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Antibiotic Resistance Profile of *Enterococcus faecalis* and *Enterococcus faecium* Isolates from Urine and Pleural Fluid in Two Hospitals of Cameroon

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Abstract

Enterococcus faecalis and *Enterococcus faecium* rank among the leading causes of nosocomial bacteremia and urinary tract infections. They often persist on hospital surfaces due to their ability to withstand adverse environmental conditions (low or high temperatures, high pH, and high salinity). The global *Enterococcus faecalis-Enterococcus faecium* ratio is currently shifting towards *Enterococcus faecium*. *Enterococci* present variable levels of resistance to certain families of antibiotics. This is the case for aminoglycosides, beta-lactams and cephalosporins. In 2017, WHO ranked *Enterococci* among priority pathogens for research and development of new antibiotics. The objective of our study was to determine the antibiotic resistance profile of *Enterococcus faecalis* and *Enterococcus faecium* isolates from urine and pleural fluid in two hospitals in Cameroon. This cross-sectional and analytic study was carried out between June to August 2023 on hospitalized and day patients in which a cytobacteriological test of urine and pleural fluid was done. The samples were inoculated on CLED Agar for urine and on Chocolate + polyvitex and blood agar (prepared from Columbia agar) for pleural fluid samples and incubated at 37˚C for 18 to 24 hours. Identification of isolates was carried out using the API 20 STREP micro gallery (Biomerieux, France) and tested for antimicrobial susceptibility. The data on socio-demographical and

potential risk factors were recorded using self-administered questionnaires and data from laboratory analyses of the specimen were collected in a data capture sheet. Potential risk factors associated with the presence of *Enterococci*, were evaluated using the logistic regression in univariate and multivariate analysis. P value < 0.05 was considered as significant. A total of 511 patients were recruited who were predominantly females. *Enterococcus spp* were isolated in 27.79% of our samples with *Enterococcus faecalis* mostly encountered. *Enterococcus* spp showed a high level of resistance to penicilline (99.3% to Ampicilline), macrolides (66.2% to Erythromycin) and cyclines (85.2% to Doxycycline). Hospitalisation, access to health facilities, contact with urine specimen and hand hygiene practices were risk factors related to infection with *Enterococcus spp* while hospitalisation, health facility and hand hygiene were related to glycopeptide resistant *Enterococcus*. Strict compliance with hygiene rules and appropriate antibiotic consumption could help in the fight against these infections.

Keywords

Enterococcus faecalis, *Enterococcus faecium*, Healthcare-Associated Infections, Antimicrobial Resistance, Cameroon

1. Introduction

Enterococci are facultative anaerobic Gram-positive bacteria, immobile and non-encapsulated, present in the digestive tract of animals and humans [1]. Some species are useful in the manufacture of dairy products, playing a role in the conservation and bacteriological quality of food while maintaining their nutritional and organoleptic properties. But others are often opportunistic and cause infections in adults and newborns especially *Enterococcus faecalis* and *Enterococcus faecium* which rank among the leading causes of nosocomial bacteremia and urinary tract infections [2] [3]. They often persist on hospital surfaces due to their ability to withstand adverse environmental conditions (low or high temperatures, high pH, and high salinity). The global *Enterococcus faecalis—Enterococcus faecium* ratio is currently shifting towards *Enterococcus faecium*. The acquired antibiotic resistance traits of this latter species can explain this evolution [4].

The advent of antibiotic therapy in the 1940s allowed a significant evolution in the management of infectious diseases. Unfortunately, antimicrobial resistance has quickly become a global health problem [5]. Enzymatic inhibition, reducing membrane permeability, modification of antibiotic targeted binding sites and the production of efflux pumps are some of the mechanisms used by organisms to fight against antibiotics. Some of the main reasons for antimicrobial resistance are self-medication, incorrect use of antibiotics (wrong dosage and prolonged use), lack of standards for health workers and misuse in animal husbandry [5].

