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MRSA screening and *spa* gene detection in isolates from healthcare workers at ophthalmology hospital in Egypt

Maha G. Haggag[1,2*], Amal E. Aboelnour[3] and Mai Al-Kaffas[1]

Abstract

Background: *Staphylococcus aureus* has a major role in different types of eye infections as conjunctivitis, keratitis, and endophthalmitis. Methicillin-resistant *Staphylococcus aureus* (MRSA) was almost restricted to hospitals, but its prevalence has been increased in people outside hospitals. The cell wall of *Staphylococcus aureus* has protein A which can bind to the Fc portion of IgG. This ptnA is encoded by surface protein A of *Staphylococcus aureus* (*spa*) gene that contains a highly polymorphic sequence which is composed of repeats of 24-bp. Sequence typing of the *spa* gene repeat region is used to study the epidemiology of MRSA. The purpose of this study was screening of MRSA strains among healthcare workers (HCWs) in the Hospital of the Research Institute of Ophthalmology (RIO), Giza, Egypt, and detecting *spa* gene in their DNAs by PCR.

Results: In the present study, 81 samples from healthcare providers in the hospital of the Research Institute of Ophthalmology, Egypt, were screened for MRSA. Out of these 81 samples, 41 isolates (50.6%) were identified as coagulase-positive *Staphylococcus aureus*. Twelve staphylococcal isolates were resistant to both oxacillin and cefoxitin, and those were identified as MRSA with a percentage of 14.8% (12/81). Conventional PCR could detect *spa* gene in 10 out of 12 DNA MRSA with a percentage of 83.3% (10/12).

Conclusion: In the present study, the prevalence of MRSA in HCWs was 14.8%. Since amplification of *spa* gene by PCR is a necessary preliminary step for *spa* typing of MRSA and since using different primers for *spa* gene amplification might affect PCR results, then proper selection of the primers and thermal cycling reaction conditions are recommended for PCR performance and *spa* typing.

Keywords: *S. aureus*, MRSA, Epidemiology, HCWs, *spa* typing, *spa* primers PCR

Introduction

Staphylococcus aureus is a crucial bacteria commonly isolated from human infections that range from minor skin and soft tissue infections to life-threatening systemic infections (Liu et al. 2016). *Staphylococcus aureus* has a major role in different types of eye infections such as conjunctivitis, keratitis, and endophthalmitis. Methicillin-resistant *Staphylococcus aureus* (MRSA) was almost restricted to hospitals and hence termed healthcare-associated (HA) strains, but its prevalence has increased in people outside hospitals which is termed community-associated (CA) strains. Infections with both types are clinically, microbiologically, and genetically different (Ritterband 2013).

MRSA strains are not essentially more virulent than methicillin-sensitive *S. aureus* strains, but some MRSA strains hold factors or genetic elements that may intensify their virulence or may allow them to induce peculiar clinical syndromes (Gordon and Lowy 2008). The cell wall of *Staphylococcus aureus* has protein A which can bind to the Fc portion of IgG at the complement-binding site thereby preventing complement activation (El-Mishad 2011). Virulence mediated by spa is not only by binding to the Fc of the Ig preventing normal phagocytosis, but also by binding to the Fab site of the B cell receptor blocking the production of antibody specific for

* Correspondence: mghaggag@gmail.com
[1]Microbiology & Immunology Unite, Research Institute of Ophthalmology, 2 El Ahram Street, Giza 12557, Egypt
[2]Allergy lab, Research Institute of Ophthalmology, 2 El Ahram Street, Giza 12557, Egypt
Full list of author information is available at the end of the article

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S. aureus and provoking B cell death (Kobayashi and DeLeo 2013; Keener et al. 2017).

