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Adaptive Strategies of *Staphylococcus Species* Isolated from Clinical and Healthcare Environments: Biofilm Formation, Methicillin and Natural Products Resistant Ability.

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Highlights

- **High prevalence of methicillin-resistant *staphylococci* (MRS) was identified among clinical isolates, particularly in CoNS.**
- **Coagulase-positive *staphylococci* (CoPS) exhibited a significantly higher capacity for biofilm formation than CoNS.**
- **No direct association was observed between biofilm-forming ability and specific antimicrobial susceptibility profiles.**
- **Arabic gum (*Acacia gummifera*) extract showed no significant antibacterial effect against highly resistant *Staphylococcus* strains**

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ABSTRACT

Background and Aim: *Staphylococcus* species, Gram-positive cocci, are implicated in both nosocomial and commensal infections, attributed to biofilm formation on medical device surfaces and contaminated areas, often aggravated by insufficient cleaning practices. This study investigates biofilm formation, methicillin resistance, and the impact of Arabic gum extraction on *Staphylococcus* species in healthcare settings, offering epidemiological insights for infection prevention and control strategies. **Patients and Methods:** *Staphylococcus spp*. Were isolated from healthcare workers and outpatients, involving samples from skin, nasal, urine, and hospital environment sources. The isolates were identified by standard phenotypic methods, including colony characteristics, Gram staining, catalase, and coagulase tests. Antibiotic susceptibility and biofilm formation tests were conducted, and data were analyzed using Excel and Minitab Info. **Results**: - The findings indicate that 29.7% of *Staphylococcus spp.* are coagulase-negative, while 70.29% are coagulase-positive. Biofilm formation varied among the isolates, with coagulase-positive strains showing stronger biofilm production than coagulase-negative strains. Methicillin resistance was observed in healthcare workers, outpatients, and hospital environments. However, aqueous Arabic Gum extract demonstrated no impact on resistant strains, and 43% revealed biofilm formation. **Conclusion:** Furthermore, the study reveals that *Staphylococcus spp*. isolates from hospital surfaces, urine, and nasal sources exhibit higher methicillin resistance, with coagulase-negative *Staphylococcus* displaying lower biofilm formation. The study utilized a consistent concentration of Arabic Gum, and its effect on resistant strains remains uncertain.

1. Introduction

Staphylococcus species (spp) are a complex group of Gram-positive cocci that constitute both commensal microbiota and opportunistic pathogens in humans and animals (Nakaminami *et al.*, 2021). Coagulase-negative *Staphylococcus* (CoNS) and Coagulase-positive *Staphylococcus* (CoPS*)* species are regarded as part of the normal flora and can be found in various parts of the human and animals' body, such as skin and mucosal surfaces of the respiratory and gastrointestinal tracts (Piette and Verschraegen, 2009). However, these bacteria can attach to and colonize environmental surfaces, particularly those associated with long-term infections involving medical devices, and are therefore responsible for both nosocomial and community-acquired infections (Proctor, 2000; Rogers, Fey, and Rupp, 2009; Piette and Verschraegen, 2009). As previously reported, commensal flora in the host body frequently includes opportunistic pathogens such as *Staphylococcus spp*, particularly on the epidermis (Bierowiec *et al*., 2016). Although these bacteria normally coexist peacefully with their hosts and contribute to microbial homeostasis, *Staphylococcus spp*, may shift from a commensal to pathogenic state under specific conditions, leading to infection (Smythe *et al*, 2023).

Biofilms are complex structured communities of microorganisms embedded within a self-produced extracellular polymeric matrix. *Staphylococcus spp* are well known for their ability to form biofilms (Harapanahalli *et al.,* 2015); biofilm development significantly enhances their virulence (Bierowiec *et al.,* 2016). Biofilm formation enables bacterial persistence, particularly on abiotic surfaces such as biomaterial implants and medical devices including pacemakers, knee replacements and hip prostheses. Biofilm formation begins with the attachment of individual *Staphylococcus* cells to a surface, followed by cellular proliferation and release of

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extracellular polymeric materials that function as the foundation for the biofilm matrix (Jee, S. *et al.,* 2020).

