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RESEARCH ARTICLE

Staphylococcal biofilm on wedding rings worn by laboratory workers

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ABSTRACT

Hands of healthcare workers play essential role in the spreading of antimicrobial-resistant microorganisms in and out of the healthcare settings. Less is known about the role of laboratory workers (LWs). The aim of our study was to evaluate the presence of biofilm-forming staphylococci on the surface of jewelry rings of LWs and their antimicrobial susceptibility pattern.

A total of 79 LWs from eight different microbiology laboratories that process and analyze specimens from the tertiary care hospitals in Belgrade, Serbia participated in the study. The study was reviewed and approved by the institutional review boards at hospitals. Samples were taken after hand washing. Bacteria on LWs wedding rings were detected with the rolling method, and further analyzed in order to determine the number of colony forming unit (CFU) per ring, species of bacteria and their antimicrobial susceptibility pattern, methicillin resistance and biofilm-producing capacity *in vitro*.

Staphylococci were recovered from 60.8% of wedding rings. All strains produced biofilm (25% weak, 56.2% moderate and 18.8% large amount), with significant difference between species ($P < 0.001$). *Staphylococcus aureus* and *Staphylococcus epidermidis* formed the largest amount of biofilm and had the largest number of CFU per ring. Staphylococci were most commonly resistant to penicillin (66.7%), tetracycline (50.0%), and erythromycin (45.8%); 41.7% of isolates was multidrug resistant and *mec*A gene was detected in five strains. All strains were susceptible to linezolid, vancomycin, teicoplanin and tigecycline.

Staphylococci colonize LWs wedding rings, form biofilm on it, have multidrug resistant phenotype and/or carry *mec*A gene, representing a significant reservoir for the spreading of microorganisms and resistance. As far as we know, our study is the first that address this topic in laboratory workers.

KEYWORDS

staphylococcal biofilm, hand hygiene, jewelry rings, laboratory workers, methicillin resistance, mecA

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1. INTRODUCTION

Hand hygiene is considered to be the most important step in the prevention of infections and wearing rings and other jewelry is strongly discouraged during health care [1]. Several studies reported an increase in the bacterial colonization of hands in healthcare workers (HCWs) who wear rings [2–5]. Besides hospital wards, microbiology laboratories can also be a source of various pathogenic microorganisms. Some of the most important microorganisms that can be found in the microbiology laboratory are multidrug-resistant (MDR), such as methicillin-resistant *Staphylococcus aureus* (MRSA) [6], vancomycin-resistant *Enterococcus* spp., and

multidrug-resistant *Enterobacteriaceae* [7]. This implies the importance of hand hygiene for laboratory workers (LWs), as a prevention method for the spreading of MDR bacteria. To the best of our knowledge, there is only one study published on the topic of hand hygiene amongst LWs that found pathogenic microorganisms exclusively on the hands of LWs who wore jewelry [8]. Likewise, studies on hand colonization in HCWs who wear jewelry have not been focused on the significance of biofilm formation on the rings so far.

Biofilm is a surface assemblage of microbial cells enclosed in an extracellular polymeric substance matrix. Through different phases of biofilm formation, from the attachment of planktonic bacteria, proliferation, maturation, and dissociation of biofilm microcolonies, bacteria express different sets of genes and undergo disparate metabolic states relative to their planktonic counterparts. Specific microarchitecture of biofilm and dormant state of bacteria are the major reasons for its resistance to the effector mechanisms of immunity, as well as to the action of antimicrobial agents. Aside from being protected in biofilm from the direct effects of antibiotics, bacteria inside biofilms are under extensive interchange of genetic material responsible for various mechanisms of bacterial resistance [9].

