ORIGINAL ARTICLE

Study of biofilm formation, structure and antibiotic resistance in *Staphylococcus saprophyticus* strains causing urinary tract infection in women in Ahvaz, Iran

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Abstract

Staphylococcus saprophyticus is the second most frequent community-acquired causative agent of acute urinary tract infection (UTI). Some strains of *S. saprophyticus* can create biofilms, increasing their virulence. Once biofilms have been produced, antibiotic resistance is exacerbated. Hence, the aims of the present study were the study of biofilm formation, structure and antibiotic resistance in *S. saprophyticus* strains causing UTIs in women in Ahvaz, Iran. Overall, 43 *S. saprophyticus* isolates were recovered from UTIs. Antibiotic resistance pattern and the biofilm production and structure were determined using phenotypic methods. Most *S. saprophyticus* isolates were resistant to erythromycin, but all isolates were sensitive to linezolid and vancomycin. Fifty-eight per cent of *S. saprophyticus* were multidrug resistant. Twenty-one per cent of *S. saprophyticus* isolates harbored the *mecA* gene. Biofilm formation was observed in 65% of *S. saprophyticus* isolates and most had polysaccharide matrix. Our data indicate high rates of antibiotic resistance and the capability of biofilm formation among *S. saprophyticus* isolates. The emergence of antibiotic resistance in the management of UTIs is a serious public health issue. The findings of this study could be used to improve treatment plans to control UTIs. Consequently, increased awareness of the mechanisms underlying biofilm formation and the development of drug resistance will allow UTIs to be more efficiently controlled and treated.

Keywords: Antibiotic resistance, antimicrobial, biofilm formation, *Staphylococcus saprophyticus*, urinary tract infection
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Introduction

Urinary tract infections (UTIs) are the most common infections in a clinical setting and the second most prevalent infection after respiratory traction infections [1]. Worldwide, UTIs affect about 150 million people every year [2]. In most cases the infectious agents are *Enterobacteriaceae*, including *Escherichia coli*, *Klebsiella* sp., *Enterobacter* sp. and *Proteus* sp., and Gram-positive bacteria such as *Enterococcus faecalis*, *Streptococcus agalactiae* and *Staphylococcus* spp. *Staphylococcus saprophyticus* is a member of the coagulase-negative staphylococci, which are commonly responsible for 5%–10% of UTIs [3]. This microorganism is the second most frequent cause of uncomplicated UTIs, especially in sexually active women [4]. UTIs are more common in women than men because of their anatomical differences: the distance between the anus and the urethra and the shortness of the urinary tract [5]. *Staphylococcus saprophyticus* colonization occurs via several different types of adhesins such as hemagglutinins with autolytic and adhesive properties, as well as surface-associated lipase that forms fimbria-like surface appendages, helping the bacteria to maintain tight adherence to these surfaces. This high ability of S. saprophyticus to colonize

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within the tract is partly thanks to the adhesins permitting the microorganism to colonize the uroepithelium, at the side of the urease, giving rise to severe infections. [6]. *S. saprophyticus* can produce biofilms, which increases its virulence and shows a 100-fold to 1000-fold increase in its antibiotic tolerance in comparison with non-biofilm-producing isolates [7]. The biofilm matrix comprises extracellular material consisting of proteins, extracellular DNA and polysaccharides, which facilitate attachment to any surfaces. The biofilm confers antibiotic resistance through processes that include encoding antibiotic-resistant genes, restricting antibiotics, and even counteracting host immunity [8]. *MecA*-positive isolates of *S. saprophyticus* were first reported from Japan and have subsequently been described from different parts of the world [9–11]. Although these are predominantly reports of sporadic cases, the incidence of *mecA* among *S. saprophyticus* was found to be 7.9% [12]. In many developing countries, including Iran, the UTIs caused by *S. saprophyticus*, as well as the biofilm formation and structure of this species, are not well studied. To our knowledge, this is the first study of biofilm formation and structure in *S. saprophyticus* isolates. The present study aimed to study biofilm formation, structure and antibiotic resistance in *S. saprophyticus* strains that cause UTIs in women in Ahvaz, Iran.

Materials and methods

Ethical approval

The study was approved by the Research Ethics Committee of the Ahvaz Jundishapur University of Medical Sciences (IR.AJUMS.REC.1399.465) Iran, and the necessary permission was granted for the work.