According to the European Centre for Disease Prevention and Control, approximately three million cases of nosocomial infections are recorded annually within the European Union, with nearly 50,000 resulting in death (ECDC 2007). These infections can also affect not only patients but also healthcare workers (HCWs) and visitors (Lis *et al.,* 2009). Between 60% and 70% of nosocomial (hospital-based) infections, the use of different medical equipment with surfaces contaminated with pathogenic bacteria is assumed to play a role (Bryers *et al.,* 2008). Furthermore, it is believed that hand contamination among HCWs contributes to the spread of pathogen transmission. It has been demonstrated that applying disinfectants correctly can significantly reduce microbial load, which in turn reduces the risk of HCIs by over 40% (Kampf *et al.,* 2009). However, biofilm-forming bacteria exhibit increased tolerance to antibiotics and disinfectants, contributing to the apparent inefficiency of conventional skin disinfection strategies (Nieshimura *et al.,* 2006; Presterl *et al.,* 2007; Høiby *et al.,* 2009). Therefore, to reduce the risk of infections associated with these microorganisms, it is imperative to undertake more stringent disinfection methods to remove biofilm strains from the general hospital environment. Numerous bacteria are grouped in biofilm forms in the natural world (Costerton *et al.,* 1999).

In clinical settings, biofilm formation presents a serious threat because it helps microorganisms withstand antimicrobial drugs, evade immune responses, and survive in harsh environments (Alzahrani *et al*., 2017; Lu *et al.,* 2022). Biofilm is a complex structure created by microorganisms on both biotic and abiotic surfaces (Riaz et al., 2023). Biofilms can adhere to a variety of medical surfaces, including wounds, water lines, tubing, endoscopes, intravenous and urinary catheters, and medical equipment and prostheses (Topka-Bielecka *et al.,* 2021). These biofilms work as harbors for harmful bacteria, which makes it simple for them to proliferate and infect susceptible individuals (Hill & Liz-Marzán, 2017). Because it is closely linked to the increase in nosocomial or hospital-acquired infections, the production of biofilms in healthcare settings has gained significant attention (Minarini *et al.,* 2020).

The rapid development of bacterial adaptive strategies has significantly contributed to the emergence and dissemination of antibiotic resistance (Assouma *et al*., 2023). Consequently, considerable effort has been directed toward identifying herbal antibacterial agents that may counteract resistant bacteria. Arabic gum, obtained primarily from *Acacia senegal* and related species within the Leguminosae family, has long been used in Africa for its medicinal properties (Ali and EL Said, 2020). It has been employed in traditional medicine for treating inflamed tissue and intestinal mucosal inflammation (Ali and EL Said, 2020). Biofilm formation is widely recognized as a major virulence factor contributing to the persistence and resistance of bacterial infections. Therefore, this study aimed to determine the prevalence of biofilm-forming *Staphylococcus spp*. (CoNS and CoPS) isolated from hospital environments and HCWs by detecting their biofilm-producing ability, methicillin resistance profiles, and the effect of aqueous AG extract. The findings of this study may provide valuable epidemiological data to support prevention and control strategies, alongside antibiotic susceptibility profiling of isolated strains.

2. Materials and Methods

2.1. Bacterial isolates

A total of 101 *Staphylococcus* isolates were collected from infectious and non-infectious sources, including healthcare workers (HCWs) and outpatients attending Brack Hospital in southwestern Libya, between August 1, 2018 and December 31, 2018. The isolates were obtained from skin samples (n = 64), nasal swabs (n = 22), urine samples (n = 10), and hospital environmental samples (n = 5). Ethical approval was obtained from the Ethics Committee of the Faculty of Engineering and Technology Sciences and the relevant hospital review board before sample collection.

Identification of isolates

The isolates were identified using standard microbiological and biochemical methods, including growth on routine culture media at 37°C, Gram staining, catalase testing, and coagulase testing. Based on these results, the isolates were classified as coagulase-negative *staphylococci* (CoNS) or coagulase-positive *staphylococci* (CoPS). Identified isolates were preserved in glycerol (20% v/v) at -70°C according to Oskouei *et al*. (2010).

2.2. Antibiotic susceptibility testing (AST)

All *Staphylococcus* isolates were tested for susceptibility to cefoxitin (FOX, 30 µg; Oxoid Ltd., Basingstoke, Hampshire, England) using the Kirby–Bauer disk diffusion method in accordance with Clinical and Laboratory Standards Institute guidelines (CLSI, 2020). The bacterial suspension was adjusted to a 0.5 McFarland standard, and the inoculum was evenly swabbed onto Mueller–Hinton agar plates. A cefoxitin disk was placed on the agar surface, and plates were incubated at 37°C for 24 h. Inhibition zone diameters were measured and interpreted according to CLSI criteria (CLSI, 2020). *Staphylococcus aureus* ATCC 25923 was included as the quality control strain (CLSI, 2020).

