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RESEARCH ARTICLE

Phenotypic and molecular characterization of biofilm formation, antibiotic resistance, and disinfectant tolerance in *Staphylococcus saprophyticus* isolated from urinary tract infections

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Abstract

Purpose This study investigates the phenotypic and genotypic characteristics of *Staphylococcus saprophyticus* isolates from urinary tract infections (UTIs), focusing on antibiotic resistance, biofilm formation, and disinfectant susceptibility.

Methods Antimicrobial susceptibility was assessed using the disk diffusion method, while biofilm formation was quantified via the microtiter plate assay. The minimum inhibitory and minimum bactericidal concentrations of four hospital disinfectants were determined using broth microdilution, and their antibiofilm efficacy was evaluated using the crystal violet assay. Antibiotics resistance and biofilm-associated genes were detected by polymerase chain reaction.

Results Among the isolates, 50% were multidrug-resistant, 10.86% carried the *mec*A gene and were classified as methicillin-resistant *S. saprophyticus*, and 89.1% exhibited biofilm formation, with 43.9% classified as moderate biofilm producers. Sodium hypochlorite (2.6%) and a benzalkonium chloride (10%)–glutaraldehyde (5%) mixture demonstrated the highest antimicrobial efficacy, while hydrogen peroxide (3%) exhibited the weakest antibacterial effect. Biofilm inhibition and eradication were most effective with sodium hypochlorite (2.6%) and hydrogen peroxide (3%), whereas benzalkonium chloride–glutaraldehyde mixture showed the least antibiofilm activity. The *qac*A/B gene, associated with disinfectant resistance, was detected in 28.27% of isolates, while *qac*C was present in 6.66%. Notably, subinhibitory disinfectant concentrations stimulated biofilm formation, potentially enhancing bacterial persistence in healthcare environments.

Conclusions These findings highlight the dual challenge posed by *S. saprophyticus* UTIs, where antibiotic resistance and biofilm formation contribute to treatment difficulties. The inappropriate use of disinfectants may select for resistant strains, emphasizing the need for evidence-based disinfection protocols to prevent bacterial persistence in clinical settings.

Keywords *Staphylococcus saprophyticus* · Urinary tract infection · Antibiotic resistance · Biofilm formation · Disinfectants

Introduction

Staphylococcus saprophyticus is a Gram-positive, coagulase-negative *Staphylococcus* (*CoNS*) and a notable uropathogen, particularly in community-acquired urinary tract infections (UTIs) among young, sexually active women [1]. It is the second most common cause of UTIs after *Escherichia coli*, accounting for approximately 5–10% of cases [2]. Recent studies suggest that the global dissemination of *S. saprophyticus* may be influenced by foodborne transmission, particularly through the meat production chain, which may serve as a reservoir for human infections [3].

Over the past five years, increasing antibiotic resistance in *S. saprophyticus* has become a growing concern. Resistance to commonly used antibiotics such as

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trimethoprim-sulfamethoxazole, fluoroquinolones, and tetracyclines has been reported in various regions, with biofilm formation playing a crucial role in enhancing resistance mechanisms [4]. Biofilm development is among the most significant virulence factors that increase the pathogenic potential of *S. saprophyticus* and play a pivotal role in the establishment of chronic infections [5]. Biofilms provide a protective niche that shields bacteria from the immune defenses and significantly reduces the effectiveness of antimicrobial agents [6]. These organized bacterial communities are encased in an extracellular polymers matrix that not only facilitates horizontal gene transfer but also promotes the development of antimicrobial resistance and enables eradication, especially in clinical environments [7]. Therefore, understanding the mechanisms of biofilm formation is critical for developing effective therapeutic and preventive strategies [6]. Notably, biofilm-forming isolates exhibit significantly increased minimum inhibitory concentrations (MICs) for antibiotics, with resistance levels often increasing more than two-fold compared to planktonic cells [8]. Furthermore, methicillin-resistant *S. saprophyticus* (MRSS) strains carrying novel staphylococcal cassette chromosome *mec* (SCCmec) elements have emerged, further complicating treatment options and highlighting the need for continuous surveillance [9].

Despite its clinical significance, comprehensive data on the prevalence and antimicrobial resistance mechanisms of *S. saprophyticus* in both community and hospital settings remain limited. Understanding the distribution and genetic determinants of resistant strains is crucial for guiding infection control strategies and improving treatment outcomes.

The World Health Organization emphasizes the importance of infection control measures, including stringent hygiene protocols, routine disinfection, and antimicrobial stewardship, to prevent the spread of multidrug-resistant (MDR) pathogens such as *S. saprophyticus* [10]. In this context, the Centers for Disease Control and Prevention have recommended standardized procedures for surface disinfection in healthcare environments, advocating for the use of effective disinfectants to reduce bacterial persistence on frequently touched surfaces [11].

Among the most widely used disinfectants in hospitals and medical laboratories (Alcohol-based, hydrogen peroxide-based, chlorine-based, and chlorine-glutaraldehyde combination) have demonstrated varying degrees of efficacy against *S. saprophyticus*. However, sub-inhibitory concentrations (sub-MICs) of certain disinfectants have been shown to paradoxically enhance biofilm formation, thereby increasing bacterial tolerance and persistence in clinical environments [12].

The aim of this study was to characterize, both phenotypically and genotypically, the antibiotic resistance patterns and disinfectant tolerance of *S. saprophyticus* isolates from UTIs, as well as their capacity for biofilm formation. Additionally, we evaluated the effectiveness of commercially available disinfectants against these isolates, with a specific focus on their antibacterial and antibiofilm properties. By elucidating the resistance mechanisms and biofilm dynamics of *S. saprophyticus*, this study contributes to the development of improved infection control strategies and informs the rational use of disinfectants in healthcare settings.

Materials and methods

Isolation and identification of *S. saprophyticus* strains

A total of 46 *S. saprophyticus* isolates, each representing a distinct case of UTIs, were collected from both public and private medical laboratories in the Greater Casablanca region, Morocco, between January 2020 and December 2022. Clinical data was systematically compiled through a multi-source approach, integrating patient medical records, laboratory reports, and complementary information obtained from urologists and affected individuals.

Among the 198 Gram-positive isolates, *Staphylococcus* spp. accounted for 130 (65.66%), including 112 CoNS (86.15%), of which *S. saprophyticus* represented 41.07% (46/112).

The isolates were initially characterized using a combination of standard morphological and biochemical assays. Mannitol fermentation was assessed on mannitol salt agar (MSA; Biokar Diagnostics, Beauvais, France), and classical microbiological tests—including Gram staining, catalase activity, coagulase production, hemolysin expression, and novobiocin susceptibility—were performed to distinguish *S. saprophyticus* from other staphylococcal species. To ensure precise species identification, all isolates were subsequently analyzed using the Vitek 2® Compact 15 automated system (bioMérieux, Marcy-l'Étoile, France), employing the Gram-Positive Identification card according to the manufacturer's protocol.

Following confirmation, the strains were preserved in brain heart infusion (BHI) broth (Oxoid, Thermo Scientific™), supplemented with 30% glycerol and stored at −20 °C to maintain viability for downstream analyses.

Antibiotic susceptibility and resistance determinants

The antimicrobial susceptibility of *S. saprophyticus* isolates was performed using the disk diffusion method on Mueller–Hinton agar (MH; Oxoid, Thermo Scientific™), following the guidelines of the Committee on Antibiograms of the French Microbiology Society (CASFM, 2021) [13]. The

antibiotic panel included penicillin-G (1 unit), cefoxitin (30 µg), levofloxacin (5 µg), gentamicin (10 µg), tobramycin (10 µg), erythromycin (15 µg), clindamycin (2 µg), tetracycline (30 µg), tigecycline (15 µg), linezolid (1 µg), trimethoprim/sulfamethoxazole (1.25/23.75 µg), and fusidic acid (10 µg). MICs for cefoxitin, teicoplanin and vancomycin were determined using the broth microdilution method in MH broth (MHB; Oxoid, Thermo Scientific™), with interpretation based on CASFM breakpoints [13]. Isolates exhibiting resistance to three or more antibiotic classes were classified as MDR [14].

To investigate β-lactam resistance, phenotypic β-lactamase activity was assessed using the edge effect method, which examines the inhibition zone around an ampicillin disk (1 unit) (Oxoid, Thermo Fisher Scientific™), as recommended by the Clinical and Laboratory Standards Institute (CLSI) [15]. Methicillin resistance was evaluated via the cefoxitin disk diffusion test (30 µg) following standard procedures [13]. Isolates were inoculated onto MH agar, incubated at 35 ± 2 °C for 20 ± 4 h, and inhibition zones were measured according to CASFM breakpoints [13]. *Staphylococcus aureus* ATCC 25923 (methicillin-susceptible) and ATCC 29213 (methicillin-resistant) were used as quality controls.

To confirm the genetic basis of β-lactam resistance, polymerase chain reaction (PCR) was performed to detect the presence of *bla*Z (encoding β-lactamase) and *mec*A (encoding penicillin-binding protein 2a, PBP2a) in all *S. saprophyticus* isolates resistant to penicillin and cefoxitin [16, 17] (Supplementary Table 1 et 2).

Biofilm formation and genetic determinants

The ability of *S. saprophyticus* isolates to form biofilms was assessed using the quantitative microtiter plate assay, as described by Christensen et al. [18]. A single colony from a fresh culture was inoculated into 3 mL of BHI broth and incubated overnight at 35 ± 2 °C under agitation (150–250 rpm). Following incubation, 180 µL of fresh BHI broth was dispensed into individual wells of a sterile 96-well microplate (Citotest, Jiangsu, China), and 20 µL of the bacterial suspension was added. A negative control, consisting of 200 µL of sterile, cell-free BHI broth, was included to verify medium sterility. The microplates were incubated at 35 ± 2 °C for 48 h to allow biofilm formation.

