INTRODUCTION

Staphylococcus aureus is a leading human pathogen of significant clinical importance, responsible for a wide array of infections from superficial skin infections to more serious invasive infections, including pneumonia, sepsis, and endocarditis. It is also one of the most common ophthalmic pathogens recovered from conjunctivitis and other ocular infections. Since the isolation of the first methicillin-resistant S. aureus (MRSA) in 1961, the increasing prevalence of MRSA worldwide has become a growing concern, prompting the typing of S. aureus in order to support infection control measures, investigate suspected outbreaks, and evaluate nosocomial transmission.

Historically, MRSA pathogens were almost exclusively isolated from hospitals or hospital-associated facilities. However, there have been an increasing number of MRSA cases reported in individuals with...
no known risk factors for MRSA colonization, such as admission to a hospital, surgery, contact with a MRSA-colonized patient, intravenous drug use, or previous antibiotic exposure.5–7 These isolates, termed community-acquired MRSA (CA-MRSA), have become a global concern and have been found worldwide not only in the community setting but also in healthcare facilities.8 In fact, some hospitals have reported a predominance of CA-MRSA isolates over hospital-acquired MRSA (HA-MRSA) isolates.9,10 Although the term “acquired” implies that the location of transmission is known, the HA- and CA-designations have also been used to describe the phenotypic and molecular traits of MRSA isolates, as we have done in this study.

HA-MRSA strains, exemplified by the USA100 clone, are typically associated with nosocomial infections including bacteremia,11 whereas CA-MRSA strains, exemplified by the USA300 clone, have been more commonly associated with skin and soft tissue infections.9,12,13 The two groups are also distinguished by differences in their susceptibilities to antimicrobial agents, the composition of the gene cassette coding for methicillin resistance, and associated exotoxin profiles. Because CA-MRSA and HA-MRSA isolates are different with respect to virulence and antimicrobial susceptibility profiles, this information could be useful in the design of future strategies to prevent and treat ocular infections.

In contrast to HA-MRSA, which generally possess multiple antimicrobial resistance determinants and are thus multidrug-resistant, CA-MRSA are typically susceptible to non–β-lactam antibiotics.14 Resistance to β-lactam antibiotics, including methicillin, is conferred by a low affinity penicillin-binding protein (PBP) 2a, encoded by the mecA gene. The mecA gene is found on a mobile genetic element known as the “staphylococcal cassette chromosome mec” (SCCmec).15–17 To date, eight major variants of SCCmec (type I to VIII) have been identified,18 with SCCmec type II and type IV found predominantly in HA-MRSA and CA-MRSA, respectively.19–21 The Panton-Valentine leukocidin (PVL) genes, coding for a pore-forming cytotoxin known to cause tissue necrosis and leukocyte destruction, are frequently present in CA-MRSA and have been shown to be stable markers of CA-MRSA cases worldwide.20,22–24 In fact, CA-MRSA has been shown to be more virulent compared to HA-MRSA due to the presence of various virulence factors, such as PVL.4,17,25,26 Both SCCmec typing and detection of the PVL locus are useful tools for the molecular characterization of HA- and CA-MRSA isolates.

A different tool used for the typing of both MRSA and MSSA is single locus DNA sequencing of the S. aureus Protein A gene variable repeat region (spa typing). The spa gene contains a hypervariable region that differs in the number of repeats (1 to 23) and the number of base pairs (21 to 30) in each repeat (http://spaserver.ridom.de, accessed 20 Jul 2010).27,28 The nucleotide composition of each distinct repeat is determined and subsequently given a spa type designation based on the unique succession of repeats. To date, pulsed-field gel electrophoresis (PFGE) is frequently used to determine clonal relationships between bacterial isolates. However, the method is cumbersome and PFGE results cannot be easily compared among multiple laboratories.29 In contrast, the spa typing method, along with the recently described spa grouping algorithm BURP (Based Upon Repeat Patterns), provides a rapid and accurate method to determine clonal relationships among S. aureus strains.29–31

While much attention has been paid to MRSA isolates from skin, soft tissue, and invasive infections, less is known about the prevalence and epidemiology of MRSA in infections of the eye. Since commensal bacteria from the skin and nasopharynx are often the source of ocular infections and CA-MRSA often cause skin and soft tissue infections, it was of interest to determine whether strains that have traits similar to those of HA-MRSA or those of CA-MRSA are more prevalent among drug-resistant isolates from ocular infections. As part of an ongoing study to characterize fluoroquinolone (FQ) resistance in ocular S. aureus isolates, we chose to further characterize such isolates with respect to the microbiological and molecular features of CA- and HA-MRSA isolated in the USA. Accordingly, SCCmec typing, the presence of the PVL gene, and antimicrobial susceptibility testing were used to characterize these strains for traits typical of either CA-MRSA or HA-MRSA. Spa typing was also performed to explore its use as a potential method for characterizing HA-MRSA and CA-MRSA strains.

