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CHARACTERIZATION OF *STAPHYLOCOCCUS AUREUS* ISOLATED FROM BURN WOUND; STRONG ANTIBACTERIAL ACTIVITY OF PHAGE COCKTAIL AGAINST VANCOMYCIN INTERMEDIATE-RESISTANT *STAPHYLOCOCCUS AUREUS*

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ABSTRACT

A universal problem is about spread of *Staphylococcus aureus* infections in burn patients. The present study aimed to learn about the molecular characteristics and the resistance pattern of *S. aureus* strains isolated from burn patients. In this cross-sectional study, we investigated 100 unique *S. aureus* isolated from burn patients by antimicrobial activity and biofilm formation and evaluated the effect of Complex Pyobacteriophage, a commercial bacteriophage cocktail, against the isolates mentioned above. Methicillin-resistant *Staphylococcus aureus* (MRSA) comprised 76%, and methicillin-susceptible *Staphylococcus aureus* (MSSA) comprised 24% of 100 *S. aureus* strains. The resistance rate among MRSA isolates was higher than compared of MSSA. Mupirocin resistance was found in 30% of isolates, with 28 (93.3%) and 2 (6.7%) strains of MRSA and MSSA, respectively, found. Vancomycin intermediate resistance in *S. aureus* (VISA) was 13% of MRSA strains. Two isolates were confirmed as vancomycin-resistant *S. aureus* (VRSA) strains and carried *vanA*. 31 and 62% of the total isolates showed inducible and constitutive resistance phenotypes. The rate of inducible and constitutive clindamycin resistance among MRSA strains was higher than MSSA strains. Biofilm production was detected in 66% of isolates. Strong, moderate, and weak producers accounted for 25%, 17%, and 24% of isolates. Phage analysis showed that 81% were susceptible to the phage cocktail, and only 19% were resistant to the phage cocktail. Our data indicated that VISA strains prevalence in the burn unit was mainly from *S. aureus* infections. Present work recommended that vancomycin treatment be closely monitored to prevent the spread of VISA and VRSA strains. Observations also highlighted the role of bacteriophage cocktails in eradicating S. aureus-related infections.

Keywords: Methicillin-Resistant *Staphylococcus aureus*, Vancomycin-Resistant *Staphylococcus aureus*, Biofilm, Bacteriophage, Wound infection

INTRODUCTION

Burning can cause of disability and sometimes mortality in different ages in developed and developing countries (**Robben, Ayalew, Chung, & Ressner, 2021; Savetamal, 2021**). Damage to skin barrier function and weakening of immune responses in burned patients lead to increase susceptibility to the general systemic disorder, bacterial colonization, and different infections, which cause complicated the management of the disease in those hospitalized (**Dou & Zhang, 2018**). Patients with burn wound infections are at high risk for infections by various microorganisms, leading to death in more than 70% of burn patients (**Dou & Zhang, 2018; Savetamal, 2021**). *Staphylococcus aureus* remains increasingly common among infectious agents in burn patients (**Robben et al., 2021; Yali et al., 2014**). Mentioned bacterium causes a wide range of illnesses. Symptoms can range from small infections on the skin to life-threatening conditions like bacteremia, endocarditis, and osteomyelitis. Recent data has indicated a remarkable increase and a heavy burden of *S. aureus* burn infections worldwide (**Dou & Zhang, 2018; Robben et al., 2021; Savetamal, 2021**). Besides various virulence factors expression and emergence of drug-resistant strains, biofilms production is most critical abilities that makes *S. aureus* infection as remarkable problem in the patients (**Tahaei *et al.*, 2021**).
Some studies have revealed an increase in multidrug-resistant (MDR) *S. aureus* strains, with a limit in the chemotherapeutic agents availability and complicating disease management. Using mupirocin to treat skin and soft tissue infections (SSTIs) and eliminate MRSA from patients, healthcare-workers, nurses and the control MRSA outbreaks is an excellent use of this topical antibiotic. The common use of mupirocin has conseqenced to increased resistance, documented in many countries worldwide. Vancomycin is a choice to treat severe MRSA infections. Globally, a majour increase in VRSA prevalence strains over the last few decades has become a cause for concern, imposing high economic costs to governments and patients. Data worldwide indicate that macrolide-lincosamide-streptogramin group B (MLSB) antibiotics have been known as drugs choice to treat SSTIs caused by this pathogen. Clindamycin using can cause inducible and constitutive resistance, making it a hard pathogen to treat. Hence, reasonable use of antibiotics is essential.
Infections by *S. aureus* are scandalously problematic to treat (**Gordillo Altamirano &Barr, 2019**). Recent data noted the critical role of phage therapy as a promising tool in controlling and treating bacterial infections (**Azam & Tanji, 2019**).
Unlike traditional therapy, phage therapy has some advantages (**Duplessis & Biswas, 2020**). They are self-propagating and self-limiting viruses that kill prokaryotes without harming humans (**Dabrowska, 2019; Moore & Moore, 2021; Picoli et al., 2021**; **Topka-Bielecka et al., 2021**).
However, the acquisition of knowledge on the prevalence, characterization, biofilm production, and phage therapy of *S. aureus* strains in Iran is limited. The objective of our study was to investigate antibiotic susceptibility and biofilm formation ability, including specific biofilm-responsible genes of *S. aureus* isolated from wound infections, and to study the effectiveness of phage therapy.