After incubation, non-adherent planktonic cells were carefully removed, and the wells were washed four times with 200 µL of sterile phosphate-buffered saline (PBS, pH 7.2) to eliminate residual bacteria. The plates were air-dried, and adherent biofilms were stained with 1% crystal violet (Sigma-Aldrich, Dorset, UK) for 15 min. Excess dye was removed by rinsing the wells three times with deionized water, and the stained biofilms were solubilized in 96% ethanol for 15 min. Optical density (OD) was measured at 590 nm using an automated microplate reader (Biotek Elx800, USA). All experiments were performed in triplicate to ensure reproducibility. The OD values were used to classify isolates into four categories: negative, weak, moderate, or strong biofilm producers, according to the criteria established by Stepanovic et al. with slight modifications [19].

To investigate the genetic basis of biofilm formation, PCR was performed to detect genes associated with intercellular adhesion polysaccharides (*ica*A, *ica*D, *ica*B, and *ica*R) and microbial surface components recognizing adhesive matrix molecules (*atl*, *aas*, *and uaf*A), as described by [1, 20] (Supplementary Table 1 et 2). The presence of these genes was analyzed in all *S. saprophyticus* isolates, providing molecular insights into their biofilm-forming potential.

Table 1 Disinfectants used in this study, classified according to their active compounds and contact time

Products	Active components	Concentration (%)	Contact time (min)
Product 1	Ethyl alcohol	70	15
Product 2	Benzalkonium chloride Glutaraldehyde	10 5	30
Product 3	Hydrogen peroxide	3	15
Product 4	Sodium hypochlorite	2.6	30

Antistaphylococcal activity of disinfectants and molecular detection of *qac* genes

To evaluate the antimicrobial efficacy of commonly used hospital disinfectants against *S. saprophyticus*, four commercially available products were selected based on their active components and safety profile. These disinfectants, representing alcohol-based, hydrogen peroxide-based, chlorine-based, and mixed formulations (chlorine and glutaraldehyde), were collected from hospitals and medical laboratories in Casablanca, Morocco. Aseptic sampling was performed, with 50 mL of each product transferred into sterile Falcon conical tubes (Corning, Arizona, United States) for further analysis. The disinfectants'compositions and recommended contact times are detailed in Table 1.

The antibacterial efficacy of disinfectants against *S. saprophyticus* isolates was evaluated using both disk diffusion and broth microdilution assays, adhering to international standards with slight modifications to enhance precision.

The disk diffusion method was performed on MH agar, following ISO 20645:2004 guidelines [21]. Bacterial suspensions, adjusted to a 0.5 McFarland standard (~ 10^8 CFU/mL), were evenly spread onto agar plates, and sterile filter paper discs impregnated with 10 µL of each disinfectant were placed on the surface. Gentamicin (10 µg) served as a

positive control, while sterile physiological water (0.85%) was used as a negative control. After incubation at 35 ± 2 °C for 18–24 h, inhibition zone diameters (IZD) were measured and classified as follows: no activity (IZD < 6 mm), weak (6–12 mm), moderate (12–20 mm), or strong activity (> 20 mm).

To further characterize the antimicrobial properties of disinfectants, the MIC and minimum bactericidal concentration (MBC) were determined using a broth microdilution assay in 96-well microtiter plates, following established protocols [22]. Serial two-fold dilutions of each disinfectant were prepared in MHB, and standardized bacterial suspensions (5×10^5 CFU/mL) were inoculated into each well. Bacterial viability was assessed using resazurin staining, with colorimetric changes indicating growth. The MBC was defined as the lowest disinfectant concentration capable of eliminating 99.9% of the bacterial population, confirmed by subculturing on agar plates. The bactericidal or bacteriostatic nature of each disinfectant was determined based on the MBC/MIC ratio, where a ratio ≤ 4 was indicative of a bactericidal effect, whereas a ratio > 4 suggested a bacteriostatic effect [23].

To investigate potential resistance mechanisms, PCR was performed to detect *qac* genes, specifically *qac*A/B and *qac*C, which confer resistance to quaternary ammonium compounds (QACs), as previously described [24, 25] (Supplementary Table 1 et 2).

Antibiofilm activity of disinfectants

The impact of disinfectants on *S. saprophyticus* biofilms was assessed by evaluating their ability to both inhibit biofilm formation and eradicate preformed biofilms. These assays were conducted using a quantitative microtiter plate method under standardized conditions.

To assess biofilm inhibition, biofilm-forming *S. saprophyticus* isolates were incubated in BHI broth at 35 ± 2 °C for 48 h without agitation in the presence of sub-MICs of disinfectants (MIC/2, MIC/4, MIC/8, and MIC/16). A negative control, consisting of BHI broth without disinfectants or bacteria, was included in each experiment. Following incubation, biofilm quantification was performed as described previously. The inhibition rate was determined using the formula [26]:

$$\text{Biofilm inhibition rate}(\%) = \frac{(ODc_1 - ODt_1)}{ODc_1} \times 100$$

ODc_1: the optical density of the control (without disinfectants); ODt_1: the optical density of the test sample (with disinfectants).

To evaluate biofilm eradication, preformed biofilms were allowed to develop over 48 h at 35 ± 2 °C in 96-well microtiter plates, with medium replenishment every 24 h to ensure structural maturation. Non-adherent planktonic cells were carefully removed by washing the wells three times with sterile PBS. The mature biofilms were then treated with different concentrations of disinfectants (MIC, 2MIC, and 4MIC) for their respective recommended contact times at room temperature. After incubation, residual

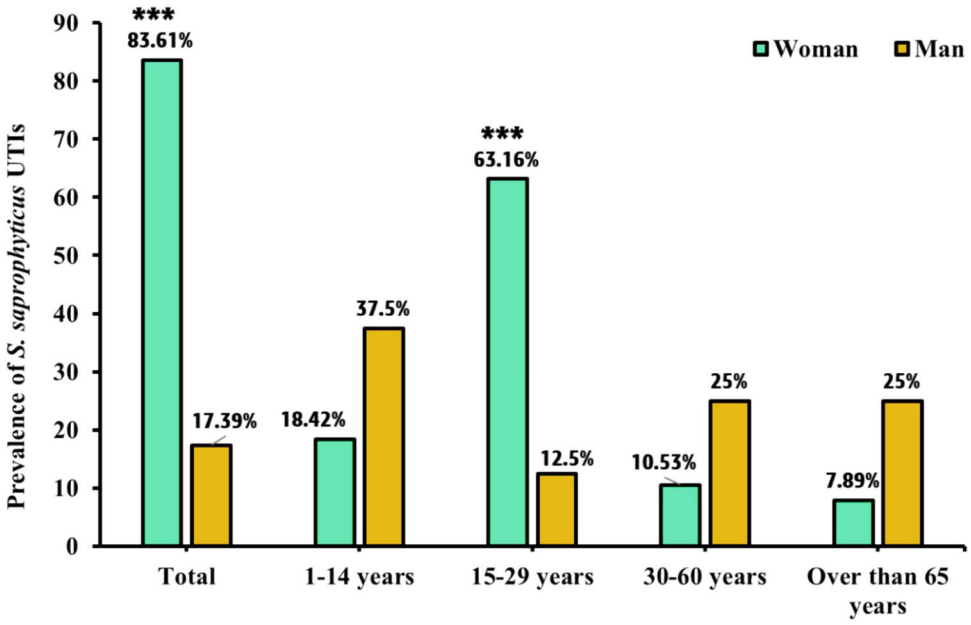


Fig. 1 Distribution of *S. saprophyticus* UTIs by sex and age; *** $p \leq 0.001$

biofilm biomass was quantified using the method described previously. The percentage of biofilm eradication was calculated as follows [27]:

$$\text{Eradication biofilm rate}(\%) = \frac{(ODc_2 - ODt_2)}{ODc_2} \times 100$$

ODc_2: the optical density of the preformed biofilm untreated with disinfectants; ODt_2: the optical density of the preformed biofilm treated with disinfectants.

Statistical analysis

All quantitative data were expressed as mean ± standard deviation (SD) and processed using Microsoft Excel. Statistical analyses were performed using SPSS software (version 26; IBM Corp., Armonk, NY, USA).

Comparisons of antimicrobial resistance and biofilm formation between groups were conducted using the Chi-square test or Fisher's exact test, depending on data distribution. The effectiveness of disinfectants against *S. saprophyticus* was evaluated using Dunnett's test, comparing the antibacterial activity of each product to that of a control. The Mann–Whitney *U* test was applied for non-parametric variables, including biofilm biomass intensity and its association with antibiotic resistance patterns.

To assess the predictive performance of the risk assessment model for *S. saprophyticus* UTIs, Receiver Operating Characteristic (ROC) curve analysis was conducted. The Area Under the ROC Curve (AUC-ROC) was computed to quantify the model's discriminatory power and to establish the optimal threshold.

For correlation analyses, Pearson's correlation coefficient (ρ) was used to assess the relationships between biofilm-associated genes and antibiotic resistance phenotypes. Hierarchical cluster analysis was performed to classify *S. saprophyticus* isolates based on their phenotypic and genetic characteristics. Clustering was conducted using Ward's linkage method with a Euclidean distance matrix, ensuring robust differentiation of bacterial subpopulations. Statistical significance was set at $p < 0.05$.

Results

Risk groups associated with *S. saprophyticus* UTIs

All *S. saprophyticus* isolates were catalase-positive, coagulase-negative, and resistant to novobiocin. The overall prevalence of *S. saprophyticus* UTIs was significantly higher in women (83.61%) than in men (17.39%) ($p < 0.001$). Among age groups, the prevalence in women was highest in the 15–29 years category (63.16%), while in men, the highest prevalence was observed in the 1–14 years (37.5%) and 30–60 years (25%) age groups (Fig. 1).