MATERIALS AND METHODS

Bacterial Strains and Growth Conditions

As part of a separate study to characterize the molecular basis of high-level FQ resistance among staphylococci, a total of 56 ocular S. aureus strains including MRSA and MSSA were obtained from Eurofins Medinet (Chantilly, Virginia, USA). Ocular isolates were collected between 2006 and 2008, representing 24 hospitals from 14 different U.S. states. Strains were isolated from one of four different ocular sources (aqueous fluid, vitreous fluid, conjunctiva, or cornea) from patients aged < 1 to 90 years (61% female). Care was taken to ensure that no duplicate isolates were included in this study.
All strains were grown for 18–24 hr at 37°C under ambient conditions. For genomic DNA extractions, bacteria were grown in Tryptic Soy Broth (Difco, Sparks, Maryland). Susceptibility testing was performed with Mueller-Hinton Broth II (Difco). ATCC 29213 (American Type Culture Collection [ATCC], Manassas, Virginia, USA) was the S. aureus quality control strain used for Clinical Laboratory and Standards Institute (CLSI) compliant susceptibility testing. Two MRSA strains, USA300 and USA600 (ATCC), were used as controls for SCCmec typing.

Antimicrobial Susceptibility Testing

In vitro antimicrobial susceptibility testing for besifloxacin (BES), moxifloxacin (MXF), gatifloxacin (GAT), ciprofloxacin (CIP), levofloxacin (LVX), azithromycin (AZI), vancomycin (VAN), rifampin (RIF), clindamycin (CLI), tobramycin (TOB), erythromycin (ERY), tetracycline (TET), linezolid (LIN), and oxacillin (OXA) was performed according to CLSI guidelines. All agents were obtained from Bausch & Lomb Inc. (Rochester, Minnesota, USA), with the exception of BES, which was obtained in powder form from LKT Laboratories (St. Paul, Minnesota, USA). PCR was carried out as simplex reactions using previously published oligonucleotide primers. PCR was carried out as described above for MRSA and was followed by a final cycle of elongation (10 min at 72°C). PCR products were purified using the EZNA Cycle Pure Kit (Omega Bio-Tek, Norcross, Georgia, USA), 1 U of Vent DNA polymerase and its reaction buffer containing 2 mM magnesium sulfate (New England Biolabs, Ipswich, Massachusetts, USA). The initial cycle of denaturation (15 min at 94°C) preceded 30 cycles consisting of 0.5 min of denaturation at 94°C, 1 min of annealing at 59°C, and 1 min of elongation at 72°C, and was followed by a final cycle of elongation (10 min at 72°C). PCR products were purified using the EZNA Cycle Pure Kit (Omega Bio-Tek), and sequenced by ACGT Inc. (Wheeling, Illinois, USA). Clone Manager 9 (Sci-Ed Software, Cary, North Carolina, USA) was used for sequence analyses, and spa types were determined using the website (http://spaserver.ridom.de/) developed by Ridom GmbH and curated by SeqNet.org (http://SeqNet.org/). The BURP clustering tool in the Ridom StaphType 2.0.3 software package (Ridom GmbH, Würzburg, Germany) was used for spa type aligning and clustering.

Detection of Genes Encoding PVL

Genomic DNA was extracted as described above and used as the template for PCR amplification. A 945-bp region of the Panton-Valentine leukocidin genes (lukF-PV and lukS-PV) from each isolate was amplified using the oligonucleotide primers, lukS 5′-CCC ATT AGT ACA CAG TGG TTT CAA TC-3′ and lukF 5′-GTC CAG CAT TTA AGT TGC TTT GTC-3′. The primer sequences were designed from the published S. aureus strain USA300 (GenBank accession no. NC_007793) using Clone Manager 9 analysis software (Sci-Ed Software). PCR was carried out as described above in a 25 μl volume. The initial cycle of denaturation (10 min at 94°C) preceded 33 cycles consisting of 0.5 min of denaturation at 94°C, 0.5 min of annealing at 57°C, and 1 min of elongation at 72°C, and was followed by a final cycle of elongation (10 min at 72°C).

