

Epidemiological Study and Antibiotic Resistance of Strains of *Staphylococcus* spp. Isolated at the National Laboratory of Public Health, Brazzaville

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Abstract

The spread of multi-resistant community-associated *Staphylococcus* strains poses a significant public health threat, greatly limiting therapeutic options for treating severe infections. A total of 403 samples were collected, including urine, semen, and vaginal swabs, from which 106 *Staphylococcus* isolates were obtained on Mannitol Salt Agar and identified using microbiological techniques. *Staphylococcus aureus* (*S. aureus*) was predominant (77.36%), while other species (22.64%) such as *S. warneri*, *S. epidermidis*, *S. haemolyticus*, *S. cohnii*, *S. saprophyticus*, and *S. xylosus* were identified at low frequencies. The high prevalence of *S. aureus* varied according to sample type, sex, and age. A statistically significant difference ($p < 0.05$, Chi-square test) was observed in resistance frequencies among the different *Staphylococcus* strains for cefoxitin, penicillin, gentamicin, tetracycline, minocycline, and teicoplanin. Eighty-six (81.13%) *Staphylococcus* strains were resistant to methicillin, including 66 (76.74%) methicillin-resistant *S. aureus* (MRSA) and 20 (23.26%) methicillin-resistant coagulase-negative *Staphylococcus* (MRCoNS). The frequency of methicillin-resistant *Staphylococcus* spp. is increasing, along with high resistance rates to other antibiotic families. Nevertheless, rifampicin and vancomycin remained more effective against methicillin-resistant *S. aureus* and co-

agulase-negative staphylococci. This study shows that although high resistance rates were observed, some antibiotics retain their efficacy and can be used as first-line treatments for infections caused by methicillin-resistant staphylococci. It is also necessary to establish an epidemiological monitoring program to control the spread of these strains and prevent multi-resistant *Staphylococcus* epidemics.

Keywords

S. aureus, Coagulase-Negative Staphylococci, Epidemiology, Resistance, Methicillin

1. Introduction

Since the discovery of antibiotics, numerous antibacterial agents have been developed and marketed for therapeutic use. Initially, these agents were regarded as powerful weapons capable of eradicating all bacterial infections [1] [2]. However, resistance to antibiotics emerged shortly after their introduction, complicating treatment and, in some cases, rendering it ineffective. Consequently, treatment failures are increasingly observed in common infections caused by multi-drug-resistant bacteria. Alarmingly, antibiotic resistance is now rising at an unprecedented rate [3]. In the past decade, *Staphylococcus* infections have been reported globally, with a rapid and widespread emergence of multi-resistant strains [4]. This trend has made the proliferation of resistant *Staphylococcus* species a global health threat, demanding urgent and coordinated intervention [5]. The Centers for Disease Control and Prevention (CDC) and the World Health Organization (WHO) have both identified antibiotic-resistant *Staphylococcus*-particularly hospital-acquired strains-as a major public health concern due to their resistance profiles and potential for rapid dissemination [6]. *Staphylococcus* are Gram-positive cocci that typically occur in clusters. They are ubiquitous in nature and responsible for a wide spectrum of infections in both humans and animals. As members of the skin microbiota, *Staphylococcus*, alongside *Streptococcus* and *Pneumococcus*, belong to a group of opportunistic and invasive Gram-positive pathogens known as pyogenic cocci, which are implicated in various human infections. The *Staphylococcus* genus is traditionally divided into two groups based on coagulase production: coagulase-positive staphylococci, the most virulent of which is *Staphylococcus aureus*, and coagulase-negative staphylococci, such as *S. epidermidis*, commonly found on the skin. The acquisition of the *mecA* gene by these strains significantly complicates treatment, particularly in vulnerable populations such as immunocompromised patients, trauma victims, those undergoing major surgery, intensive care patients, diabetics, and the elderly. Infections in these groups can result in increased morbidity and mortality worldwide due to the emergence and spread of antibiotic-resistant strains [7]. Indeed, *Staphylococcus* species are pyogenic bacteria [8]. Their ability to survive in hostile environments, along with their

