics to treat *Enterococcus* infection also showed high rates of resistance in vitro: erythromycin (88.8% of resistance), tetracycline (77.7%), chloramphenicol (44.4%) and Amikacin (44.4%). This agrees with the data obtained by Mahto (2010) [22] and Arias and Murray (2012) [9] in their work. This sufficiently demonstrates the importance of the use of antibiotics of last resort in the treatment of enterococcal infections when conventional antibiotics are no longer effective. Vancomycin is considered an antibiotic of last resort.

It has always been used in the treatment of *enterococci* resistant to several antibiotics. However, by dint of ultimately use, this antibiotic has lost its effectiveness at the bridge of becoming very resistant at times. Thus, the emergence of VRE (Vancomycin Resistant Enterococci) is also due to the inappropriate use of antibiotics. As our results showed, 6 strains of *E. faecium* (85.7%) were resistant to vancomycin and 1 strain of *E. faecalis* (50%) was resistant to vancomycin, that is a total of 77.7% of the enterococcal population. This shows us the high proportion of resistance to this antibiotic today. These results are superior to those reported by *Coleri, A. and al* (2004) [24]. The possible reason for the emergence of VRE observed in these patients can be explained by the fact that in our study we had to observe that 68.5% of the patients coming to the enude were taking medication. Thus, the repeated use of medication could have created a selection pressure for antibiotics due to the uncontrolled taking of drugs and self-medication of these patients, which could have made the *enterococci* resistant, these being commensal of the digestive tract.

5. Conclusion

Very early on, bacteria developed many forms of resistance to antibiotics leading to treatment failures. It is in this sense that we have recently witnessed the emergence of strains of enterococci resistance to vancomycin in many countries such as Cameroon. It emerges at the end of our study, that *E. faecium* and *E. faecalis* are the most isolated *enterococci*. We have been able to observe that the proportion of *enterococci* resistance to Vancomycin is very high. This therefore constitutes a real public health problem in our country, in view of the broad spectrum of vancomycin. We must therefore take advantage of the experience of industrialized countries to reduce or curb this scourge and this by the rational use of antibiotics and epidemiological monitoring studies of multi-resistant bacteria.

Compliance with ethical standards

Acknowledgments

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Disclosure of conflict of interest

The authors declare that they have no conflicts of interests.

Statement of ethical approval

We have obtained the authorization of the national Ethics Committee for Human Health Research. N° 2020/06/68/CE/CNESRH/SP.

Statement of informed consent

Informed consent was obtained from all individual participants included in the study.

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