Study design and sample collection

This cross-sectional research included 390 midstream urine samples from women who were inpatients with suspected UTIs and women who were referred to Razi teaching hospitals from January to December 2019. Women were aged from 18 to 60 years without any previous genitourinary anomalies, underlying diseases, or antibiotic usage. Urine samples were collected using the midstream method in toilet-trained women. The samples were transferred to the laboratory at the Department of Microbiology of Ahvaz Jundishapur University. Samples were inoculated on blood agar and eosin methylene blue agar plates (Merck KGaA, Darmstadt, Germany) and were incubated at 37° C for 24 hours. Then, the urine cultures were classified as negative, positive or contaminated. When polymorphic bacterial growth (two or more bacterial species growing on one plate) was observed, the samples were classified as contaminated (exclusion criteria). The urine cultures were considered negative when bacterial growth was $<10^3$ CFU/mL (exclusion criteria). When monomorphic bacterial growth was $>10^5$ CFU/mL, the culture was classified as positive (inclusion criteria). To confirm infection with *S. saprophyticus*, after culture on blood agar and mannitol salt agar, the colonies of suspected to *S. saprophyticus* were detected based on standard microbiological tests such as Gram stain, rapid tests for catalase, coagulase, and biochemical tests like maltose, sucrose, trehalose, xylose, novobiocin test, haemolysin, urease and nitrate reduction [13]. Finally, 43 isolates were suspected to be *S. saprophyticus* isolates but for definite identification, all 43 isolates were exposed to the specific primer by PCR assay. The *S. saprophyticus* (ATCC 15305) strain was used as a positive control.

Antimicrobial susceptibility testing

The resistance of the *S. saprophyticus* isolates to 13 specific antibiotics was investigated by the disc diffusion method according to CLSI (2019). The antibiotic discs represented 13 classes of antibiotics: The antibiotic discs including 13 classes of antibiotics: chloramphenicol (chloramphenicol); ciprofloxacin (fluorinated quinolones); clindamycin (lincosamides); gentamicin (aminoglycosides); erythromycin (macrolides); sulphamethoxazole/trimethoprim (sulphonamides); tetracycline (tetracyclines); vancomycin (glycopeptides); quinupristin-dalfopristin (streptogramins); cefoxitin (penicillinase-stable penicillins); nitrofurantoin (nitrofurantoin); rifampin (ansamycins) and linezolid (oxazolidinones). As per the standardized definition of multidrug-resistance (MDR), extensively drug-resistant, and pan-drug-resistant bacteria have been well studied. Multidrug-resistance was defined as acquired resistance to at least one agent in three or more antimicrobial classes. Extensively drug-resistant was defined as resistant to at least one agent in all but two or fewer antimicrobial classes (i.e. bacterial isolates remain susceptible to only one or two antimicrobial categories). Pan-drug-resistant was well-defined as resistant to all agents in all antimicrobial classes [14,15].

Determination of biofilm formation by microtitre plate method

The biofilm formation of *S. saprophyticus* isolates was performed using the microtitre plate method. First, the *S. saprophyticus* isolates were inoculated in brain–heart infusion agar at 37°C for 24 hours. Then, these isolates were adjusted to 0.5 McFarland. A 10-µL aliquot of each suspension was then diluted 1 : 200 in 190 µL of tryptic soy broth containing 1% glucose in 96-well microtitre plates. Following incubation at 37°C overnight, the plates were washed three times with phosphate-buffered saline (PBS). The adherent cells were fixed with methanol for 10 minutes and stained with 200 µL of 0.1% crystal violet (CV) for 20 minutes at room temperature. Again,

the plates were washed with PBS and next, the unbound CV was removed by adding 200 µL of ethanol for 20 minutes, and the optical density at 570 nm (OD_{570}) was measured using a UV-visible spectrophotometer (UV-1601, Shimadzu, Kyoto, Japan). *Staphylococcus epidermidis* ATCC 35984 and tryptic soy broth broth were used as positive and negative controls (OD_c) for the biofilm formation, respectively. The results were consistent with the criteria recommended by Zhang *et al.* [16]. The isolates were classified into several groups about the bio-film formation capacity: $OD_{570} \leq OD_c$ = no bio-film producer; $OD_c < OD_{570} \leq 2 \times OD_c$ = weak biofilm producer; $2 \times OD_c < OD_{570} \leq 4 \times OD_c$ = moderate biofilm producer; and $4 \times OD_c < OD_{570}$ = strong biofilm producer, where OD_c represents the positive control. All experiments were repeated three times.