SCCmec Typing

Genomic DNA was extracted as described by Zhang et al. with modifications. Briefly, five to ten bacterial colonies were suspended in 50 μl of nuclease-free water (Promega) and heated at 99°C for 10 min. After centrifugation at 21,000 × g for 1 min, 2.5–9 μl of the supernatant was used as the template for PCR amplification. The SCCmec typing assay contained eight unique and specific pairs of primers as previously described for SCCmec types and subtypes I, II, III, IVa, IVb, IVc, IVd, and V. PCR was carried out as simplex reactions using conditions identical to those described above for PVL detection.
Gel Electrophoresis

PCR amplicons were visualized using a UV light box after electrophoresis on 1% (spa typing) or 2% (detection of PVL genes and SCCmec typing) agarose gels containing 0.1 µg/ml ethidium bromide (Omega Bio-Tek).

RESULTS

Published CLSI breakpoints for OXA-36 were used to classify all ocular S. aureus isolates as either MSSA (n = 16) or MRSA (n = 40). With the exception of two MRSA isolates, where the SCCmec type could not be determined, the remaining 38 MRSA isolates were classified as either SCCmec type II (n = 22) or type IV (n = 16) (Table 1). Two different subtypes of the SCCmec type IV cassette were found with subtype IVa identified in 15 (93.8%) of the type IV isolates and subtype IVb being found in a single isolate (6.3%). The PVL genes were absent in all SCCmec type II isolates, while 75.0% (12/16) of SCCmec type IV and 18.8% (3/16) of the MSSA isolates contained the PVL genes. Four different, but related, spa types were found among SCCmec type II isolates while five different, but related, spa types were found among SCCmec type IV isolates; MSSA was the most diverse with respect to spa type with 12 different types being observed.

Susceptibility testing was performed with 14 antimicrobial agents representing the following nine drug classes: fluoroquinolones (FQs), macrolides, lincosamides, aminoglycosides, tetracyclines, rifamycins, glycopeptides, oxazolidinones, and β-lactams. All SCCmec type II isolates were resistant to at least three drug classes, with one isolate in this group exhibiting resistance to six different drug classes. Conversely, 62.5% of SCCmec type IV isolates were resistant to at least three drug classes, with no isolate in this group possessing resistance to more than four different drug classes.

The MIC90, MIC90, and MIC range values for all antimicrobial agents tested are presented in Table 2. One hundred percent of all S. aureus isolates were susceptible to the systemic (i.e., non-ophthalmic) agents RIF, VAN, and LIN. Additionally, 100% of the SCCmec type IV isolates were susceptible to CLI and TET while 87.5% and 93.8% of MSSA isolates and 54.5% and 95.5% of the SCCmec type II isolates were susceptible to these drugs, respectively. With the exception of BES, where the percentage of susceptible isolates cannot be determined due to the lack of any established resistance breakpoints for exclusively topical agents, all SCCmec type II isolates were resistant to the FQs (MXF, GAT, CIP, and LVX) and macrolides (AZI and ERY) tested. However, 37.5% and 6.3% of SCCmec type IV isolates were susceptible to all FQs and macrolides tested, respectively; 56.3% of MSSA isolates were susceptible to the macrolides and early generation FQs (CIP and LVX), while 62.5% of MSSA isolates were susceptible to the subsequent generation of FQ agents (GAT and MXF). While only 13.6% of SCCmec type II isolates were susceptible to TOB, 93.8% of SCCmec type IV isolates and 81.3% of MSSA isolates exhibited susceptibility to this aminoglycoside.

