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Antibiotic Susceptibility of *Enterococcus Spp* Strain Isolated from Urine Culture At the Centre Hospitalier Universitaire Pédiatrique Charles De Gaulle in Ouagadougou, Burkina Faso

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Abstract: Poorly documented data on infections caused by *Enterococcus spp* in Africa, especially south of the Sahara, and the increasingly growing demand for Cytobacteriological Examination of Urine (ECBU) have made necessary to carry out this study. The study aimed to investigate the epidemiological and bacteriological aspects of *Enterococcus spp* strains isolated in urine from 2015 to 2017 at CHUP-CDG, in order to contribute to better care. The distribution of the infected participants according to age has shown that children aged from 1 month to 1 year old were the most representative (37.9%). Regarding the sex, the majority of the infected people were female (66.7%). The distribution of urine analyzed by year has shown that from 2015 to 2017 the number of analyzed samples increased. The lowest number was recorded in 2015 (24.4%) and the highest number was recorded in 2017 (41.9). The study results showed that the prevalence of *Enterococcus spp* increased between 2015 and 2017 from 1.9 to 3.1 respectively. The majority of the participants (61.4%) infected by *Enterococcus spp* were hospitalized at CHUP-CDG. The outpatients represented 38.6% of the participants. Among the hospitalized patients, the majority came from infant's unit accounting for 38.3% of the overall participants. The lowest number were recorded in the emergency and oncology services with 1.2% for each clinical service. Most of these strains were sensitive to Vancomycin. We also found sensitivity to Nitrofurans, Chloramphenicol, Lincomycin and Pristinamycin. However, we saw a decrease in their sensitivity to Lincomycin in the same period.

Keywords: *Enterococcus spp, Antibiotics, susceptibility, Burkina Faso*

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1. Introduction

Enterococci are Gram-positive cocci (GPC) which appear in the form of diplococci or cocci in chains. They are facultative anaerobes, immobile and without a capsule. This family includes around thirty species which have long been classified in the genus *Streptococcus* in view of their similarities with group D streptococci. The two main species of clinical importance are *Enterococcus faecalis* and *Enterococcus faecium*, the first being found more frequently than the second (90% versus 10%) [1,2]. Regardless of the geographic area considered, the *Enterococcus* (E) genus remains one of the major causes of nosocomial infections [3,4]. It is involved in the occurrence of urinary infections, peritonitis, intra-abdominal abscesses or endocarditis [1,2]. Enterococci represent 10 to 15% of the microorganisms responsible of infectious endocarditis worldwide. These endocarditis generally have a subacute course with a high mortality of around 20% [5]. Its role in human pathology is now clearly established since a prospective study carried out in 2009 and focusing on the prevalence of infections in intensive care units in 75 countries around the world (1265 intensive care units/intensive care units included)

made it possible to highlight evidence that enterococci were well represented with approximately 10% of infections. *E. faecium* played a predominant role with nearly 4% of infections due to a strain of Vancomycin-resistant enterococci (VRE) [6]. This significant prevalence implies a worldwide dissemination of VRE strains adapted to the hospital environment [7]. In the United States, enterococci are the third bacterial genus isolated during bacteremia [8]; and the second cause of nosocomial infections [9]. It is also the genus most often cited in surgical wound infections in intensive care units [10]. In France, according to a recently published study covering 15.803 cases of nosocomial infections, enterococci were identified as responsible for 6.4% of cases, giving the fifth place [11]. Today, enterococcal infections pose serious public health problems linked to the intrinsic capacity of these microorganisms to resist different antibiotics [12]. These pathogens are endemic and ranked third among multi-resistant bacteria [13]. For many years, the constant increase in resistance of enterococci to first-line antibiotics has led many authors to evaluate the risk factors involved in *Enterococcus spp* bacteremia [14-23]. The propensity of enterococci has led to infections from medical equipment such as catheters, and has likely contributed to the increase in nosocomial infections. These materials are ideal supports for the formation of bacterial biofilms [24,25]. Other foreign materials (urinary catheter, parenteral nutrition, mechanical ventilation) have also been identified by certain studies [2,21,24] as risk factors, in particular mechanical ventilation which could be a poor prognosis factor [26]. The entry points for enterococcal bacteremia are mainly digestive, particularly in the context of surgical intervention [27]. The first strains of *E. faecium* exhibiting high-level resistance to glycopeptides (vancomycin and teicoplanin) were reported in France and the United Kingdom from 1987 to 1988, then in the United States from 1989 to 1990 [28,29]. Their monitoring appears necessary due to the emergence of enterococci resistant to vancomycin [30]. *E. faecalis* is the most represented species in the gastrointestinal flora of humans and exhibits much greater virulence and pathogenicity than other enterococcal species [31]. It is distinguished from other species of enterococci by its high resistance to antibiotics [32,33]. Due to its high resistance to antibiotics and its increased adaptability to the hospital environment, *E. faecium* has emerged over the past twenty years and presents an infection rate which tends towards that of *E. faecalis*, particularly in the United States [34,35].