The difference in *S. saprophyticus* UTIs prevalence between sexes and across age groups was statistically significant ($p < 0.001$).

Table 2 Prevalence of *S. saprophyticus* isolates resistant to selected antibiotics

Antibiotics	MRSS (n = 5, 10.86%)	Non-MRSS (n = 41, 89.14%)	Over (n = 46)
Penicillin	5 (100%)	38 (92.68%)	43 (93.48%)
Erythromycin	5 (100%)	14 (34.15%)	19 (41.30%)
Clindamycin	5 (100%)	0	5 (10.87%)
Tetracycline	0	8 (19.51%)	8 (17.40%)
Trimethoprim-sulfamethoxazole	0	6 (14.64%)	6 (13.04%)
Fusidic acid	3 (60%)	33 (80.49%)	36 (78.3%)

Antibiotics resistance patterns

The resistance profiles of *S. saprophyticus* against selected antibiotics are summarized in Table 2. Among the isolates, 93.48% (43/46) exhibited resistance to penicillin, all carrying the *blaZ* gene and producing β-lactamase. Methicillin resistance (*mec*A-positive, Cefoxitin MIC ≥ 4 µg/mL) was detected in 10.86% (5/46) of isolates, classified as MRSS. Resistance to fusidic acid was observed in 78.3% (36/46) of isolates, while 41.3% (19/46) displayed resistance to erythromycin. Clindamycin resistance was exclusively detected in MRSS isolates. Resistance to tetracycline and trimethoprim-sulfamethoxazole was present at lower frequencies. No resistance was detected against aminoglycosides, fluoroquinolones, glycopeptides, or linezolid.

The *S. saprophyticus* isolates were classified into four groups based on their resistance profiles: fully susceptible (6.5%, Group 1), resistant to one or two antibiotics (39%, Group 2), MRSS (10.86%, Group 3), and MDR non-MRSS (43.47%, Group 4). Among the resistant phenotypes, the most frequently observed patterns included penicillin alone (14.29%), penicillin combined with fusidic acid (21.73%), and penicillin with erythromycin and fusidic acid (16.67%). The most prevalent MDR resistance patterns included penicillin, erythromycin, fusidic acid, clindamycin (7.14%), penicillin, erythromycin, tetracycline, fusidic acid (4.76%), and penicillin, fusidic acid, trimethoprim-sulfamethoxazole (14.29%).

Ability of isolates of *S. saprophyticus* to produce biofilm

Among the *S. saprophyticus* isolates, 89.1% (n = 41) exhibited biofilm formation, with 17% (n = 7) classified as strong biofilm producers, 43.9% (n = 18) as moderate, and 39% (n = 16) as weak. Notably, 10.9% (n = 4) did not form biofilms.

Biofilm biomass was significantly higher in MRSS isolates than in non-MRSS, as confirmed by the Mann–Whitney U test ($p < 0.001$).

Genotypic analysis revealed that *atl* was the most prevalent biofilm-associated gene (84.78%), followed by *aas* (82.61%) and *uafA* (58.7%). The *ica* operon was less frequent, with *ica*A and *ica*B detected in 19.57% and 17.39% of isolates, respectively (Fig. 2).

A significant correlation was found between the presence of *atl* and *aas* genes and moderate biofilm formation, while *uaf*A was associated with strong biofilm formation, and *ica*A correlated with weak biofilm production. However, no association was found between biofilm intensity (strong, moderate, or weak) and the presence of other biofilm-related genes.

Pearson's correlation analysis (n = 46) revealed several strong and moderate correlations among the studied genes. A strong positive correlation was observed between *atl* and *aas* ($\rho = 0.764$, $p < 0.01$), *ica*A and *ica*B ($\rho = 0.641$, $p < 0.01$), *ica*A and *ica*D ($\rho = 0.623$, $p = 0.002$), and *ica*D and *ica*B ($\rho = 0.844$, $p = 0.001$). Moderate correlations were also observed between *uaf*A and *aas* ($\rho = 0.310$, $p = 0.03$), *uaf*A and *ica*A ($\rho = 0.302$, $p = 0.04$), and *uaf*A and *ica*D ($\rho = 0.325$, $p = 0.02$). Additionally, *ica*R was significantly correlated with *ica*A ($\rho = 0.432$, $p < 0.01$), *ica*D ($\rho = 0.550$, $p < 0.01$), and *ica*B ($\rho = 0.465$, $p < 0.01$) (Fig. 3).

MRSS isolates (Group 3) exhibited the highest biofilm biomass, with a statistically significant difference compared to other groups ($p = 0.012$). All MRSS isolates were strong biofilm producers, and 45% of moderate biofilm producers were MDR. Weak biofilm-forming strains showed greater antibiotic susceptibility, with 67% being sensitive compared to 33% of moderate biofilm formers (Fig. 4A, B).

Higher biofilm biomass was observed in penicillin-, erythromycin-, and tetracycline-resistant isolates. However, no significant association was found between biofilm biomass and resistance to these antibiotics ($p > 0.05$). In contrast, a significant association was observed between biofilm biomass and clindamycin resistance ($p = 0.0008$) (Fig. 5).

Development of a risk assessment tool for *S. saprophyticus* UTIs.

This study aimed to develop a predictive model for assessing the risk of *S. saprophyticus*-associated UTIs based on key epidemiological and microbiological determinants. Four major risk factors were identified: patient sex, age, methicillin resistance, and biofilm formation. To evaluate the diagnostic performance of the model, a ROC curve analysis was performed, integrating both sensitivity and specificity to determine its discriminatory capability (Fig. 6).

The analysis yielded an AUC-ROC of 0.889 (95% confidence interval [CI] 0.884–0.953), demonstrating a strong predictive ability. The optimal cutoff value was established at 4, corresponding to a sensitivity of 73.9% and a specificity

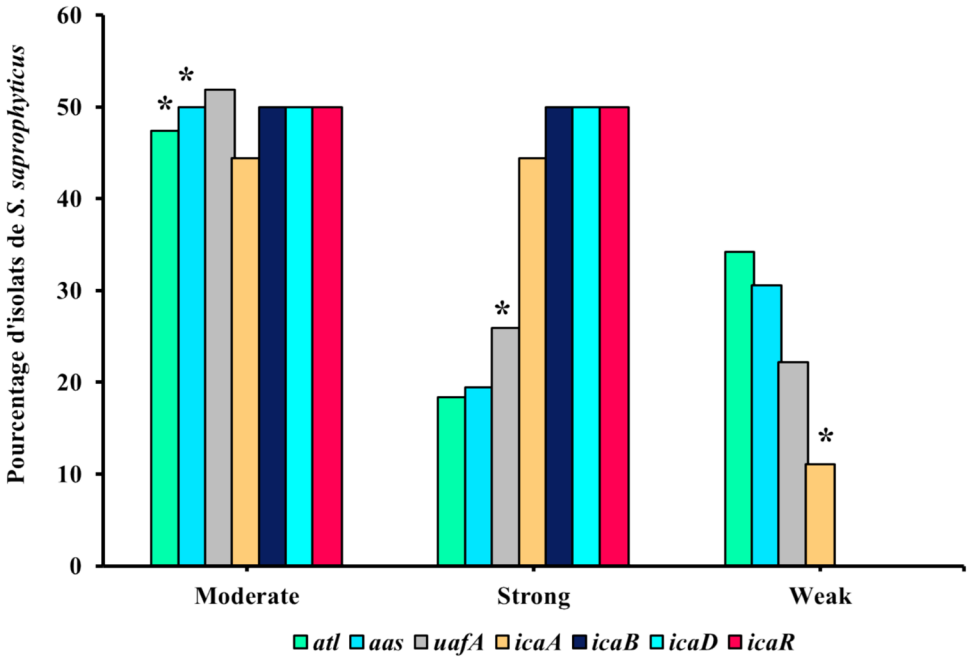


Fig. 2 Comparison of biofilm formation and the frequency of biofilm-associated genes in *S. saprophyticus* isolates. *$p \leq 0.05$

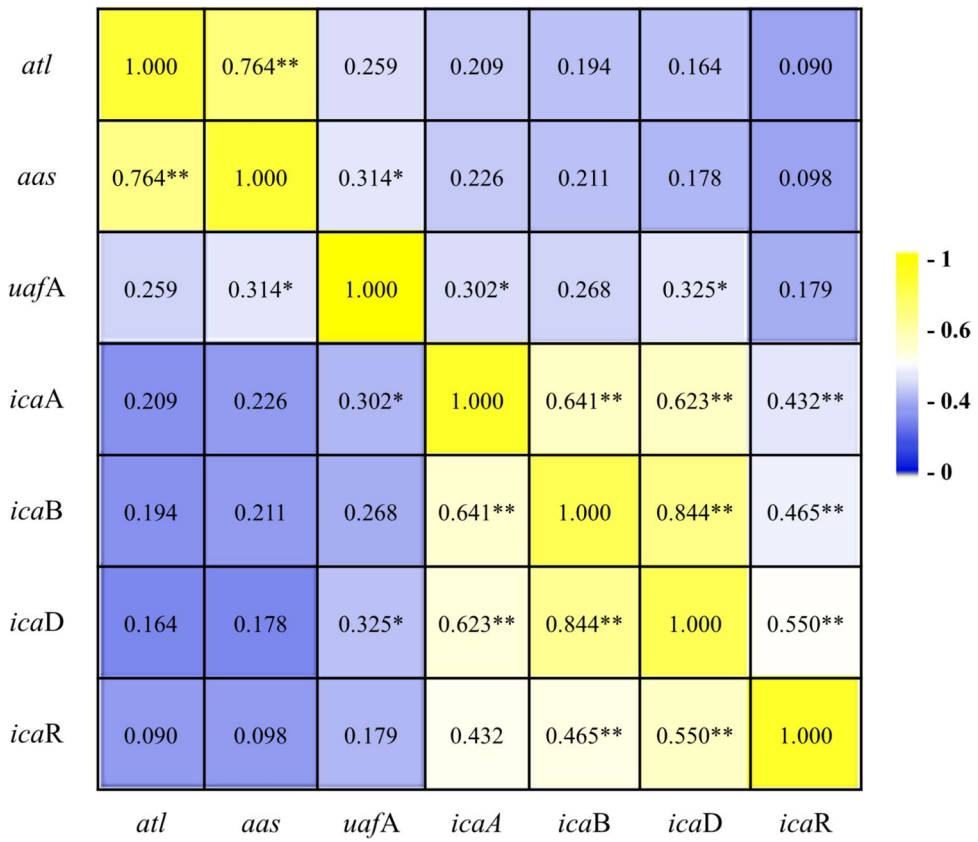


Fig. 3 Correlation matrix heatmap showing Pearson's correlation coefficients between biofilm-associated genes in *S. saprophyticus* strains. The color intensity represents the strength of the correlation, ranging from blue (weak or no correlation) to yellow (strong positive correlation). Asterisks indicate the level of statistical significance: $^{*}p \leq 0.05$, $^{**}p \leq 0.01$

of only 7.2%. These findings indicate that while the model is highly sensitive in detecting *S. saprophyticus* UTIs, its low specificity results in a high false-positive rate. Consequently, despite an overall accuracy of 88.9%, the model's clinical utility may be constrained in settings requiring higher specificity for diagnostic decision-making.