Enterococci present variable resistance levels to some antibiotics. This is the

case for aminoglycosides, beta-lactams and cephalosporins [6]. In 2017, WHO ranked *Enterococci* among priority pathogens for research and development of new antibiotics. In America, a study carried out by Faron *et al.*, in 2016 indicated that the prevalence of vancomycin-resistant *Enterococci* (VRE) over the past decade was increasing [7]. In 2015 Abamecha *et al.*; in Ethiopia evaluated the resistance profile of *Enterococci* and demonstrated that 36% of the isolated strains were resistant to ampicillin, 54.4% to streptomycin and 34.2% to gentamycin [8]. While in Algeria, Benzaid *et al.*, in 2022 reported a prevalence of 7.81% of nosocomial infections due to *Enterococcus faecalis* [9]. In Cameroon, a study conducted in 2015 by Gonsu *et al.*; on the antibiotic sensitivity profile of *Enterococci* showed that 94% of *Enterococcus faecalis* strains were resistant to erythromycin, 59% to trimethoprim + sulfonamides and 53% to tetracycline. They stated that the majority of strains were isolated from 72% of hospitalized patients, and 27.58% of non-hospitalized patients [10].

However, few studies have been carried out in Cameroon to fully understand the burden of antibiotic resistance as a result of *Enterococci* in hospital acquired infections. Therefore, the objective of our study was to determine the antibiotic resistance profile of *Enterococcus faecalis* and *Enterococcus faecium* isolates from urine and pleural fluid in two hospitals of Cameroon.

2. Materials and Methods

2.1. Study Duration and Location

A cross-sectional descriptive, laboratory and analytical study was carried out between June to August 2023 in two public hospitals; One in the Centre region (Saint Porres Dominican Hospital Center) and the other in Littoral region (Laquintinie hospital of Douala). Sampling and analysis was done in the laboratories of both hospitals.

2.2. Sampling Method and Study Population

Our study population included all hospitalized and non-hospitalized patients which a cytobacteriological test of urine and pleural fluid were done. The minimum sample size was obtained using the Lorentz formula. Data related to potential risk factors were collected using a questionnaire.

2.3. Sample Analysis

The samples were inoculated on CLED Agar for urine and on Chocolate + polyvitex and blood agar (prepared from Columbia agar) for pleural fluid samples and incubated at 37˚C for 18 to 24 hours. Identification of isolates was carried out using the API 20 STREP micro gallery (Biomerieux, France) and tested for antimicrobial susceptibility.

2.4. Antibiotic Susceptibility Testing

All isolates of *Enteroccocus spp* confirmed were inoculated using a bacterial in-

oculum (following the 0.5McFarland standard) on Müeller-Hinton medium and incubated for 24 hours at 37˚C in order to test their sensitivities to different families of antibiotics using the agar diffusion method. The interpretation was made according to the Antibiogram Committee of the French Society of Microbiology (CA-SFM-2023 v.1.0). We used 11 different antibiotic discs per strain: Ampicillin (2 µg), Gentamicin (30 µg), Vancomycin (5 µg), Teicoplanin (30 µg), Doxycycline, cotrimoxazole (1.25/23.75 µg), Erythromycin (15 µg), Streptomycin (300 µg), Norfloxacin (10 µg), Rifampicin (5 µg), and Imipenem (10 µg). The detection of *Enteroccocus* isolates resistant to glycopeptides was done by observing the reduced inhibition diameter around the Vancomycin and Teicoplanin disks.

2.5. Data Evaluation and Analysis

The data on socio-demographical and potential risk factors were recorded on the Microsoft Excel 2016 software and analysed using Epi info version 7.0. The results were expressed using descriptive statistics and associated to their confidence interval at 95%. The research of potential risk factors associated to the presence of Enterococci, was done using the logistic regression in univariate and multivariate analysis. P value < 0.05 was considered as significant.

2.6. Ethical Considerations

An ethical clearance was obtained on the basis of the evaluation and validation of the research protocol by the Institutional Ethics Committee of Université des Montagnes (Autorisation N˚ 2023/176/UdM/PR/CEAQ). Free and informed consent of patients were obtained and sample collection authorizations were equally obtained from the two hospitals.

