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A ten year study of prevalence, antimicrobial susceptibility pattern, and genotypic characterization of Methicillin resistant *Staphylococcus aureus* causing ocular infections in a tertiary eye care hospital in South India

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ABSTRACT

Methicillin-resistant *Staphylococcus aureus* (MRSA) is an important cause of vision threatening ocular infections. This study aimed to determine the prevalence and antimicrobial susceptibility pattern of MRSA and their genotypic characterization in ocular infections. The study period was from January 2007 to December 2017 in Aravind Eye Hospital, Madurai. Retrospective analysis of clinical records found a total of 1306 *Staphylococcus aureus* in various ocular infections. Among these, 274 (21%) were found to be MRSA with an increased incidence from 9% in 2007 to 38% in 2017 ($P = .007$). MRSA was isolated commonly from lacrimal sac infection 89 (32%), lid infection 55 (20%), keratitis 45 (16%) and orbital infection 34 (12%). MRSA isolates showed 100% sensitivity to vancomycin, 91% to chloramphenicol and majority of MRSA isolates were resistant to all fluoroquinolones. MSSA strains showed very minimal resistance to chloramphenicol (5%) and also there was no resistance to vancomycin. In case of the MSSA isolates, resistance to fluroquinolones (ciprofloxacin, gatifloxacin, moxifloxacin, ofloxacin and levofloxacin) was found to increase during study period. Methicillin-resistance is conferred by the carriage of Staphylococcal Cassette Chromosome *mec* (SCC*mec*) and most of our isolates were belonged to SCC*mec* type V and IV which is known to be community acquired MRSA. MLST sequencing on seven housekeeping genes revealed, sequence type ST772 was predominant followed by ST22. *Agr* typing identified most of the isolates (69) were agr type II (77%). By spa typing, there are 16 *spa* types were identified, among which 60% of the isolates had t657 *spa* type.

1. Introduction

Staphylococcus sps are normal micro-flora colonizing the skin and mucosal surfaces of humans and animals, including the anterior nasal nares and nasopharynx. Coagulase-negative *Staphylococcus* (CoNS) and *S. aureus* are the most common bacterial isolates found on the external ocular surface (Mshangila et al., 2013). Methicillin-resistant *Staphylococcus aureus* (MRSA) is known for causing high mortality all over the world due to its multi-drug resistance and the rapid progression of disease, subsequent to infection(Centers for Disease Control and Prevention, 2014; Kalwaje et al., 2012). Colonization with MRSA is normally asymptomatic in healthy individuals, but elderly, immunocompromised individuals and patients subject to surgical procedures display a significantly higher risk for developing MRSA infections with high concomitant mortality (Noskin et al., 2005). There have been various reports from India (Marangon et al., 2004) and the US demonstrating an increase in the prevalence of ocular MRSA infections in recent years (Asbell et al., 2008), while some other reports indicate a stable prevalence in Taiwan (Hsiao et al., 2012).

Methicillin-resistance in Staphylococci is conferred by the carriage of the Staphylococcal Cassette Chromosome *mec* (SCC*mec*), a mobile genetic element that encodes a penicillin binding protein (PBP2a), which have a lower affinity for beta-lactam antibiotics such as penicillin and methicillin, and there by allows survival during beta-lactam treatment (Hartman and Tomasz, 1981). MRSA infections were traditionally hospital-associated (HA-MRSA) and have been a major public health issue worldwide. However in the past 10–15 years, an increasing number of infections due to community-associated MRSA (CA-MRSA) have been reported from Europe and the US (Blomquist, 2006; DeLeo et al., 2010). CA-MRSA infections are mainly seen in healthy

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individuals who possess no recent contact with the healthcare system and display a distinct genetic signature when compared with HA-MRSA (Diep and Otto, 2008).

The knowledge about the ocular MRSA genotype, prevalence and epidemiology in India is limited (Nadig et al., 2012; Bagga et al., 2010; Khan et al., 2010). Hence, the purpose of this study is to estimate the prevalence, antibiotic resistance pattern and molecular type of ocular MRSA over a recent decade, spanning the years 2007–2017.