Antistaphylococcal potential of disinfectants and detection of resistance genes

The antibacterial efficacy of four disinfectants against *S. saprophyticus* was evaluated using the disk diffusion method (Table 3). Among the tested products, Product 2 exhibited the most potent antibacterial activity, with a strong inhibitory and bactericidal effect against all *S. saprophyticus* isolates. The MIC and MBC values for its active compounds were 0.07% (v/v) for benzalkonium chloride and 0.03% (v/v) for glutaraldehyde. The mean IZD for MRSS isolates was 31.4 ± 3.68 mm, while for MDR non-MRSS isolates, it measured 28.55 ± 2.8 mm.

In contrast, Product 4 displayed moderate antibacterial activity, with mean IZDs of 15.4 ± 0.88 mm for MRSS isolates and 16.35 ± 1.72 mm for MDR non-MRSS isolates. The antibacterial potential of Product 4 was confirmed by MIC values ranging from 0.02 to 1.3% (v/v) and MBC values between 0.04 and 1.3% (v/v). Product 1, however, exhibited a weaker antibacterial effect, inhibiting only 63% of *S. saprophyticus* isolates, with IZDs of 15.2 ± 4.56 mm for MRSS isolates and 12.85 ± 5.6 mm for MDR non-MRSS isolates (Fig. 7). The MIC values ranged from 0.54 to 17.5% (v/v), while the MBC values spanned 1.09 to 35% (v/v), indicating a limited bactericidal effect.

On the other hand, Product 3 showed no significant antibacterial activity, failing to inhibit 69.57% of *S. saprophyticus* isolates (Fig. 7). This lack of efficacy suggests an intrinsic resistance mechanism or an adaptation of *S. saprophyticus* strains to certain disinfectants.

To further elucidate the potential mechanisms underlying disinfectant tolerance, molecular screening for QAC resistance genes was performed. The *qac*A/B gene, which encodes an efflux pump conferring resistance to QACs, was detected in 13 isolates (28.27%), with the highest prevalence in Group 2 (46.16%), followed by Group 4 (30.77%), Group 3 (15.38%), and the lowest detection rate in Group 1 (7.69%). In contrast, *qac*C was detected in only 3 isolates (6.52%), equally distributed across Groups 2, 3,

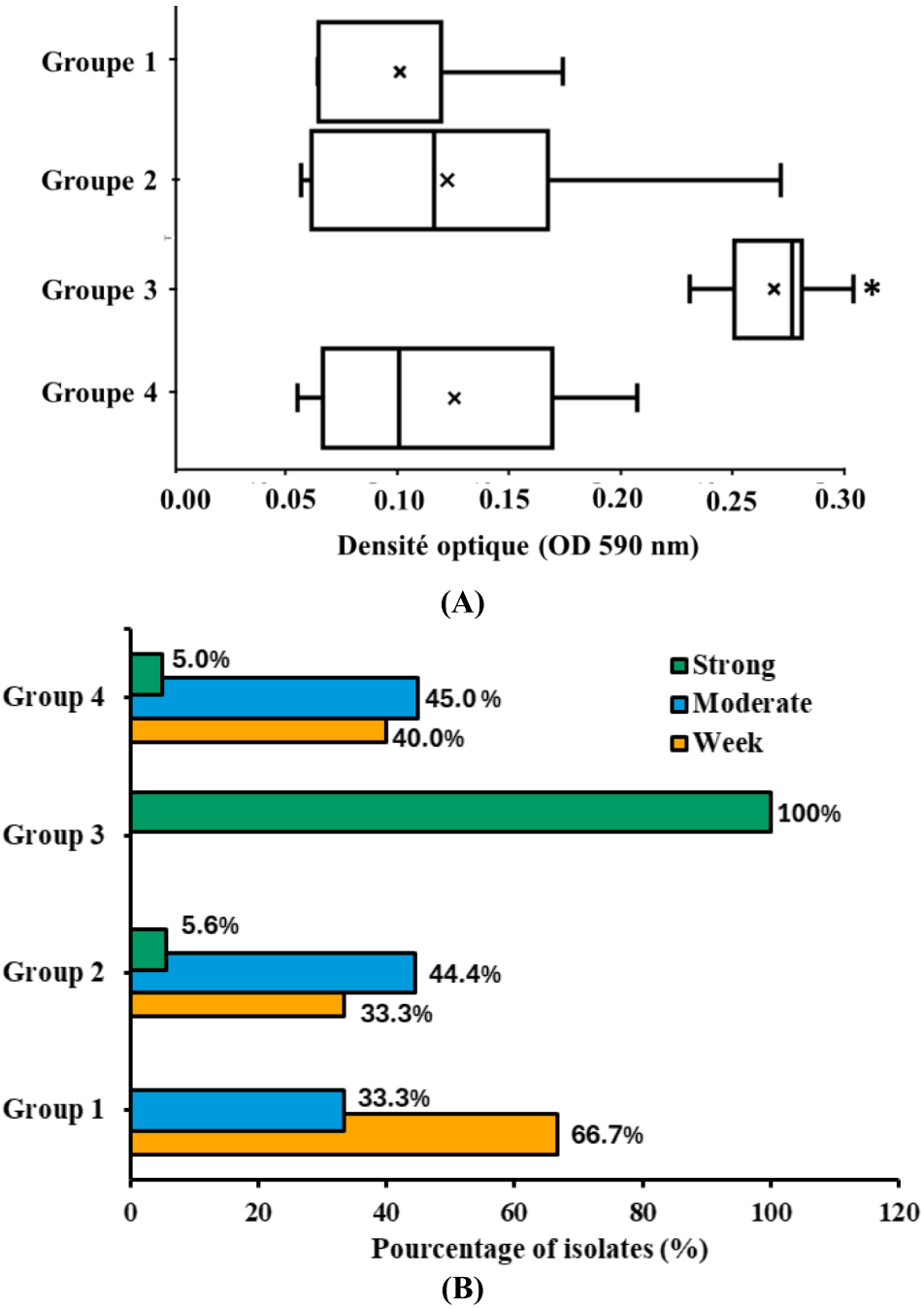


Fig. 4 Relationship between antibiotic resistance levels of the different antibiotics tested and biofilm formation capacity in *S. saprophyticus* isolates. **A** Association between biofilm biomass and antibiotic resistance. **B** Correlation between antibiotic resistance levels and biofilm formation capacity (Strong, moderate and weak). Group 1: Susceptible *S. saprophyticus* isolates; Group 2: *S. saprophyticus* isolates resistant to one or two antibiotics; Group 3: MRSS isolates; Group 4: MDR non-MRSS isolates

and 4 (each accounting for 33.33% of the positive cases) (Table 4).

Overall, 30 isolates (65.21%) were negative for both *qac*A/B and *qac*C, indicating that a significant proportion of *S. saprophyticus* isolates do not harbor these specific resistance determinants. Notably, 50% of Group 4 isolates (MDR non-MRSS strains) lacked detectable resistance genes, suggesting alternative mechanisms of disinfectant tolerance, possibly linked to biofilm formation, membrane modifications, or enzymatic degradation of disinfectant compounds.

Assessment of the antibiofilm activity of disinfectants

The ability of disinfectants to inhibit *S. saprophyticus* biofilm formation and eradicate preformed biofilms was evaluated at different sub-MICs (MIC/2, MIC/4, MIC/8, and MIC/16) (Supplementary Table 3). The findings revealed a general reduction in biofilm formation in the presence of disinfectants; however, the extent of inhibition varied depending on the product and concentration, demonstrating a dose-dependent effect.

