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Virulence and Resistance of *Staphylococcus* ssp. and *Streptococcus* spp. Strains Isolated from Suppurations at Chud-Ba (Benin)

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ABSTRACT

Background and Objective: Wound infections can cause delayed healing, leading to multiple healthcare facility visits and extended hospital stays. This study aims to identify the virulence and antibiotic resistance genes in bacteria isolated from suppuration at the Departmental University Hospital of Borgou-Alibori (CHUD-BA). **Materials and Methods:** This report was a cross-sectional study with an analytical aim. The study was spread from June, 2021 to February, 2022, including participants admitted to the CHUD-BA. The bacteria' isolation, identification, and antibiogram were carried out by standard microbiological techniques followed by molecular characterization in the search for virulence genes. A total of 107 cases of suppuration were collected. GraphPad Prism 8 identified significant differences between means ($p<0.05$). **Results:** Gram-positive bacteria dominate suppurative infections at 51.8%. Key bacteria include *Staphylococcus aureus* (69.64%), *Streptococcus* spp. (23.21%), and coagulase-negative *Staphylococcus* (7.14%). Most isolated strains showed antibiotic resistance, with 55.4% of *Staphylococcus* spp. and *Streptococcus* spp. being biofilm-forming. The mecA gene was found in 17.9% of *S. aureus* strains, while 43.6% contained the cna gene. Suppuration is common in hospital and community settings, with *Staphylococcus* spp. and *Streptococcus* spp. as the primary pathogens. **Conclusion:** Antibiotic resistance, which has become a real public health problem, can complicate the treatment of suppuration, hence the importance of rationalizing the use of antibiotics. Therefore, it is concluded that wound healing is predicated on good hygiene, proper wound infection care, and effective antimicrobial drugs.

KEYWORDS

Suppuration infections, *Staphylococcus* spp., *Streptococcus* spp., bacterial resistance, virulence factor

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INTRODUCTION

Chronic wounds present a significant and growing challenge in wound care and nursing, influenced by factors such as wound etiology, comorbidities, and environmental and socio-economic conditions[1]. Untreated wound infections, which complicate the healing process, are a major contributor to the development of chronic wounds. Understanding the dynamics of wound infections how they develop, their various presentations, and the appropriate timing and methods for initiating topical and systemic therapies is crucial for effective treatment[1,2].

The development of chronic wounds is often influenced by various factors, with wound etiology, coexisting medical conditions, and environmental and socio-economic determinants playing pivotal roles. Etiology refers to the underlying causes of wounds, which can vary significantly depending on the wound type. Common wound types, such as diabetic ulcers, venous leg ulcers, pressure ulcers, and arterial ulcers, all have distinct pathophysiologies and healing mechanisms, each contributing to the chronicity of the wound if not adequately managed. In addition to wound etiology, the presence of comorbidities, including diabetes, heart disease, and autoimmune disorders, significantly complicates wound healing and increases the risk of infection, prolonged inflammation, and tissue breakdown[3,4]. Furthermore, the socio-economic status of patients influences their access to adequate healthcare, nutrition, and wound care supplies, leading to delayed treatment and adverse outcomes. Individuals living in poverty, for example, often face barriers to timely medical intervention, wound care education, and optimal management strategies, thereby exacerbating the progression of chronic wounds[5].

Indeed, wounds can become contaminated with microorganisms, which may lead to infection and hinder healing. When a wound's protective skin barrier is breached, the underlying tissue is exposed to microbial contamination. Common pathogens, including *Staphylococcus aureus* and *Pseudomonas aeruginosa*, possess virulence factors that enable them to adhere and invade the wound site[6].