In view of the findings listed above, poorly documented data in Africa, especially south of the Sahara, and the increasingly growing demand for Cytobacteriological Examination of Urine (ECBU) with the national policy of free healthcare for children. from 0 to 5 years old in Burkina Faso, we set out to study the epidemiological and bacteriological aspects of *Enterococcus spp* strains isolated in urine from 2015 to 2017 at CHUP-CDG in order to contribute to better care.

2. Material and Methods

Study design, site and period

This was a retrospective study with a descriptive aim ranging from January 1, 2015 to December 31, 2017. This work was carried out within the bacteriology unit of the medical biology laboratory (LBM) of the CHUP-CDG.

Study population and inclusion criteria

The study population consisted of all ECBU cases received during this period. All cases of ECBU positive for *Enterococcus spp* received at the LBM of the CHUP-CDG in children aged 0 to 14 years and for which the data were available. These patients were included in the current study.

Samples analysis and data collection

We carried out a rigorous census of cases of *Enterococcus spp* recorded in the LBM registers of the CHUP-CDG. Once in the bacteriology laboratory, the macroscopic appearance of the urine is noted. Inoculation was carried out on media such as Bromo cresol purple (BCP) or uriselect medium; and incubated at 35 - 37°C for 18 to 24 hours. Leukocytes and red blood cells were also counted using a Malassez cell. The following day, samples with high leukocyte counts and monomorphic bacterial flora were identified. Then, *Enterococcus spp* strains were Gram positive microorganisms with negative catalase. These pathogens were positive on bile esculin agar with their ability to grow in the presence of 6.5% sodium chloride. Antibiotics susceptibility testing was done using disk diffusion methods in accordance with the recommendations of the antibiogram committee of the French Microbiology Society (CA-SFM). The inhibition diameters of the antibiotic's discs tested make it possible to interpret bacterial strains as susceptible, intermediate or resistant.

Study variables

Age; sex; the requesting clinical service, the bacterial genus highlighted, the antibiogram were the main variables of the study.

Data processing and analysis

Data entry was done using Microsoft Word and Excel software. Statistical analysis was performed using SPSS (Statistical Package for Social Sciences, IBM corporation) version 25.

Ethical considerations

To carry out this study, we received authorization (N° 2019-1558) from the General Directorate of CHUP-CDG to collect data. The anonymity of the patients was respected during the processing of the data.

3. Results

Overall results

From January 1, 2015 to December 31, 2017, 5.283 urine samples were received and analyzed at the CHUP-CDG bacteriology laboratory for cytobacteriological examination. Of the 5.283 ECBU requests, 132 (2.5%) were positive for *Enterococcus spp*.

Distribution of participants infected with *Enterococcus spp* according to age and sex

The distribution of the infected participants according to age has shown that children aged from 1 month to 1 year old were the most representative (37.9%). Regarding the sex, the majority of the infected people were female (66.7%). The sociodemographic characteristics of the study participants are shown in Table 1.

Table 1. Sociodemographic characteristics of participants

Age group	Number	Frequency (%)
[0-1month [	14	10.6
[1M-1 year [	50	37.9
[1A-2 years [	25	18.9
[2A-5years [	27	20.5
[5A-14 years]	16	12.1
Total	132	100
Sex		
Male	44	33.3
Female	88	66.7
Total	132	100

Distribution of urine analyzed according to year

The distribution of urine analyzed by year has shown that from 2015 to 2017 the number of analyzed samples increased. The lowest number was recorded in 2015 (24.4%) and the highest number was recorded in 2017 (41.9). The distribution of the urines analyzes by year is shown in Table 2.