Determination of biochemical characterization of biofilm structures

For the biochemical characterization of the biofilm structures, the 24-hour biofilms of *S. saprophyticus* isolates was grown in the 96-well microtitre plates and washed with PBS. The biofilms were treated for 1 hour at 37°C with (a) a solution of 10 mM sodium metaperiodate ($NaIO_4$) in 50 mM sodium acetate buffer for the disruption of the extracellular polysaccharides, (b) 100 µg/mL of proteinase K for the disruption of the extracellular proteins, or (c) 100 µg/mL of DNAsel in 150 mM of NaCl and 1 mM $CaCl_2$ for the disruption of the extracellular DNAs. After treatments, the biofilms were washed with PBS, stained with 0.1% CV, and the OD_{570} was measured, as described by Sheikh *et al.* [17].

DNA extraction

The boiling method was used to extract genomic DNA from *S. saprophyticus* isolates. A few bacterial colonies of *S. saprophyticus* strains grown overnight on nutrient agar (Merck, Germany) were resuspended in microtubes containing 500 µL of Tris–HCl–EDTA buffer, then the microtubes were placed in thermoblock (Denville Scientific, Metuchen, NJ, USA) for 5 min at 95°C, and centrifuged at 14 000 rpm for 10 min at 4°C. The supernatant was used as the DNA template in the PCR assays. UV absorbance ratios, A_{280}/A_{260} were used to evaluate DNA extract purity using a Nanodrop instrument (Thermo Scientific, Waltham, MA, USA).

Confirmation of *S. saprophyticus*-specific PCR amplification primers

For definitive identification, all 43 suspected *S. saprophyticus* isolates were exposed to *S. saprophyticus*-specific PCR amplification primers [18]. The *S. saprophyticus* (ATCC 15305) strain was used as a positive control and *Staphylococcus aureus* ATCC 25923 strain was the negative control.

Detection of oxacillin resistance by disc diffusion method and *mecA* gene

All of the *S. saprophyticus* isolates were confirmed for resistance to oxacillin using cefoxitin (30 µg) (MAST Diagnostics, Bootle, UK) disc diffusion method and results were interpreted consistent with CLSI (2019). The *S. aureus* ATCC 33591 was used as the control strain [14]. Existence of the *mecA* gene was investigated using the PCR assay, as earlier defined by Moosavian *et al.* [19], The *S. aureus* ATCC 33591 strain was used as a positive control and *S. aureus* ATCC 25923 strain was the negative control.

Statistical analysis

All data were evaluated using SPSS version 23.0 software (IBM, Armonk, NY, USA). Two-tailed P value < 0.05 was considered statistically significant. Data normality of continuous variables was initially verified using the Shapiro–Wilk test. Fisher's exact test/χ^2 and Mann–Whitney U test were used to determine the significant association between qualitative and continuous variables, respectively. Continuously distributed variables were described by reporting their mean.

Results

Overall, 43 clinical *S. saprophyticus* isolates were confirmed from UTIs based on culture, biochemical tests and PCR amplification. Details of molecular and phenotypic identification are shown in Table 1. The mean age of the study population was 35.53 years (standard deviation 8.01 years) (Table 2). Out of 43 isolates, 11 (25%) were resistant to the cefoxitin disc diffusion method and were considered as oxacillin-resistant. All oxacillin-resistant *S. saprophyticus* isolates harboured a *mecA* gene. Hence, the prevalence rates of oxacillin-resistant *S. saprophyticus* isolates and oxacillin-susceptible *S. saprophyticus* isolates were 11 (25%) and 32 (74%), respectively. According to antibiogram results, the maximum resistance was found with erythromycin (58%, 25/43), clindamycin (46%, 20/43), gentamicin (37%, 16/43), ciprofloxacin (34%, 15/43), tetracycline and chloramphenicol (25%, 11/43); and the minimum resistance was found with trimethoprim, sulfamethoxazole (9%, 4/43) and rifampin (4%, 2/43) (Table 3). The antibiotic resistance patterns of the 43 *S. saprophyticus* isolates from the UTIs are recorded in Table 4; 33 different patterns from 13 antibiotics in each combination. Out of the 43 *S. saprophyticus* isolates, none was resistant to all antibiotic classes. The present study also showed 25 (58%) *S. saprophyticus* isolates were comparatively more resistant to multiple antimicrobial agents and were MDR. None of our isolates were extensively drug-resistant or pan-drug-resistant (Table 2). All *S. saprophyticus* isolates were susceptible to vancomycin, linezolid, nitrofurantoin and quinupristin-dalfopristin (Table 3). Four