virulence and ubiquity, explains their high prevalence in both community and hospital-acquired infections [9]. These bacteria are now responsible for numerous nosocomial outbreaks in regions including France, Greece, Israel, the Maghreb, Algeria, and various parts of Asia. They are a common cause of healthcare-associated infections, many of which are preventable. A recent study in the Republic of Congo reported the isolation of methicillin-resistant *Staphylococcus* strains from both community and hospital settings [10]. In addition to methicillin resistance, these strains frequently exhibit resistance to other antibiotic classes, such as beta-lactams and macrolide-lincosamide-streptogramin B (MLSB) antibiotics, further complicating therapeutic management [7] [11]. Over the past decade, the epidemiology of methicillin resistance has evolved rapidly. Initially restricted to healthcare settings, methicillin-resistant strains have now emerged in the community. Some of these strains carry the Panton-Valentine leukocidin (PVL) gene, which is associated with severe infections such as necrotizing pneumonia [12]. Due to their opportunistic nature, *Staphylococcus* species are capable of infecting a wide range of body sites, including the respiratory and genitourinary tracts, where they can cause severe or chronic infections [13]. In Africa, the epidemiology of methicillin-resistant *S. aureus* (MRSA) has been the subject of various studies. These include investigations in Nigeria focusing on isolates from urine and semen [14], skin and soft tissue infections in Gabon [15], and bovine mastitis in the North-West region of Cameroon [16]. However, there is a notable lack of epidemiological data on methicillin-resistant *Staphylococcus* spp. in the Republic of Congo. In this context, we deemed it important to conduct the present study to investigate the epidemiology of methicillin-resistant *Staphylococcus* strains in the Republic of Congo, with the aim of establishing epidemiological surveillance and facilitating optimal therapeutic decision-making.

2. Materials and Methods

2.1. Biological Material

Various biological samples from patients were used for the isolation of staphylococcal strains. These samples included vaginal swabs, seminal fluids, and urine, collected at the National Laboratory of Public Health. A total of 403 samples were collected between February and November 2022. Any sample of urine, vaginal swab, or seminal fluid submitted for routine biological analysis at the National Public Health Laboratory was included in this study.

No approval from an ethics committee was obtained. Indeed, this institution also has a mandate for research and scientific production. Thus, after the results were returned to the patients, the bacterial strains were used for the research component.

2.2. Methods

2.2.1. Isolation and Identification of Strains

Staphylococcus strains were isolated on Mannitol Salt Agar (Bio-Rad) and identified using conventional microbiological techniques, including culture character-

istics, Gram staining, and standard biochemical tests such as catalase, coagulase, DNase, and the API Staph gallery from BioMérieux.

2.2.2. Quality Control

Quality control of antibiotic discs was performed using the reference strain *S. aureus* ATCC 29213, following CASFM recommendations [17].

2.2.3. Antibiotic Susceptibility Testing

The following antibiotics were tested: Penicillin G (P, 1 µg); Cefoxitin (FOX, 30 µg); Oxacillin (OXA, 1 µg); Amikacin (AK, 30 µg); Gentamicin (CN, 10 IU); Tobramycin (TOB, 15 µg); Erythromycin (E, 15 µg); Azithromycin (AZM, 15 µg); Minocycline (MN, 30 µg); Tetracycline (TE, 15 µg); Vancomycin (VA, 30 µg); Teicoplanin (TEC, 30 µg); Norfloxacin (NOR, 10 µg); Levofloxacin (LEV, 5 µg); Rifampicin (RD, 5 µg).

The antibiotic resistance profile of the bacterial strains was evaluated using the standard Kirby-Bauer disk diffusion method [11] [18]. The inoculum was prepared by suspending a well-isolated colony from a 24-hour pure culture on agar in 5 ml of normal saline (NaCl 0.9%). The turbidity was adjusted to 0.5 McFarland using a Vitek Densichek. Mueller-Hinton agar was inoculated using a sterile swab, as recommended by the Clinical and Laboratory Standards Institute (CLSI) [2] [19]. Antibiotic discs were then placed on the inoculated plates, which were incubated at 37°C for 18 - 24 hours. The diameters of the inhibition zones around the discs were measured after incubation, and susceptibility was interpreted according to the breakpoint values published by the Antibiogram Committee of the French Society of Microbiology [17]. The strains were classified as susceptible, intermediate, or resistant to the antibiotics tested.

2.2.4. Testing for Methicillin-Resistant Strains

Cefoxitin (30 µg) and/or oxacillin (1 µg) discs were used to detect methicillin-resistant *Staphylococcus* strains (MRSA and others), using the agar diffusion method, in accordance with CLSI guidelines [17].