Rifampin was the most potent antimicrobial agent tested for all S. aureus isolates with MIC90 values of 0.008 µg/ml. Among the FQ agents tested, BES

| Number of isolates | Type II | Type IV | MSSA |
|--------------------|---------|---------|------|
| Source of isolates by state (number of sites) | AL (2), FL (1), GA (1), ID (1), MA (2), MD (1), MI (3), NY (2), OH (2), PA (4), SC (1), TN (2) | FL (1), GA (1), ID (1), MA (1), MD (4), MI (1), NC (1), NY (2), OH (4) | AL (1), FL (2), GA (1), MA (2), MD (1), MI (1), MO (1), NY (3), OH (2), PA (2) |
| SCCmec type (n) | II (22) | IVa (15) | NA |
| PVL positive, n (%) | 0 (0.0%) | 12 (75.0%) | 3 (18.8%) |
| Number of spa types | 4 | 5 | 12 |
| % Resistant to 1+ drug class | 100.0% | 100.0% | 62.5% |
| % Resistant to 2+ drug classes | 100.0% | 93.8% | 37.5% |
| % Resistant to 3+ drug classes | 100.0% | 62.5% | 18.8% |
| % Resistant to 4+ drug classes | 95.5% | 6.3% | 6.3% |
| % Resistant to 5+ drug classes | 36.4% | 0.0% | 0.0% |
| % Resistant to 6+ drug classes | 4.5% | 0.0% | 0.0% |
| % Resistant to 7+ drug classes | 0.0% | 0.0% | 0.0% |

aDrug classes tested included fluoroquinolones, macrolides, lincosamides, aminoglycosides, tetracyclines, rifamycins, glycopeptides, oxazolidinones, β-lactams.
TABLE 2 Antimicrobial susceptibilities of ocular S. aureus, including 16 MSSA, 22 MRSA SCCmec type II, and 16 MRSA SCCmec type IV isolates.

| Agent       | Organism | MIC (μg/ml)       | % Susceptible | Range | 50% | 90% |
|-------------|----------|------------------|---------------|-------|-----|-----|
| Besifloxacin| MSSA     | 0.016–4          | 0.03          | 4     | NA  |
|             | MRSA (type II) | 0.5–8        | 4             | 4     | NA  |
|             | MRSA (type IV) | 0.016–1      | 0.25          | 0.5   | NA  |
| Moxifloxacin| MSSA     | 0.03–64         | 0.06          | 32    | 62.5|
|             | MRSA (type II) | 2–64         | 32            | 64    | 0   |
|             | MRSA (type IV) | 0.016–2      | 2             | 2     | 37.5|
| Gatifloxacin| MSSA     | 0.06–64         | 0.125         | 32    | 62.5|
|             | MRSA (type II) | 2–128        | 64            | 128   | 0   |
|             | MRSA (type IV) | 0.031–2      | 2             | 2     | 37.5|
| Ciprofloxacin| MSSA    | 0.125–256       | 0.5           | 256   | 56.3|
|             | MRSA (type II) | 32–256        | 256           | 256   | 0   |
|             | MRSA (type IV) | 0.125–64     | 16            | 32    | 37.5|
| Levofloxacin| MSSA     | 0.125–512       | 0.25          | 512   | 56.3|
|             | MRSA (type II) | 8–1024        | 512           | 512   | 0   |
|             | MRSA (type IV) | 0.125–8      | 4             | 8     | 37.5|
| Azithromycin| MSSA     | 0.5–256         | 0.5           | > 256 | 56.3|
|             | MRSA (type II) | > 256         | > 256         | > 256 | 0   |
|             | MRSA (type IV) | 0.5–256     | 128           | 128   | 6.3 |
| Erythromycin| MSSA     | 0.25–256        | 0.5           | > 256 | 56.3|
|             | MRSA (type II) | > 256         | > 256         | > 256 | 0   |
|             | MRSA (type IV) | 0.25–64     | 64            | 64    | 6.3 |
| Clindamycin | MSSA     | 0.063–256       | 0.125         | > 256 | 87.5|
|             | MRSA (type II) | 0.063–256     | 0.25          | > 256 | 54.5|
|             | MRSA (type IV) | 0.063–0.125 | 0.063         | 0.063 | 100 |
| Tobramycin  | MSSA     | 0.25–256        | 0.5           | 256   | 81.3|
|             | MRSA (type II) | 0.5–256       | 256           | 256   | 13.6|
|             | MRSA (type IV) | 0.5–32       | 1             | 2     | 93.8|
| Tetracycline| MSSA     | 0.5–32          | 0.5           | 4     | 93.8|
|             | MRSA (type II) | 0.25–64       | 0.5           | 0.5   | 95.5|
|             | MRSA (type IV) | 0.25–0.5     | 0.5           | 0.5   | 100 |
| Rifampin    | MSSA     | 0.002–0.016     | 0.008         | 0.008 | 100 |
|             | MRSA (type II) | 0.004–0.125   | 0.008         | 0.008 | 100 |
|             | MRSA (type IV) | 0.004–0.008 | 0.004         | 0.008 | 100 |
| Vancomycin  | MSSA     | 1               | 1             | 1     | 100 |
|             | MRSA (type II) | 1             | 1             | 1     | 100 |
|             | MRSA (type IV) | 0.5–1        | 1             | 1     | 100 |
| Linezolid   | MSSA     | 2–4             | 2             | 4     | 100 |
|             | MRSA (type II) | 2–4          | 4             | 4     | 100 |
|             | MRSA (type IV) | 2            | 2             | 2     | 100 |
| Oxacillin   | MSSA     | 0.25–0.5        | 0.25          | 0.5   | 100 |
|             | MRSA (type II) | > 8          | > 8           | > 8   | 0   |
|             | MRSA (type IV) | 4–> 8       | > 8           | > 8   | 0   |