Jewelry rings are suitable for biofilm formation due to their close contact with commensal colonizers of the skin, like coagulase-negative staphylococcal (CoNS) species. Most of CoNS isolates (75.4%) in US medical centers are methicillin-resistant (MRCoNS) [10]. The presence of MRCoNS biofilm on rings could potentially enhance the interhuman transmission of these strains, not only in HCWs that have direct contact with patients, but also in LWs that process and analyze the microbiology specimens.

The staphylococcal colonization of the palm skin is common, and it is expected that skin colonizers also populate the internal surface of the LWs wedding rings. However, the common skin microbiota can easily be reduced in quantity after hand hygiene or application of disinfectants. As biofilms are very resistant to the external factors and cannot be removed with plane hand washing or disinfection, we wanted to design the experiment that will test the hypothesis that external surface of the jewelry can become a reservoir for antimicrobial-resistant microorganisms through biofilm formation, and that wedding rings and other jewelry are important objects for transmission of such microorganisms.

Accordingly, our study aimed to evaluate the presence of biofilm-forming staphylococci on the surface of jewelry rings of laboratory workers, their antimicrobial susceptibility pattern and biofilm-forming capacity.

2. MATERIAL AND METHODS

Eight different microbiology laboratories in Belgrade with a total of 79 laboratory workers that process and analyze specimens obtained from tertiary care hospitals were included in the study. The study was reviewed and approved by the institutional review boards at hospitals. Participation in the study was voluntary, participants were given oral and written information before consenting to participate and all data were treated anonymously.

2.1. Isolation and identification of staphylococci and antimicrobial susceptibility testing

Specimens from the wedding rings of LWs were obtained for culture during the workers' coffee breaks. Before sampling, all participants washed their hands with soap and water in order to remove common skin colonizers and dried them with a single-use paper hand-rub. According to the European Society for Clinical Microbiology and Infectious Diseases and the Infectious Diseases Society of America current recommendations, the Maki semi-quantitative method was used for detection of biofilms on surface of the wedding rings [11, 12]. Wedding rings were taken off with sterile gloves, and the external surface of the rings was rolled back and forth over the surface of a blood agar plates (bioMérieux, France). Plates were incubated for 24 h at 37 °C and a total bacterial load (i.e., number of colony forming units, CFU) per ring was counted on the Colony counter (Boeco, Germany).

Identification of the strains and antimicrobial susceptibility were determined by the Vitek2 automated system (bioMérieux, France) in accordance with the European Committee on Antimicrobial Susceptibility Testing recommendation [13]. After identification, only staphylococcal isolates were included in further investigation. Molecular identification of the methicillin resistance was confirmed by PCR for the *mec*A gene. DNA was isolated from staphylococcal strains using the thermolysis method and PCR was performed in accordance with Jonas et al. [14].

2.2. Quantification of biofilm

Biofilm-producing capacity was investigated in 96-well microtiter plates with a quantitative method previously described by Stepanovic et al. [15], A 20 µl of 0.5 McFarland bacterial suspension was added to 180 µl of Trypticase soy broth (bioMérieux, France) with the addition of 1% glucose in triplicates. Negative controls (medium without bacteria) were set in two triplicates. After overnight incubation at 37 °C, wells were thoroughly rinsed three times with a 300 µl of sterile phosphate buffer solution per well, and air-dried. Biofilm was fixed with absolute methanol for 20 min and colored with 2% crystal violet (Alfapanon, Serbia) for 15 min. Crystal violet that was bonded to the bacterial cells was diluted with a 150 µl of 96% ethanol per well and optical density was measured at 570 nm using a microtiter plate reader (ICN Flow Titertek Multiscan Plus). Each assay was repeated three times in three consecutive days. and the results were calculated according to Stepanović et al. as mean value of all measurements for each isolate (total of nine values per isolate). To calculate the category of biofilm production, the cut-off optical density (ODc) was determined as three standard deviations above the mean OD of

the negative control. According to the obtained results all tested strains were clustered into four groups:

OD ≤ ODc - category 0 (no biofilm producer)
ODc < OD ≤ 2 × ODc - category 1 (weak biofilm producer)
2 × ODc < OD ≤ 4 × ODc - category 2 (moderate biofilm producer)
4 × ODc < OD - category 3 (strong biofilm producer)

The data obtained in this study were analyzed in SPSS statistical program (version 24, SPSS, Inc., Chicago, USA) using the Pearson Chi-Square test, Pearson's and Spearman's correlation coefficient, and *P*-value less than 0.05 was considered statistically significant.