MATERIAL AND METHODS

Collection of samples and isolates

In the present research, 100 *S. aureus* strains from burn wounds were obtained from September 2018 to December 2019. Ethics Committee of the Shahid Beheshti University of Medical Sciences confirmed the study (IR. SBMU. MSP.REC. 1398.

561) as well as consent was obtained from participants. Burned (high level) patients were sampled from wound area with sterile cotton swabs. Detection of *S. aureus* was done by standard bacteriological tests and coagulase production (Sigma, St. Louis, USA), catalase, and DNase (Merck, St. Louis, Germany). An PCR assay used to confirm the nuc gene presence in all isolates (Eftekhar et al., 2017). Confirmed isolates stored in trypticase soy broth (TSB, Merck, St. Louis, Germany), containing 30% glycerol, and stored at -70ºC.

Antimicrobial susceptibility testing

Antibiogram was done by Kirby Baur method based on clinical and the laboratory standards institute (CLSI) instructions against tetracycline (TET 30µg), amikacin (AMK 30µg), clindamycin (CLI 2µg), penicillin (PEN 10 units), gentamicin (GEN 10 µg), erythromycin (ERY 15µg), and rifampicin (RIF 5µg) (Mast Diagnostics Ltd, Merseyside, UK). To classify the strains as highly sensitive, intermediate, or highly resistant, they were incubated for 24 hours at 37°C and then measured and compared to the CLSI guidelines (CLSI 2021).

The screening of vancomycin (VAN) and mupirocin (MUP) resistance strains was performed by standard broth micro-dilution procedure in concurrence with CLSI guideline (CLSI 2021). Mupirocin had three different MIC breakpoints: susceptible (1 mg/L), low-level resistant (2–256 mg/L), and high-level resistant (>512 mg/L).

The ability of these strains to grow in Mueller-Hinton agar (Merck, Germany) containing cefoxitin (30 g) disc and PCR amplification of the *mecA* gene identified them as MRSA or MSSA (**Eftekhar *et al*., 2017**). *S. aureus* isolates with the zone of inhibition ≤21 mm around the cefoxitin disc were confirmed as MRSA strain. *S. aureus* ATCC 25923 was applied as positive control of antibiotic susceptibility testing.

Inducible and constitutive screening

D-test was used to determine isolates with inducible resistance phenotype ($iMLS_B$). The screening of isolates with constitutive and inducible resistance phenotype ($cMLS_B$ and $iMLS_B$) was performed under CLSI guideline (CLSI 2021). MS phenotype was defined for isolates with resistance to macrolides and streptogramin B.

VISA and VRSA screening

The MIC for vancomycin (VAN) was determined as previously described (**Park, Lee, Kim, & Kim, 2019**). The MIC titer obtained was compared to the MIC breakpoints according to CLSI 2021 recommendations, and the isolate was classified as resistant, intermediate or susceptible. According to the CLSI guideline, negative (ATCC 25923) and positive (*Enterococcus faecalis* ATCC 51299) strains were used.