2. Materials and methods

2.1. Specimen collection and S. aureus isolation

All cases of culture proven *S. aureus* seen between January 2007 and December 2017 were identified from the records of the Ocular Microbiology Laboratory at Aravind Eye Hospital, Madurai. Ethical clearance was obtained from the Institutional Review board. Specimens including pus from lacrimal sac abscesses, corneal scrapings from keratitis patients, vitreous or aqueous fluid from cases of end ophthalmitis and buckle infection, and conjunctival or lid swabs were collected. Corneal scrapings were collected from all patients with microbial keratitis using a platinum spatula. For preseptal cellulitis samples, pus accumulated in the conjunctiva was collected using a sterile swab. In case of patients with post-cataract wound infection, pus was collected from the site of infection. The specimens were inoculated on blood agar, chocolate agar and thioglycollate broth (Hi-Media) and grown for 24 to 48 h at 37 °C. Typical staphylococcal colonies were examined under the microscope following Gram's staining; identities were further confirmed by standard biochemical tests.

2.2. MRSA identification by phenotypic method

Methicillin-resistance was confirmed on Mueller Hinton agar by standardized disk diffusion susceptibility testing against 1 μg oxacillin and 30 μg cefoxitin disks incubated at 35 °C. Zones of inhibition ≤13 mm around oxacillin (1 μg) disc and ≤ 19 mm around cefoxitin (30 μg) were deemed methicillin-resistant, as per Clinical Laboratory and Standards Institute (CLSI)standards M100-S22 (CLSI M100-S22, 2012).

2.3. Antibiotic susceptibility testing

All of the isolates were subjected to antimicrobial susceptibility testing by the Kirby-Bauer disk diffusion method against a range of antibiotics, which are commonly used in the treatment of ocular infections. The antibiotics tested include: chloramphenicol (30 μg), cefotaxime (30 mg), ciprofloxacin (5 μg), ofloxacin (5 μg), gatifloxacin (5 μg), moxifloxacin (5 μg), levofloxacin (5 μg), gentamicin(10 μg) and tobramycin(10 μg). For vancomycin MIC was determined using by the *E*-test (0.016 to 256 MCG). Antibiotic zones of inhibition around the disks were measured and recorded as sensitive, intermediate and resistant, in accordance with CLSI Standards M100-S22 (CLSI M100-S22, 2012).

2.4. Genomic DNA extraction

Over-night culture of *S. aureus* in brain heart infusion broth (Himedia) was prepared and pelleted by centrifugation at (3000) ×*g*. The cells were then suspended in lysis buffer containing phosphate buffered saline, 0.5% sodium dodecyl sulfate and 100 μg/ml proteinase K (P2308,Sigma) and lysostaphin (L9043, Sigma USA) and incubated at 37 °C for 1 h (Zhao et al., 2012). An equal volume of phenol:chloroform (1:1) mixture was then added to the cell suspension and it was vortexed. The samples were centrifuged and the aqueous phase was transferred to a fresh tube. The DNA was precipitated by the addition of 30 μl of 3 M sodium acetate and three volumes of ice cold ethanol (99%). The DNA pellet was washed twice with ice cold 70% ethanol, air-dried, and then suspended in 200 μl of TE buffer (10 mM Tris-HCl (pH 8.0), 1 mM EDTA (pH 8)). The quantity (ng/μl) and quality (260/280) of DNA was measured using the nano drop spectrophotometer (ND 1000, Thermo Scientific).

Table 1
List of primers used in this study.