3. RESULTS

Among 79 tested wedding rings of laboratory workers, staphylococci were recovered from 48 (60.8%) rings. The most frequently detected microorganism was *S. epidermidis* (27.1%), followed by *S. haemolyticus* (18.7%), *S. hominis* (18.7%), *S. warneri* (16.7%), *S. aureus* (12.5%), and *S. lentus* (6.3%). There was no more than one species of staphylococci per ring. The number of isolated CFU per ring was the largest in *S. aureus* (22.3 ± 4.4) and *S. epidermidis* (17.5 ± 4.6), followed by *S. hominis*, *S. haemolyticus*, *S. warneri,* and *S. lentus* (Table 1, Fig. 1).

Table 1. Number of CFU per ring and antimicrobial resistance of different *Staphylococcus* spp. isolated from rings of laboratory workers

Species % (number of isolates)							
		S. aureus 12.5 (6/48)	*S. epidermidis* 27.1 (13/48)	*S. haemolyticus* 18.7 (9/48)	*S. hominis* 18.7 (9/48)	*S. lentus* 6.3 (3/48)	*S. warneri* 16.7 (8/48)
N° of CFU per ring		22.3 ± 4.4	17.5 ± 4.6	11.7 ± 3.9	12.6 ± 4.6	6.7 ± 1.5	11.8 ± 3.9
Susceptibility pattern*							
Penicillin (%)	S	0.0	15.4	66.7	22.2	33.3	62.5
	R	100.0	84.6	33.3	77.8	66.7	37.5
Cefoxitin (%)	S	83.3	100.0	100.0	55.6	100.0	100.0
	R	16.7	0.0	0.0	44.4	0.0	0.0
Gentamicin (%)	S	83.3	61.5	100.0	77.8	100.0	100.0
	R	16.7	38.5	0.0	22.2	0.0	0.0
Ciprofloxacin (%)	S	83.3	100.0	100.0	100.0	100.0	100.0
	I	16.7	0.0	0.0	0.0	0.0	0.0
Erythromycin (%)	S	83.3	61.5	66.7	66.7	33.3	0.0
	R	16.7	38.5	33.3	33.3	66.7	100.0
Clindamycin (%)	S	83.3	76.9	100.0	66.7	100.0	62.5
	R	16.7	23.1	0.0	33.3	0.0	37.5
Tetracycline (%)	S	83.3	15.4	66.7	66.7	0.0	62.5
	R	16.7	84.6	33.3	33.3	100.0	37.5
Fusidic acid (%)	S	100.0	84.6	66.7	66.7	33.3	100.0
	I	0.0	0.0	33.3	33.3	66.7	0.0
	R	0.0	15.4	0.0	0.0	0.0	0.0

*Susceptibility to linezolid, vancomycin, teicoplanin, tigecycline, and trimethoprim-sulfamethoxazole was 100% in all tested microorganisms; CFU – colony-forming unit; S - Susceptible, standard dosing regimen, I - Susceptible, increased exposure, *R* – Resistant.