2.3. Arabic gum susceptibility test

Arabic gum was purchased from a traditional market and ground into powder, then dissolved in distilled water to prepare a 50% (w/v) Arabic gum aqueous extract according to Perumal Samy & Ignacimuthu (2006). Sterile filter paper discs (7 mm) were impregnated with 20 µL of the aqueous gum extract and placed on Mueller-Hinton agar plates previously inoculated with the bacterial isolates. Plates were incubated at 37°C for 24–48 h, and the inhibition zone diameter was measured in millimeters (Tohidpour *et al.* 2010).

2.4. Biofilm formation assay

Biofilm formation was assessed according to Stepanovic *et al.* (2007) and Nepean *et al.* (2018) using the tissue culture plate method in Falcon® 96-well flat-bottom microplates (Corning, USA). Overnight bacterial cultures grown on blood agar were inoculated into tryptic soy broth and incubated at 37°C. The bacterial suspension was adjusted to the required density and diluted 1:100. Then, 200 µL of the inoculum was added to each well in duplicate and incubated at 37°C for 24 h. After incubation, the contents of the wells were removed, and the wells were washed three times with 300 µL sterile phosphate-buffered saline (pH 7.3). The adhered cells were fixed with 150 µL of 95% ethanol for 20 min, air-dried, and stained with 150 µL of 1% crystal violet for 30 min. Excess stain was removed, the wells were rinsed with tap water, and the plate was dried. Then, 150 µL of 33% glacial acetic acid was added to each well to solubilize the stain. Optical density (OD) was measured using an ELISA plate reader at 450 and 630 nm. The results were interpreted based on the OD cut-off value (ODc), calculated as the mean OD of the negative control plus three standard deviations (Stepanovic *et al.,* 2007; Nepean *et al.,* 2018). Isolates were classified as follows: OD ≤ ODc = no biofilm production (0); ODc < OD ≤ 2×ODc = weak biofilm production (+); ODc < OD ≤ 4×ODc = moderate biofilm production (++); and OD > 4×ODc = strong biofilm production (+++).

2.5. Statistical analysis

All experiments were performed in duplicate. Data were analyzed using Microsoft Excel and Minitab Info version 16.1. Categorical variables were compared using the Chi-square test, and correlation analysis was performed using Pearson's correlation test. A p-value < 0.05 was considered statistically significant (Sokal and Rohlf, 1995).

3. Results

3.1. Sample distribution and methicillin resistance

101 isolates of *Staphylococcus* were found in hospital environments, outpatients, and healthcare workers. The majority of isolates were cefoxitin-resistant, suggesting that methicillin-resistant *staphylococci* (>90%) were highly prevalent in the environment under study (Table 1). Isolates obtained from hospital environments showed the highest level of resistance, followed by outpatients and medical personnel (Table 1). When isolates were recognized by sample type, skin swabs yielded the greatest number of isolates (>50%); however, nasal swabs, urine samples, and hospital surfaces also contributed to the overall collection. The isolates from nasal swabs, urine, and hospital surfaces exhibited the highest cefoxitin resistance among all sample types (Table 2).

Table 1

Source of isolation, coagulase reaction and cefoxitin susceptibility among *Staphylococcus* isolates.

Sample sources	*Sensitive to Cefoxitin No. (%)	*Resistant to Cefoxitin No. (%)	Total (No).
HCW	7(13.46)	45 (86.53)	**52**
Out patients	1(2.27)	43(97.72)	**44**
Hospital environment∞	0(0)	5(100)	**5**
Total CoPS	6(8.45)	65(91.54)	**71**
Total CoNS	2 (6.67)	28(93.33)	**30**

*Chi-Sq = 10.75, *P*-Value = 0.005 ∞Hospital Kitchen surface.

Values are presented as numbers (%). Cefoxitin susceptibility was determined by disk diffusion according to the CLSI (2020) guidelines. CoPS, coagulase-positive staphylococci; CoNS, coagulase-negative staphylococci

Table 2

Sample types and Cefoxitin susceptibility of *Staphylococcus* isolates.