Primer	Sequence	Reference
MecA1	GTA GAA ATG ACT GAA CGT CCG ATA A	Pérez-Roth et al. (2001)
MecA2	CCA ATT CCA CAT TGT TTC GGT CTA A	
MECI P2	ATCAAGACTTGCATTCAGGC	Oliveira and de Lencastre (2002)
MECI P3	GCGGTTTCAATTCACTTGTC	
RIF4 F3	GTGATTGTTCGAGATATGTGG	
RIF4 R9	CGCTTTATCTGTATCTATCGC	
RIF5 F10	TTCTTAAGTACACGCTGAATCG	
RIF5 R13	GTCACAGTAATTCCATCAATGC	
IS431 P4	CAGGTCTCTTCAGATCTACG	
pT181 R1	GAAGAATGGGGAAAGCTTCAC	
ccrC F2	GTACTCGTTACAATGTTTGG	Milheiriço et al. (2007)
ccrC R2	ATAATGGCTTCATGCTTACC	
SCCmec V J1 F	TTCTCCATTCTTGTTCATCC	
SCCmec V J1 R	AGAGACTACTGACTTAAGTGG	
CIF2 F2	TTCGAGTTGCTGATGAAGAAGG	
CIF2 R2	ATTTACCACAAGGACTACCAGC	
kdp F1	AATCATCTGCCATTGGTGATGC	
kdp R1	CGAATGAAGTGAAAGAAAGTGG	
dcs F2	CATCCTATGATAGCTTGGTC	
dcs R1	CTAAATCATAGCCATGACCG	
arcC-Up	TTGATTCACCAGCGCGTATTGTC	Enright et al. (2000)
arcC-Dn	AGGTATCTGCTTCAATCAGCG	
aroE-Up	ATCGGAAATCCTATTTCACATTC	
aroE-Dn	GGTGTTGTATTAATAACGATATC	
glpF-Up	CTAGGAACTGCAATCTTAATCC	
glpF-Dn	TGGTAAAATCGCATGTCCAATTC	
gmk-Up	ATCGTTTTATCGGGACCATC	
gmk-Dn	TCATTAACTACAACGTAATCGTA	
pta-Up	GTTAAAATCGTATTACCTGAAGG	
pta-Dn	GACCCTTTTGTTGAAAAGCTTAA	
tpi-Up	TCGTTCATTCTGAACGTCGTGAA	
tpi-Dn	TTTGCACCTTCTAACAATTGTAC	
yqiL-Up	CAGCATACAGGACACCTATTGGC	
yqiL-Dn	CGTTGAGGAATCGATACTGGAAC	
spa-1113f	TAA AGA CGA TCC TTC GGT GAG C	Aires-de-Sousa et al. (2006)
spa-1514r	CAG CAG TAG TGC CGT TTG CTT	
pan	ATG CAC ATG GTG CAC ATG C	Gilot et al. (2002)
agr1	GTC ACA AGT ACT ATA AGC TGC GAT	
agr2	TAT TAC TAA TTGAAA AGT GGC CAT AGC	
agr3	GTA ATG TAA TAG CTT GTATAA TAA TAC CCA G	
agr4	CGA TAA TGC CGT AAT ACCCG	

2.5. Molecular characterization of MRSA

2.5.1. Identification of mecA gene

All *S. aureus* strains that showed resistance to oxacillin and cefoxitin were further screened by targeting the gene coding for methicillin resistance (*mecA*) by PCR. Primer sequence of this mecA gene is listed in Table 1. *S. aureus* strains with an amplified product length of 310 bp were confirmed as MRSA.

2.5.2. Multiplex PCR for SCCmec typing

The multiplex PCR targeting eight loci (A through H) was performed for the SCC *mec* typing (Ito et al., 2001). The target genes includes *pls* in locus A (type I); *kdp* operon in locus B (type II); *mecI* in locus C (SCC *mec* types II and III); *dcs* region in locus D (types I, II, and IV); locus E located in the region between integrated plasmid pI258 and transposon Tn*554* (type III); locus F located in the region between Tn*554* and the chromosomal right junction *orfX* (type III). Loci G and H were included to distinguish structural variants IA and IIIA, respectively. Locus G is in

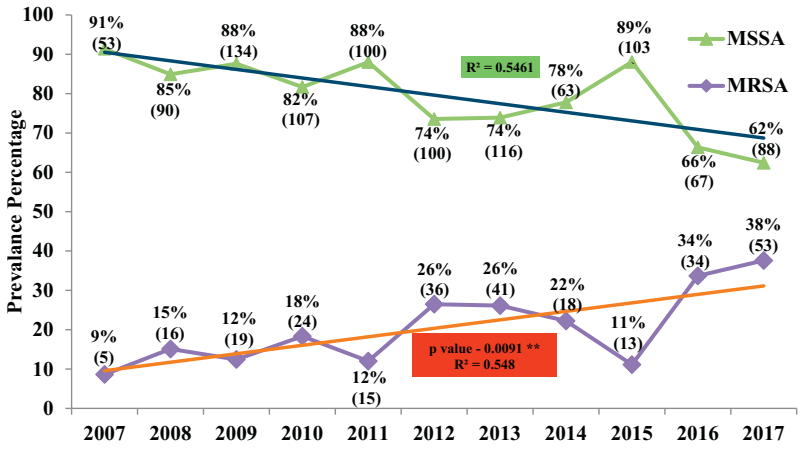

Fig. 1. Year-wise prevalence percentage of MRSA and MSSA isolates causing ocular infections from 2007 to 2017.The number within the bracket indicate the actual number of cases reported in that particular year. Trend line was drawn and the relevant Pearson $R^2$and *p* values are mentioned within the box.