TABLE 1. **Results of *Staphylococcus saprophyticus* identification by phenotypic and genotypic tests**

IDNumber	Novobiocin test	Coagulase	Haemolysin	Urease production	Mannitol	Maltose	Trehalose	Sucrose	Xylose	Nitrate	Thioglycolate	Phenotypictests	Specific gene
1	R	—	—	—	—	+	+	—	—	—	+	*S. saprophyticus*	*S. saprophyticus*
2	R	—	—	—	—	+	+	—	—	—	+	*S. saprophyticus*	*S. saprophyticus*
3	R	—	—	—	—	+	+	—	—	—	+	*S. saprophyticus*	*S. saprophyticus*
4	R	—	—	—	—	+	+	—	—	—	+	*S. saprophyticus*	*S. saprophyticus*
5	R	—	—	—	—	+	+	—	—	—	+	*S. saprophyticus*	*S. saprophyticus*
6	R	—	—	—	—	+	+		—	—	+	*S. saprophyticus*	*S. saprophyticus*
7	R	—	—	—	—	+	+	—	—	—	+	*S. saprophyticus*	*S. saprophyticus*
8	R	—	—	—	—	+	+	—	—	—	+	*S. saprophyticus*	*S. saprophyticus*
9	R	—	—	—	—	+	+	—	—	—	+	*S. saprophyticus*	*S. saprophyticus*
10	R	—	—	—	—	+	+	—	—	—	+	*S. saprophyticus*	*S. saprophyticus*
11	R	—	—	—	—	+	+	—	—	—	+	*S. saprophyticus*	*S. saprophyticus*
12	R	—	—	—	—	+	+	—	—	—	+	*S. saprophyticus*	*S. saprophyticus*
13	R	—	—	—	—	+	+	—	—	—	+	*S. saprophyticus*	*S. saprophyticus*
14	R	—	—	—	—	+	+	—	—	—	+	*S. saprophyticus*	*S. saprophyticus*
15	R	—	—	—	—	+	+	—	—	—	+	*S. saprophyticus*	*S. saprophyticus*
16	R	—	—	—	—	+	+	—	—	—	+	*S. saprophyticus*	*S. saprophyticus*
17	R	—	—	—	—	+	+	—	—	—	+	*S. saprophyticus*	*S. saprophyticus*
18	R	—	—	—	—	+	+	—	—	—	+	*S. saprophyticus*	*S. saprophyticus*
19	R	—	—	—	—	+	+	—	—	—	+	*S. saprophyticus*	*S. saprophyticus*
20	R	—	—	—	—	+	+	—	—	—	+	*S. saprophyticus*	*S. saprophyticus*
21	R	—	—	—	—	+	+	—	—	—	+	*S. saprophyticus*	*S. saprophyticus*
22	R	—	—	—	—	+	+	—	—	—	+	*S. saprophyticus*	*S. saprophyticus*
23	R	—	—	—	—	+	+	—	—	—	+	*S. saprophyticus*	*S. saprophyticus*
24	R	—	—	—	—	+	+	—	—	—	+	*S. saprophyticus*	*S. saprophyticus*
25	R	—	—	—	—	+	+	—	—	—	+	*S. saprophyticus*	*S. saprophyticus*
26	R	—	—	—	—	+	+	—	—	—	+	*S. saprophyticus*	*S. saprophyticus*
27	R	—	—	—	—	+	+	—	—	—	+	*S. saprophyticus*	*S. saprophyticus*
28	R	—	—	—	—	+	+	—	—	—	+	*S. saprophyticus*	*S. saprophyticus*
29	R	—	—	—	—	+	+	—	—	—	+	*S. saprophyticus*	*S. saprophyticus*
30	R	—	—	—	—	+	+	—	—	—	+	*S. saprophyticus*	*S. saprophyticus*
31	R	—	—	—	—	+	+	—	—	—	+	*S. saprophyticus*	*S. saprophyticus*
32	R	—	—	—	—	+	+	—	—	—	+	*S. saprophyticus*	*S. saprophyticus*
33	R	—	—	—	—	+	+	—	—	—	+	*S. saprophyticus*	*S. saprophyticus*
34	R	—	—	—	—	+	+	—	—	—	+	*S. saprophyticus*	*S. saprophyticus*
35	R	—	—	—	—	+	+	—	—	—	+	*S. saprophyticus*	*S. saprophyticus*
36	R	—	—	—	—	+	+	—	—	—	+	*S. saprophyticus*	*S. saprophyticus*
37	R	—	—	—	—	+	+	—	—	—	+	*S. saprophyticus*	*S. saprophyticus*
38	R	—	—	—	—	+	+	—	—	—	+	*S. saprophyticus*	*S. saprophyticus*
39	R	—	—	—	—	+	+	—	—	—	+	*S. saprophyticus*	*S. saprophyticus*
40	R	—	—	—	—	+	+	—	—	—	+	*S. saprophyticus*	*S. saprophyticus*
41	R	—	—	—	—	+	+	—	—	—	+	*S. saprophyticus*	*S. saprophyticus*
42	R	—	—	—	—	+	+	—	—	—	+	*S. saprophyticus*	*S. saprophyticus*
43	R	—	—	—	—	+	+	—	—	—	+	*S. saprophyticus*	*S. saprophyticus*