The pathogenicity of *Staphylococcus aureus* and *Pseudomonas aeruginosa* is closely linked to their ability to form biofilms clusters of microorganisms encased in an extracellular matrix that protect the bacteria from both host immune defenses and antibiotic therapy. Biofilms are particularly problematic in chronic wounds, contributing to persistent infection and tissue damage. This microbial community formation makes chronic wound infections challenging to treat, as the biofilm matrix acts as a physical barrier to antimicrobial agents, rendering conventional treatment strategies less effective[7,8]. Additionally, biofilms contribute to increased bacterial resistance, as the interactions among microbial species within the biofilm can lead to synergistic resistance mechanisms. As a result, chronic wound infections involving biofilm-forming organisms often necessitate more aggressive and prolonged antimicrobial treatments. These complexities underscore the importance of understanding wound infection dynamics and implementing appropriate therapeutic strategies to manage infection and promote healing.

The prevalence of wound infections is widespread and varies by geographical region, host skin flora, and factors such as clothing and the timing of wound assessment[9]. The rise of antibiotic resistance, particularly concerning Methicillin-Resistant *Staphylococcus aureus* (MRSA), has made the management of these infections increasingly challenging[4,10]. This study aims to isolate, identify, and characterize the virulence and molecular resistance of *Staphylococcus* and *Streptococcus* species associated with suppurations at the Departmental University Hospital of Borgou-Alibori (CHUD-BA) in Benin.

Materials and Methods

Study area and duration: It was a prospective study of bacterial strains isolated from infected wounds in the Departmental University Hospital of Borgou-Alibori patients over nine months (June, 2021 to February, 2022).

Sample collection: The 107 bacterial strains were randomly collected at the Departmental University Hospital of Borgou-Alibori (CHUD-BA). This center was chosen because it is a public center, has many patients, and is equipped to perform microbiological examinations on various biological samples from patients. These strains were isolated from the suppurations of patients admitted to this center, especially from the operating room during the study period. Each time a case of suppuration is noted, the pus is collected with a sterile swab and immediately sent to the center's biomedical analysis laboratory.

Bacteriological examinations: Once in the laboratory, the swabs were used to inoculate Blood Agar, Blood Agar+ANC, and Chapman agar media, which were incubated in the oven (Memmert UNE 200, Germany) at 37°C for 24 hrs. The next day, the colonies are described on each agar. Gram staining is performed from the obtained settlements using the classical method[11]. Then, the strains were identified with the API Staph Gallery.

Antibiotic susceptibility testing of strains: It was evaluated by the solid-state diffusion method. Each bacterial inoculum was standardized (10^6 CFU/mL) after a 10^{-2} dilution of Mc Farland's solution and then inoculated by flooding (1 mL) the surface of Muller Hinton (MH) agar. The plates were allowed to dry at laboratory temperature (25±2°C) for 15 min for the *Staphylococcus* spp. strains, 15 antibiotics tested were: fusidic acid (FD, 10 µg), aztreonam (ATM, 30 µg), cefoxitin (Fox, 30 µg), chloramphenicol (C, 30 µg), ciprofloxacin (CIP, 5 µg), erythromycin (E, 15 µg), Fosfomycin (FOS, 50 µg), Fosfomycin/trometamol (FOT, 200 µg), gentamyicin (CN, 10 µg), levofloxacin (LEV, 5 µg), netilmicin (NET, 10 µg), oxacillin (OX, 5 µg), penicillin (P, 1IU), sulfamethoxazole-trimethoprim (SXT, 25 µg), and tetracycline (TE, 30 µg). Finally, 17 antibiotics were tested on the *Streptococcus* spp. strains, namely: fusidic acid (FD, 10 µg), cefixime (CFM, 5 µg), cefoxitin (Fox, 30 µg), ceftriaxone (CTR, 30 µg), chloramphenicol (C, 30 µg), ciprofloxacin (CIP, 5 µg), erythromycin (E, 15 µg), ertapenem (ETP, 15 µg), gentamyicin (CN, 10 µg), imipenem (IPM, 10 µg), levofloxacin (LEV, 5 µg), meropenem (MEM, 10 µg), netilmicin (NET, 10 µg), nitrofurantoin (F, 100 µg), penicillin (P, 1IU), pristinamycin (PT, 15 µg), and sulfamethoxazole-trimethoprim (SXT, 25 µg).