the left junction between IS*431* and pUB110, locus H is in the left junction between IS*431* and pT181 (Oliveira and de Lencastre, 2002).

The multiplex PCR was performed in a 50 µl reaction volume with the PCR mixture containing the following components: 10× PCR buffer; 200 µM (each) dNTPs; 400 nM concentration each of primers CIF2F2, CIF2R2, MECI P2, MECI P3, RIF5 F10, RIF5 R13, SCC*mec* VJ1F, SCC*mec* VJ1R, PT181 R1; 200 nM concentration each of primers KDP F1, KDP R1, RIF4 F3, RIF4 R9; 800 nM concentration each of primers IS431 P4, ccrC F2, ccrCR2, dcs F2, dcs R1; 1.25 U of *Taq*; and 20 ng of template DNA. PCR amplifications were performed with the following parameters: initial denaturation at 94 °C for 4 min; 30 cycles at 94 °C for 30s, 53 °C for 30 s, and 72 °C for 1 min; and final extension at 72 °C for 4 min.

2.5.3. Multi locus sequence typing

Multi locus sequence typing (MLST) was performed targeting ~450-bp internal fragments of seven housekeeping genes, namely carbamate kinase (*arcC*), shikimate dehyrogenase (*aroE*), glycerol kinase (*glpF*), guanylate kinase (*gmk*), triosephosphateisomerase (*tpi*), phosphate acetyltransferase (*pta*) and acetyl coenzyme A acetyltransferase (*yqiL*). PCRs were carried out in 50 µl reaction volumes containing 10 ng of chromosomal DNA, 10 pmol of each primer, 1 U of *Taq* DNA polymerase, 5 µl of 10× PCR buffer, and 200 µM each of dNTPs. PCR was performed with an initial denaturation at 95 °C for 5 min, followed by 30 cycles of denaturation at 95 °C for 1 min, annealing at 55 °C for 1 min, extension at 72 °C for 1 min, followed by a final extension step of 72 °C for 5 min (Enright et al., 2000). After amplification, the PCR products were purified using QiaQuick PCR purification kit (QIAGEN GmbH, Hilden, Germany) and sequenced by Sanger sequencing chain termination method. The consensus sequences were assembled and the allelic profile was matched using the MLST database (http://saureus.mlst.net/).The seven housekeeping gene sequences of the isolates were aligned using MUSCLE, followed by phylogenetic trees construction using the PhyML 3.0 implementing a fast aLRT for branches (Anisimova and Gascuel, 2006).

2.5.4. Spa typing to sub-type MRSA isolates

S. aureus-specific staphylococcal protein A (Spa) typing was performed according to the procedure described by Shopsin et al., 1999. The *spa* gene was amplified using the SpaF1 and SpaR1 primers followed by sequencing. The PCR protocol included an initial denaturation at 95 °C for 10 min, followed by 30 cycles of denaturation at 95 °C for 30 s, annealing at 60 °C for 30 s, extension at 72 °C for 45 s, and a final extension step of 72 °C for 10 min. The consensus sequences were matched using both forward and reverse sequences and the repeat units were identified using the Ridom database.

2.5.5. Agr group-specific multiplex PCR

Accessory gene regulator of *S. aureus* (*agr)*sequences were amplified from 10 ng of purified nucleic acid in a 25 µl reaction mixture containing 0.3 µM of the following individual primers: *pan, agr1, agr2, agr3* and *agr4*. The *agr* were identified based on the amplified product size of 441 bp for *agr* group-1, 575 bp for *agr* group-2, 323 bp for *agr* group-3 and 659 bp for *agr* group-4 strains. PCR amplification was done with the following thermal cycling program: initial denaturation at 94 °C for 5 min; 26 cycles at 94 °C for 30s, 55 °C for 30s and 72 °C for 60s; and final extension at 72 °C for 10 min (Gilot et al., 2002). Amplification products was electrophoresed in a 1.5% agarose gel containing ethidium bromide and visualized under UV trans-illumination.