3. Results

3.1. Sociodemographic Characteristics of the Study Population

A total of 511 patients made up of (63.99%). females were recruited for the study. The majority 90.22% of the patients were not hospitalised and 80.63% had not consumed antibiotics for at least two weeks. Our specimen were mostly made up of wound (67.71%) (**Table 1**).

3.2. Distribution of *Enterococcus spp* Infection among Patients

Enterococcus spp were isolated in 27.79% of our samples with *Enterococcus faecalis* mostly encountered (**Figure 1**).

3.3. Antibiotic Resistant Pattern of *Enterococcus spp* Isolates

3.3.1. Antibiotic Susceptibility Testing

Enterococcus spp showed high level of resistances to penicilline (99.3% to Ampicilline), macrolides (66.2% to Erythromycin) and cyclines (85.2% Doxycycline) (**Figure 2**). Eleven antibiotics were tested following EUCAST 2023.

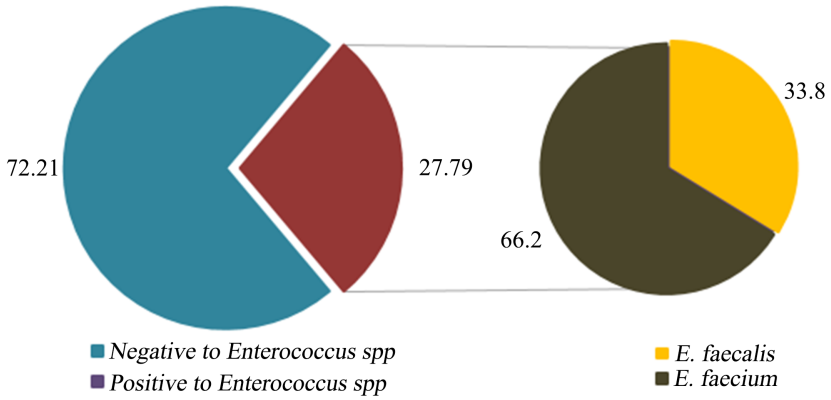

Figure 1. Distribution of *Enterococcus spp* infection among patients.

Table 1. Distribution of the study population according to their socio-demographic characteristics.

Variable	Categories	Frequency	
		n	%
Age (years)	<30	127	24.85
	[30 - 49[	221	43.25
	≥50	163	31.90
Sex	Female	327	63.99
	Male	184	36.01
Scholar level	Primary	16	3.13
	Secondary	145	28.38
	University	350	68.49
Marital status	Single	262	51.27
	Married	249	48.73
Health facility	Laquintinie	252	49.32
	CHDSMP	259	50.68
Occupation	Student	142	27.79
	Worker	369	72.21
Hospitalised	No	461	90.22
	Yes	50	9.78
Specimen	Blood	9	1.76
	Wound	346	67.71
	Urine	156	30.53
Antibiotic consumption ≤2 weeks	No	412	80.63
	Yes	99	19.37
Hand hygiene	No	127	24.85
	Yes	384	75.15
Total		**511**	**100**

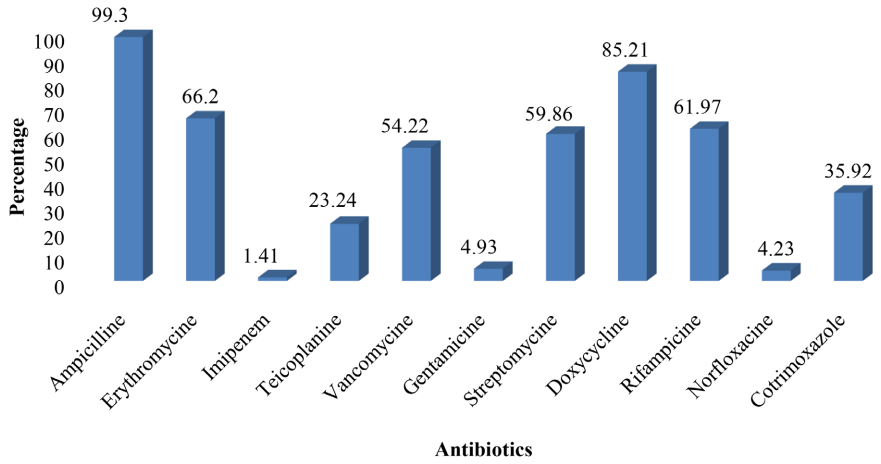

Figure 2. Antibiotic susceptibility test of *Enterococcus spp.*

3.3.2. Prevalence of Glycopeptide Resistant *Enterococcus*

A few 6.65% of *Enterococcus spp* isolates were resistant to Glycopeptide (**Figure 3**).