Molecular detection of resistance and virulence genes

Extraction of bacterial DNA from strains: The DNA extraction was performed following the method adapted from Rasmussen and Morrissey[12]. The preserved strains were grown on MH agar and incubated at 37°C for 24 hrs. DNA was extracted from the 24 hrs bacterial cultures. For this purpose, a 24 hrs preculture was performed in 1 mL of liquid Muller Hinton in an Eppendorf tube. After 24 hrs, the precultures were centrifuged at 12000 rpm for 5 min, and the supernatant was removed. Then, 500 µL of sterile distilled water was added to the pellet, and the whole was mixed and heated (Barnstead/Thermolyne, Iowa, USA) in a dry bath at 100°C for 30 min. Centrifugation at 12000 rpm for 5 min was performed, followed by recovery of the supernatant in another Eppendorf tube, and 500 µL of 96° alcohol kept at 4°C was added to the mixture. Finally, the mixture was centrifuged at 12000 rpm for 5 min. After centrifugation, the supernatant was drained and allowed to air dry. After drying, the DNA pellets were suspended in 50 µL of sterile distilled water and stored at 4°C for immediate use or at -20°C for long-term storage.

Search for the *mecA* gene in *S. aureus*: Thus, DNA extracted from our *S. aureus* strains was used to determine the mecA gene by using primers previously designed (*mecA* F: 5'- TCCAGGAATGCAGAAAGACC-3'; *mecA* R: 5'-TCACCTGTTTGAGGGTGGAT-3') by Malik *et al.*[13].

The reaction was performed in a 25 µL volume, which contained 20 µL of 10x GoTaq mix (PROMEGA, Madison, WI USA), 1 µL of primer F (10 pmol), 1 µL of primer R (10 pmol), and 3 µL of previously exacted DNA sample. The amplification program included the following steps: initial denaturation at 94°C for 3 min, 35 cycles of denaturation at 94°C for 1 min, hybridization at 55°C for 1 min, and elongation at 72°C for 1 min. Finally, there was a final extension at 72°C for 10 min. The expected size of the PCR product fragment is 675 bp.

Table 1: Sequences of *cna* and *edinB* primers used for the polymerization chain reaction

Genes	Sequences	Size
cna	*cna* 1: 5'-CGGGAGATATGCTACCAGAAGATA-3'	278
	cna 2: 5'-ATAGCCTTGTGGAATTGTTACATCA-3'	
edinB	edinb 1: 5'-ACAGACTTAGTTGAAGCTACTAAATG-3'	522
	edinb 2: 5'-TGTCCCTGTAGGCAAAAGAACTTCTTG-3'	

Search for virulence factors in *S. aureus* species: Multiplex PCR on purified DNA using primers (Table 1) previously used by Sina *et al.*[14] searched for the presence of genes encoding collagen binding protein (*cna*) and toxin edin factors (*edinB*).

The reaction mixture was made in a total volume of 20 µL containing 2 µL of 10X buffer, 2 µL of $MgCl_2$ (25 mM), 0.2 µL dNTP (10 mM), 1 µL of each primer (10 pmol), 0.3 µL of Taq polymerase (5 U/µL), and 5 µl of the DNA sample. The amplification program was as follows: Initial denaturation (94°C) for 5 min; 35 cycles of denaturation (94°C) for 1 min; hybridization (50°C) for 1 min and elongation (72°C) for 1 min and finally final extension (72°C) for 10 min.

Data analysis: Data coding was performed using Microsoft Excel 2013 spreadsheet software. GraphPad Prism 8 software determined significant differences between calculated means at the 5% threshold ($p<0.05$).

RESULTS

Microbial diversity of suppurations: Of the 107 cases of suppuration studied, 51.8% were positive for Gram-positive bacteria. 36.5% of samples were contaminated with *S. aureus* strains and 12.2% with *Streptococcus* spp. Table 2 shows the frequency of bacteria isolated from suppurative samples.