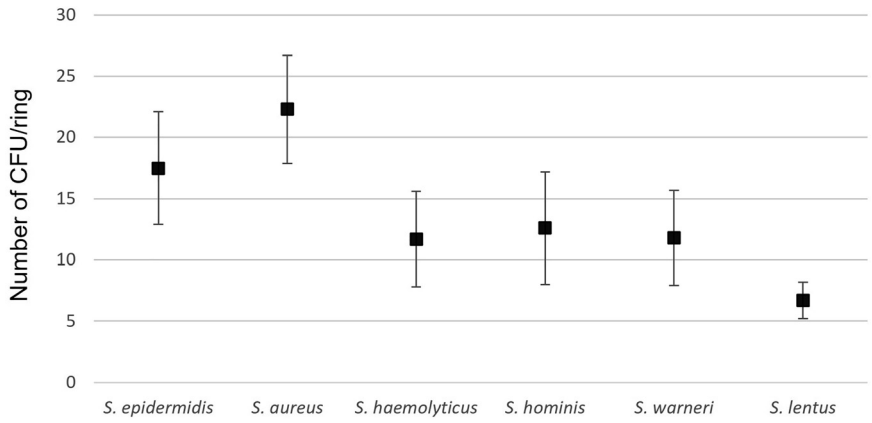

Fig. 1. Number of colony forming units (CFU) per ring in different species of staphylococci isolated from the rings of laboratory workers. Results are present as mean ± SD

All isolated strains produced biofilm, with different biofilm-forming capacities: 25% were weak biofilm producers, 56.2% were moderate biofilm producers and 18.8% were strong biofilm producers (Fig. 2). *S. aureus* and *S. epidermidis* were species that produced the largest amount of biofilm and had the largest number of CFU per ring. A significant difference in biofilm production was observed between different staphylococcal species ($P < 0.001$; Fig. 2).

Antimicrobial resistance patterns of analyzed staphylococcal strains are presented in Tables 1 and 2. All isolated strains were susceptible to linezolid, vancomycin, teicoplanin, tigecycline, and trimethoprim-sulfamethoxazole. Minimum Inhibitory Concentration (MIC) for vancomycin ranged from 0.5 to 2.0 mg L^{-1} and for teicoplanin from 0.5 to 4.0 mg L^{-1}. Even though all isolates were susceptible to vancomycin and teicoplanin, significantly higher MIC values were observed in strains of *S. lentus* (vancomycin 2.00 ± 0.00, teicoplanin 2.83 ± 2.02) and *S. epidermidis* (vancomycin 0.96 ± 0.52, teicoplanin 3.54 ± 0.88), compared to other strains ($P < 0.01$ and $P < 0.001$, respectively). *Staphylococcus* spp. isolates were most commonly resistant to penicillin (66.7%), tetracycline (50.0%), and erythromycin (45.8%). Multidrug resistance was found in 20 isolates (41.7%). The occurrence of MDR was different between species, it was more common in *S. epidermidis, S. haemolyticus,* and *S. lentus* ($P < 0.01$).

In five strains of staphylococci that were methicillin-resistant, the *mec*A gene was detected in four *S. hominis* and one *S. aureus* strains, although there was no significant difference in methicillin resistance between *S. aureus* and CoNS ($P > 0.05$). Staphylococci resistant to penicillin formed a significantly higher amount of biofilm, compared to the penicillin-susceptible isolates ($P < 0.01$). Staphylococci with moderate biofilm production were more commonly resistant to erythromycin ($P < 0.05$). There was no difference in biofilm production between multidrug-resistant (MDR) and non-MDR isolates ($P > 0.05$).

4. DISCUSSION

Due to the nature of their job, LWs are in contact with various pathogenic and opportunistic microorganisms daily. Wearing different items of jewelry might interfere in the process of hand hygiene and disinfection. However, guidelines for hand hygiene do not provide exact instructions regarding the removal of rings in healthcare workers, both those in direct contact with patients and LWs [16].