3.4. Analysis of Risk Factors Related to Infection with *Enterococcus spp*

Risk factors related to infection with *Enterococcus* spp were sought with a 95% confidence interval. P-values were significant for values less than 0.05 (**Table 2**).

Hospitalization, Health facility, type of urine specimen and hand hygiene were risk factors related to infection with *Enterococcus spp.*

3.5. Univariate Analysis of Risk Factors to Glycopeptide Resistant *Enterococcus spp* Infection

Risk factors to glycopeptide resistant *Enterococcus* spp were sought with a 95% confidence interval. P-values were significant for values less than 0.05 Hospitalization, health facility and hand hygiene were risk factors related to glycopeptide resistant *Enterococcus* spp (**Table 3**).

4. Discussion

The current study highlights the significance of the fight against antibiotic resistance, contribute to the care of patients suffering from multi-resistant bacteria and reduce nosocomial infections. The study revealed a high frequency of *Enterococcus faecium* mostly encountered as compared to *Enterococcus* infection with significant resistance to 11 antibiotics.

The average age of the participants in the current study was 35 years given that the African population is mainly made up of young people. Furthermore, our participants were predominantly women (66.99%) than men (36.01%). This is explained by the fact that women are more exposed to urinary infections due to the anatomical particularities of the urinary system; their urethra is shorter, making it easier for bacteria to enter the bladder [11].

Figure 3. Prevalence of glycopeptide resistant *Enterococcus* spp.

Table 2. Univariate analysis of risk factors related to *Enterococcus spp* infection.

Variables		GRE n (%)	OR (CI95%)	P-value
Age (years)	<30	10 (7.87)	1	Ref
	[30 - 49[	17 (7.69)	0.97 (0.43 - 2.20)	0.95
	≥50	7 (4.29)	0.52 (0.19 - 1.42)	0.20
Sex	Female	20 (6.12)	1	Ref
	Male	14 (7.61)	1.26 (0.62 - 2.57)	0.52
Scholar level	Primary	0 (0.00)	-	-
	Secondary	12 (8.28)	1	Ref
	University	22 (6.29)	0.74 (0.36 - 1.54)	0.43
Marital status	Single	17 (6.49)	1	Ref
	Married	17 (6.83)	1.06 (0.53 - 2.12)	0.88
Professional category	Student	8 (5.63)	1	Ref
	Worker	26 (7.05)	1.27 (0.56 - 2.87)	0.57
Hospitalised	No	23 (4.99)	1	Ref
	Yes	11 (22.00)	5.37 (2.44 - 11.83)	**0.00**
Health facility	Laquintinie	9 (3.57)	1	Ref
	CHDSMP	25 (9.65)	2.88 (1.32 - 6.31)	**0.01**
Specimen	Blood	0 (0.00)	1	Ref
	Wound	17 (4.91)	1	Ref
	Urine	17 (10.90)	2.37 (1.17 - 4.77)	**0.01**
Antibiotic consumption ≤2 weeks	No	27 (6.55)	1	Ref
	Yes	7 (7.07)	1.08 (0.46 - 2.57)	0.85
Hand hygiene	Yes	19 (4.95)	1	Ref
	No	15 (11.81)	2.57 (1.27 - 5.23)	**0.01**
Specie	*E. faecalis*	14 (29.17)	1	Ref
	E. faecium	20 (21.28)	0.66 (0.30 - 1.45)	0.30

Table 3. Univariate analysis of risk factors to glycopeptide resistant *Enterococcus spp* infection.