TABLE 2. Characteristic of *Staphylococcus saprophyticus* isolated from women with urinary tract infections

ID number	Age	Biofilm formation	Dnase	PK	SM	*mecA*	ARPs	MDR
1	29	Strong	—	—	D	—	C, CD, E	+
2	33	Strong	—	—	D	—	E, T	—
3	39	Weak	—	D	—	—	CIP, E, GN, T	+
4	27	Moderate	—	D	—	—	C, CIP, E, GN	+
5	27	Strong	—	D	—	+	CIP, CD, T, FOX	+
6	28	Strong	—	—	D	—	CD, E, GN	+
7	45	Moderate	—	—	D	+	CD, E, T, FOX	+
8	35	Weak	—	D	—	+	SXT, CD, T, FOX	+
9	33	Weak	—	D	—	—	CD, T	—
10	42	Strong	—	—	D	—	CIP, CD, E	+
11	47	—	—	—	—	—	CD, E, T	+
12	43	—	—	—	—	—	C, CD, E, GN, T	+
13	22	Strong	—	—	D	+	SXT, C, CIP, CD, T, FOX	+
14	28	Weak	—	—	D	—	CIP, CD, E, T	+
15	32	—	—	—	—	—	CD, E	—
16	36	Moderate	—	—	D	+	CIP, CD, FOX	+
17	21	—	—	—	—	—	CD, GN	—
18	51	Moderate	—	—	D	+	CIP, CD, GN, FOX	+
19	53	Strong	—	—	D	+	SXT, C, CD, GN, FOX	+
20	36	Weak	—	D	—	—	CIP, GN	—
21	37	Weak	—	D	—	+	C, RP, FOX	+
22	40	—	—	—	—	—	E	—
23	34	—	—	—	—	—	E	—
24	22	Moderate	—	—	D	—	C, CD	—
25	26	Moderate	—	—	D	+	SXT, CD, FOX	+
26	39	—	—	—	—	—	CIP, CD, E	+
27	43	Moderate	—	—	D	—	CIP, C, CD, E, GN	+
28	27	Strong	—	—	D	+	E, GN, RP, T, FOX	+
29	41	Weak	—	D	—	—	CIP, C, E, GN	+
30	25	—	—	—	—	—	E, GN	—
31	36	Moderate	—	—	D	+	E, T, FOX	+
32	33	—	—	—	—	—	CIP, GN	—
33	30	Moderate	—	—	D	—	E, GN, T	+
34	30	Strong	—	—	D	—	E, GN, FOX	+
35	28	Weak	—	D	—	—	—	—
36	39	Weak	—	D	—	—	E, GN	—
37	47	Strong	—	—	D	—	C, CIP, E	+
38	46	—	—	—	—	—	—	—
39	45	—	—	—	—	—	E	—
40	35	—	—	—	—	—	—	—
41	42	—	—	—	—	—	C, E	—
42	39	—	—	—	—	—	—	—
43	37	—	—	—	—	—	E	—