Many studies addressed the issue of the influence of wearing jewelry during hand hygiene [2, 3, 17, 18], but there are no consistent results on this topic. Salisbury et al. presented a correlation between wearing jewelry and increased bacterial load, before and after hand disinfection [17]. However, some authors state that after an adequate surgical hand preparation, there is no significant discrepancy in bacterial count in surgeons with or without jewelry [18]. On the other hand, a study of Fagernes et al. presented the higher count of *Enterobacteriaceae* in HCWs who wore rings, with no difference in the count of *S. aureus* and other staphylococci [2]. Also, no differences were found in bacterial colony counts when dominant and non-dominant hands were compared, irrespective of whether rings were worn [19]. A comprehensive study that tested the impact of

Table 2. Susceptibility of different *Staphylococcus* spp. isolated from rings of laboratory workers to glycopeptide antibiotics

Staphylococcus species	Vancomycin (mg L^{-1})		Teicoplanin (mg L^{-1})	
	MIC range	Average ± SD	MIC range	Average ± SD
S. aureus	0.5–1	0.58 ± 0.20	0.5	0.50 ± 0.00
S. epidermidis	0.5–2	0.96 ± 0.52	2–4	3.54 ± 0.88
S. haemolyticus	0.5	0.50 ± 0.00	0.5–2	1.50 ± 0.75
S. hominis	0.5–1	0.72 ± 0.26	0.5	0.50 ± 0.00
S. lentus	2	2.00 ± 0.00	0.5–4	2.83 ± 2.02
S. warneri	0.5–1	0.69 ± 0.26	0.5	0.50 ± 0.00

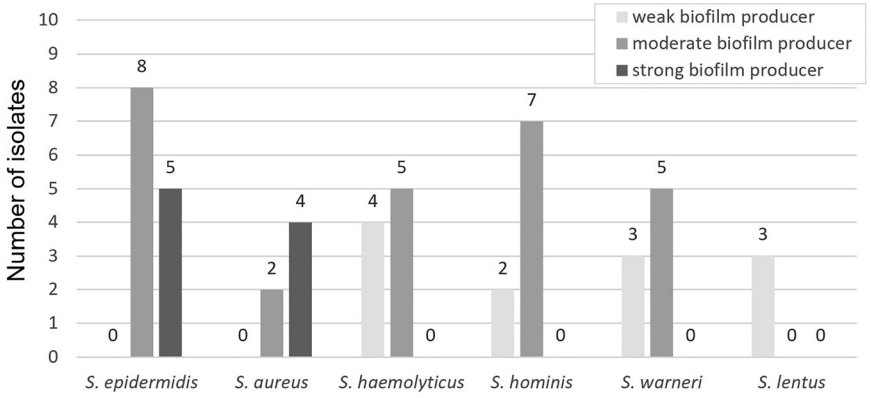

Fig. 2. Category of biofilm production in different species of staphylococci. Results are presented as the number of strains that produce a specific category of biofilm: weak biofilm producer (light gray); moderate biofilm producer (medium gray) and strong biofilm producer (dark gray)

ring wearing on hand hygiene in intensive care unit nurses, showed that wearing rings results in a 10-fold increase in the count of *S. aureus*, Gram-negative bacilli, and *Candida* spp. on the hands of nurses. MRCoNS were most prevalent on the uncleaned hands of nurses, but ring wearing wasn't a risk factor for contamination with these bacteria [3].

Hand hygiene is a concern in clinical practice since the hands of medical staff can be the transmission path for many nosocomial pathogens. Only one study addressed this concern to LWs. Alp et al. found a higher count of both commensal and pathogenic bacteria on LWs' hands who wore jewelry [8]. In our study, we informed LWs about the results and encourage them to avoid wearing rings at work. Nevertheless, strong cultural and religious beliefs in our society dictate that married people should wear wedding rings all the time. More studies showed that many HCWs wear wedding rings during work, and this behavior is based on assumption that plain wedding rings are less likely to harbor bacteria on their surface, in contrast to other jewelry [20]. More than 60% of tested wedding rings in our study were positive for staphylococcal colonization. This finding should enhance LWs to follow strict rules for hand washing and disinfection while wearing rings during laboratory work.