Variables		Adj OR (CI 95%)	P-value
Hospitalised	No	1	Ref
	Yes	4.44 (1.97 - 9.96)	**0.00**
Health facility	Laquintinie	1	
	CHDSMP	2.38 (1.07 - 5.31)	**0.03**
Specimen	Wound	1	Ref
	Urine	1.40 (0.64 - 3.05)	0.40
Hand hygiene	Yes	1	Ref
	No	2.18 (01.05 - 4.53)	**0.04**

The *Enterococcus spp* were isolated in 27.79% of our samples. This result is higher than the 8.44% found by Akhter *et al.* in Tunisia in 2014 in which they isolated *Enterococci* from urine only [12]. *Enterococci* ranks among the leading bacteria responsible for urinary tract infection [13] [14].

In the current study, *E. faecium* predominates over *E. faecalis* (66.2%); these results are similar to those of Gonsu *et al.*; who also demonstrated the predominance of 52% of *E. faecium* [10]. Recent studies have demonstrated that the worldwide ratio of *Enterococcus faecalis—Enterococcus faecium* is changing in favor of *E. faecium* due to the Intrinsic and acquired antimicrobial resistance traits of this species [15] [16].

The antibiotic susceptibility testing of the current study revealed of a strong resistance to penicillins, tetracyclines, and macrolides. This is similar to that of Muylaert and Mainil who reported this resistance to macrolides, tetracycline betalactams. Intrinsic resistance to beta lactams is maintained in *Enterococci* through the overproduction of penicillin binding proteins. Concerning aminoglycoside resistance, the low permeability of the well to the large aminoglycoside molecules could explain the moderate resistance [17]. Furthermore, 6.65% of *Enterococcus spp* isolates were resistant to Glycopeptide. Resistance to glycopeptides is mainly the result of the presence of resistance genes carried by plasmids. The most common being vanA, vanB and vanC [18].

The univariate analysis of risk factors in our study allowed us to highlight the main factors linked to infection due to *Enterococcus spp* such as hospitalization (OR = 5.37; P-value = 0.00), hand hygiene (OR = 2.57; P-value = 0.01) and the specimen type (OR = 2.37; P-value = 0.01). Our results corroborate with a similar study carried out by Stucki *et al.* in Switzerland in 2014 [19]. These values could be explained on the one hand by the fact that when hygiene and environmental conditions are not favorable, the promiscuity of patients in hospital rooms favors transmissible infections; during their stay at the hospital, patients are exposed to bacteria through several sources including infected patients especially when hygiene rules are not respected [20]. On the other hand, the length

of stay hospital is also a factor because the more a hospitalized person is bedridden, the more they are exposed to the risk of infections due to *Enterococcus spp* [21]. These factors were equally linked to glycopeptide resistant *Enterococcus*.

5. Conclusions

Our study revealed that the frequency of *Enterococcus* infection was 27.79% with *Enterococcus faecium* mostly encountered (66.2%). Significant resistance to penicillin (99.3% Ampicillin), macrolides (66.2% Erythromycin) and cyclines (85.2% Doxycycline) were observed. Equally, 6.65% of *Enterococcus spp* isolates were resistant to Glycopeptide.

Risk factors related to infection with *Enterococcus spp* were related to the type of health facility, urine specimen and hand hygiene practices while risk factors related to infection with *Enterococcus spp* were hospitalisation, health facility and hand hygiene being related to glycopeptide resistant *Enterococcus*.

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Conflicts of Interest

Authors declare no competing interests.

Authors' Contributions

Conceptualization: CID; Project administration: CID, and AMA; Resources: CID, LPM, EMNM, NMN; Methodology: CID, FW, GYK, CSN, CPK, LPM, EMNM, NMN and AMA; Investigation: FW, GYK, CSN, CPK, LPM, EMNM, NMN, HKT, MJTT, and YVYM; software: CID, and CSN; Formal analysis: CSN and FW; Visualization: CID, and AMA; Writing-original draft: CID, FW, GYK, and CPK; Writing-review and editing: CID, FW, and CSN; Validation: CID, and AMA; Supervision: AMA; Data curation: CID. All authors have read and agreed to the published version of the manuscript.

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