2.6. Statistical analysis

All statistical analyses were performed using Graphpad Prism V5.0, STATA 11.1 and Microsoft Excel. Statistical significance was set at *p* value of < 0.05. Chi-square trend test and chi-square test were used for comparison across two sets of data, Pearsons' correlation R^2 with p value was used for trend analysis.

3. Results

3.1. Prevalence of MRSA

During the period of ten years (January 2007 to December 2017), a total of 1306 isolates of *S. aureus* were isolated from ocular infections in Aravind Eye Hospital, Madurai. Out of these, 274 (21%) isolates were confirmed as methicillin-resistant by antibiotic disk diffusion test and the remaining 1032 (79%) were methicillin-susceptible *Staphylococcus aureus*. The total number of MRSA isolates obtained increased significantly over the course of the ten year study with the percentage of ocular MRSA infections increasing from 9% to 38% (Fig. 1). The majority of the MRSA strains were isolated from lacrimal sac (32%) infections, followed by lid disorder (20%) which includes external hordeolum and blepharitis (Table 2). Infectious keratitis by MRSA contributed to 16% of total cases while orbital infections, including cellulitis, dacryocystitis and canaliculitis accounted for 12% of total cases (Table 2; Fig. 2).

3.2. Antiobiotic resistance pattern of MRSA

Drug resistance pattern against various antibiotic agents was found to be highly variable between MRSA and MSSA isolates. Overall, MRSA strains exhibited highest percentage of resistance to ciprofloxacin (96%) and ofloxacin (92%) followed by moxifloxacin (88%),

Table 2
Clinical diagnosis associated with ocular MRSA infections for the period under study (2007 to 2017).

Ocular disorder	No of MRSA isolates (%)
Lacrimal disorder[a]	89 (32)
Lid disorder[b]	55 (20)
Keratitis	45 (16)
Orbital infection	34 (12)
Conjunctival disorder	16 (6)
Wound Infection	14 (5)
Suture infection	7 (3)
Endophthalmitis	6 (2)
Device-induced[c]	6 (2)
AC exudate	2 (1)
Total	274

[a] Lacrimal disorder includes dacryocystitis and canaliculitis.
[b] Lid disorder includes cellulitis, lid abscess and hordeolum.
[c] Device-induced includes infected sling, buckle infection.

Table 3
Overall resistance pattern towards the commonly used antibiotics for MSSA and MRSA, averaged over a ten year period, from 2007 to 2017.

Antibiotics	MSSA (1027)		MRSA (274)	
	No	%	No	%
Ciprofloxacin	679	66	263	96
Ofloxacin	582	57	252	92
Moxifloxacin	561	55	243	88
Levofloxacin	532	52	239	87
Cefotaxime	288	28	236	86
Tobramicin	347	34	235	85
Gentamycin	319	31	228	83
Gatifloxacin	520	51	216	79
Chloramphenicol	54	5	24	9
Vancomycin	0	0	0	0

levofloxacin (87%), cefotaxime (86%), tobramycin (85%), gentamicin (83%) and gatifloxacin (79%) (Table 3). The lowest resistance (9%) was observed for chloramphenicol and none of the MRSA strains were resistant to vancomycin. MSSA isolates also exhibited a broadly similar pattern of antibiotic resistance, but the resistance levels were lower when compared with the MRSA strains (Table 3). The highest resistance was found for ciprofloxacin (66%) followed by ofloxacin (57%), moxifloxacin (55%), levofloxacin (52%), gatifloxacin (51%), cefotaxime (28%), tobramycin (34%), and gentamicin (31%). MSSA strains showed very minimal resistance to chloramphenicol (5%)and similar to MRSA, there was no resistance to vancomycin (Table 3; Fig. 3).