Antibiotic susceptibility of cocci strains isolated from suppurations

Antibiotic resistance of *Staphylococcus* spp.: The antibiotic susceptibility of *Staphylococcus* spp. was variable based on the results depicted in Fig. 1. The test results indicate that all the strains of *Staphylococcus* spp. (100%) are resistant to penicillin (P), nitrofurantoin, and netilmicin. However, less than half of the strains (14.3% for Fosfomycin and 26.7% for Fosfomycin/Trometamol) of *Staphylococcus* spp. exhibit resistance to Fosfomycin and Fosfomycin/Trometamol, respectively.

Antibiotic resistance of *Streptococcus* spp.: The susceptibility of different *Streptococcus* spp. strains to various antibiotics were evaluated, and the results were illustrated in Fig. 2. The findings of this study suggested that not all *Streptococcus* spp. strains are resistant to antibiotics. Among the antibiotics tested, fusidic acid, cefixime, ceftriaxone, ertapenem, levofloxacin, sulfamethoxazole-trimethoprim, penicillin, and netilmicin were found to be ineffective against all *Streptococcus* spp. strains. However, meropenem and nitrofurantoin were effective against all *Streptococcus* spp. strains.

Biofilm formation of gram-positive cocci: The biofilm formation test reveals that 55.4% of gram-positive cocci were biofilm-forming (Fig. 3). Considering species, it is observed that 66.67% of *Streptococcus* spp. The isolated species were biofilm-forming bacteria, followed by *Staphylococcus aureus* (53.7%). 33.3% of the coagulase-negative *Staphylococcus* species in our study were biofilm-forming. Variance analysis shows no significant difference between biofilm-forming and non-biofilm-forming cocci+($p>0.9999$).

Detection of the *mecA* gene in isolated *Staphylococcus* spp.: The detection of the *mecA* gene reveals that 17.9% of the *Staphylococcus* strains possess this gene. Considering their biochemical character, it is observed that 33.3% of the coagulase *Staphylococcus* strains possess the *mecA* gene. At the same time, 16.7% of *S. aureus* have the *mecA* gene (Fig. 4).

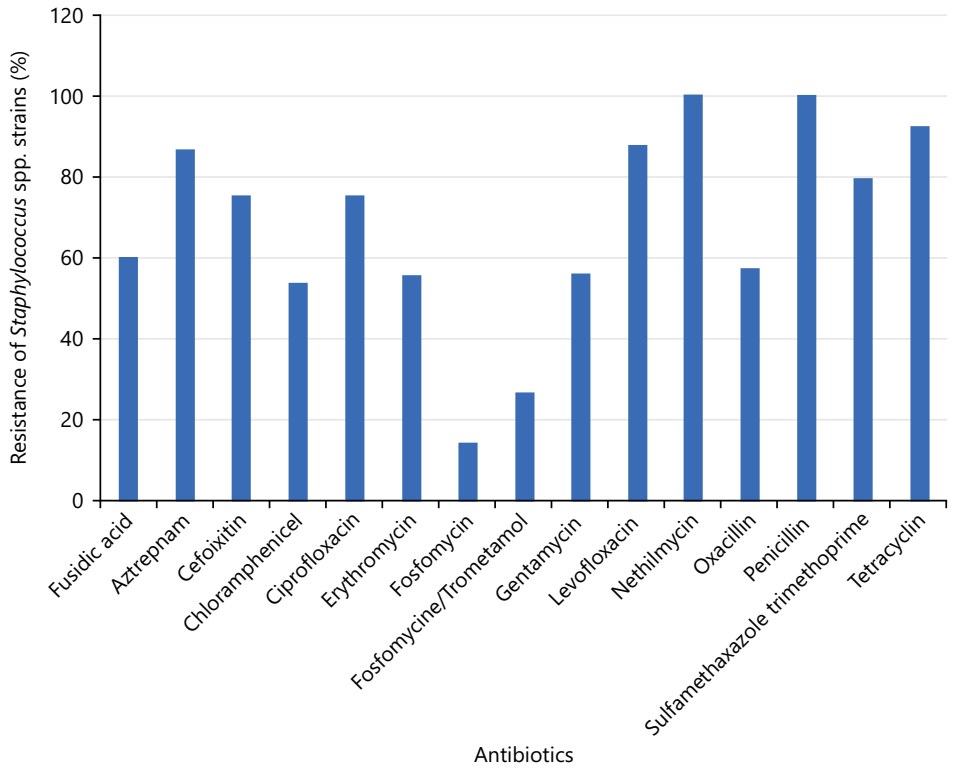

Fig. 1: Antibiotic resistance of *Staphylococcus* spp.