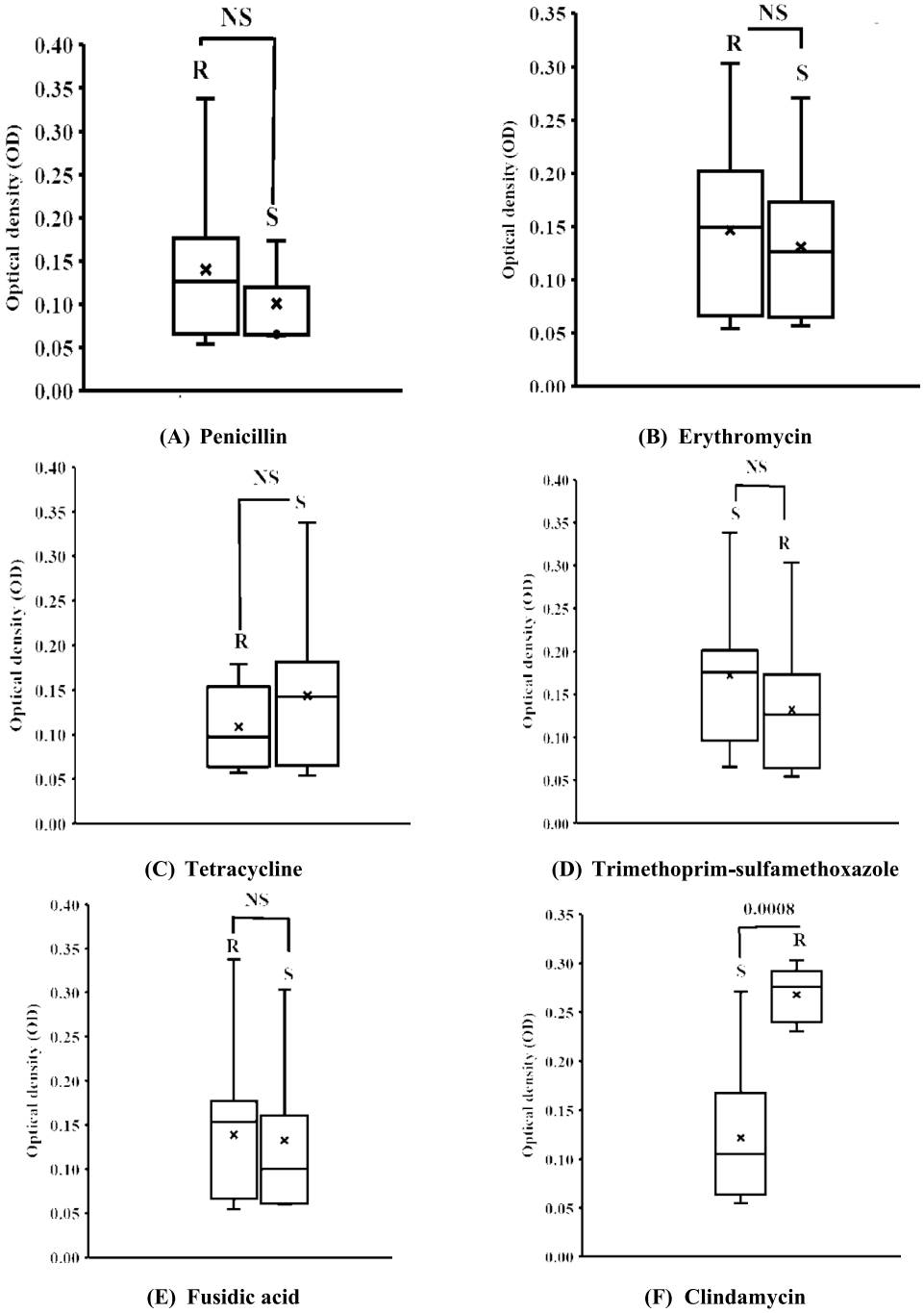


Fig. 5 Association between biofilm formation and different antibiotic resistance profiles. The data are presented as box plots. NS: non significative

Among the tested disinfectants, Products 1, 3, and 4 exhibited significant anti-biofilm activity, particularly against *S. saprophyticus* isolates that formed strong and moderate biofilms. These disinfectants maintained their inhibitory effects across all sub-MICs, suggesting their potential as biofilm control agents. Product 1 reduced biofilm formation in 94% of isolates at MIC/2, 81% at MIC/4, and 69% at both MIC/8 and MIC/16, while Product 4 exhibited inhibitory effects in 81, 75, and 69% of isolates at MIC/2, MIC/4, and MIC/8, respectively.

Product 2 showed low antibiofilm activity at all sub-inhibitory concentrations. In contrast, it strongly induced biofilm formation at MIC/4, MIC/8, and MIC/16, with enhancements observed in 69, 88, and 100% of isolates, respectively. This suggests that sub-MICs of Product 2 may induce a stress response that enhances biofilm development, potentially promoting bacterial persistence and resistance in contaminated environments.

Overall, the results showed that disinfectants significantly reduced biofilm biomass at sub-inhibitory concentrations

(MIC/2, MIC/4, MIC/8, and MIC/16) compared to untreated controls (Fig. 8). However, isolates treated with MIC/2, MIC/4, and MIC/8 of Products 1, 3, and 4 exhibited significantly reduced biofilm formation, particularly for strong or moderate biofilm-producing strains, whereas Product 2 consistently stimulated biofilm formation at MIC/8 and MIC/16.

Beyond their inhibitory effects, disinfectants were also evaluated for their ability to eradicate mature *S. saprophyticus* biofilms. Products 1, 3, and 4 demonstrated potent antibiofilm activity, effectively disrupting established biofilms at all tested concentrations. Their efficacy was statistically significant compared to untreated biofilms ($p < 0.05$), highlighting their potential for biofilm eradication. Product 2 demonstrated moderate biofilm eradication at MIC and MIC/2, with eradication rates ranging between 20 and 40% ($p < 0.05$). However, at lower concentrations (MIC/4 and MIC/8), its antibiofilm effects were weak, with eradication rates falling below 20%, further reinforcing its paradoxical biofilm-stimulatory effect at subinhibitory levels.

These results emphasize the variability in disinfectant effectiveness for both biofilm prevention and eradication, underscoring the importance of proper concentration selection to maximize efficacy. While Products 1, 3, and 4 demonstrated consistent antibiofilm activity, Product 2 not only failed to effectively inhibit biofilm formation but also enhanced it at lower concentrations, raising concerns about its potential role in promoting biofilm-associated resistance (Table 5).

Hierarchical clustering of *S. saprophyticus* isolates

The hierarchical clustering of *S. saprophyticus* isolates, based on their antibiotic resistance profiles, biofilm-forming ability, and tolerance to disinfectants, revealed considerable heterogeneity within the studied population (Fig. 9). The dendrogram allowed for the identification of five distinct clusters, each characterized by specific phenotypic and genetic traits.

Group 1 comprised isolates with high resistance to multiple antibiotic classes, particularly penicillin and erythromycin. These isolates exhibited a strong biofilm-forming capacity, likely linked to the presence of *atl*, *aas*, and *uafA* genes, which are associated with cell adhesion and biofilm development. Additionally, the frequent detection of *qac*A/B and *qac*C in this group suggests an enhanced tolerance to disinfectants commonly used in hospital environments, potentially contributing to their persistence in clinical settings .

Group 2 was characterized by a high resistance to penicillin, trimethoprim-sulfamethoxazole, and fusidic acid, while remaining more susceptible to macrolides. The biofilm-forming ability varied among isolates, with some showing

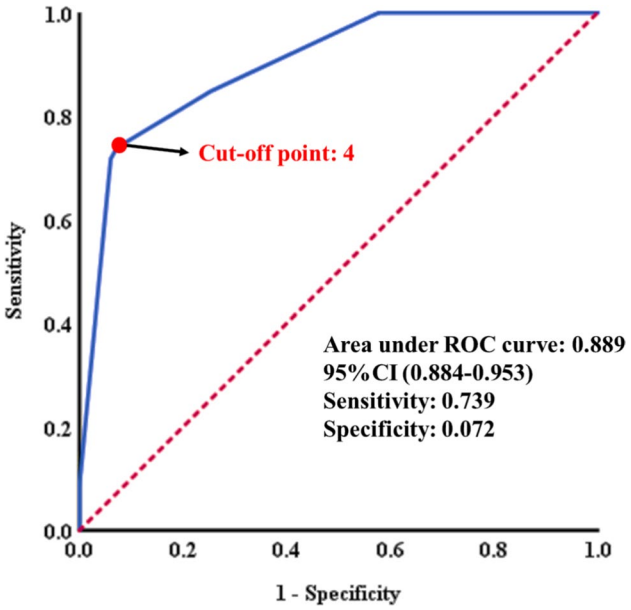


Fig. 6 Receiver operating characteristic (ROC) analysis for prediction of *S. saprophyticus* UTIs

weak adhesion and others exhibiting strong biofilm formation. The frequent presence of *atl* and *aas* genes suggests that cell adhesion mechanisms contribute to their ability to colonize abiotic surfaces.

Group 3 comprised isolates with moderate resistance profiles, exhibiting partial resistance to macrolides and penicillins while remaining relatively susceptible to fluoroquinolones. The biofilm formation capacity in this group was moderate; however, the presence of the *qacA/B* gene in some isolates suggests a potential tolerance to quaternary ammonium compounds, which may influence their ability to persist in disinfectant-treated environments.

Group 4 consisted of isolates with low antibiotic resistance and lacking both biofilm-forming ability and biofilm-associated genes. This suggests that these isolates may utilize alternative adhesion mechanisms instead of biofilm formation to persist on surfaces. Unlike the previous groups, these isolates were more susceptible to disinfectants, possibly reflecting an evolutionary trade-off between biofilm formation and chemical biocide resistance.

Group 5 consisted of isolates with marked resistance to methicillin and strong biofilm-forming ability. However, these isolates displayed lower tolerance to disinfectants, supporting the hypothesis that biofilm formation may act as a compensatory survival strategy in specific environmental conditions.