Spot test assay

The sensitivity of isolates to Complex Pyobacteriophage (Microgen, Russia) was explained by Kutter *et al*. with minor modifications (**Kutter *et al*. 2009**). Briefly, isolates were cultured overnight in TSB (Merck, St. Louis, Germany) at 37°C with gentle shaking. Then 50 µl of each isolate was added to 3-4 mL molten top agar, LB medium with 0.4% agarose (Merck, Germany), mixed well, and poured on bottom agar (LM medium with 1.2% agarose). Subsequently, 5 µl of Complex Pyobacteriophage and SM buffer were spotted on the lawn of bacteria with proper distance; then plates incubated in 37°C for 18-24 hours. Spots examined and graded according to the morphology of the spot zone. The clear spots without turbidity are considered as "++", substantial turbidity in spots or individual plaques as "+", and no recognizable zone as "0" (**Mahmoud, Ahmed, Abo-senna, Riad, & Abo-Shadi, 2020**).

Determination of biofilm production by microtiter plate (MtP) assay

MtP assay performed based on Yousefi *et al*. protocol with no significant modification (**Yousefi *et al*., 2016**). The biofilm measured by ELISA reader absorbance (OD) at 490 nm. All tests were performed four times, and the results were then re-run triplicate to ensure duplicability. Strain (ATCC 35984) of *Staphylococcus epidermidis* and 1% glucose TSB-supplemented were recruited as positive and negative control strains. The degree of biofilm production (without biofilm, weak, moderate, and strong) was interpreted according to the study of Yousefi *et al*. (**Yousefi *et al*., 2016**).

Deoxyribonucleic acid (DNA) extraction and screening for *vanA*, and *icaABCD* genes

DNA extraction done by Goudarzi *et al*. method, (**Goudarzi *et al*., 2017**). Strains were screened for *vanA*, and *icaABCD* genes by PCR assay (Chen *et al*., 2020; Park *et al*., 2019). Also, *Enterococcus faecalis* BM6217 was used for *vanA* genes as positive controls in each run of amplification. The entire isolates were investigated for the presence of *icaA* and *icaD* genes by multiplex PCR assay. *Enterococcus faecalis* BM6217 was used for *vanA* genes, as positive controls in each run of amplification. Agarose gel electrophoresis was applied to analyze PCR products at 90 V for 60 min. Amplified DNAs were observed with gel documentation, and their size was compared with 100bp DNA ladder (BioFact, Korea). Data analyzed by SPSS software (SPSS Inc no. 20).

RESULTS

Based on our results, 76% of isolates were MRSA and all harbored *mecA* gene (Figure 1).

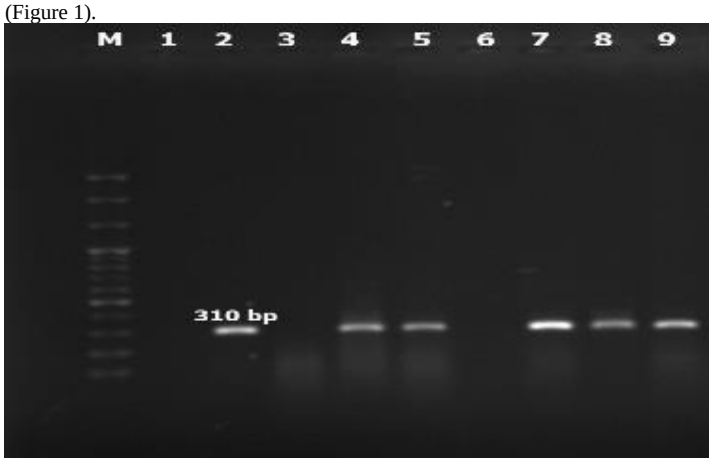

Figure 1 PCR result of *mecA* encoding gene. Lane M, 100-bp DNA Ladder (Fermentas, UK); Lane 1, negative control; Lane 2 positive control; Lane 4, 5, 7, 8 ,9, the 310bp PCR product of *mecA* encoding.

Our finding revealed resistance of 95% of isolates for ERY, followed by 88% for AMK, 62% for CLI, 61% for TET, 58% for GEN, 40% for RIF 55%, 30% for MUP, and 15% for VAN. Resistance prevalence among MRSA and MSSA isolates to antibiotics provided in Table 1.