The percentage of antibiotic resistance for each of the antibiotics under study was calculated for the duration of the study. Over the ten year period, there was no statistically significant increase in antibiotic resistance among the MRSA isolates. In the case of the MSSA isolates, resistance to the fluroquinolones (ciprofloxacin, gatifloxacin,

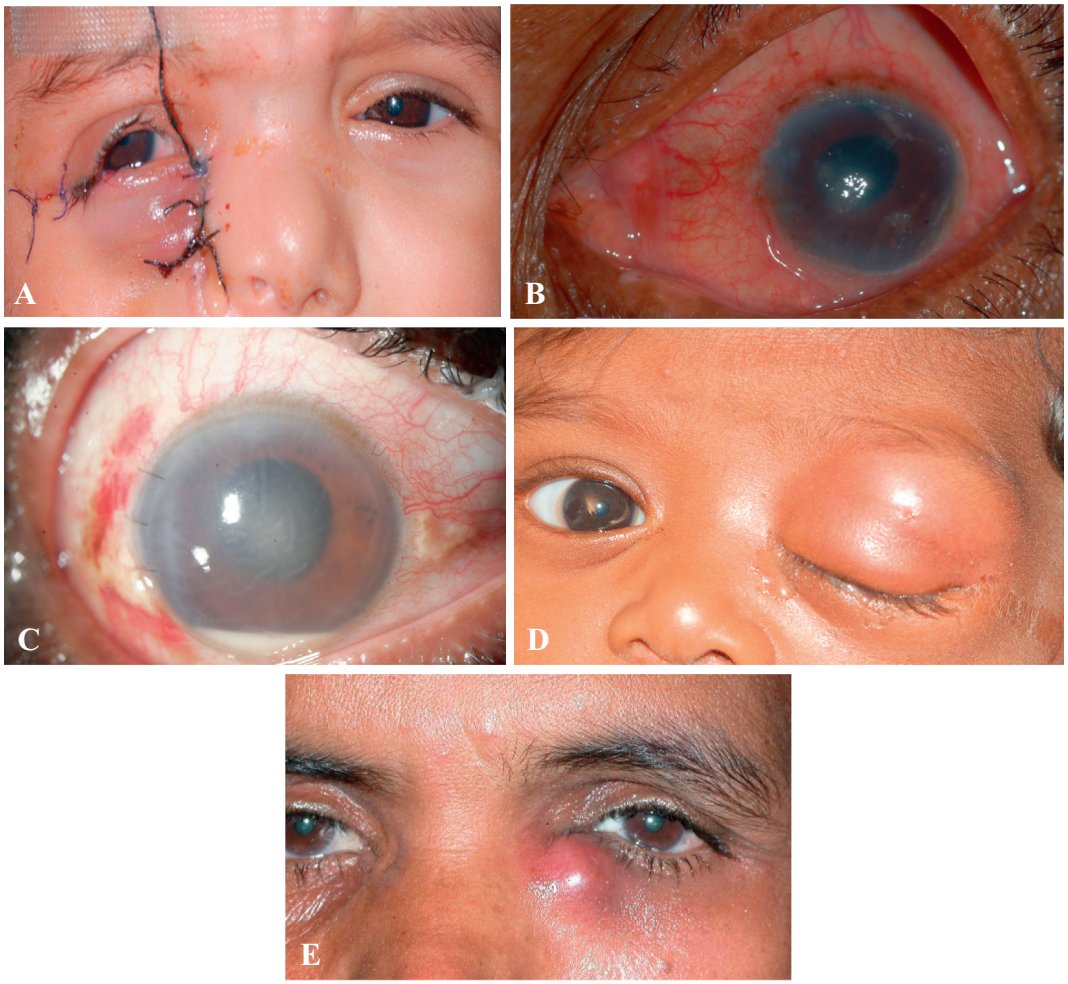

Fig. 2. Clinical pictures of ocular *S. aureus* infections. A. Suture infection caused by MRSA after lower lid slit correction; B. Slit-lamp picture showing central corneal ulcer with neovascularization; C. Slit-lamp picture of a case of endophthalmitis showing central corneal edema with hypopyon; D. Swollen erythematous eyelid in a case of orbital cellulitis; E. Abscess of lacrimal sac secondary to acute dacryocystitis.