Abbreviations: +, positive; –, negative; ARP,; C, Chloramphenicol; CD, clindamycin; CIP, ciprofloxacin; D, dissolve; E, erythromycin; FOX, cefoxitin; GN, gentamicin; MDR, multidrug resistance; mecA, methicillin-resistant gene; Nd, not done; PK, proteinase K; RP, rifampin; SM, sodium metaperiodate; SXT, sulfamethoxazole-trimethoprim; T, tetracycline.

TABLE 3. Results of antimicrobial resistance tests by disc diffusion method

Antimicrobial category	Antimicrobial agent	susceptible	resistant
Oxazolidinones	Linezolid	43 (100%)	—
Folate pathway inhibitors	Trimethoprim-sulphamethoxazole	39 (90%)	4 (9%)
Phenicols	Chloramphenicol	32 (74%)	11 (25%)
Fluoroquinolones	Ciprofloxacin	28 (65%)	15 (34%)
Lincosamides	Clindamycin	23 (53%)	20 (46%)
Macrolides	Erythromycin	18 (41%)	25 (58%)
Aminoglycosides	Gentamycin	27 (62%)	16 (37%)
Ansamycins	Rifampin	41 (95%)	2 (4%)
Tetracyclines	Tetracycline	32 (74%)	11 (25%)
Glycopeptides	Vancomycin	43 (100%)	—
Penicillinase-stable penicillins	Cefoxitin	32 (74%)	11 (25%)
Nitrofurantoins	Nitrofurantoin	43 (100%)	—
Streptogramins	Quinupristin-dalfopristin	43 (100%)	—

S. saprophyticus isolates were sensitive to all antimicrobial agents used in this study. The biofilm formation of 43 *S. saprophyticus* isolates was performed using the microtitre plate method. The OD_{570} values of positive control and negative control were 0.410 ± 0.043 and 0.066 ± 0.006, respectively. The OD_{570} values for the *S. saprophyticus* isolates ranged from 0.137 ± 0.054 to 1.543 ± 0.050. Generally, 28 (63%) *S. saprophyticus* isolates were biofilm positive, and among them 10/28 (35%) isolates showed strong biofilm formation, 9/28 (32%) showed moderate biofilm formation, 9/28 (32%) were weak biofilm producers and 15/28 (53%) could not form any detectable biofilm (Table 2). The biofilm structures in 18/28 (64%) *S. saprophyticus* isolates were composed of polysaccharide structures, in 10/28 (35%) they were composed of a combination of proteins, and none were isolated that did not dissolve with DNAase treatment. The composition of the biofilm structure of *S. saprophyticus* isolates is shown in Table 2. Consistent with our results, the antibiotic resistance of the *S. saprophyticus* polysaccharide biofilm structure was higher than that of the *S. saprophyticus* protein biofilm structure. The prevalence and rate of antibiotic resistance in the polysaccharide biofilm structure and protein biofilm structure in *S. saprophyticus* isolates are shown in Table 2 and Fig. 1.

TABLE 4. Antibiotic resistance patterns among *Staphylococcus saprophyticus* isolates

No. of resistant antibiotics	Antibiotics	No.of species
6	SXT, C, CIP, CD, T, FOX	1
5	SXT, C, CD, GN, FOX	1
	CIP, CD, GN, FOX	1
	E, GN, RP, T, FOX	1
4	SXT, CD, T, FOX	1
	CIP, CD, T, FOX	1
	CIP, C, CD, E, GN	1
	C, CD, E, GN, T	1
	CD, E, T, FOX	1
	C, CIP, E, GN	1
	CIP, C, E, GN	1
	E, GN, FOX	1
	CIP, E, GN, T	1
3	SXT, CD, FOX	1
	CIP, CD, FOX	1
	C, RP, FOX	1
	CIP, CD, E	2
	E, T, FOX	1
	CD, E, GN	1
	C, CIP, E	1
	E, GN, T	1
	CD, E, T	1
	C, CD, E	1
2	CIP, GN	1
	CD, GN	1
	CIP, GN	1
	E, GN	2
	C, CD	1
	CD, T	1
	CD, E	1
	E, T	1
	C, E	1
1	E	4

Antibiotic abbreviations: C, chloramphenicol; CD, clindamycin; CIP, ciprofloxacin; E, erythromycin; FOX, cefoxitin; GN, gentamicin; RP, rifampin; SXT, sulfamethoxazole-trimethoprim; T, tetracycline.