*Since besifloxacin was developed as an exclusively topical ophthalmic agent, no breakpoints exist.

was the most potent agent, with MIC$_{90}$ values of 4μg/ml for SCCmec type II and MSSA isolates and 0.5μg/ml for SCCmec type IV isolates. For all FQ agents tested, the MSSA isolates displayed the broadest MIC ranges. The highest MIC$_{90}$ values observed among the SCCmec type II isolates and MSSA isolates were 512μg/ml for LVX, > 256μg/ml for AZI, ERY, and CLI, and 256μg/ml for TOB and CIP. In contrast the highest MIC$_{90}$ values for SCCmec type IV isolates were 128μg/ml for AZI, 64μg/ml for ERY, and 32μg/ml for CIP.

The spa typing data presented in Table 3 revealed that all 22 SCCmec type II isolates, representing four different spa types, occurred within the same BURP cluster (spa-CC002) with spa type 002 observed most frequently (81.8%; 18/22). Similarly, the 16 SCCmec
type IV isolates, representing five different spa types, occurred within the same BURP cluster (spa-CC008) with spa type t008 observed most frequently (75.0%; 12/16). In contrast, the 16 MSSA isolates displayed by far the most diversity in terms of the number of different spa types found; with the exception of spa type t002, which occurred five times, no other type was represented more than once. The 12 different spa types found among the MSSA isolates comprised four BURP clusters (spa-CC002, spa-CC008, spa-CC084, and spa-CC240/773) in addition to three singletons.

**DISCUSSION**

Molecular and microbiological characterizations were conducted to determine whether ocular isolates have traits characteristic for CA-MRSA or HA-MRSA. To our knowledge, no SCCmec typing study\(^\text{13}\) has been conducted on an exclusively ocular set of MRSA isolates. Because these isolates were also part of separate FQ resistance characterization studies among ocular isolates, a substantial fraction of strains tested here (41/56) had elevated FQ MIC values and therefore may not be fully representative of ocular MRSA in general. Nevertheless, because most ocular infections are treated empirically this report of multidrug-resistant strains isolated from the eye with genetic traits of both CA-MRSA and HA-MRSA should be of interest to the ophthalmic community.

The results of the current study showed that all 38 typeable ocular MRSA isolates tested could be classified as either SCCmec type II or SCCmec type IV, the predominant cassette types of HA-MRSA and CA-MRSA, respectively.\(^{21,37}\) Consistent with previous reports,\(^{38}\) all the ocular SCCmec type II strains examined here exhibited traits typical for HA-MRSA, including the absence of the PVL toxin and resistance to multiple drug classes (Table 1). Over 95% of the SCCmec type II isolates were resistant to at least four different classes of antimicrobial agents and MIC\(_{50}\) and MIC\(_{90}\) values against such agents were elevated in comparison to values observed among the SCCmec type IV isolates. The increased resistance to non-β-lactam antibacterials found in the SCCmec type II isolates may be partly due to the fact that these cassettes contain a variety of additional drug resistance gene elements not found within SCCmec type IV cassettes.\(^{39}\)

CA-MRSA isolates have usually been defined as containing SCCmec type IV cassettes, expressing PVL, and exhibiting susceptibility to non-β-lactam antimicrobial agents.\(^{21,40}\) In this study, 16 MRSA isolates were characterized as SCCmec type IV isolates and of these 75% were found to contain the PVL genes. In contrast to the SCCmec type II isolates, which all showed high level resistance to several classes of antimicrobial agents, all SCCmec type IV isolates shared only resistance to β-lactam agents. There were, however, high levels of resistance to other drug classes observed; among the SCCmec type IV isolates, susceptibility to the FQ and macrolide classes was only 37.5% and 6.3%, respectively.