Table 1 Distribution of resistance among MRSA and MSSA strains

Antibiotic	*S. aureus* Resistance %		Total %
	MRSA	MSSA	
Penicillin	71	14	85
Amikacin	72	16	88
Gentamicin	52	6	58
Erythromycin	73	22	95
Tetracycline	55	6	61
Clindamycin	53	9	62
Rifampin	33	7	40
Mupirocin	21	9	30
Vancomycin	12	3	15
Total	76	24	100

The MIC test for vancomycin showed that 18 isolates (18%) inhibited by 0.5 µg/mL, 30 (30%) by 1 µg/mL, and 37 (37%) by 2µg/mL. VISA strains were detected in 13 (13%) isolates which 3 (23.1%) isolates were MRSA. Of 13 VISA strains, 8 strains were inhibited in MIC titer of 4 µg/mL of vancomycin and 5 strains in 8 µg/mL. Two isolates were resistant to vancomycin, of which one had MIC 32 µg/mL and another exhibited MIC titer of 128 µg/mL. Both VRSA isolates harbored *vanA* gene (Figure 2).

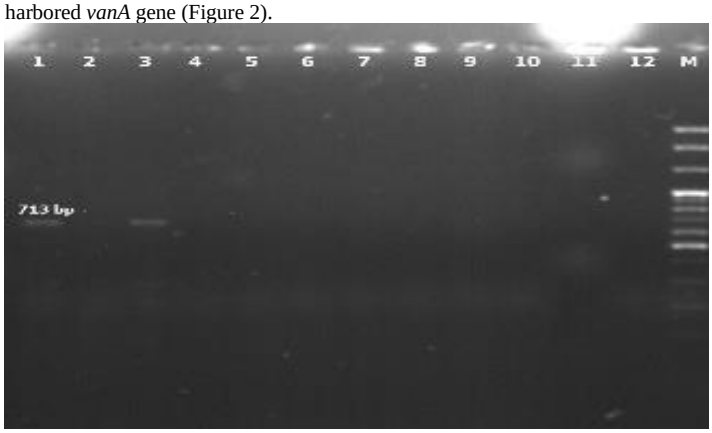

Figure 2 PCR result of *vanA* encoding gene. Lane 1, the 713bp PCR product of *vanA* gene (positive control); Lane 2, negative control; Lane 3, the 713bp PCR product of *vanA* gene, Lane M, DNA Ladder (100-bp, Fermentas, UK).

According to the MIC test, 30% of examined isolates were resistant to mupirocin (mup-R). Of these, 8 and 22 isolates displayed high (HLMUPR) and low-level (LLMUPR) resistance pheotypes. Of 100 isolates under study, 25 (25%) inhibited by 0.5 µg/mL of mupirocin, 10 isolates (10%) by 1 µg/mL, 13 isolates (13%) by 2 µg/mL, 22 isolates (22%) by 4 µg/mL, 15 isolates (15%) by 64 µg/mL, 7 isolates (7%) by 128 µg/mL, 5 isolates (5%) by 512 µg/mL and 3 isolates (3%) by more than 512µg/mL. Of the *S. aureus* strains that had mupirocin-resistant, 28 (93.3%) and 2 (6.7%) strains were MRSA and MSSA. Thoroughly, 90% of isolate were multidrug resistance (MDR). Of the total isolates, 62, 31, and 7 isolates showed $cMLS_B$, iMLSB, and MS phenotypes accounting for 62%, 31%, and 7% respectively. As shown in Table 2, twelve patterns of resistance identified in AMK, CLI, ERY, GEN, PEN, TET, (28%), AMK, CLI, ERY, GEN, PEN, RIF, TET (20), and AMK, PEN, ERY, MUP (15%).

Table 2 The resistant pattern in 100 *S. aureus* strains isolated from patients with burn wound

MDR	Resistance pattern	Percentage
Nine	PEN, GEN, AMK, TET, ERY, CLI, RIF, MUP, VAN	5
Seven	PEN, GEN, TET, AMK, ERY, RIF, CLI	20
Six	PEN, GEN, AMK, TET, ERY, CLI	28
	PEN, GEN, AMK, ERY, RIF, MUP	5
	AMK, TET, ERY, CLI, RIF, MUP	2
Five	PEN, AMK, TET, ERY, VAN	4
	PEN, AMK, RIF, MUP, VAN	3
	PEN, AMK, ERY, CLI, RIF	5
Four	PEN, AMK, ERY, MUP	15
	TET, ERY, CLI, VAN	2
Three	AMK, ERY, VAN	1
One	ERY	8
without	-	2

S. aureus biofilm production was found in 66% of the 100 strains studied. There were 25 (25%) strong, 17 (17%) moderate, and 24 (24%) weak producers of biofilm among the isolates analyzed in the study. Our results showed that icaA was present in 94 strains (94%) and *icaD* in 95 (95%) strains. Four isolates were negative for both *icaA* and *icaD* genes and confirmed as non-biofilm producer isolates. One isolate was negative only for *icaD* and 2 isolates were negative only for *icaA* (Figure 3).