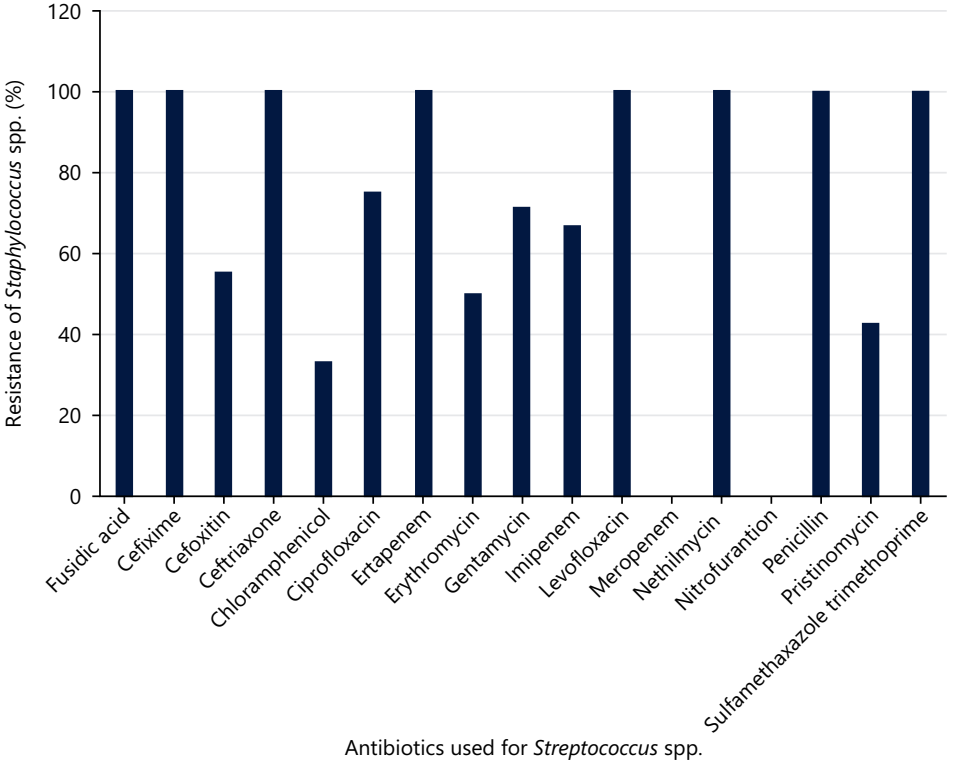

Fig. 2: Antibiotic resistance of *Streptococcus* spp.

Table 2: Frequency of bacteria isolated according to species

Bacteria isolated	Percentage (%)
Staphylococcus aureus	36.5
Streptococcus spp.	12.2
Coagulase-negative *Staphylococci-*	3.7

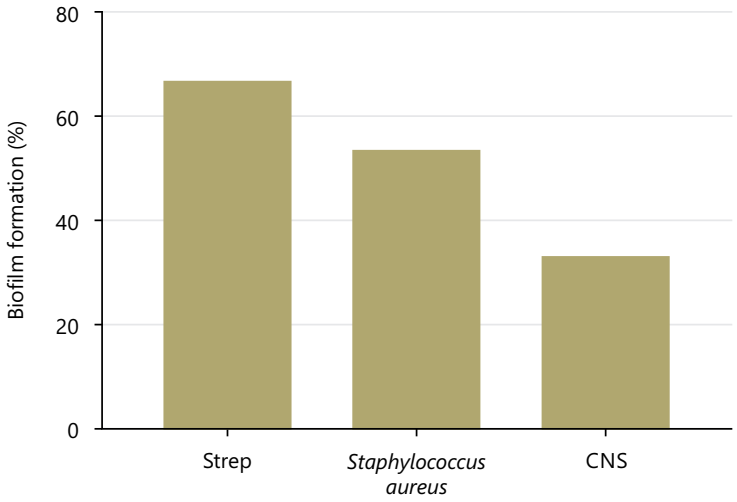

Fig. 3: Biofilm formation pattern of isolated gram-positive cocci
Strep: *Streptococcus* spp, CNS: coagulase-negative *Staphylococcus*