According to our results, antibiotic resistance in *S. saprophyticus* biofilm producers was higher than in *S. saprophyticus* non-biofilm producers (Fig. 2).

Statistical analysis showed that the relationship between biofilm formation and antibiotic resistance among *S. saprophyticus* isolates was significant (p 0.0002). Moreover, our results indicated that biofilm formation in MDR *S. saprophyticus* isolates was significantly higher than that of non-MDR *S. saprophyticus* isolates (p 0.0003) (Table 2). Overall, 46.8% of MDR *S. saprophyticus* isolates had the ability to form a strong biofilm. However, no significant relationship was detected between biofilm formation intensity (strong, moderate and weak) and MDR *S. saprophyticus* isolates. Moreover, our results showed that all oxacillin-resistant *S. saprophyticus* isolates exhibited biofilm formation ($p \leq 0.05$).

Discussion

Urinary tract infections are the most frequent types of hospital and community infections, and account for >30% of hospital infections [20]. *Staphylococcus saprophyticus* is one of the main pathogens of UTIs; however, little is known about antibiotic-resistant patterns and biofilm production in this species. The prevalence of *S. saprophyticus* in UTIs was 17%. This is higher than the reports by Onyemelukwe *et al.* and Magliano *et al.* [21,22]. In some studies, the term methicillin resistance is used, and in others the term oxacillin resistance is used. In both methods, a cefoxitin disc is used and the result is read according to the CLSI guideline. Therefore, resistance to methicillin or oxacillin both seem to be used for *S. saprophyticus* isolates [7,12]. Resistance to oxacillin was determined using a cefoxitin disc. Our results were confirmed using a *mecA* gene-based PCR method as a reference standard. The incidence of the *mecA* gene in *S. saprophyticus* isolates was 25%. All oxacillin-resistant *S. saprophyticus* isolates harbured the *mecA* gene. In previous studies, the prevalence of *mecA* among *S. saprophyticus* was 7.9% [12].

Unfortunately, UTIs are often treated with a broad-spectrum antibiotic without performing culture and sensitivity tests. This inappropriate usage of antibiotics has increased antibiotic

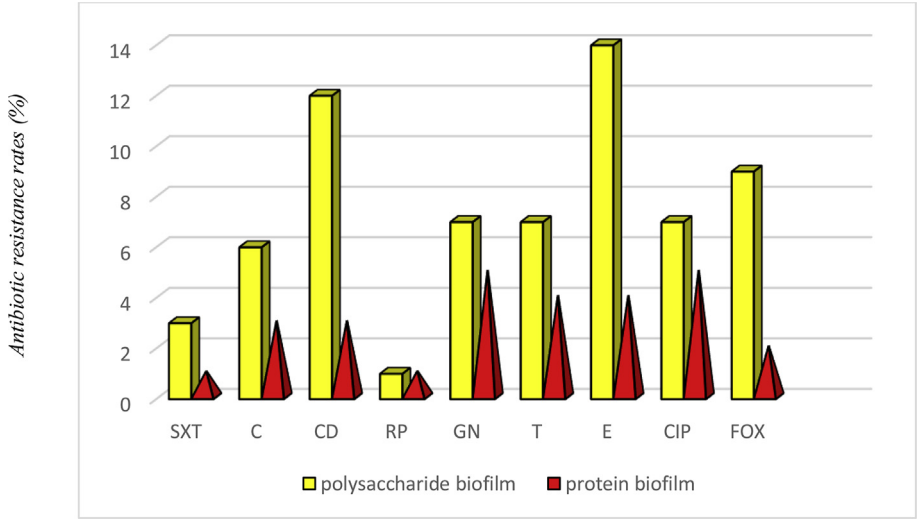

FIG. 1. The frequency of antibiotic resistance in polysaccharide biofilm and protein biofilm.