This Fc-binding region, the so-called X-region of protein A gene (*spa*) contains a highly polymorphic sequence which is composed of repeats of 24-bp. Most epidemic MRSA strains contain more than seven repeats, while most nonepidemic MRSA strains have seven or fewer repeats. It is reasonable that a longer X region leads to a better exposition of the Fc-binding region of protein A and hence increasing colonization of host surfaces and responsible for the epidemic phenotype (Frénay et al. 1994, 1996).

For epidemiological investigations, prevention and control of infections molecular typing is crucial. Choosing the method for molecular typing is based on being consistent, cost-effective, quick, simple, and easy to understand results. Sequence typing of the *spa* gene repeat region was used to study the epidemiology of MRSA (Harmsen et al. 2003; Fasihi et al. 2017).

Strain typing of microbial pathogens aims to detect genetic microvariation for outbreak investigations and to sign genetic macrovariation for phylogenetic identification and population-based analyses. However, there has been no evidence that one genetic character can be used efficiently for indexing micro-and macro-variation until Koreen et al. (2004) in their study showed that genetic analysis of the repeat region of protein A (*spa* typing) was able to measure genetic variations that accumulate both rapidly and slowly (micro- and macro-variation) by two independent mechanisms, so this genetic character of repeat region of protein A is efficient for both local and global long-term epidemiologic- and population-based studies.

The aim of this study was screening of MRSA strains among healthcare workers (HCWs) in the hospital of the Research Institute of Ophthalmology, Giza, Egypt, and detection of *spa* gene in their DNAs by PCR.

Material and methods

The work in the present study involved 81 nasal swabs collected from healthcare providers including doctors, nurses, and workers in the hospital of the Research Institute of Ophthalmology, Egypt.

The first step was identification of MRSA isolates as follows.

The specimens were cultured on nutrient agar, blood agar, and mannitol salt agar and incubated at 37 °C for 18–24 h. Hemolytic colonies from blood agar and yellow colonies from mannitol salt agar and nutrient agar were identified microscopically as *staphylococci.*

Coagulase test was done by Dry spot Staphytect Plus which is a latex agglutination slide test for identification of *Staphylococcus aureus* by detection of clumping factor, Protein, A and capsular polysaccharides, the latter may be held by MRSA and may mask both the clumping factor and protein A (Fournier et al. 1989). The tested colony was emulsified in a drop of distilled water onto the reagent of the card and mixed, and agglutination within a minute identifies coagulase-positive *Staphylococcus aureus* (Fig. 1).

MRSA was identified by disc diffusion method according to the Clinical Laboratory Standards Institute (CLSI) Wayne (2013) criteria by culturing the coagulase-positive *Staphylococcus aureus* isolates on Muller Hinton agar plates to test their sensitivity to oxacillin (1 μg) and cefoxitin (30 μg), and incubation at 35 °C for a full 24 h was done. Plates were observed carefully in transmitted light for any growth. Any growth after 24 h was considered oxacillin resistant. MRSA was identified when the diameter of the inhibition zone to oxacillin is ≤ 12 mm and for cefoxitin is ≤ 21 mm (Ferreira et al. 2012) (Fig. 2). Cefoxitin is a crucial back-up due to heteroresistance phenomenon, which stated that all cells in a culture may possess genes for resistance, but only a small number may express the resistance in vitro. This phenomenon is termed heteroresistance and occurs in staphylococci resistant to penicillinase-stable penicillins, such as oxacillin. However, cells expressing heteroresistance grow more slowly than the oxacillin-susceptible isolates and may be missed at temperatures above 35 °C. This is why CLSI recommends incubating isolates being tested against oxacillin or cefoxitin at 33–35 °C (not more than 35 °C) for a full 24 h before reading (Wayne 2013).