2.2.5. Statistical Analyses

Data were processed using Excel 2016 (Microsoft Corporation, USA). Percentages were calculated based on the measured inhibition diameters. Resistance rates were compared using the Chi-square test, with a significance level of $p < 0.05$ and a 95% confidence interval, using GraphPad Prism 2008.

3. Results

3.1. Sample Collection

Of the 403 samples collected, 251 were urine, 117 were vaginal swabs, and 35 were seminal fluids. These results are presented in **Table 1**.

3.2. Isolation and Identification

Among the 403 samples collected, 106 were positive, representing an isolation rate

of 26.30%. A positive result indicates bacterial growth on Mannitol Salt Agar. Coagulase test results were confirmed by identification using the API Staph gallery.

Of these, 82 coagulase-positive strains were identified as *S. aureus* (77.36%), and 24 (22.64%) were coagulase-negative staphylococci, including 8 (7.55%) *S. warneri*, 6 (5.66%) *S. epidermidis*, and 4 (3.77%) *S. haemolyticus*. Two strains each were identified as *S. cohnii*, *S. saprophyticus*, and *S. xylosum*, representing 1.88% each. **Figure 1** shows the distribution of the 106 *Staphylococcus* strains isolated and identified.

Table 1. Number of samples collected.

Type of sampling	Samples (%)
Urine	251 (62.28%)
Vaginal samples	117 (29.03%)
Sperm fluid	35 (8.68%)

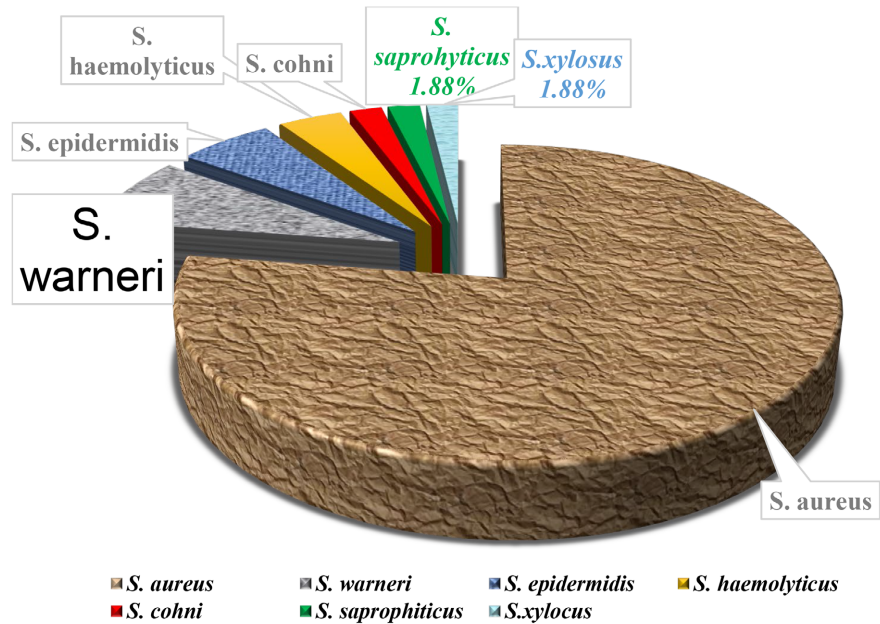


Figure 1. Distribution of *Staphylococcus* strains by species.

3.3. Distribution by Sampling Site

The results show that 46 (43.39%) *Staphylococcus* spp. strains were isolated from urine, 38 (35.84%) from vaginal swabs, and 22 (20.75%) from seminal fluids (**Figure 2**).

3.4. Distribution by Sex

A total of 62 (58.49%) *Staphylococcus* spp. strains were isolated from female patients, 40 (37.73%) from male patients, and 4 (3.78%) from patients of undetermined sex (**Figure 3**).