Table 2. Distribution of urine analyzed according to year

Year	Number	Frequency (%)
2015	1287	24.4
2016	1783	33.7
2017	2213	41.9
TOTAL	5283	100

Prevalence of *Enterococcus spp* infection according to year

The study results showed that the prevalence of *Enterococcus spp* increased between 2015 and 2017 from 1.9% to 3.1% respectively. The frequency of *Enterococcus spp* according to year is shown in Table 3.

Table 3. Frequency of *Enterococcus spp* strains by year

Year	Frequency (%)
2015	1.9
2016	2.1
2017	3.1

Distribution of participants by hospitalization status

The majority of the participants (61.4%) infected by *Enterococcus spp* were hospitalized at CHUP-CDG. The outpatients represented 38.6% of the participants. Among the hospitalized patients, the majority of the patients came from infant's unit accounting for 38.3% of the overall participants. The lowest number were recorded in the emergency and oncology services with 1.2% for each clinical service. The distribution of infected participants by clinical department is shown in Table 4

Antibiotic susceptibility of *Enterococcus spp*

Most of these strains were sensitive to Vancomycin. We also found sensitivity to Nitrofurans, Chloramphenicol, Lincomycin and Pristinamycin. However, we saw a decrease in their sensitivity to Lincomycin in the same period.

Table 4. Distribution of *Enterococcus* by hospitalization departments

Hospitalization department	Number	Frequency %
Infants	31	38.3
Paediatric emergencies	1	1.2
Infectious diseases	7	8.6
Neonatology	8	9.9
Medical emergencies	13	16.1
Children	10	12.3
Surgical emergencies	5	6.2
Resuscitation	5	6.2
Oncology	1	1.2
Total	81	100

Table 5. Antibiotic susceptibility of *Enterococcus spp* strains

Antibiotics tested	Susceptibility					
	2015		2016		2017	
	S/T	%	S/T	%	S/T	%
Vancomycin (VA)	13/13	100	30/30	100	49/50	98
Nitrofurane (F/N)	15/16	93.8	20/21	95.2	36/39	92.3
Gentamicin (CN)	3/12	25	6/30	20	7/47	14.9
Ampicillin (AMP)	3/18	16.7	2/19	10.5	11/51	21.6
Chloramphénicol (C)	5/10	50	11/21	52.4	36/50	72
Érythromycin (E)	2/19	10.5	5/30	16.7	11/65	16,9
Kanamycin (K)	0/9	-	2/12	16.7	9/38	27,7
Lincomycin (L)	20/23	87	16/22	72.7	20/51	39,2
Oxacillin (OXA)	1/18	5.6	1/16	6.3	3/21	14,3
Pristinamycin (P)	0	-	10/13	77	24/29	82,8
Cotrimoxazole (SXT)	3/18	16.7	5/13	38.5	2/37	5,4

S = sensitive T= tested

4. Discussion

From January 1, 2015 to December 31, 2017, 5.283 urine samples were received at the CHUP-CDG laboratory for cytobacteriological examinations. Of the 5283 ECBU requests, 132 (2.5%) were positive for *Enterococcus spp*. The females were the most representative in all the age groups with a frequency of 65.9% of cases, i.e. a sex ratio (M/F) of 0.52. This result is similar to that of Bouazza A. et al., in 2017, in Algeria, who reported a female predominance of 66% with a sex ratio of 0.51 [36]. This result is different to that of Vuke-Weledji S.A., in 2014, in Morocco, who reported in a study of *Enterococcus* infections and colonizations at the Mohamed V Military Instruction Hospital (HMIMV) in Rabat, and found 52% of males, i.e. a M/F sex ratio of 1.07 [37]. This female predominance could be explained by the shortness and straightness of the female urethra on the one hand and the contiguity between the urethra and the vagina on the other hand, contributing to infection via the ascending route. The most represented age group was 1 month to 1 year old with 37.9% of patients. This result is lower than the finding of Ouédraogo et al. in 2016 in Burkina Faso (BF), who found that the age group from 0 to 2 years was the most affected with 50.3% of cases [38]. This result could be linked to the reception characteristics of our study site. Indeed, this study was carried out in the laboratory