The second step was DNA extraction from 12 MRSA isolates (resistant to both oxacillin and cefoxitin), 5 isolates

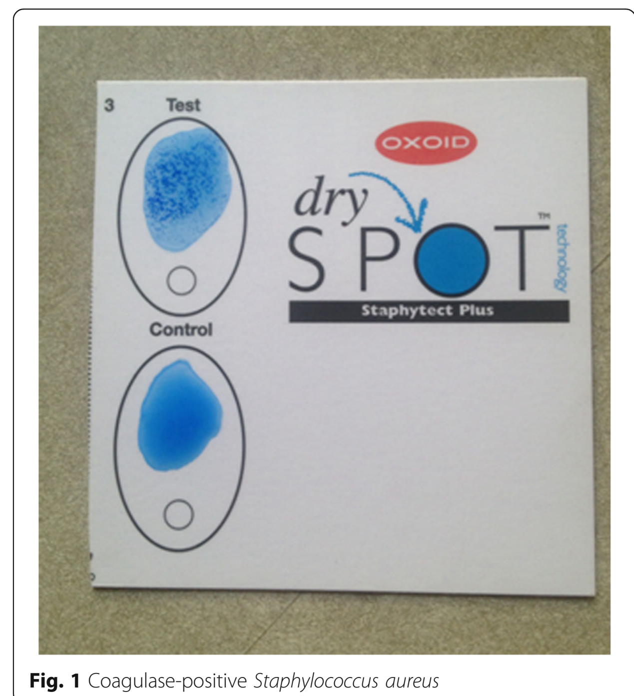

Fig. 1 Coagulase-positive *Staphylococcus aureus*

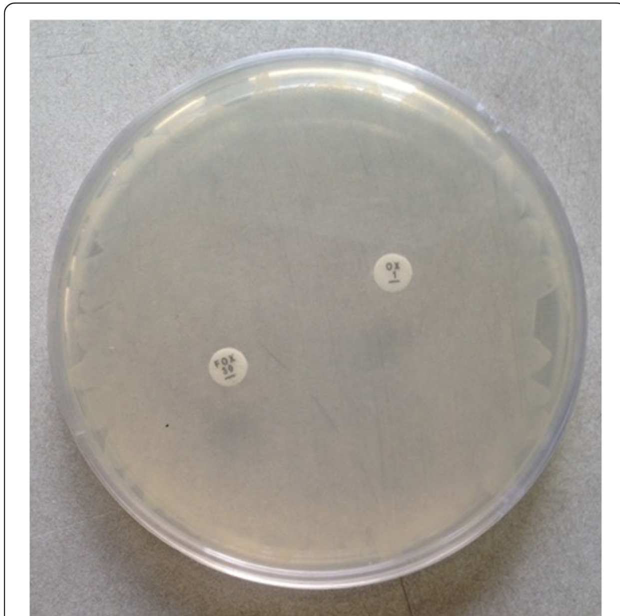

Fig. 2 Identification of MRSA. Coagulase positive *S. aureus* showed resistance to oxacillin (1 μg) ≤ 12 mm and cefoxitin (30 μg) ≤ 21 mm by disc diffusion method

which were resistant to oxacillin only, and 5 isolates which showed no resistance to any (sensitive to both oxacillin and cefoxitin); however, those last 10 isolates were studied for comparison as controls. Qiagen DNeasy kit (Qiagen, USA) was used for DNA extraction from bacterial cultures according to the manufacturer's instructions (Qiagen DNeasy handbook, July 2006) as follows.

Preparation of Qiagen Protease solution was done by adding 1.2 ml protease solvent into the vial containing lyophilized Qiagen Protease and stored at − 20 °C. Buffer AL (lysis buffer) was mixed thoroughly by shaking before use and stored at room temperature. Buffer AW1 (wash buffer 1) and buffer AW2 (wash buffer 2) were supplied as a concentrate before using for the first time; appropriate amount of ethanol (96–100%) was added and stored at room temperature.