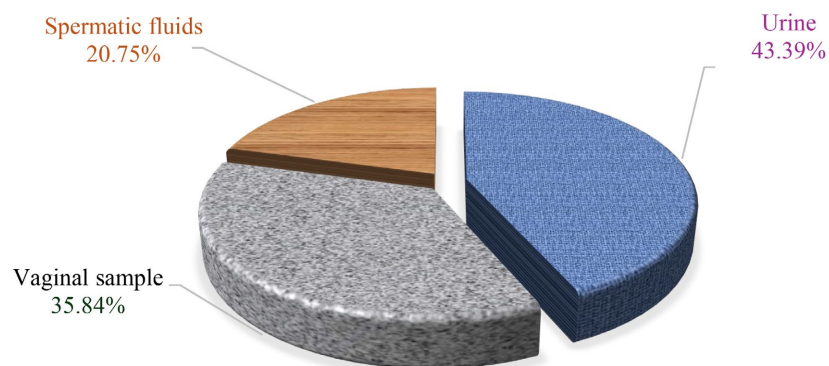


Figure 2. Distribution of *Staphylococcus* spp. strains by sample type.

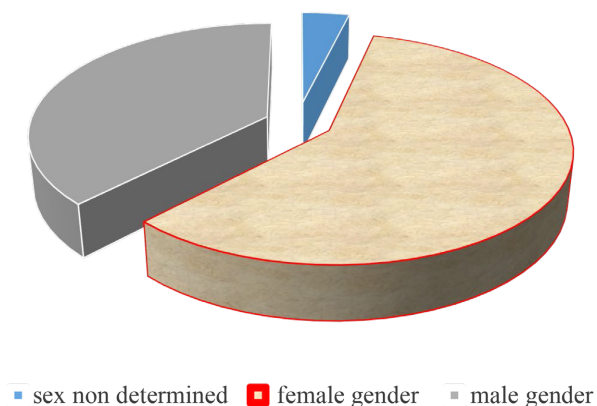


Figure 3. Distribution of *Staphylococcus* spp. strains by gender.

3.5. Distribution by Age Group

The age groups included were: 4 - 18 years, 18 - 36 years, 36 - 54 years, and 54 - 67 years. The highest frequency of *Staphylococcus* spp. strains was observed in the 18 - 36 age group, with 50 isolates (47.17%). The 4 - 18 and 54 - 67 age groups showed similar distributions, with 13 (12.26%) and 15 (16.03%) isolates, respectively. Eleven strains (10.39%) were from patients of undetermined age (**Figure 4**).

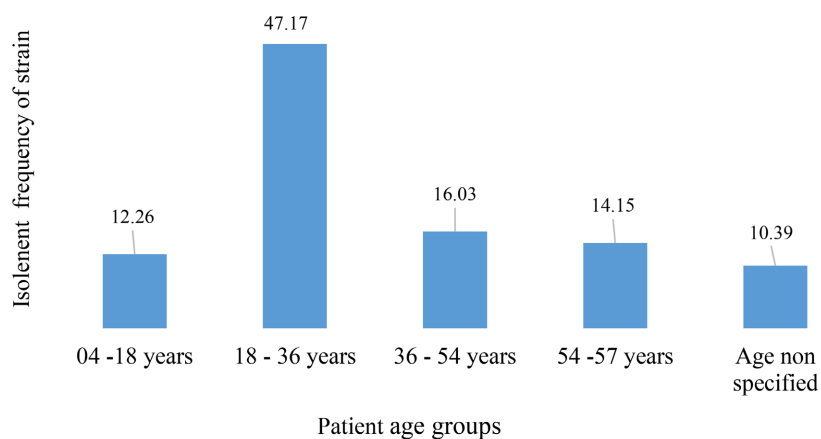


Figure 4. Distribution of *Staphylococcus* spp. strains by age group.

3.6. Antibiotic Susceptibility by Species

Staphylococcus spp. strains exhibited varying levels of resistance depending on the species. The data show that for *S. aureus*, rifampicin was the most effective antibiotic, with a resistance rate of only 14.63% (12 strains). *S. warneri* was sensitive to vancomycin. All *S. epidermidis* strains were susceptible to vancomycin and minocycline. In other coagulase-negative staphylococci (CoNS), vancomycin remained the most active molecule (Table 2).

Among the 106 strains tested, rifampicin and vancomycin showed good efficacy, with sensitivity rates of 84.91% and 77.36%, respectively. Oxacillin exhibited the highest resistance rate (69.81%) among the β -lactam antibiotics. Similar resistance rates were observed with aminoglycosides such as gentamicin and tobramycin (Figure 5).

In total, 86 (81.13%) of the 106 strains were resistant to ceftioxin and/or oxacillin, compared to 20 (18.87%) methicillin-susceptible strains (Table 3). A statistically significant difference ($p < 0.05$) in resistance frequencies was observed among different bacterial species for ceftioxin, penicillin, gentamicin, tetracycline, minocycline, and teicoplanin.