As *Staphylococcus* spp. is one of the most abundant species of the human skin microbiota, the findings in our study weren't surprising. Since *S. epidermidis* and *S. hominis* are the most common staphylococcal species in humans [21], we can conclude that most frequently isolated species on laboratory workers' rings are the same as those most found in microbiota of human skin. Although there are more than 50 different species in the genus *Staphylococcus*, we found a relatively small number of different staphylococcal species on LWs rings in our study.

The presence of staphylococci on the rings can be explained by the production of extracellular slime in various staphylococcal species [22]. Slime-producing strains could remain attached to the ring surface regardless of handwashing, which we observed in our study since our participants washed their hands before sampling. All staphylococcal species that were isolated in our study showed the ability to form a biofilm with different capacities. Regardless of frequent hand washing during the work, biofilms have pronounced resistance to surface-active compounds, like soaps and detergents, compared to their planktonic forms [23]. This indicates that wedding rings of LWs could be the reservoir of biofilm-producing bacteria.

Methicillin resistance (MR) in staphylococci is emerging both in clinical and extra hospital environments [24], and in our study, MR was present in 10% of isolated staphylococci, including one MRSA strain. In these strains, resistance to other groups of antibiotics is often present simultaneously. We found MDR staphylococci on more than 40% of tested wedding rings. Also, MRCoNS possess a class of mobile genetic elements, Staphylococcal Cassette Chromosome *mec* (SCC*mec*) [25] that could transfer resistance gene to more virulent *S. aureus*, posing a serious threat to human health. These findings support that wedding rings could be reservoirs and a source of antibacterial resistance.

In the previous study performed among patients in a Belgrade tertiary care hospital, susceptibility patterns of coagulase-negative staphylococci were investigated, and the obtained results showed that 70% of the strains were resistant to penicillin, 50% to erythromycin, 40% to clindamycin and 55% to tetracycline [26]. Although similar susceptibility profile was detected in both research, advantages of our investigation is identification of the coagulase-negative staphylococci to the species level and, additionally, determination of the strains sensitivity only to antistaphylococcal antibiotics. Similar results were obtained in another study that evaluate susceptibility of community-associated staphylococcal strains to macrolides and lincosamide [27].

To prevent the spreading of these microorganisms, thorough hand hygiene must be highlighted. In the last two years, there is an increasing awareness of the importance of hand hygiene and disinfection because of the COVID-19 pandemic [28]. The education and implementation of adequate hand hygiene are some of the most important tasks in the prevention of infections.

5. CONCLUSIONS

In conclusion, several studies examined rings as objects successfully colonized by bacteria, and as such, could represent a significant reservoir for the spreading of microorganisms. As far as we know, this is the first study tackling this topic in LWs, in which we directly sampled the wedding rings. Based on this investigation, we can conclude that staphylococci colonize laboratory workers' jewelry, successfully form biofilm on it, express multidrug resistance phenotype and harbor resistance genes. The most commonly isolated species was *S. epidermidis*, and a particular attention should be paid to isolation of MRSA as the most virulent staphylococcal strain.

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REFERENCES

1. WHO guidelines on hand hygiene in health care: first global patient safety challenge clean care is safer care. Geneva: World Health Organization; 2009. Available from: https://pubmed.ncbi.nlm.nih.gov/23805438/.
2. Fagernes M, Lingaas E. Factors interfering with the microflora on hands: a regression analysis of samples from 465 healthcare workers. J Adv Nurs 2011; 67: 297–307. Available from: https://pubmed.ncbi.nlm.nih.gov/20946569/.