Bacterial sources	*Sensitive to Cefoxitin No. (%)	*Resistant to Cefoxitin No. (%)	Total (No).
Skin swab	8(12.5)	56(87.5)	**64**
Nasal swab	0(0)	22(100)	**22**
Urine	0(0)	10(100)	**10**
∞Hospital Kitchen surface	0(0)	5(100)	**5**
Total	**8**	**93**	**101**

*Chi-Sq = 24, *P- =0.000 Value, Values are presented as number (%). Cefoxitin susceptibility was interpreted according to the CLSI (2020)* guidelines.

3.2. Biofilm, Resistance, and Arabic gum effect

The tissue culture plate method of biofilm technique revealed that over half (>50%) of the isolates did not generate biofilms. The other isolates, however, had weak, moderate, or strong biofilm-forming capacity (Table 3). Whereas coagulase-positive *staphylococci* showed a greater capacity for biofilm production than coagulase-negative *staphylococci* (Table 4). No significant correlation was found between the cefoxitin inhibition zone diameter and the biofilm optical density or between biofilm development and cefoxitin resistance (Table 4). The aqueous extract of Arabic gum in the present study did not inhibit the *Staphylococcus* isolates studied (Table 4 and Fig. 1).

Table 3

Biofilm formation capacity of *Staphylococcus* isolates

Biofilm category	No. of isolates (%)
Non-biofilm formation	58 (57%)
Weak biofilm formation	19 (19%)
Moderate biofilm formation	12 (12%)
Strong biofilm formation	12 (12%)

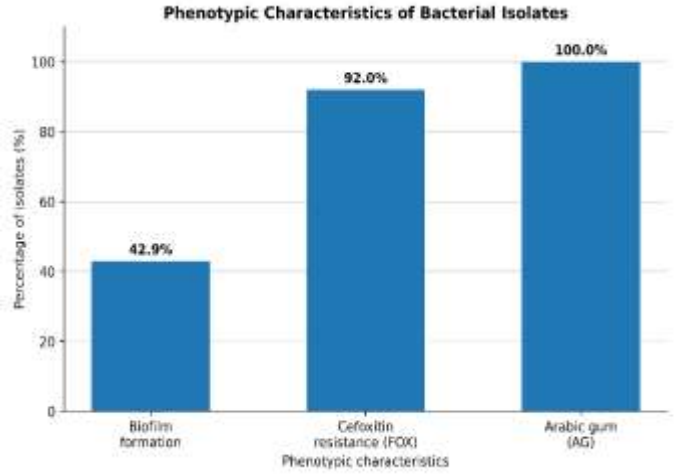

Fig.1-Biofilm formation and resistance to Cefoxitin and Arabic Gum among *Staphylococcus* strains

Table 4

Biofilm formation and Cefoxitin susceptibility of *Staphylococcus* isolates.

Biofilm* formation	Total CoNS No. (%).	Total CoPS No. (%)	*Cefoxitin-sensitive No. (%)	*Cefoxitin-resistant No. (%)
Non formation (0)	24(80)	34(47.9)	7(87.5)	**51(54.83)**
Weak (+)	20(6)	37(52.1)	1(12.5)	**18(19.35)**
Moderate (++)			0(0)	**12(12.9)**
Strong (+++)			0(0)	**12(12.9)**
Total (%) NO.			**8(7.9)**	**93(92)**

*Person correlation= r= -0.139, *P*-Value =0.166(relation between zone diameter and OD)

4.Discussion

Our findings presented a high proportion of methicillin-resistant *Staphylococcus spp*. Among our isolates, those that are coagulase-positive have a greater ability to form biofilms compared to coagulase-negative isolates. These findings suggest that both methicillin resistance and biofilm formation ability may contribute to the persistence of the target bacterial species in medical environments.

Most of the *Staphylococcus* species that were isolated for this investigation were members of the CoPS group. This was anticipated as COPS is frequently linked to some clinical disorders in people. Despite this, CoNS were also commonly found. The results of Martineau *et al*. (2000), who identified CoNS as urine pathogens, are in line with this. Most *Staphylococcus* isolates obtained in this study were recovered from skin specimens, followed by nasal swabs. These anatomical sites serve as primary reservoirs for *staphylococcal* colonization; the bacteria persist consistently. Other studies show the nasopharyngeal tract is usually the best place to detect the target bacterial species, yet our findings suggest colonization patterns might differ substantially across different populations or that how samples are collected could really change the results (Pereira *et al.,* 2010, and Braga *et al.,* 2017). *Staphylococcus spp.,* found in urine, require careful interpretation because such isolates may reflect contamination or colonization rather than a true urinary infection (Sigudu *et al.,* 2023).