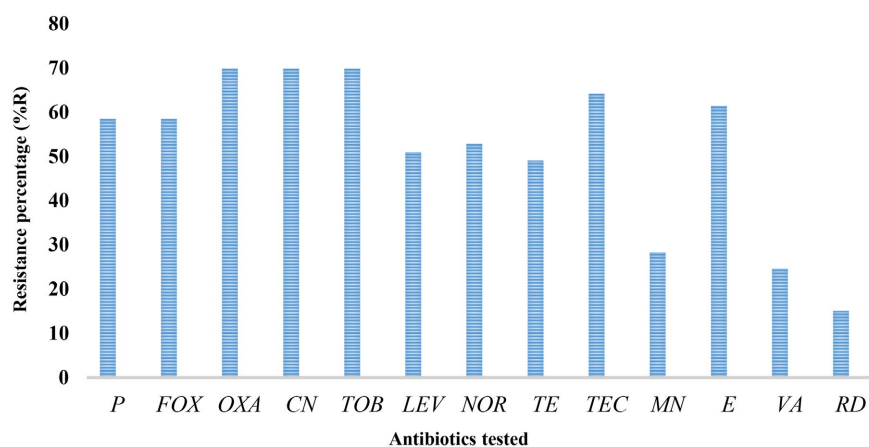
Table 2. Antibiotic resistance rates for *Staphylococcus* species.

ATB tested	<i>S. aureus</i> 82 (%R)	<i>S. warneri</i> 8 (%R)	<i>S. epidermidis</i> 6 (%R)	<i>S. haemolyticus</i> 4 (%R)	<i>S. saprophyticus</i> 2 (%R)	<i>S. cohnii</i> 2 (%R)	<i>S. xylosus</i> 2 (%R)	<i>p-value</i>
FOX	52 (63.41)	2 (12.5)	2 (33.33)	2 (50)	2 (100)	0 (0)	2 (100)	0.04697*
OXA	62 (75.60)	6 (75)	2 (33.33)	4 (100)	2 (100)	2 (100)	2 (100)	0.2716
P	48 (58.53)	2 (25)	4 (66.66)	4 (100)	2 (100)	0 (0)	2 (100)	0.04522*
NOR	42 (51.21)	4 (50)	4 (66.66)	4 (100)	2 (100)	2 (100)	0 (0)	0.1374
LEV	40 (48.78)	4 (50)	4 (66.66)	4 (100)	2 (100)	2 (100)	0 (0)	0.1046
TOB	58 (70.73)	4 (50)	4 (66.66)	4 (100)	2 (100)	2 (100)	0 (0)	0.1802
CN	60 (73.17)	2 (25)	6 (100)	4 (100)	2 (100)	2 (100)	0 (0)	0.004012*
TE	44 (53.65)	2 (25)	4 (66.66)	0 (0)	2 (100)	2 (100)	0 (0)	0.02811*
MN	22 (26.82)	2 (25)	0 (0)	4 (100)	2 (100)	2 (100)	0 (0)	0.000635*
VA	24 (29.26)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0.2721
TEC	54 (65.85)	1 (12.5)	4 (66.66)	4 (100)	1 (100)	0 (0)	2 (100)	0.00479*
E	48 (58.53)	3 (37.5)	4 (66.66)	2 (50)	1 (100)	0 (0)	2 (100)	0.5235
RD	12 (14.63)	2 (25)	0 (0)	0 (0)	0 (0)	2 (100)	0 (0)	0.1082

Legend: % R: Resistance Percentage, * $p < 0.05$: statistically significant difference, ATB = Antibiotic, P = Penicillin G, FOX = Ceftioxin, OXA = Oxacillin, CN = Gentamicin, VA = Vancomycin, TOB = Tobramycin, NOR = Norfloxacin, LEV = Levofloxacin, TE = Tetracycline, MN = Minocycline, TEC = Teicoplanin, VA = vancomycin, E = Erythromycin, RD = Rifampicin.

Table 3. Distribution of *Staphylococcus* spp. isolates for methicillin resistance.