The procedure was done by adding 20 μl of Qiagen Protease into the bottom of a 1.5-ml microcentrifuge tube, and the bacterial suspension was added after heated at 100 °C for 10 min. Two hundred microliters of buffer AL was added to the sample and mixed by pulse-vortexing for 15 s then incubated at 56 °C until the suspension became clear. Two hundred microliters of ethanol 96% was added to the sample and mixed again then centrifuged. The mixture was applied to the QIAamp Mini spin column and centrifuged at 6000 rpm for 1 min; then, the column was placed in a clean 2-ml collection tube, and the tube containing the filtrate was discarded. Five hundred microliters of buffer AW1 was added and centrifuged for 1 min (same as above). Five hundred microliters of buffer AW2 was added and centrifuged at full speed 20,000 rpm for 3 min. The QIAamp Mini spin column was placed in a new 2 ml collection, and the old collection tube was discarded with the filtrate and centrifuged at full speed for 1 min. The QIAamp Mini spin column was placed in a clean 1.5-ml micro-centrifuge tube, and the collection tube containing the filtrate was discarded and 200 μl buffer AE was added and incubated at room temperature for 1 min then centrifuged for 1 min.

The third step was the amplification of *spa* gene by polymerase chain reaction for the extracted 22 DNA. The primers used were:

Forward: 5 ATCTGGTGGCGTAACACCTG-3′ and the reverse: 5-C GCTGCACCTAACGCTAATG-3 (Shakeri et al. 2010).

The reaction was carried out by *Taq* PCR Master Mix. The PCR mixture consisted of 1 mmol/l magnesium chloride, 0.2 mmol/l dNTPs, PCR buffer, 1 μmol/l of primers, and 1 unit of Taq-DNA polymerase in a final volume of 50 μl. Samples were denaturated at 94 °C for 4 min followed by 35 cycles using the following parameters: denaturation at 94 °C for 1 min, annealing at 56 °C for 1 min, and extension at 72 °C for 3 min, with a final extension at 72 °C for 5 min.

The fourth step was detection of the amplified product using agarose gel electrophoresis. Agarose gel was prepared by adding 2 g of agarose powder then dissolved in 100 ml of Tris-acetate EDTA buffer, mixed thoroughly, and heated in the microwave until totally dissolved then left to cool down to 50 °C. The agarose was then poured into the electrophoresis tray containing the comb fixed in place and left to cool at room temperature for 30 min. Comb was removed from the solidified agarose and placed into the electrophoresis tray. Amplified samples were mixed with loading buffer, and each sample was dispensed into one well in the agarose gel (total volume 15–20 μl per well). Ladder was added in the first well of the agarose gel, and one drop of ethidium bromide was put per tray. The tray was covered with the electric electrodes cover. Reading was done under UV light gel documentation system.

Results

In the present study, 81 samples from healthcare providers in the hospital of the Research Institute of Ophthalmology (RIO), Egypt, were screened for MRSA. Out of these 81 samples, 41 isolates (50.6%) were identified as coagulase-positive *Staphylococcus aureus*. Twelve staphylococcal isolates were resistant to both oxacillin and cefoxitin, and those were identified as MRSA with a percentage of 14.8% (12/81). Out of 12 MRSA DNA, *spa* gene could be detected in 10 of them by conventional PCR with a percentage of 83.3% (10/12) (Fig. 3).