These findings highlight the phenotypic and genotypic diversity of *S. saprophyticus* isolates, suggesting complex adaptive mechanisms shaped by selective pressure in clinical

Table 3 Antibacterial activities of the four disinfectants tested in this study against *S. saprophyticus*

Group	Strain	Product 1		Product 2		Product 3		Product 4	
		IZD (mm)	MBC (%)/ MIC (%)	IZD (mm)	MBC (%)/MIC (%)	IZD (mm)	MBC (%)/ MIC (%)	IZD (mm)	MBC (%)/ MIC (%)
Group 1: Susceptible *S. saprophyticus* isolates	USS 4	28 ± 1.8	2.18/1.09	21 ± 1.8	0.17: 0.07/0.17: 0.07	8 ± 1.8	0.18/0.09	19 ± 1.8	0.32/0.16
	USS 18	10 ± 1.5	2.18/1.09	30 ± 1.5	0.17: 0.07/0.17: 0.07	0	> 3/3	16 ± 1.5	0.65/0.32
	USS 32	16 ± 1	2.18/1.09	23 ± 1	0.17: 0.07/0.17: 0.07	10	0.09	18 ± 1	0.16/0.08
Group 2: *S. saprophyticus* isolates resistant to one or two antibiotics	USS2	23 ± 2	2.18/1.09	20 ± 2	0.17: 0.07/0.17: 0.07	7 ± 2	0.18/0.09	26 ± 2	0.32/0.16
	USS6	18 ± 1.5	2.18/1.09	25 ± 1.5	0.17: 0.07/0.17: 0.07	7 ± 1.5	0.09/0.04	30 ± 1.5	0.32/0.16
	USS7	8 ± 1.9	35/17.5	25 ± 1.9	0.17: 0.07/0.17: 0.07	0	3/1.5	15 ± 1.9	0.32/0.16
	USS9	11 ± 1	2.18/1.09	26 ± 1	0.17: 0.07/0.17: 0.07	0	3/1.5	16 ± 1	0.08/0.04
	USS10	24 ± 2.3	2.18/1.09	26 ± 2.3	0.17: 0.07/0.17: 0.07	8 ± 2.3	0.09/0.04	23 ± 2.3	0.16/0.08
	USS16	7 ± 1	2.18/1.09	30 ± 1	0.17: 0.07/0.17: 0.07	0	> 3/3	15 ± 1	0.65/0.32
	USS19	8 ± 2	2.18/1.09	27 ± 2	0.17: 0.07/0.17: 0.07	0	> 3/3	17 ± 2	0.65/0.32
	USS20	9 ± 1	8.75/4.37	29 ± 1	0.17: 0.07/0.17: 0.07	0	> 3/3	16 ± 1	0.65/0.32
	USS21	9 ± 1.4	2.18/1.09	28 ± 1.4	0.17: 0.07/0.17: 0.07	0	> 3/3	16 ± 1.4	0.65/0.32
	USS22	12 ± 1	2.18/1.09	26 ± 1	0.17: 0.07/0.17: 0.07	0	> 3/3	15 ± 1	0.65/0.32
	USS23	11 ± 2	4.37/2.18	30 ± 2	0.17: 0.07/0.17: 0.07	0	> 3/3	16 ± 2	0.65/0.32
	USS24	11 ± 2.5	2.18/1.09	31 ± 2.5	0.17: 0.07/0.17: 0.07	9 ± 2.5	0.18	19 ± 2.5	0.32/0.16
	USS30	9 ± 1.5	17.5/8.75	34 ± 1.5	0.17: 0.07/0.17: 0.07	0	> 3/3	14 ± 1.5	0.32/0.16
	USS34	8 ± 2.5	17.5/8.75	30 ± 2.5	0.17: 0.07/0.17: 0.07	0	> 3/3	20 ± 2.5	0.32/0.16
	USS35	11 ± 1	4.37/2.18	29 ± 1	0.17: 0.07/0.17: 0.07	8 ± 1	0.04	16 ± 1	0.32/0.16
	USS37	9 ± 1	17.5/8.75	29 ± 1	0.17: 0.07/0.17: 0.07	0	> 3/3	16 ± 1	0.32/0.16
	USS38	9 ± 1.4	17.5/8.75	28 ± 1.4	0.17: 0.07/0.17: 0.07	0	> 3/3	16 ± 1.4	0.65/0.32
	USS44	9 ± 1.5	35/17.5	34 ± 1.5	0.17: 0.07/0.17: 0.07	0	> 3/3	14 ± 1.5	0.32/0.16
Group 3: Methicillin-resistant S. saprophyticus isolates	USS8	25 ± 2	1.09/0.54	28 ± 2	0.17: 0.07/0.17: 0.07	8 ± 2	0.09/0.04	16 ± 2	0.32/0.16

Table 3 (continued)

Group	Strain	Product 1		Product 2		Product 3		Product 4	
		IZD (mm)	MBC (%)/ MIC (%)	IZD (mm)	MBC (%)/MIC (%)	IZD (mm)	MBC (%)/ MIC (%)	IZD (mm)	MBC (%)/ MIC (%)
	USS13	10 ± 2.5	35/17.5	29 ± 2.5	0.17: 0.07/0.17: 0.07	0	3/1.5	14 ± 2.5	0.16/0.08
	USS27	16 ± 2	4.37/2.18	36 ± 2	0.17: 0.07/0.17: 0.07	0	3/1.5	15 ± 2	0.16/0.08
	USS36	9 ± 1.5	35/8.75	28 ± 1.5	0.17: 0.07/0.17: 0.07	0	> 3/3	17 ± 1.5	0.65/0.32
	USS41	16 ± 2	4.37/2.18	36 ± 2	0.17: 0.07/0.17: 0.07	0	3/1.5	15 ± 2	0.65/0.32
Group 4: MDR S. saprophyticus isolates	USS5	24 ± 2.4	2.18/1.09	26 ± 2.4	0.17: 0.07/0.17: 0.07	0	3/1.5	17 ± 2.4	0.32/0.16
	USS1	31 ± 2.5	1.09/0.54	26 ± 2.5	0.17: 0.07/0.17: 0.07	12 ± 2.5	0.18/0.09	28 ± 2.5	1.3/1.3
	USS3	8 ± 1.4	2.18/1.09	26 ± 1.4	0.17: 0.07/0.17: 0.07	0	> 3/3	19 ± 1.4	0.32/0.16
	USS11	9 ± 1.5	4.37/2.18	24 ± 1.5	0.17: 0.07/0.17: 0.07	7 ± 1.5	0.09/0.04	14 ± 1.5	0.08/0.04
	USS12	17 ± 2	2.18/1.09	26 ± 2	0.17: 0.07/0.17: 0.07	0	> 3/3	18 ± 2	0.16/0.08
	USS14	8 ± 1.4	17.5/8.75	26 ± 1.4	0.17: 0.07/0.17: 0.07	8 ± 1.4	0.09/0.04	15 ± 1.4	0.04/0.02
	USS15	8 ± 2	1.09/0.54	24 ± 2	0.17: 0.07/0.17: 0.07	0	3/1.5	16 ± 2	0.65/0.32
	USS17	7 ± 1.2	2.18/1.09	29 ± 1.2	0.17: 0.07/0.17: 0.07	10 ± 1.2	0.18/0.09	15 ± 1.2	0.65/0.32
	USS25	13 ± 2.3	4.37/2.18	30 ± 2.3	0.17: 0.07/0.17: 0.07	0	> 3/3	17 ± 2.3	0.16/0.08
	USS26	16 ± 1	4.37/2.18	40 ± 1	0.17: 0.07/0.17: 0.07	0	> 3/3	16 ± 1	0.16/0.08
	USS28	8 ± 1	8.75/4.37	28 ± 1	0.17: 0.07/0.17: 0.07	0	> 3/3	14 ± 1	0,32/0.16
	USS29	9 ± 2.5	8.75/4.37	31 ± 2.5	0.17: 0.07/0.17: 0.07	0	> 3/3	16 ± 2.5	0.32/0.16
	USS31	20 ± 1.2	1.09/0.54	35 ± 1.2	0.17: 0.07/0.17: 0.07	0	> 3/3	15 ± 1.2	0.32/0.16
	USS33	25 ± 2	1.09/0.54	26 ± 2	0.17: 0.07/0.17: 0.07	6 ± 2	0.18/0.09	16 ± 2	0.65/0.32
	USS39	12 ± 1	4.37/2.18	26 ± 1	0.17: 0.07/0.17: 0.07	0	> 3/3	15 ± 1	0,65/0.32
	USS40	11 ± 2	4.37/2.18	30 ± 2	0.17: 0.07/0.17: 0.07	0	> 3/3	16 ± 2	0.65/0.32
	USS42	8 ± 1	17.5/8.75	28 ± 1	0.17: 0.07/0.17: 0.07	0	3/1.5	14 ± 1	0.65/0.32
	USS43	9 ± 2.5	17.5/8.75	31 ± 2.5	0.17: 0.07/0.17: 0.07	0	> 3/3	16 ± 2.5	0.65/0.32
	USS45	7 ± 1	35/17.5	30 ± 1	0.17: 0.07/0.17: 0.07	0	> 3/3	15 ± 1	0.16/0.08
	USS46	7 ± 1.2	35/17.5	29 ± 1.2	0.17: 0.07/0.17: 0.07	10 ± 1.2	0.09/0.04	15 ± 1.2	0.16/0.08

IZD Zones of Inhibition, *MIC* Minimum Inhibitory Concentrations, *MBC* Minimum Bactericidal Concentrations

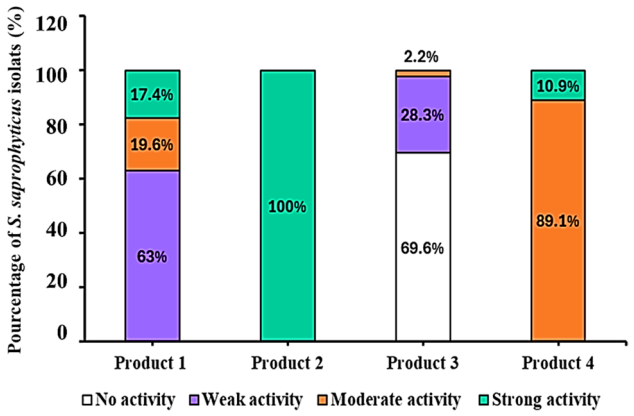


Fig. 7 Types of antibacterial activity of the four disinfectants tested in this study against *S. saprophyticus*

environments. The identification of distinct clusters provides valuable insights into bacterial survival strategies and may support refinement of disinfection protocols.