Recently, in a large MRSA surveillance study conducted in San Francisco, Diep et al. found that almost 90% of their USA300 strains (n=188) were resistant to ERY, over 60% were resistant to CIP, over 24% were resistant to TET, and over 10% were resistant to CLI.\(^{41}\) These data, taken together with our resistance data, indicate that resistance to non-β-lactam antibiotics might be increasing among SCCmec type IV isolates. The increased resistance and the fact that 25% of our MRSA isolates might be increasing among SCCmec type IV isolates.
or lower for SCCmec type IV isolates than for SCCmec type II isolates.

PFGE or spa typing can reveal clonal relationships among MRSA isolates. The most observed types in this study were spa types t008 and t002 representing the clones USA300 and USA100, respectively. These spa types have been frequently reported as common spa types found in large surveillance studies worldwide.\(^4\)\(^2\)\(^4\)\(^2\) In the relative global frequencies database on the Ridom SpaServer website, spa types t003 (12.5%), t032 (10.7%), t008 (6.6%), and t002 (5.9%) are listed as the most commonly isolated spa types (http://spaserver.ridom.de/frequencies.shtml, accessed 07 Jun 2010). Although the Ridom database contains isolates from all over the world, the data set is dominated by European isolates. For example, our analysis of the Ridom database revealed that of the 50 most frequent spa types (those with a prevalence of 0.25% or higher), 95.5% of the 59,811 isolates with country of origin listed were from Europe, while only 1.5% originated in the United States. The most common spa types among the 898 U.S. isolates were t008 (69.7%), t002 (11.8%), t064 (4.7%), t045 (2.8%), t024 (2.0%), and t242 (1.6%); the same six spa types were also identified among the 54 ocular isolates described here. These data support the hypothesis that the spa types of ocular S. aureus are similar to those isolated from other body sites.

All MRSA isolates containing the SCCmec type II element were either of spa type t002 or one of three similar spa types that belong to cluster spa-CC002. Type t002 was the founder of this cluster and was, with 18 isolates, the most prevalent spa type. Similarly, all strains containing a SCCmec type IV element were either spa type t008 or one of four spa types that are part of the spa-CC008 cluster. Type t008 was the founder of the spa-CC008 cluster and was, with 12 isolates, the second most prevalent spa type among MRSA isolates.

Spa types t002 and t242 (cluster spa-CC002) and t008 (cluster spa-CC008) were present in the MRSA and the MSSA groups. Several previous reports have documented the in vivo conversions of clinical MSSA to MRSA and vice versa.\(^4\)\(^5\)\(^6\) The acquisition or loss of DNA encoding the SCCmec cassette may occur more frequently in SCCmec type IV isolates than in SCCmec type II isolates, presumably due to the smaller, more mobile SCCmec type IV cassette.\(^1\)\(^9\)\(^\)\(^2\)\(^1\)\(^4\)\(^4\) It remains to be determined if an ancestral strain acquired a specific SCCmec element and then diversified into clones with similar spa types, or if related strains independently converted from MSSA to MRSA by integrating SCCmec elements.\(^4\)\(^8\)

In conclusion, the molecular characterization and, to some extent, the antimicrobial phenotypes of the MRSA isolates tested in this study demonstrate that SCCmec type II and SCCmec type IV isolates generally fit the classical definition of HA- and CA-MRSA strains, respectively. In contrast to MRSA, MSSA isolates were more diverse in their PVL, spa type, and antimicrobial susceptibility profiles, whereas the MRSA isolates were less varied with respect to these traits. In particular, the presence of a dominant spa type and the similarity between spa repeats among isolates with the same SCCmec type suggests that spa typing may be an additional useful tool when molecularly investigating and classifying ocular MRSA isolates as either CA- or HA-MRSA.

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**Declaration of interest:** All authors are employees of Bausch & Lomb, Inc., Rochester, NY. The authors alone are responsible for the content and writing of the paper.

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