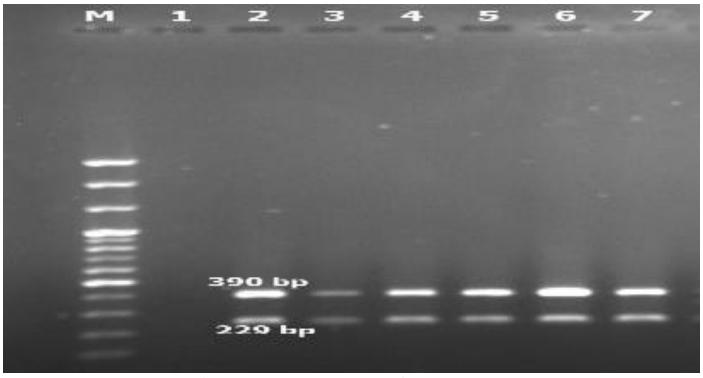

Figure 3 The results of multiplex PCR assay for *icaA* and *icaD* encoding gene in *S. aureus* isolated from burn patients. Lane M, DNA Ladder; Lane 2, negative control; Lane 3, the 390bp PCR product of *icaA* and the 229bp PCR product of *icaD* gene.

Cocktail Complex Pyobacteriophage showed an effective lytic activity against examining *S. aureus* isolates. According to our analysis, the phage cocktail exhibited an effective inhibitory activity against *S. aureus* strains isolated from burn patients. So 81 isolates were susceptible to the phage cocktail, and only 19 isolates were resistant to the phage cocktail (Figure 4).

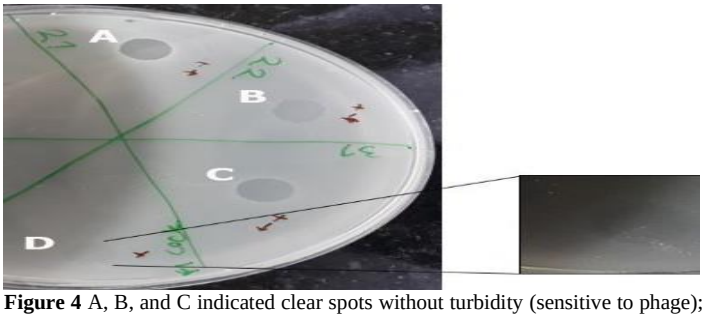

Figure 4 A, B, and C indicated clear spots without turbidity (sensitive to phage); D indicated a zone of inhibition with turbidity.

All phage-resistant isolates were MRSA with simultaneous resistance to nine (2 isolates, 10.5%), seven (12 isolates, 63.2%), and six (5 isolates, 26.3%) antibiotics. Out of 19 phage-resistant strains, 10 isolates belonged to weak-biofilm producer isolates and 9 isolate to moderate producers. All strong biofilm producers were susceptible to the phage cocktail. Also, all VISA and VRSA isolates were susceptible to the phage cocktail. Table 3 represents the analysis of VISA and VRSA strains.

Table 3 Characterization of VISA and VRSA strains isolated from burn wounds

Strain number	Biofilm	Resistance	Mupirocin resistance	MLS_B phenotype	Cocktail spot test	*mecA*	*vanA*/ MIC(µg/mL)
IR1-VRSA	Strong producer	PEN, GEN, AMK, TET, ERY, CLI, RIF, MUP	High level	Constitutive	+	+	+/32
IR2-VRSA	Strong producer	PEN, GEN, AMK, TET, ERY, CLI, RIF, MUP	High level	Constitutive	++	+	+/128
IR1-VISA	Weak producer	PEN, AMK, TET, ERY, VAN	Low level	Inducible	++	+	-/4
IR2-VISA	Weak producer	TET, ERY, CLI, VAN	Low level	Constitutive	++	-	-/4
IR3-VISA	Moderate producer	PEN, AMK, RIF, MUP, VAN	High level	-	++	+	-/4
IR4-VISA	Strong producer	PEN, AMK, RIF, MUP, VAN	High level	-	++	+	-/8
IR5-VISA	Strong producer	PEN, GEN, AMK, TET, ERY, CLI, RIF, MUP	High level	Constitutive	++	+	-/8
IR6-VISA	Strong producer	PEN, AMK, RIF, MUP, VAN	High level	-	++	+	-/8
IR7-VISA	Weak producer	PEN, AMK, TET, ERY, VAN	Low level	Inducible	++	+	-/4
IR8-VISA	Strong producer	PEN, GEN, AMK, TET, ERY, CLI, RIF, MUP	High level	Constitutive	++	+	-/8
IR9-VISA	Moderate producer	TET, ERY, CLI, VAN	Low level	Constitutive	++	-	-/4
IR10-VISA	Weak producer	PEN, AMK, TET, ERY, VAN	Low level	Inducible	++	+	-/4
IR11-VISA	Moderate producer	AMK, ERY, VAN	Low level	Inducible	++	-	-/4
IR12-VISA	Weak producer	PEN, AMK, TET, ERY, VAN	Low level	Inducible	++	+	-/4
IR13-VISA	Strong producer	PEN, GEN, AMK, TET, ERY, CLI, RIF, MUP	High level	Constitutive	+	+	-/8