services of the CHUP-CDG which mainly welcomes patients aged between 0 and 14 years. Thus, during the study period, data from the hospital planning and information service (SPIH) showed a consultation peak of 3968 patients in the age group of 1 to 11 months, or 20.88 %. Also, the high frequency in the age group of 1 to 11 months could be justified by the fact that the national policy of free care further favors the care of newborns and infants. Finally, it should be remembered that at this age group, the child's immune system is still being built; anything that exposes it to infections including urinary tract infections. *Enterococcus spp* infections were more frequent among hospitalized patients than among outpatients with a rate of 61.4%. These results are similar to those of Gonsu Kamga and al., in Cameroon in 2013, and of Ait Miloud et al., in 2011, in Morocco, who reported in their studies that the majority of strains were isolated from patients hospitalized with respective rates of 72% and 87.1% [38,39]. This predominance of *Enterococcus spp* infection in hospitalized patients could be explained by the fact that it is most often nosocomial in nature. Indeed, Lavigne et al., in 2005, in France, found that these infections were nosocomial in 60.6% of cases [40]. Similarly, Dali et al. in 2015, in Algeria, reported that *E. faecium* and *E. faecalis* were germs responsible for nosocomial infections in the Hospital and University Establishment of Oran (EHUO) with 38.1% [41]. In our hospital environment, the high frequency of *Enterococcus spp* infections could highlight the deficit in hospital hygiene; the role of risk factors linked to urinary tract infection (UTI) in hospitalized patients such as bladder catheterization and other instrumental maneuvers.