Antibiotic disc	Number	Frequency
Methicillin-resistant strains	86	81.13%
Methicillin-sensitive strains	20	18.87%
Total	106	100%



Legend: P = Penicillin G, FOX = Cefoxitin, OXA = Oxacillin, CN = Gentamicin, VA = Vancomycin, TOB = Tobramycin, NOR = Norfloxacin, LEV = Levofloxacin, TE = Tetracyclin, MN = Minocyclin, TEC = Teicoplanin, VA = vancomycin, E = Erythromycin, RD = Rifampicin.

Figure 5. Sensitivity status of *Staphylococcus* spp. strains.

3.7. Methicillin Resistance by Species

Among the 86 methicillin-resistant strains, 66 (76.74%) were identified as *S. aureus* (MRSA), while 20 (23.26%) belonged to other species, including *S. warneri*, *S. epidermidis*, *S. haemolyticus*, *S. cohnii*, *S. saprophyticus*, and *S. xylosus* (Figure 6).

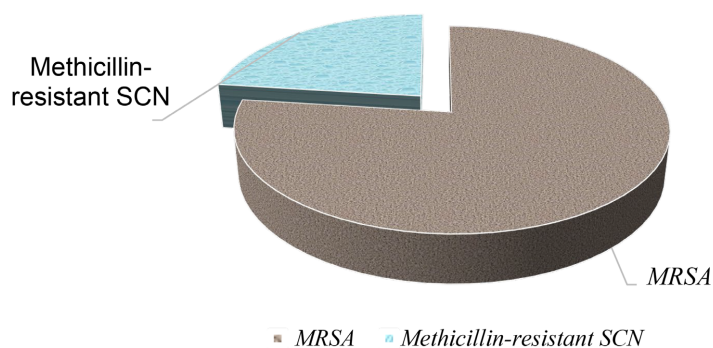


Figure 6. Distribution of methicillin-resistant *Staphylococcus* strains.

4. Discussion

This study focused on the epidemiology of methicillin-resistant *Staphylococcus* spp. strains isolated at the National Laboratory of Public Health. A total of 403 samples were collected, from which 106 *Staphylococcus* strains were isolated and identified. These belonged to seven species: *S. aureus*, *S. warneri*, *S. epidermidis*, *S. haemolyticus*, *S. cohnii*, *S. saprophyticus*, and *S. xylosus*. The distribution of these strains by sample type showed that most were isolated from urine (43.39%), followed by vaginal samples (35.84%). These findings are consistent with those reported by [20], who found a predominance of *S. aureus* in urine samples (48.57%). Similarly, [21] reported a high urinary isolation rate of *S. aureus* at

13.06%. The gender distribution showed a predominance of *Staphylococcus* spp. isolates in female patients (58.49%). These results are higher than those reported by [20] at the National Public Health Laboratory of Brazzaville (48.57% in females), but lower than those found by [22] in Algeria, where *S. aureus* was more frequently isolated in males (65%) in community settings. Patients aged 18 - 36 years were the most affected by *Staphylococcus* infections, with a frequency of 52.83%. This high rate may be explained by poor hygiene practices and unprotected sexual activity. However, this finding differs from the literature, which generally identifies the extremes of age—infants under one year and adults over 75 years—as populations at higher infectious risk due to immature or weakened immune systems [22]. Coagulase results were confirmed using the API Staph identification system. Among the 106 isolates, *S. aureus* was the most frequently identified species (77.36%), followed by *S. warneri* (7.55%). These results agree with those of [23], who reported a 66, 66% frequency for *S. aureus*. According to the literature, *S. aureus* is the most commonly isolated *Staphylococcus* species in humans, with approximately 30% of adults being persistent carriers and 50% intermittent carriers [24]. Regarding antibiotic susceptibility, resistance was observed across most antibiotic classes. For β -lactams, oxacillin showed the highest resistance rate (69.81%). These results corroborate those of [10], who reported a 53.06% resistance rate. The β -lactam resistance was primarily associated with the P-OX-FOX phenotype, with varying rates. Similar resistance rates were found among aminoglycosides, with gentamicin and tobramycin showing resistance rates close to 70%. This high resistance may result from selection pressure due to inappropriate or excessive antibiotic use, the production of β -lactamase enzymes, or altered penicillin-binding proteins (PBP2a) encoded by the *mecA* gene [25]. Comparable results were reported by [26] in *S. aureus* isolates from Brazzaville University Hospital, where resistance reached 80%. In Morocco, resistance to methicillin reached 59% in 2007 [27]. The mechanisms of aminoglycoside resistance include ribosomal protein mutations, reduced antibiotic permeability, and the production of modifying enzymes. In this study, 66 of the 86 methicillin-resistant strains were *S. aureus* (76.74%), while 20 were coagulase-negative *Staphylococcus* (CoNS) (23.26%). These results are higher than those found by [28] in Shanghai, where MRSA accounted for 64% of *S. aureus* bacteremia. In the Americas, MRSA prevalence ranges from 36% to 62.6% [29]. In contrast, a study in Morocco by [30] reported a much lower MRSA rate of 13.5%, a trend attributed to improved infection control measures such as patient isolation [31]. Our study, which recorded an MRSA prevalence of 81.13% in the Republic of Congo, provides important epidemiological insights into *Staphylococcus* spp. Cross-resistance to penicillin was observed in MRSA at a rate of 72.72%, and 100% resistance was observed in methicillin-resistant CoNS. These findings match those of [26], who reported a 100% resistance rate. This resistance likely results from PBP2a-mediated penicillinase production [32]. Among aminoglycosides tested, MRSA showed high resistance to gentamicin and tobramycin (\approx 80%). In methi-