DISCUSSION

In the present research, we reported several main findings. A high VISA rate among *S. aureus* isolated from burn patients was primarily found. Second, a high prevalence of MDR rate emphasized improper prescription of antibiotics in infected with related to burn patients. Third, biofilm formability was observed in two-thirds of the isolates. Fourthly, the phage cocktail exhibited an effective inhibitory effect on *S. aureus* strains isolated from patients who burned; meanwhile, all VISA and VRSA isolates were susceptible to the phage cocktail.
Data relating to antimicrobial activity revealed that 76% of *S. aureus* isolates were resistant to methicillin and MRSA confirmed strains. Different frequency of MRSA strains in burn patients (between 69% to 80%) was reported in researches

performed from Iran (**Abbasi-Montazeri *et al*., 2013; Emaneini *et al*., 2018; Sahebnasagh, Saderi, & Owlia, 2014**). Recently published data by Dadashi *et al* reported a 43% prevalence of MRSA clinical isolates, which was lower than the study (**Dadashi *et al*., 2018**). However, various frequencies of MRSA, including 45%, 93.7%, 42.9%, 53.4%, and 11.8% were reported in data published from Palestine (**Biber *et al*., 2012**), Iran (**Eftekhar *et al*., 2017**), China (**Yang *et al*., 2017**), Africa (**Wangai, Masika, Maritim, & Seaton, 2019**), and Serbia (**Cirkovic *et al*., 2015**) respectively.

Based on published data, a remarkable prevalence increases in mupirocin resistance declared by several investigators. In our survey, one- thirds of strains were mupirocin-resistant (30%), which was close to the reported rate in the USA (31.3%) and India (25.5%) (**Antonov *et al*., 2015**). However, lower rates reported from France (2.1%) and China (17.6%) (**Desroches *et al*., 2013; Liu et al., 2017**). Shittu et al. presented a relatively low prevalence (14%) of mup-R MRSA strains (**Shittu *et al*., 2018**).

In the current study, 8% of examined isolates were confirmed as HLMUPR *S. aureus*. Our results were in parallel by Dadashi et al. study, who indicated an 8.1% prevalence of HLMUPR MRSA with a high prevalence in Asia comparing Europe and the USA (12.1% vs. 8% and 5.9%) (**Dadashi, Hajikhani, Darban-Sarokhalil, van Belkum, & Goudarzi, 2020**). Different data were reported from Iran (25.5%), Africa (0.5-38%), India (26.1%), USA (26.9%), and Africa (23.5%) (**Dadashi *et al*., 2020; Shittu *et al*., 2018**).

According to our research, irrational use, different prescription policies, drug availability, low cost, and the spread of dominant types are all potential causes of the high resistance rate in Asian countries. Earlier findings have demonstrated an increase in the prevalence of VISA and VRSA strains. Data showed that 13% of *S. aureus* isolates were resistant to vancomycin at an intermediate level. Previous research conducted by Shariati and colleagues revealed an ascending trend of VRSA and VISA in different regions. They found that 1.5% and 1.7% of isolates were VRSA and VISA, respectively, and also demonstrated a remarkable increase in the prevalence of VRSA and VISA after 2010 compared to before 2010. Shariati et al. found that the prevalence rate of VISA in Asia, especially in Iran and India (67%), was higher than in other countries (**Shariati *et al*., 2020**). These discrepancies could be excessive use, irrational policies in the consumption, drug availability, low public hygiene standards, and dissemination of specific clones in these regions.