3. Trick WE, Vernon MO, Hayes RA, Nathan C, Rice TW, Peterson BJ, et al. Impact of ring wearing on hand contamination and comparison of hand hygiene agents in a hospital. Clin Infect Dis 2003; 36: 1383–90. Available from: https://pubmed.ncbi.nlm.nih.gov/12766832/.
4. Khodavaisy S, Nabili M, Davari B, Vahedi M. Evaluation of bacterial and fungal contamination in the health care workers' hands and rings in the intensive care unit. J Prev Med Hyg 2011; 52: 215–8. Available from: https://pubmed.ncbi.nlm.nih.gov/22442928/.
5. Saxena S, Singh T, Agarwal H. Bacterial colonization of rings and cell phones carried by health-care providers: are these mobile bacterial zoos in the hospital? Trop Doct 2011; 41: 116–8. Available from: https://pubmed.ncbi.nlm.nih.gov/21273217/.
6. Ng LSY, The WT, Ng SK, Eng LC, Tan TY. Bacterial contamination of hands and the environment in a microbiology laboratory. J Hosp Infect 2011; 78: 231–3. Available from: https://pubmed.ncbi.nlm.nih.gov/21481970/.
7. Collins SM, Hacek DM, Degen LA, Wright MO, Noskin GA, Peterson LR. Contamination of the clinical microbiology laboratory with vancomycin-resistant enterococci and multidrug-resistant *Enterobacteriaceae*: implications for hospital and laboratory workers. J Clin Microbiol 2001; 39: 3772–4. Available from: https://pubmed.ncbi.nlm.nih.gov/11574615/.
8. Alp E, Haverkate D, Voss A. Hand hygiene among laboratory workers. Infect Control Hosp Epidemiol 2006; 27: 978–80. Available from: https://pubmed.ncbi.nlm.nih.gov/16941327/.
9. Hall CW, Mah TF. Molecular mechanisms of biofilm-based antibiotic resistance and tolerance in pathogenic bacteria. FEMS Microbiol Rev 2017; 41: 276–301. Available from: https://pubmed.ncbi.nlm.nih.gov/28369412/.
10. Sader HS, Jones RN. Antimicrobial susceptibility of Gram-positive bacteria isolated from US medical centers: results of the Daptomycin Surveillance Program (2007–2008). Diagn Microbiol Infect Dis 2009; 65: 158–62. Available from: https://pubmed.ncbi.nlm.nih.gov/19748426/.
11. Hoiby N, Bjarnsholt T, Moser C, Bassi GL, Coenye T, Donelli G, et al. ESCMID guideline for the diagnosis and treatment of biofilm infections 2014. Clin Microbiol Infect 2015; 21: S1–25. Available from: https://pubmed.ncbi.nlm.nih.gov/25596784/.
12. Mermel LA, Allon M, Bouza E, Craven DE, Flynn P, O'Grady NP, et al. Clinical practice guidelines for the diagnosis and management of intravascular catheter-related infection: 2009 update by the infectious diseases society of America, IDSA guidelines. Clin Infect Dis 2009; 49: 1–45. Available from: https://pubmed.ncbi.nlm.nih.gov/19489710/.
13. The European Committee on Antimicrobial Susceptibility Testing. Breakpoint tables for interpretation of MICs and zone diameters, 2021. Version 11.0. http://www.eucast.org.
14. Jonas D, Speck M, Daschner FD, Grundmann H. Rapid PCR-based identification of methicillin-resistant *Staphylococcus aureus* from screening swabs. J Clin Microbiol 2002; 40: 1821–3. Available from: https://pubmed.ncbi.nlm.nih.gov/11980967/.
15. Stepanović S, Vuković D, Hola V, Di Bonaventura G, Djukić S, Ćirković I, et al. Quantification of biofilm in microtiter plates: overview of testing conditions and practical recommendations for assessment of biofilm production by staphylococci. APMIS 2007; 115: 891–9. Available from: https://pubmed.ncbi.nlm.nih.gov/17696944/.