cillin-resistant CoNS, resistance to these antibiotics reached 100%. Our results exceed those of [10], who reported resistance rates of 27.27% (tobramycin) and 72.23% (gentamicin). This resistance may be due to ribosomal mutations, altered permeability, efflux mechanisms, or enzymatic modifications (acetyltransferases, phosphotransferases, nucleotidyltransferases). In the fluoroquinolone group, norfloxacin and levofloxacin were less active, with resistance rates of $\approx 60\%$ in MRSA and 100% in methicillin-resistant CoNS. In the fluoroquinolone group, norfloxacin and levofloxacin were less active, with resistance rates of $\approx 60\%$ in MRSA and 100% in methicillin-resistant CoNS. These results are different for norfloxacin and are similar for levofloxacin to those reported by [33] in *S.aureus* isolates from the University Hospital center of Brazzaville, with respective resistance rates of 70.37% and 59.25%. Resistance in this class is usually caused by mutations in DNA gyrase or topoisomerase IV [5]. For tetracyclines, minocycline showed better activity with 30.30% resistance in MRSA, but 100% resistance in CoNS. Tetracycline showed a resistance rate of 63.63% in MRSA, and 100% in CoNS. Glycopeptides performed better: vancomycin was the most effective antibiotic against MRSA (36.36% resistance) and showed no resistance in CoNS. Teicoplanin, however, showed a resistance rate of 81.81%, higher than rates reported by [20] (45.71% for teicoplanin, 11.42% for vancomycin). These data highlight the need for increased vigilance to prevent the spread of multidrug-resistant strains. Tetracycline resistance may involve efflux pumps (TetK) or ribosomal protection proteins [34]. As for macrolides-lincosamides, erythromycin showed low activity, with a resistance rate of 72.72% in MRSA. This is close to the rate reported by [35], who found 62.5% resistance linked to the *erm* gene. Resistance in this group is caused by plasmid-encoded methylases that modify ribosomal targets [5] [11]. According to [36], MRSA strains are often resistant to multiple drug classes, including macrolides, lincosamides, fluoroquinolones, tetracyclines, aminoglycosides, and chloramphenicol. These findings align with our results, as all strains were resistant to MLSB antibiotics. The high rate of inducible resistance may be linked to the inappropriate use of erythromycin in *Staphylococcus* infections.

5. Conclusion

This study contributes to the ongoing investigation of methicillin resistance and cross-resistance among *Staphylococcus* spp. in the Republic of Congo. Using classical microbiology techniques, we isolated 106 strains from urine, vaginal, and seminal samples, among which 86 strains (81.13%) were methicillin-resistant. Of these, 66 were MRSA (76.74%) and 20 were methicillin-resistant CoNS (23.26%). The prevalence of methicillin-resistant *Staphylococcus* spp. is increasing, and high resistance rates to other antibiotic families were also observed. However, rifampicin and vancomycin remained highly effective against MRSA and CoNS. Despite this, the rising resistance trends are alarming, especially due to cross-resistance across multiple antibiotic classes-excluding glycopeptides, which retained their activity. These findings underscore the urgent need for stricter hygiene pro-

protocols and better antibiotic stewardship. This study builds on ongoing research within our laboratory aimed at understanding the epidemiology of methicillin-resistant *Staphylococcus* strains and guiding public health interventions.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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