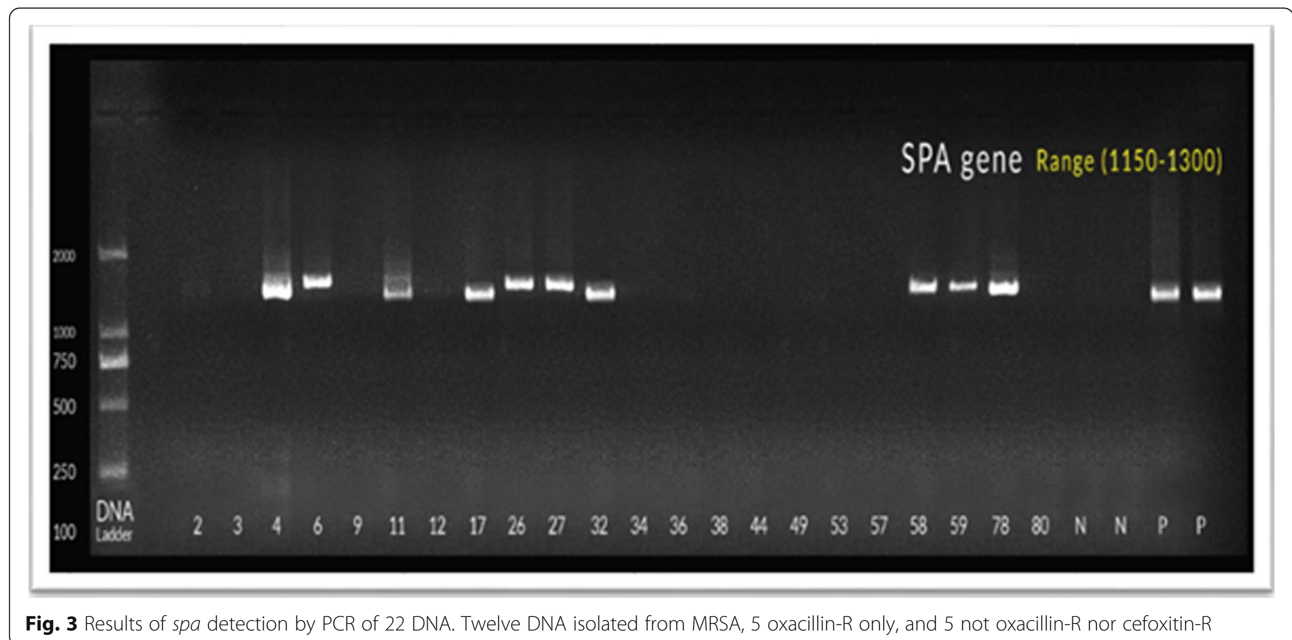

Fig. 3 Results of *spa* detection by PCR of 22 DNA. Twelve DNA isolated from MRSA, 5 oxacillin-R only, and 5 not oxacillin-R nor cefoxitin-R

Discussion

In the present study, the prevalence of *Staphylococcus aureus* was 50.6% (41/81) among healthcare workers (HCWs) providing nasal swabs, and the prevalence of MRSA was 14.8% (12/81).

Regarding the prevalence of *Staphylococcus aureus*, Price et al. (2017) observed during their study that 58% of healthcare workers yielded *S. aureus* at least once from nasal swabs; however, their results are more than ours. While Sakr et al. (2018) reported that *Staphylococcus aureus* permanently colonized up to 30% of asymptomatic humans and although it is a commensal of the nose and skin, yet it may induce opportunistic and even life-threatening infections such as surgical site infections (SSIs). However, their results are lower than ours.

Chuang et al. (2012) in their 10 years retrospective study in Chang Gung Memorial Hospital, Taiwan, determined that MRSA account for 52.8% of ocular *S. aureus* infections per year with nearly half of ocular MRSA infections were sight-threatening, while in the USA, Weiner et al. (2013) reported that MRSA accounts for about 30% of all severe *S. aureus* eye infections, and the percentage is rising every year. American Nurse Today (2010) reported that about one third of the general population harbor staphylococci and the carrier status of healthcare workers ranges from 50 to 90% of which the commonest site is the anterior nares. They also reported that MRSA is able to withstand or recover quickly from difficult conditions and able to live outside the host for some time which is not exactly known. Smith (2008) reported that 1 in every 20 healthcare workers was MRSA carrier (5%) and stated that healthcare workers are usually vectors, rather than the main sources of MRSA transmission.