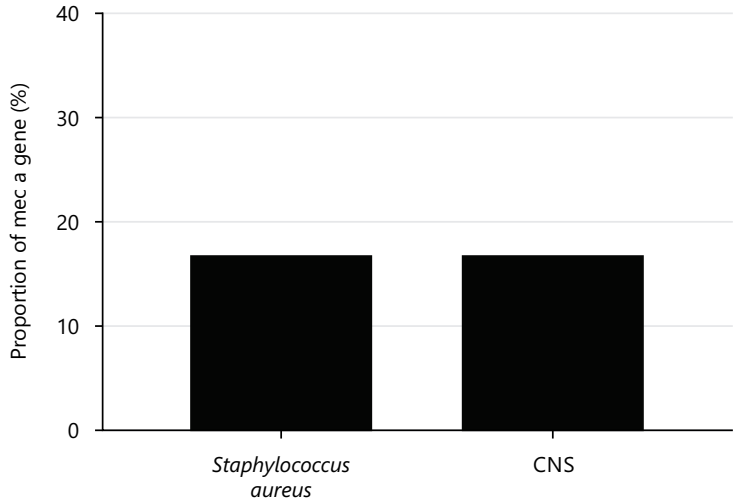

Fig. 4: Percentage of identified *Staphylococcus* species with *mec* A genes
CNS: coagulase negative *Staphylococcus*

Detection of the virulence genes *edin* B and *cna* in isolated *Staphylococcus* spp.: The detection of the *cna* gene reveals that 43.6% of *Staphylococcus* strains have this gene. Considering their biochemical character, 44.4% of *Staphylococcus aureus* strains possess the *cna* gene. At the same time, 33.3% of the coagulase-negative *Staphylococcus* strains have the *cna* gene (Fig. 5). Nevertheless, none of the strains possessed the *edin B* gene.

DISCUSSION

Bacteriologically, 51.8% of the suppuration samples tested positive for Gram-positive bacteria. The study results were lower than those reported by a study done in 2020 in the Department of Microbiology, SMS Medical School, India, which reported an overall positivity rate of 85.02% in patients with pus or wound infection[15,16]. The sample size could explain this difference. In addition, suppuration is defined by the urgent nature of the management, which did not ensure rigorous asepsis through proper patient preparation.