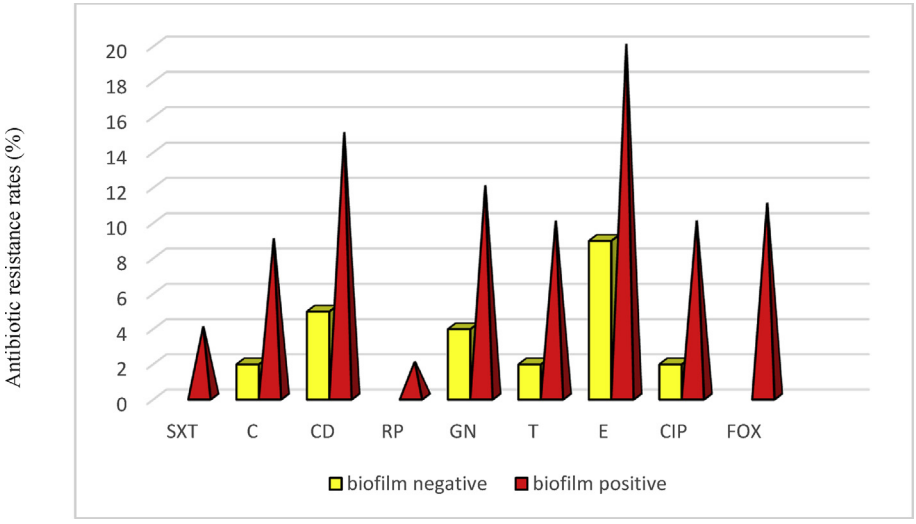

FIG. 2. The frequency of antibiotic resistance in biofilm positive and biofilm negative isolates.

resistance, leading to the development of MDR bacterial pathogens. Changing patterns of antibiotic resistance in the aetiological agents of urinary tract pathogens have been reported [23,24]. According to the CLSI, routine susceptibility testing of urinary *S. saprophyticus* isolates to choose antibiotics is not recommended as this microorganism is normally susceptible to trimethoprim/sulfamethoxazole [25]. However, in our study, 9% of the *S. saprophyticus* isolated from UTIs were resistant to sulfamethoxazole/trimethoprim. Similar to our results, 17.6% of the *S. saprophyticus* isolates were resistant to sulfamethoxazole/trimethoprim [26]. In addition, the *S. saprophyticus* isolates described in this study were susceptible to vancomycin, linezolid and quinupristin/dalfopristin [27]. In *S. saprophyticus* isolates, the maximum resistance has been observed against clindamycin (46%), followed by ciprofloxacin and gentamicin (37%). The results are comparable to studies conducted in other parts of the country [28,29]. Our results are alarming as they reveal the high rate of MDR *S. saprophyticus* in the majority of Ahvaz hospitals. The highest percentage of MDR in our study might be a result of the improper use of antibiotics. The virulence is connected with this species ability to form biofilms on host surfaces and its resistance to antibiotics. A similar result of high antimicrobial resistance in biofilm-forming bacterial isolates has been found in other studies [30–32]. Generally, 51% of MDR and 9% of non-MDR isolates can produce biofilms. The results of this study indicated that biofilm formation in MDR *S. saprophyticus* isolates was greater than in non-MDR *S. saprophyticus* isolates. However, our results are not supported by de Campos *et al.* [33]. In *S. saprophyticus* isolates that did not produce biofilm, less resistance was observed.

In conclusion, it is important to take into consideration specific local resistance patterns when choosing appropriate antibiotic coverage. It seems that MDR *S. saprophyticus* strains have emerged and antimicrobial susceptibility testing of these strains is therefore necessary. The development of antibiotic resistance in UTIs is a serious issue, particularly in developing countries where in addition to a high level of poverty, poor hygienic practices are a serious concern. Our results can be used to improve treatment plans to control UTIs. Increased awareness of the mechanisms underlying biofilm formation and the development of drug resistance allow more efficient control and treatment of UTIs.

Authors' contributions

The concept and the design of the study were developed by AAZD and MH. The methodology was designed by MH. Data collection and the experimental work were carried out by AAZD and SH. Formal analyses and interpretation of data were carried out by FJ. The original draft was prepared by AAZD and reviewed by MH. All the authors have read and approved the final manuscript for submission.

Conflicts of interest

The authors report no conflicts of interest in this work.

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