Parks and Croce (2012) estimated an elevated percentage of asymptomatic carriers of MRSA in the general population to be 5–10%; they stated that in spite of possible decreasing in the percentage of MRSA infections in hospitals, yet it is of great concern as asymptomatic carriers might be exposed to skin and soft tissues infections, including serious surgical site infections (SSIs). The same average was also obtained by Nair and coauthors (2013) when they investigated 251 *S. aureus* isolates that were collected by the National Center for Communicable Diseases (NCCD) in Ulaanbaatar, Mongolia, and demonstrated methicillin resistance in 8.8% of isolates (22/251). Dulon et al. (2014) estimated the prevalence of MRSA in HCWs to be 4.6%, predicting that MRSA carriage in HCWs in non-outbreak settings is thought to be higher than in an outbreak situation, due to increased hygiene awareness in outbreaks. However, they found that MRSA prevalence among HCWs in non-outbreak settings was not higher than carriage rates estimated for outbreaks. Similar results were published by Sassmannshausen et al. (2016) who investigated MRSA prevalence and the risk factors for MRSA colonization among HCWs by gathering nasopharyngeal swabs from 9 hospitals with different care levels within the German part of a Dutch-German border region (EUREGIO). *Spa* typing of the isolated MRSA strains was done, and the overall MRSA prevalence among HCWs in a non-outbreak situation was 4.6%; it was higher in nurses (5.6%) than in physicians (1.2%). However, the results in the present study are higher than all of them. Very recently in the USA, Kavanagh et al. (2018) reported the carriage rate of *S. aureus* in the general population approximates 33% and that of MRSA is approximately 2% as estimated by the CDC; still, the carriage rate of MRSA

in healthcare workers approximates 5% with concerns of transmission of this pathogen to patients. Also, these recent results reported by Kavanagh et al. (2018) are lower than our results regarding *S. aureus* and MRSA.

On the other hand, in Iran, a study was done by Shakeri et al. (2010) reported the prevalence of MRSA from healthy carriers and patients as 28%. A higher prevalence of MRSA was reported later by Afrough et al. (2013) as 52.5% in staff and 83.7% in patients' specimens. More recent study in a burn hospital in Tehran by Abbasian et al. (2018) reported MRSA prevalence as 50% among HCWs and 64.2% among patients. And hence, all three studies in Iran showed higher MRSA prevalence than the prevalence in the present study which was 14.8%.

When comparing an old method of MRSA typing which was phage typing with more recent molecular techniques as *spa* typing the latter is more sensitive allowing differentiation of strains within a particular phage type (Frénay et al. 1996). MRSA typing is a crucial step for an effective surveillance system to outline epidemiological trends and infection control strategies. Therefore, it is important to focus on choosing the proper technique in terms of accuracy, authenticity, simplicity, clarity, and cost-effectiveness regarding MRSA typing (Mehndiratta and Bhalla 2012).

Amplification of spa gene by PCR is a necessary preliminary step for *spa* typing of MRSA. In the present study, the results of detection of *spa* gene in MRSA DNA by conventional PCR were 83.3% (10/12).

Different primer sequences for detection of *spa* gene by PCR have been used in different parts of the world. However, although we used the same primer sequences and the same thermal cycling reaction conditions that Shakeri et al. (2010) had used for *spa* gene detection by conventional PCR, yet we could not detect s*pa* gene in 16.7% of the DNA MRSA while their percentage was much lower as 3.4% only. Meanwhile, they found that frequencies of strains with short *spa* bands in strains isolated from patients were significantly more than those isolated from healthy carriers, and they concluded that the length of *spa* gene depends either on resistance to methicillin or the source of *S. aureus* isolation, while in earlier study by Koreen et al. (2004) suggested that repeat composition and organization, and not the number of repeats, allow *spa* typing to correlate with the DNA microarray data. Peacock et al. (2002) detected spa gene by PCR in 90% of *S. aureus* isolated from carriers which was a better result than in the present study as 83.3%; they reported the presence of different gene codes for virulence in natural populations of *S. aureus* and clarified that the effect of those genes were separately increasing the chances for a disease and reported that it might be inaccurately to regard virulence in relation to the presence or absence of a given bacterial factor. It is worthwhile to mention that they used different primers than that used in the present study. Baum et al. (2009) also used different primers to analyze eight non-*spa*-typeable strains that were isolated from blood cultures of patients with invasive *S. aureus* infections in Denmark; however, they found that only two strains actually failed to give a positive result, suggesting that those two strains were true *spa*-deficient strains.