16. Boyce JM, Pittet D. Guideline for hand hygiene in health-care settings: recommendations of the Healthcare infection control practices advisory committee and the HICPAC/SHEA/APIC/IDSA hand hygiene task force. Infect Control Hosp Epidemiol 2002; 23: S3–40. Available from: https://pubmed.ncbi.nlm.nih.gov/12515399/.
17. Salisbury DM, Hutfilz P, Treen LM, Bollin GE, Gautam S. The effect of rings on microbial load of health care workers' hands. Am J Infect Control 1997; 25: 24–7. Available from: https://pubmed.ncbi.nlm.nih.gov/9057940/.
18. Al-Allak A, Sarasin S, Key S, Morris-Stiff G. Wedding rings are not a significant source of bacterial contamination following surgical scrubbing. Ann R Coll Surg Engl 2008; 90: 133–5. Available from: https://pubmed.ncbi.nlm.nih.gov/18325213/.
19. Fagernes M, Lingaas E. Impact of finger rings on transmission of bacteria during hand contact. Infect Control Hosp Epidemiol 2009; 30: 427–32. Available from: https://pubmed.ncbi.nlm.nih.gov/19344265/.
20. Fagernes M, Lingaas E, Bjark P. Impact of a single plain finger ring on the bacterial load on the hands of healthcare workers. Infect Control Hosp Epidemiol 2007; 28: 1191–5. Available from: https://pubmed.ncbi.nlm.nih.gov/17828698/.
21. Chiller K, Selkin BA, Murakawa GJ. Skin microflora and bacterial infections of the skin. J Investig Dermatol Symp Proc 2001; 6: 170–4. Available from: https://pubmed.ncbi.nlm.nih.gov/11924823/.
22. Boynukara B, Gulhan T, Gurturk K, Alisarli M, Ogun E. Evolution of slime production by coagulase-negative staphylococci and enterotoxigenic characteristics of *Staphylococcus aureus* strains isolated from various human clinical specimens. J Med Microbiol 2007; 56: 1296–300. Available from: https://pubmed.ncbi.nlm.nih.gov/17893164/.
23. Gilbert P, McBain AJ. Biofilms: their impact on health and their recalcitrance toward biocides. Am J Infect Control 2001; 29: 252–5. Available from: https://pubmed.ncbi.nlm.nih.gov/11486267/.
24. Tenover FC, McDougal LK, Goering RV, Killgore G, Projan SJ, Patel JB, et al. Characterization of a strain of community-associated methicillin-resistant *Staphylococcus aureus* widely disseminated in the United States. J Clin Microbiol 2006; 44: 108–18. Available from: https://pubmed.ncbi.nlm.nih.gov/16390957/.
25. Katayama Y, Ito T, Hiramatsu K. A new class of genetic element, Staphylococcus cassette chromosome mec, encodes methicillin resistance in *Staphylococcus aureus*. Antimicrob Agents Chemother 2000; 44: 1549–55. Available from: https://pubmed.ncbi.nlm.nih.gov/10817707/.
26. Protic D, Savic D, Andjelkovic D, Djukanovic N, Zdravkovic M, Djurasevic SF, et al. Nosocomial coagulase-negative staphylococci in Belgrade: between scylla and charybdis. J Infect Dev Ctries 2016; 10: 907–12. Available from: https://pubmed.ncbi.nlm.nih.gov/27694722/.
27. Aleksandra AD, Misic MS, Mira ZV, Violeta NM, Dragana IT, Zoran BM, et al. Prevalence of inducible clindamycin resistance among community-associated staphylococcal isolates in central Serbia. Indian J Med Microbiol 2014; 32(1): 49–52. Available from: https://pubmed.ncbi.nlm.nih.gov/24399388/.
28. Nimer R, Swedan S, Kofahi H, Khabour O. Increased adherence to infection control practices among medical laboratory technicians during the COVID-19 pandemic: a self-reported survey study. Ann Glob Health 2021; 87: 56. Available from: https://pubmed.ncbi.nlm.nih.gov/34221909/.