Discussion

Staphylococcus saprophyticus is a major cause of UTIs in young women, accounting for 54.35% of cases in our study. This high frequency is linked to biological factors, including the anatomical and behavioral factors, such as sexual activity and may be influenced by hormonal regulation [2]. Its pathogenicity relies on adhesion to uroepithelial cells and the production of lipase and urease [2], with high detection rates of biofilm formation genes such as *atl* (84.78%) and *aas* (82.61%).

Despite susceptibility to several antibiotic classes, *S. saprophyticus* exhibited notable resistance to penicillin G (93.48%), linked to the *bla*Z gene. The prevalence of MRSS (10.86%) was higher than in some regions (e.g., Japan and North America) but lower than in others (e.g., Iran), reflecting geographic variation in antibiotic use [4, 9, 28]. Resistance to fusidic acid (78.3%) and erythromycin (41.3%) remains concerning, reinforcing the need for ongoing surveillance [29–32].

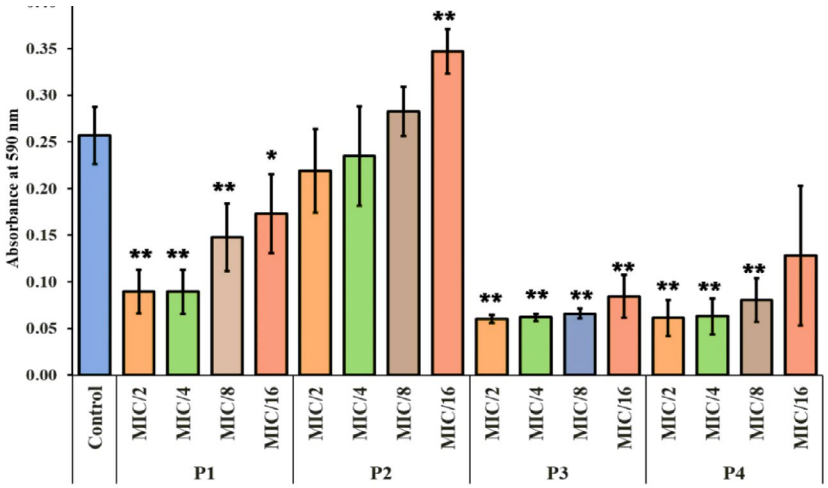
Hierarchical clustering of *S. saprophyticus* isolates revealed distinct resistance phenotypes. Group 1 exhibited high resistance to penicillins, correlating with *bla*Z detection, while Group 5 was marked by methicillin resistance and frequent *mec*A gene detection. These patterns highlight the phenotype and genotypic heterogeneity of *S. saprophyticus* population and emphasize the importance of genomic monitoring.

Biofilm formation, observed in 89.1% of isolates, plays a critical role in persistence and antimicrobial tolerance, with

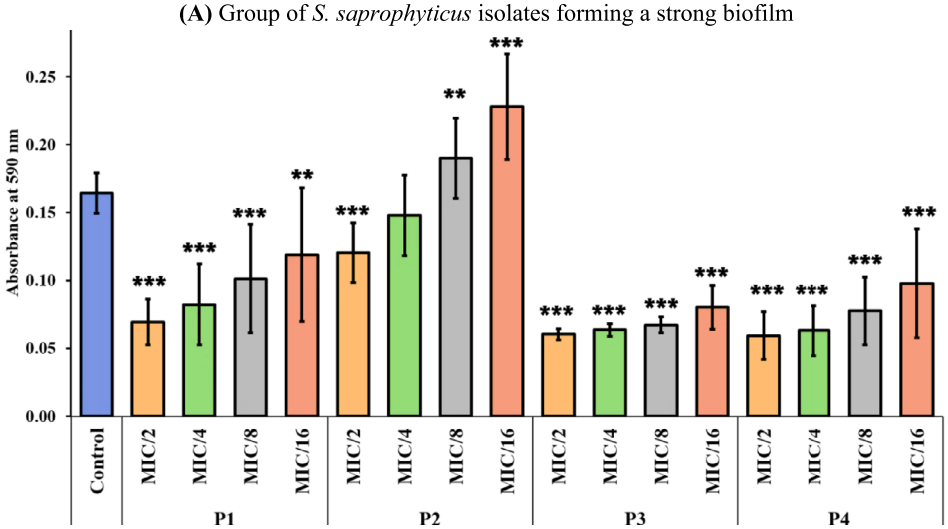
Table 4 Distribution of disinfectant resistance genes in *S. saprophyticus* isolates

Disinfectant resistance genes	Group 1	Group 2	Group 3	Group 4	Total (n = 46)
*qac*A/B	1 (7.69%)	6 (46.16%)	2 (15.38%)	4 (30.77%)	13 (28.27%)
*qac*C	0	1 (33.33%)	1 (33.33%)	1 (33.33%)	3 (6.52%)
Negative	2 (6.66%)	11 (36.68%)	2 (6.66%)	15 (50%)	30 (65.21%)

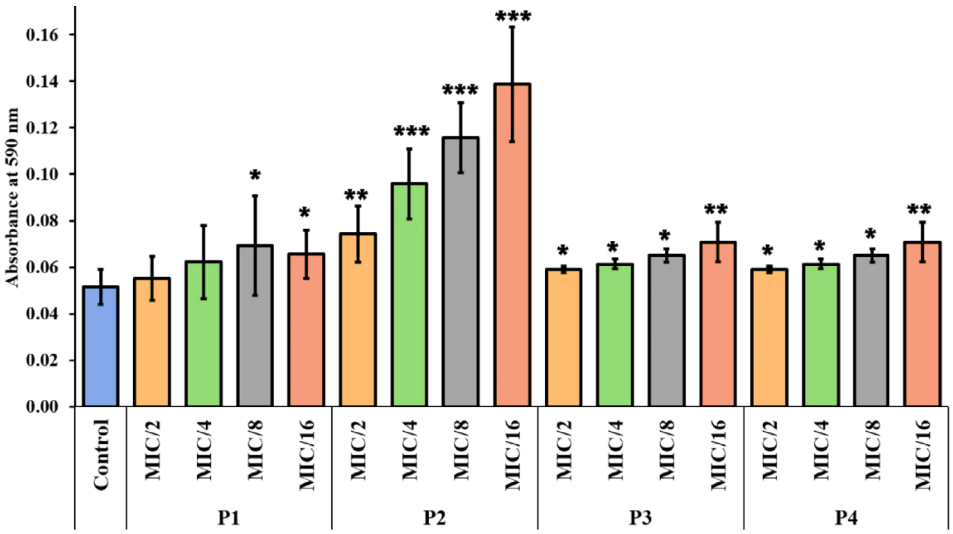
Group 1: Susceptible *S. saprophyticus* isolates; Group 2: *S. saprophyticus* isolates resistant to one or two antibiotics; Group 3: MRSS isolates; Group 4: MDR non-MRSS isolates



(A) Group of *S. saprophyticus* isolates forming a strong biofilm



(B) Group of *S. saprophyticus* isolates forming a moderate biofilm



(C) Group of *S. saprophyticus* isolates forming a weak biofilm

Fig. 8 Antibiofilm activities of disinfectants against strong (**A**), moderate (**B**) and weak (**C**) biofilm-forming *S. saprophyticus* isolates. Error bars represent the mean and standard deviation of three replicates. Asterisks indicate a significant difference between EO concentrations applied to each bacterial biofilm compared with untreated biofilm (*$p < 0.05$, **$p < 0.01$, ***$p < 0.001$)

Table 5 Biofilm eradication activities of disinfectants against *S. saprophyticus* isolates forming strong biofilms

Disinfectants	Concentrations	Biofilm eradication activities of disinfectants (%)							*p* value
		USS7	USS8	USS13	USS25	USS27	USS36	USS41	
Product 1	MIC	79.64 ± 4.5	66.52 ± 3.8	76 ± 6.8	80.22 ± 5.8	72.83 ± 3.8	72.83 ± 3.8	77.54 ± 4.6	0.002
	MIC/2	75.71 ± 3.5	62.61 ± 4.1	69.2 ± 2.8	72.64 ± 5.4	49.64 ± 2.7	72.28 ± 5.3	71.38 ± 3.4	0.001
	MIC/4	72.5 ± 3.2	57.39 ± 2.8	60.4 ± 6.8	64.73 ± 5	22.83 ± 3	74.46 ± 2	67.03 ± 2	0.003
	MIC/8	69.29 ± 5.5	78.26 ± 3	47.60 ± 4	44.29 ± 3	19.57 ± 4	71.20 ± 5.9	61.85 ± 6.1	0.001
Product 2	MIC	33.21 ± 6.7	28.26 ± 4.8	27.2 ± 4.8	37.69 ± 4.8	40.22 ± 2.9	37.5 ± 3.2	40.94 ± 3.1	0.005
	MIC/2	26.07 ± 4.8	15.22 ± 3.6	21.2 ± 1.8	33.08 ± 2.4	30.80 ± 4.8	16.85 ± 2.9	28.26 ± 1.9	0.021
	MIC/4	17.50 ± 3.1	9.57 ± 0.8	8.40 ± 1.3	17.91 ± 2.2	11.23 ± 1.6	4.35 ± 1	17.39 ± 3	NS
	MIC/8	2.5 ± 4.1	7.83 ± 3	7.2 ± 3.2	11.98 ± 2.1	4.71 ± 2.1	1.63 ± 0.9	8.7 ± 3.2	NS
Product 3	MIC	79.29 ± 2.5	67.39 ± 1.8	76.4 ± 6.1	69.01 ± 5.1	80.8 ± 3.8	64.67 ± 2.5	75.36 ± 8.2	0.002
	MIC/2	76.79 ± 1.5	69.57 ± 2.5	68.4 ± 2.3	65.71 ± 8.3	73.91 ± 2.4	57.61 ± 3.5	79.35 ± 2.5	0.002
	MIC/4	75 ± 4.2	62.17 ± 2.2	65.6 ± 4.1	61.43 ± 5.9	70.29 ± 2.1	48.37 ± 6.5	76.81 ± 8.2	0.002
	MIC/8	68.21 ± 4.3	58.26 ± 3.1	57.2 ± 3.2	58.79 ± 3.2	63.04 ± 5.2	42.39 ± 3.5	75.72 ± 5.5	0.002
Product 4	MIC	80.36 ± 5.8	76.09 ± 2.2	80 ± 8.1	81.21 ± 4.3	77.17 ± 4.1	64.67 ± 7.1	80.8 ± 6.1	0.001
	MIC/2	79.64 ± 5.1	69.13 ± 2.8	74 ± 4.2	77.25 ± 8.2	72.46 ± 3.7	60.33 ± 5.5	76.81 ± 4.2	0.002
	MIC/4	76.79 ± 4.1	63.04 ± 3.2	71.2 ± 4.1	71.98 ± 5.1	69.2 ± 5.1	53.80 ± 5.4	64.86 ± 4.8	0.002
	MIC/8	73.21 ± 3.8	56.96 ± 4.2	64.4 ± 4.2	65.32 ± 4.1	66.67 ± 3.9	41.30 ± 3.9	61.59 ± 5.1	0.002