Regarding the clinical départements, the infant's unit was the most affected with 38.3%. This result is close to that of Tinguéri et al. in 2017 in Burkina Faso, who reported in their study carried out in the same laboratory that the infant's unit was the 2nd unit with 23.1% (after Medical Emergencies (31.8%) where the cultures positive for *Enterococcus spp* came from [42]. These results could be explained by the fact that there were more patients hospitalized in this unit during the study period, as evidenced by data from the hospital planning and information department (SPIH) of the CHUP-CDG who found that the CHUP-CDG recorded a consultation peak at the level of the infant's unit of 5493 patients, or 28.9% and this in relation to the national policy of free healthcare introduced for children aged 0 to 5 and pregnant women. In our series, we observed an increase in the prevalence of *Enterococcus spp* strains responsible for urinary tract infections over the three years. Indeed, we noticed 1.9% in 2015, 2.1% in 2016 and 3.1% in 2017. Our results are similar to those of Boukadida et al. in 2000, in Tunisia, who reported a frequency of *Enterococcus spp* strains of 1% [43]. This growth in *Enterococcus spp* infections is closely related to the increase in urine cultures analyzed. This is justified by the fact that the majority of our study population is targeted by the national policy of free healthcare which thus makes ECBU accessible. Regarding the antibiotic susceptibility, the study results showed an intermediate case with vancomycin in 2017. This situation does not seem worrying, but it represents an alert for monitoring the sensitivity profile of antibiotics. *Enterococcus spp* strains showed sensitivity to the following antibiotics: Vancomycin (100%: 2015 and 2016), Nitrofuran (92.3 to 95.2%), Lincomycin (87%), Pristinamycin (82, 8%), and Chloramphenicol (52.4 to 72%). These results are close to those of Vuke-Weledji and al. in 2014 in Morocco, which reported that strains of *Enterococcus spp* remained sensitive (100%) to Teicoplanin and Vancomycin. They were also sensitive to Chloramphenicol (78%) and Nitrofuran (64%). [37]. In addition, Sissoko and al. in 2006 in Mali reported a sensitivity of 80% to Pristinamycin and 67% to Erythromycin [44]. We noticed a decrease in the sensitivity of *Enterococcus spp* strains to Lincomycin (72.7% to 39.2%). These results are comparable to those of Ouédraogo and al. in 2015 in Burkina Faso, who found, in their work on the bacteriological profile and sensitivity to antibiotics of germs isolated from pathological products in the same laboratory, a reduction in sensitivity from 85% to 50 % to Pristinamycin between 2013 and 2014; and that of 100% to 95% with Vancomycin in the same period [45]. This could be explained by the fact that the antibiotic was commonly prescribed for urinary infections; which can inevitably lead to selection pressure. The epidemiology of resistance of *Enterococcus spp* strains to different classes of antibiotics is often complex and varies from one country to another and even from one hospital to another [40]. In the current study, we found high resistance to the following antibiotics: Cotrimoxazole (80.28%), Penicillin G (100%), Erythromycin (83.19%), Oxacillin (85%) and Ampicillin (81.82%) .This is corroborated by the following several authors such as Tinguéri L. in 2017, in Burkina Faso, determined the presence of enterococci phenotype resistant to Vancomycin (ERV) at 87.5%; strong resistance to Cotrimoxazole (75%), Ampicillin (79.41%), Erythromycin (75.05%) and moderate to Chloramphenicol (21.21%) [42]. Ramde and al. summarized the antibiotic resistance of strains of *Enterococcus faecalis*, in 2017, in Burkina Faso, in a Systematic review and found that *Enterococcus faecalis* presented high resistance to Oxacillin (100%), Erythromycin (84.6%), Lincomycin (100%), Cotrimoxazole (100%), Chloramphenicol (66.7%) [46]. Gonsu et al. in 2013, in Cameroon, reported significant resistance of *Enterococcus spp* strains to Erythromycin (88%), Lincomycin (86%) and Ampicillin (60%), average resistance to sulfonamides, cyclins and glycopeptides: 46% for Sulfamethoxazole +Trimethoprim, 42% for tetracyclin [39]. Vuke-Weledji and al. in 2014, in Morocco, reported that strains of *Enterococcus spp* are very resistant (100%) to Oxacillin 5 µg. They are also resistant (resistance rate between 80 and 100%) to Lincomycin. They are also resistant (resistance rate between 50 and 70%) to Kanamycin 1000, Pristinamycin, Erythromycin, and Sulfamethoxazole +Trimethoprim [37]. Ouédraogo and al. in 2011 in Burkina Faso, classified Aminopenicillins and Cotrimoxazole in the list of antibiotics that are the most inactive on most bacterial strains. they found that the evolution of antibiotic resistance in this context showed that the antibiotics mentioned no longer have a place in probabilistic antibiotic therapy, and their use should be abandoned.

Fromage and al. in 2007, in France, reported that the strains of *Enterococcus faecalis* and *E. faecium* had good sensitivity to Linezolid (95.1 to 98.2%) while they present

discrepancies regarding the other antibiotics. Thus, *Enterococcus faecalis* was sensitive to Vancomycin (97.9%), Teicoplanin (98.8%) and Cotrimoxazole (85.4%), while *Enterococcus faecium* was sensitive to Tetracycline (98.1%), Gentamycin (54%) and Pristinamycin (96.6%) [47].

5. Conclusion

Urinary tract infections caused by *Enterococcus spp* remain poorly documented in sub-Saharan Africa in general and particularly Burkina Faso. The current study carried out at the CHUP-CDG laboratory showed that 132 patients were infected by this pathogen from 2015 to 2017 with a female predominance. The age group from 1 month to 1 year old was the most affected. In addition, most patients came from hospitalization departments. Among the hospitalization units, the infant's unit was the most affected. Considering the data by year, the greatest demand for ECBU was recorded in 2017. Furthermore, strains of *Enterococcus spp* presented a sensitivity above 80% for antibiotics such as Vancomycin, Nitrofurans, Lincomycin and Pristinamycin. As for Chloramphenicol, it presented a sensitivity of between 52.4 and 72%. However, activity of less than 20% was noted for antibiotics such as Cotrimoxazole, Erythromycin, Oxacillin and Ampicillin. We found that the Penicillin G molecule was inactive on all the strains tested. Large scale studies are suggested to be conducted on *Enterococcus spp* urinary tract infections to confirm the current findings.

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