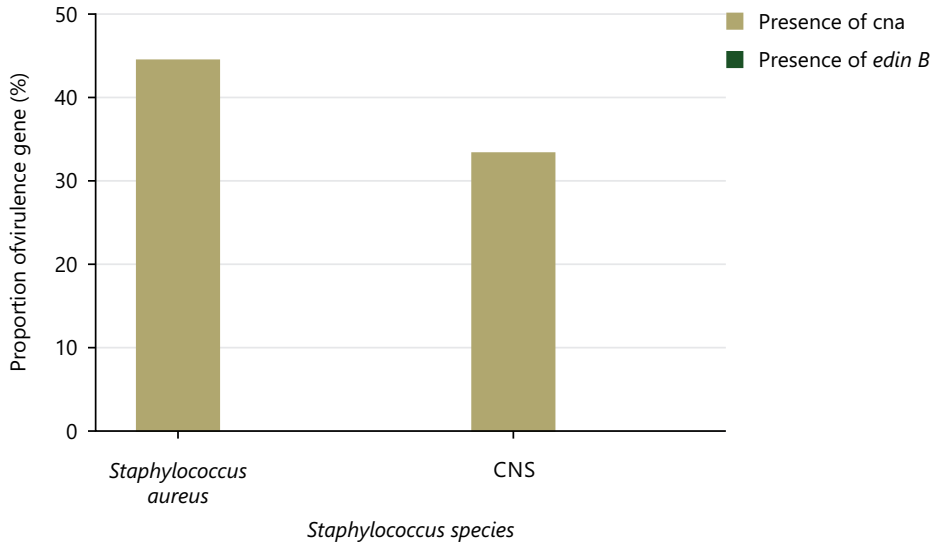

Fig. 5: Percentage of identified *Staphylococcus* species with *edin* B and cna genes
CNS: coagulase negative *Staphylococcus*

Despite growing concerns about antibiotic-resistant bacteria, systemic antibiotics are recommended when there is clear evidence of infection[17]. Bacterial isolates have been examined for their sensitivity profile to the most used antibiotics in therapy. In this study, *S. aureus* strains were resistant to oxacillin (57.1%). Oxacillin resistance is significant because it can give us the percentage of Methicillin-resistant *S. aureus* (MRSA). *S. aureus* strains (100%) are resistant to 8 antibiotics (ticarcillin, kanamycin, nalidixic acid, tazobactam/piperacillin, doxycycline, norfloxacin, penicillin, nitrofurantoin, netilmicin, and pefloxacin). This high resistance of *S. aureus* strains could be explained by the patient's environment favoring the diffusion of bacterial resistance. These decreases in strain sensitivity could also result from the increasing consumption of antibiotics and the easy accessibility of certain antibiotics without a medical prescription, which would cause the emergence of multi-resistant bacterial strains.

Regarding the isolated *Streptococcus* spp. strains, total resistance to 12 antibiotics (levofloxacin, sulfamethoxazole-trimethoprim, aztreonam, oxacillin, penicillin, netilmicin, pefloxacin, nalidixic acid, optochin, amikacin, cefepime, cefixime, and to ertapenem) was recorded. This elevated resistance may be attributed to the improper or ineffective use of antibiotics, which plays a significant role in developing antibiotic resistance. Furthermore, fluoroquinolone resistance is mainly linked to mutations in the quinolone resistance-determining regions of the genes that code for DNA gyrase (*gyrA* and *gyrB*) and topoisomerase IV (*parC* and *parE*)[18]. Additionally, mutations in genes related to membrane-associated efflux pumps may also play a role in the emergence of fluoroquinolone resistance[18].

Coagulase-negative *Staphylococci* (SCN) strains are all resistant to 10 antibiotics (ampicillin, ciprofloxacin, spiramycin, sulfamethoxazole-trimethoprim, amoxicillin+clavulanic acid, meropenem, tetracycline, levofloxacin, and ticarcillin). Multidrug-resistant SCNs can adhere to medical devices and surfaces through the slime secreted by the cell and have a mucopolysaccharide structure. This way, they can quickly colonize and spread in the hospital environment[19]. In addition, the viscosity factor protects coagulase-negative *Staphylococci* from antibiotics, phagocytosis, and chemotaxis[18].

Regarding biofilm formation, 55.4% of gram-positive cocci were biofilm formers. Among these gram-positive cocci, 66.7% of *Streptococcus* spp. species, 53.7% of *Staphylococcus aureus*, and 33.3% of coagulase-negative *Staphylococcus* species were biofilm-forming. Our results are lower than the 87.7% obtained by Naorem *et al.*[20] in Hungary on clinical strains and the 88.7% biofilm formation reported in

Algeria on staphylococcal strains isolated from human, animal, and food infections[20]. This difference would be because biofilm formation is a complex phenomenon influenced by many factors, including the surrounding environment and genetic regulatory factors[21]. The strains of *S. aureus* and the biofilm-forming coagulase-negative *staphylococci* are dangerous germs since their virulence also resides in the capacity to produce an extracellular matrix and form a biofilm[22]. Indeed, biofilms play an essential role in infection by creating a barrier that allows cocci to evade the host immune system and resist antibiotics, thereby impeding wound healing and prolonging illness[23]. The growing interest in bacterial biofilms stems from their natural ability to withstand antimicrobial agents and host inflammatory responses. Biofilms exhibit various resistance characteristics in their growth patterns, and studies indicate that higher mutation rates within biofilms contribute to the development of resistance[24].