Our analysis revealed the prevalence of iMLSB phenotype in 31% of isolates. The inducible resistance reported in the present work is lower than study conducted in Jordan (76.7%) (**Jarajreh, Aqel, Alzoubi, & Al-Zereini, 2017**) and higher report from Brazil (7.9%) (**Bottega *et al*., 2014**) and Nepal (21%) (**Adhikari, Shrestha, Barakoti, & Amatya, 2017**). Our study demonstrated that 62% of isolates had constitutive phenotype. The results obtained were contrary to 29.3% reported from Nepal (**Adhikari *et al*., 2017**) and 82.9% from Iran (**Khashei, Malekzadegan, Ebrahim-Saraie, & Razavi, 2018**), and 20.1% from Greece (**Doudoulakakis *et al*., 2017**). This can be inferred from the present results that the improper use of erythromycin and clindamycin in treating staphylococcal infections in our settings raises the clinicians' attention to reducing the prescription of drugs, as mentioned above for patients infected with *S. aureus*.

The emergence of resistance to the antibiotics mentioned above is associated with several factors such as study population and design, unrestricted prescription and consumption of this antibacterial agent, sociodemographic differences, and dissemination of particular MRSA clones, which important choices that available for control and prevention of infections in burn patients.

According to the literature, there is a diversity in producing of biofilm genes in the MRSA strains and specific genotypes (**Yousefi *et al*., 2016**). According to previous studies, *S. aureus* biofilm formation rates ranged from 43 to 88 percent (**Luther *et al*., 2018; Yousefi *et al*., 2016**). The prevalence of biofilm producers was high in the current study (66%). The magnitude of biofilm producers in the present study is consistent with the study findings reported from China (66%) (**Wang *et al*., 2010**) and Iran (62.9%) (**Yousefi *et al*., 2016**). As referenced in study of Hosseini et al., biofilm-formation prevalence exceeds to 80% in MRSA strains. As well, they exhibited that 22.5%, 45.3% and 52.9% of MRSA could produce weak, moderate and strong biofilms (**Hosseini *et al*., 2020**). Past research has been indicated that some *S. aureus* isolates need polysaccharide intercellular adhesin (PIA) for producing of biofilm and its regulation is controlled by the *icaADBC* operon. In this study, 94% and 95% of the isolates were found to have *icaA* and *icaD*, which is in agreement with the findings of Azmi et al. (**Azmi, Qrei, & Abdeen, 2019**) and supports those of Sharma and coworkers (**Sharma et al., 2017**) who reported that *icaA/D* was found abundantly among biofilm-producing isolates. However, these findings can suggest an indirect role for *ica* in biofilm formation.

Our study investigated the in-vitro effectiveness of phage cocktail against *S. aureus* isolated from burn patients. Our findings indicated that the phage cocktail had an inhibitory effect on 81% of isolates, suggesting the role of its potent inhibitory against MDR *S. aureus*.

This agreed with Chhibber et al. from India that showed Phage Cocktail could be an effective treatment against MRSA strains related to SSTIs (**Chhibber *et al*., 2017**). In another study, Taha et al. investigated the effect of Phage Cocktail against biofilm-producing MRSA clinical strains and indicated that planktonic cell growth was reduced significantly in the presence of Phage Cocktail (**Taha *et al*., 2021**). In a 2020 study, Kifelew et al. assessed the efficacy of *Myoviridae* phages cocktail on three *S. aureus* in treatment wound of diabetic mouse caused by MDR *S. aureus* infections compared with vancomycin. They indicated that *S. aureus* was eradicated from the wounds of phage-treated compared to saline-treated mice, while the bacterum was detected in all the vancomycin-treated mice (**Kifelew *et al*., 2020**). This study successfully justified *in-vitro* effectiveness of phage cocktail against *S. aureus* isolated from burn patients; it has certain limitations. Pulsed-field gel electrophoresis and next-generation sequencing were not performed. The number of tested strains for characterization of VRSA and VISA strains was small, which may only display regional data and not reflect the perfect epidemiological picture of the variability of these isolates.

CONCLUSION

We showed evidence to effectiveness of Phage Cocktail against *S. aureus* strains isolated from burn patients. Prevalence of VISA strains in our country indicates that studying phages as topical antimicrobials against these isolates more in-depth can help us minimize the spreed of the isolates.

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