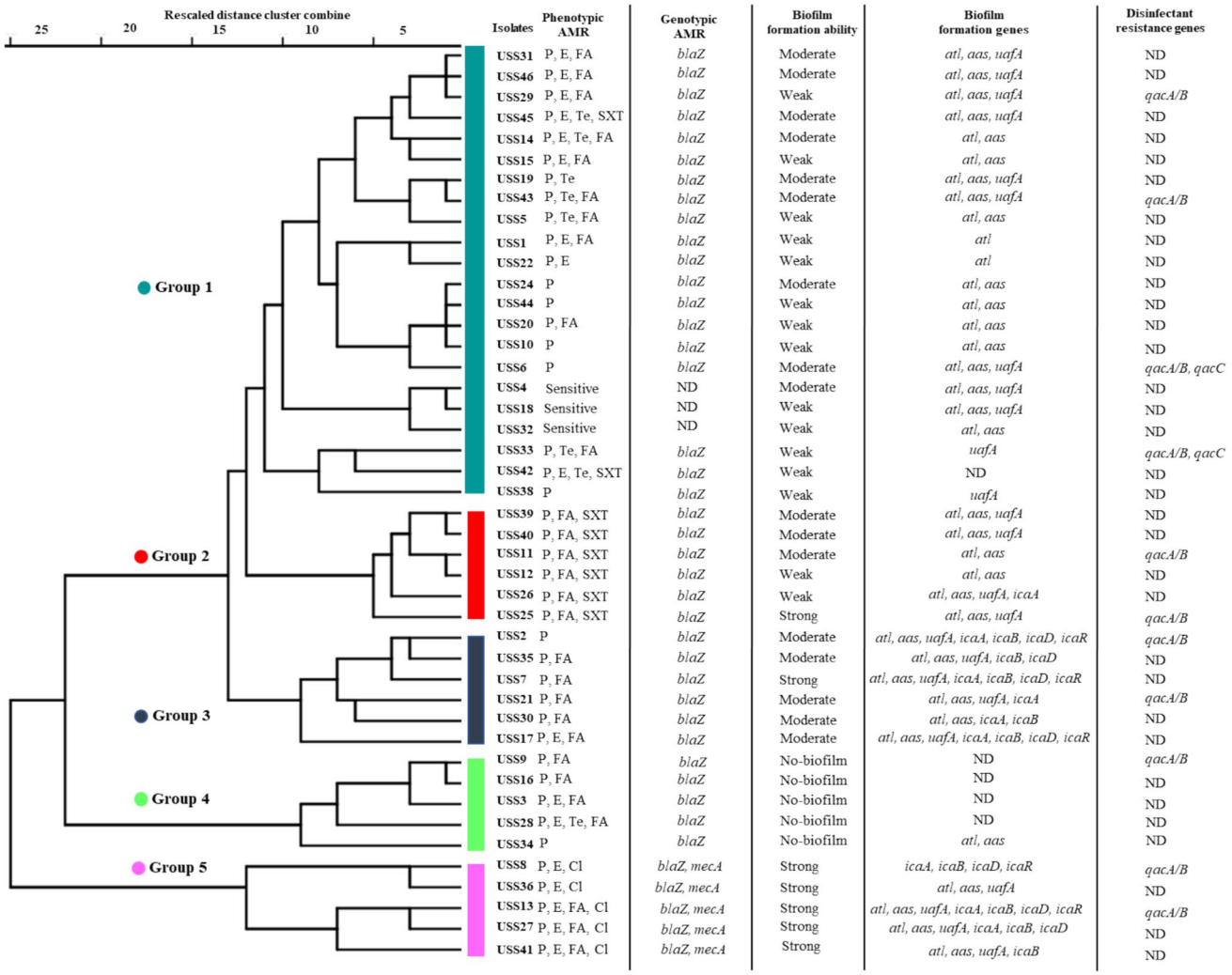


Fig. 9 Hierarchical clustering of *S. saprophyticus* isolates based on antibiotic resistance profiles, biofilm formation capacity, disinfectant tolerance, and the presence of associated resistance and biofilm-related genes. The dendrogram reveals five distinct clusters, each represented by a different color, indicating phenotypic and genotypic diversity among the isolates

prevalence rates reported in the literature ranging from 70 to 91% [4, 33]. MRSS isolates showed greater biofilm biomass, consistent with previous studies linking methicillin resistance to enhanced biofilm formation [34, 35]. Moreover, strains with *atl*, *aas*, *uaf*A, and *ica* operon genes exhibited stronger biofilm capabilities, suggesting that adhesion factors can compensate for lower antibiotic resistance.

Biofilm biomass positively correlated with resistance to clindamycin ($p = 0.0008$), and moderate biofilm producers harbored *uaf*A and *aas* more frequently, underlining their role in adhesion and maturation [33]. Conversely, weak biofilm-forming strains were more antibiotic-susceptible (67%), supporting the concept that biofilms protect against antimicrobials through reduced permeability and altered metabolism [36].

Additionally, biofilm-associated bacteria often exhibit reduced metabolic activity, making them less susceptible to bactericidal agents targeting actively dividing cells [34]. Effective treatment of *S. saprophyticus* infections, particularly MDR and MRSS strains, remains challenging due to biofilm-mediated persistence. Anti-biofilm therapies, such as diflunisal, show promise in disrupting biofilm integrity and may enhance antibiotic effectiveness [36].

Our predictive model, while useful for early risk stratification, lacks specificity due to broad predictors like biofilm formation and methicillin resistance. Future models should incorporate clinical data (e.g., UTIs recurrence, immune status, virulence profiles) and leverage machine learning to improve predictive performance and therapeutic guidance.

In the disinfection context, sodium hypochlorite (2.6%) and a benzalkonium chloride (10%)–glutaraldehyde (5%)

combination were most effective. In contrast, hydrogen peroxide-based agents were less potent, likely due to catalase-mediated degradation [37, 38]. Alcohol-based disinfectants showed inconsistent efficacy (only 17% highly susceptible isolates), indicating the need for optimized application strategies [29, 39].

Disinfectant tolerance genes (*qacA/B*, *qacC*), were frequently found in Groups 1, 2, 3 and 5, suggesting selective pressure from disinfection practices. Interestingly, some isolates lacking these genes still showed reduced susceptibility, indicating alternative mechanisms such as biofilm protection or membrane modifications [40, 41].

At sub-MIC levels, benzalkonium chloride and glutaraldehyde paradoxically enhanced biofilm formation, likely via stress responses that increase extracellular polymeric substances production and adhesion gene expression [38, 42]. Quorum sensing may also facilitate biofilm adaptation and resistance to sublethal disinfectant exposure [43].

Lastly, the study's limitations include the relatively small sample size (n = 46) and in vitro conditions that may not fully replicate in vivo environments. Additional resistance mechanisms, such as efflux pumps and membrane alterations, warrant investigation via transcriptomic and proteomic approaches. Enhancing our model with external validation and clinical biomarkers will improve its diagnostic utility.

Conclusions

This study provides new insights into the antimicrobial resistance and biofilm-forming capacity of *S. saprophyticus* in UTIs. The high prevalence of *blaZ*-mediated β-lactam resistance and the presence of MRSS highlight the growing challenge of treatment failure. Notably, biofilm formation was strongly linked to clindamycin resistance, suggesting that bacterial persistence may be a key factor in recurrent infections.

Among the tested disinfectants, sodium hypochlorite and benzalkonium chloride-glutaraldehyde combinations demonstrated the strongest antibacterial effects, while sodium hypochlorite and hydrogen peroxide most effectively inhibited and eradicated biofilms. However, sub-CMI of certain disinfectants paradoxically enhanced biofilm formation, raising concerns about bacterial adaptation under inadequate disinfection conditions.

These findings underscore the importance of using disinfectants at effective concentrations to prevent resistance selection and persistent colonization. Further research should focus on understanding the molecular mechanisms driving these adaptive responses and developing targeted strategies to disrupt biofilm-associated resilience in *S. saprophyticus*.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s00345-025-05704-3.

Author Contribution R.A., B.A., and N. K. drafted the manuscript and conducted molecular data analysis. D.A., R.H., R.A., isolated and provided the strain with routine phenotypical data. T.M., B.A., and N. K. supervised and validated the study, and revised the manuscript.

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Data availability No datasets were generated or analysed during the current study.

Declarations

Conflict of interest The authors declare no competing interests.

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