The *mecA* gene in bacterial cells allows them to resist antibiotics such as methicillin, penicillin, and other penicillin-like antibiotics[25]. The bacterial strain best known to carry the *mecA* gene is methicillin-resistant *S. aureus* (MRSA). With *Staphylococcus, mecA* is spread via the genetic element of the staphylococcal chromosome cassette *SCCmec*[26]. In this study, 17.95% of *S. aureus* strains possess the *mecA* gene, of which 33.3% of coagulase-negative *Staphylococcus* strains have the *mecA* gene. At the same time, 16.7% of *Staphylococcus aureus* strains possess the *mecA* gene. Current results contradicted Naorem *et al.*[19] study obtained in Hungary. These authors showed that 94.3% of clinical *S. aureus* carried the *mecA* gene. This disparity may be attributed to the limited transfer of the *mecA* gene among *S. aureus* strains in pus. However, many studies indicate that the *mecA* gene also exists in coagulase-negative staphylococci[27].

The *cna* gene, which encodes a protein that plays a role in the infectious process of pathogenic *S. aureus*, has been identified as a virulence factor in various animal models of staphylococcal infections, such as arthritis, keratitis, endocarditis, mastitis, and osteomyelitis[28]. In our study, 43.6% of *S. aureus* strains possess this gene. These results were similar to those obtained by Baba-Moussa *et al.*[29]. in Benin, who showed that genes encoding agglutination factor B, collagen, and laminin-binding proteins (*clfB, cna, lbp,* respectively) were frequent (30-55%). Nevertheless, none of the strains possessed the *edinB* gene. The prevalence of these genes in *S. aureus* needs to be better described. However, in diabetic foot ulcers, *S. aureus* isolates were positive for the *edin* genes (A and B) in 7.2% of patients[30]. A 14.0% prevalence of EDIN-encoding genes was found in *S. aureus* isolates from various clinical sites of infection in Nice[31]. Interestingly, the association between PVL and EDIN in MRSA has been observed with a prevalence ranging from 12-100%[32].

The presence of virulence and antibiotic-resistance genes emphasizes the need for ongoing surveillance and a deeper understanding of these pathogens in hospital and community settings.

Identifying biofilm-forming strains suggests the necessity for tailored treatment strategies, potentially incorporating anti-biofilm agents. Additionally, the study's insights can contribute to developing public health policies to control antibiotic resistance.

Promote education on proper wound care and hygiene to prevent infections in healthcare and community settings. And Encourage further research into alternative treatments and preventive measures for wound infections, particularly in resource-limited settings.

While 107 cases were analyzed, a larger sample may provide more comprehensive insights into the diversity of pathogens and resistance mechanisms. In addition, Findings are specific to the CHUD-BA and may not be generalizable to other regions or healthcare facilities with different demographic or environmental contexts.

CONCLUSION

This study emphasizes that effective wound healing depends on good hygiene, proper management of infections, and the use of effective antimicrobial treatments. It highlights the prevalence of *Staphylococcus* spp. and *Streptococcus* spp. in suppurative infections and the pressing issue of antibiotic resistance. Strengthening infection control measures in healthcare settings is essential, along with educating patients and providers on optimal wound care practices. Future research should explore targeted therapies and alternative strategies.

SIGNIFICANCE STATEMENT

This study highlights the prevalence of Gram-positive bacteria, particularly *Staphylococcus* aureus and *Streptococcus* spp., in suppurative infections at the Departmental University Hospital of Borgou-Alibori. The findings underscore a critical public health issue: the high levels of antibiotic resistance among these bacteria complicating treatment and prolonging healing times. By identifying key virulence and antibiotic resistance genes, the research emphasizes the need for improved hygiene practices, careful management of wound infections, and the rational use of antibiotics to enhance healing outcomes.

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