A significant virulence factor that increases bacterial persistence on biotic and abiotic surfaces and plays a role in chronic and device-related illnesses is biofilm development. While the remaining isolates showed weak, moderate, or great biofilm-forming potential, over half of the isolates in this investigation did not develop biofilms. Compared to coagulase-negative *staphylococci*, coagulase-positive *staphylococci* exhibited a higher propensity to develop biofilm. The idea that biofilm expression varies depending on species, source, and environmental conditions is supported by this trend, which deviates from certain publications that demonstrate a higher proportion of powerful biofilm makers (Otto, 2008; Høiby *et al.,* 2010). Furthermore, no significant association was observed between biofilm-forming ability and antimicrobial susceptibility profiles, contrasting with previous findings that link biofilm production directly to increased resistance (Høiby *et al.,* 2010). In contrast to previous reports (O'Gara *et al.,* 2001; Nishimura *et al.,* 2006; Høiby *et al.,* 2010) that directly linked biofilm production to increased phenotypic resistance, this finding suggests that reduced drug susceptibility in biofilm producers may be physically mediated by matrix-restricted drug penetration rather than distinct genetic resistance pathways.

Of the 43 isolates of *Staphylococcus spp*. that were assessed for biofilm morphologies, 12% had moderate-to-strong biofilm capacity and 19% were weak producers. This is less than the significant biofilm prevalence of 46% that Oliveira and Cunha (2010) reported. Conversely, non-biofilm-producing CoNS were more prevalent than non-biofilm-producing CoPS (80% vs. 47.9%). Clinical strains show a variety of antibiotic resistance patterns in the hospital microbiota, which is extremely dynamic (Kochman, 2005). Compared to the 23.8% reported by Kateete *et al.* (2020), the overall prevalence of methicillin-resistant *staphylococci* (MRS) in our group was 92% (93/101). In a similar vein, the overall MRCoNS prevalence was 93.3% (28/101), which was far higher than the 20.9% (67/320) found in the same Ugandan study. High resistance to penicillin and elevated rates of nasopharyngeal MRS colonization reflect global trends of rising community and hospital MRS dissemination (Nazareth *et al.,* 2012), contrasting sharply with the lower prevalence reported in Nordic countries (Widerström, 2011). Because *staphylococci* are opportunistic pathogens, identifying virulent and resistant strains is critical for guiding clinical management (Chessa *et al.,* 2016). We observed a higher prevalence of virulence and MRS markers in our isolates compared to earlier African reports (Kateete *et al.,* 2011; Okee *et al.,* 2011), underscoring a heavy burden of resistance genes in both hospital and outpatient settings.

To place these results in a local context, our findings align with and expand upon regional data from Libya. Previous observations at Tripoli Hospital revealed that among clinical *staphylococcal* isolates, the MRSA rate was 28.4% (62/218) and the MRCoNS rate was 21.5% (47/218) (Aetrugh *et al.,* 2017). Furthermore, studies carried out in Benghazi hospitals found an 8% MRSA frequency on ambient surfaces and a 32% MRS phenotype in *S. aureus* clinical strains (Al-Abdli & Baiu, 2016). Acacia gummifera (Arabic Gum/AG) extract did not inhibit *Staphylococcus species* that were totally resistant to aminoglycosides, according to our investigation of alternative antimicrobial agents. These findings run counter to research by Ali (2020) and Lawrence *et al.* (2015), which found that AG extract significantly inhibited S. aureus. These findings were usually linked to bioactive substances including flavonoids, tannins, and alkaloids (Biswas & Roymon, 2013; Godghate *et al.,* 2014). The lack of efficacy observed here suggests that high-level methicillin and multidrug resistance mechanisms may confer cross-protection or tolerance against these natural phytochemical compounds.

5. Conclusion

In conclusion, our study focused on the behavior of isolated *Staphylococcus spp.*, particularly in relation to methicillin resistance and biofilm formation. Higher methicillin resistance was observed among isolates from clinical sources, especially CoNS. In contrast, CoPS showed a greater capacity for biofilm formation, whereas CoNS isolates generally exhibited weak or absent biofilm-forming ability. The potential antibacterial and anti-inflammatory effects of Arabic gum were also evaluated; however, its impact on highly resistant *Staphylococcus* strains was not significant, and its precise role in modulating resistance remains inconclusive, indicating that high-level resistance profiles limit the therapeutic applicability of this natural agent.

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Conflict of interest

The authors declare no conflict of interest

Data Availability

The data is shared only with authorized individuals.

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