They concluded that mutations in the primer binding region might have missed spa-positive strains. They also concluded that in spite of *spa* having a strong role in the pathogenesis of *S. aureus* infections, the two *spa*-deficient strains were isolated from patients with severe *S. aureus*-related infections, denoting that the strains were still virulent and invasive in spite of being *spa*-deficient. That was confirmed later in a study also made in Denmark as part of the national surveillance of *S. aureus* bacteraemia and MRSA by Sorum et al. (2013); sequencing of the whole *spa* including the promoter region was amplified by PCR using three different primer sets, which included primers previously described by (Strommenger et al. 2006) and their novel primers. They notice naturally occurring mutants secreting spa into the extracellular environment were sporadically present among isolates involved in disease and carriage, suggesting that cell wall-bound *Staphylococcus* protein A is not necessary for survival and virulence of *S. aureus* in the host. They stated that with the plenty and overflow of virulence factors in *S. aureus*, including surface proteins removing just one factor is not necessarily enough to leave this bacterium unable to cause disease.

Votintseva and coauthors (2014) designed an improved primers that were capable of sequencing of all strains, containing any type of genetic rearrangement, in a large study among community carriers and hospital inpatients in Oxfordshire, UK, three of these rearrangements would be designated non-typeable using current *spa*-typing methods; they occurred in 1.8% *S. aureus* strains isolated from asymptomatic carriers and in 0.6% strains isolated from patients. They concluded that a weakness of current *spa*-typing primers is that rearrangements in the IgG-binding region of the gene cause 1–2% of strains to be designated as non-typeable and accounting for hidden diversity of *S. aureus* in both community and hospital environments.

Conclusion

In the present study, the prevalence of MRSA in HCWs was 14.8%. Since amplification of *spa* gene by PCR is a necessary preliminary step for *spa* typing of MRSA and since using different primers for *spa* gene amplification might affect PCR results, then proper selection of the primers and thermal cycling reaction conditions are recommended for PCR performance and *spa* typing.

Abbreviations
CA: Community-associated; CDC: Centers for disease control; CLSI: Clinical Laboratory Standards Institute; FcIgG: Fragment crystallizable of immunoglobulin G; HA: Healthcare-associated; HCWs: Health care workers; MRSA: Methicillin-resistant *Staphylococcus aureus*; PCR: Polymerase chain reaction; Ptn A: protein A; RIO: Research Institute of Ophthalmology; *spa*: Surface protein A of *Staphylococcus aureus*; SSIs: Surgical site infections

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Authors' contributions
MH designed the plan of the work, participated in lab work, and wrote and submitted the manuscript. AA participated in the lab work and shared in writing it and participated in the development and implementation of the research plan. MA participated in the lab work and shared writing lab work. All authors read and approved the final manuscript.

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Availability of data and materials
The materials and the generated and analyzed data during this study are included in this published article.

Ethics approval and consent to participate
The work is ethically approved by the Scientific Research Committee of the Research Institute of Ophthalmology RIO, Egypt, prior to the beginning of the study. Nasal swab samples used in the study were obtained after individuals' consent.

Consent for publication
Not applicable.

Competing interests
The authors declare that they have no competing interests.

Author details
[1]Microbiology & Immunology Unite, Research Institute of Ophthalmology, 2 El Ahram Street, Giza 12557, Egypt. [2]Allergy lab, Research Institute of Ophthalmology, 2 El Ahram Street, Giza 12557, Egypt. [3]Infection Control Unit, Research Institute of Ophthalmology, Giza, Egypt.

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