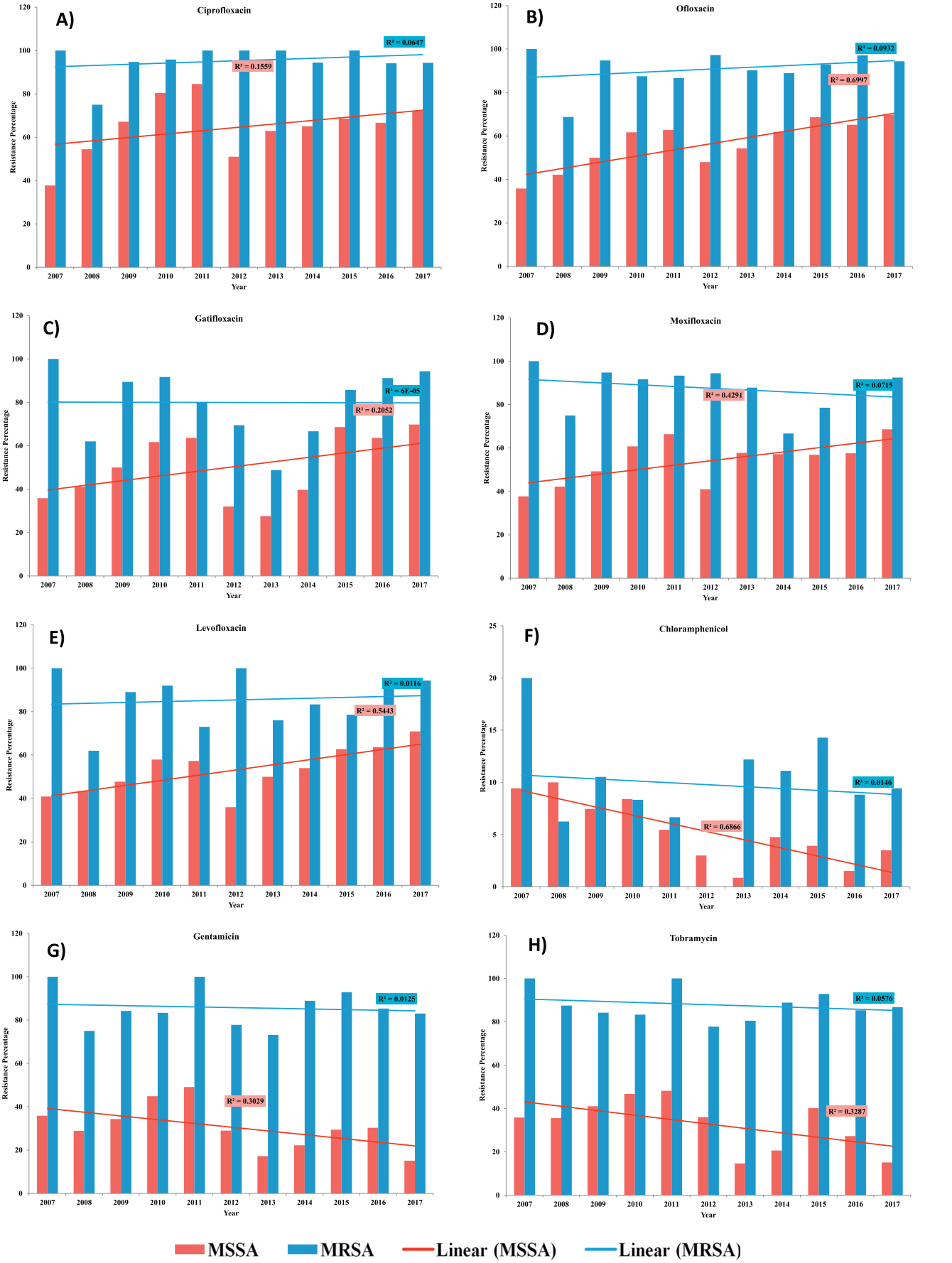

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Fig. 3. Extent of antibiotic resistance of MRSA and MSSA isolates shown for a few select antibiotics, from 2007 to 2017 A) Ciprofloxacin, B) Ofloxacin, C) Gatifloxacin, D) Moxifloxacin, E) Levofloxacin, F) Chloramphenicol, G)Tobramycin and E) Gentamicin. Linear trend line was drawn and the Pearson R2value was calculated to examine correlation trends. Only significant p values are mentioned in the graph along with the R2value. p value * - < 0.05; **- < 0.001.

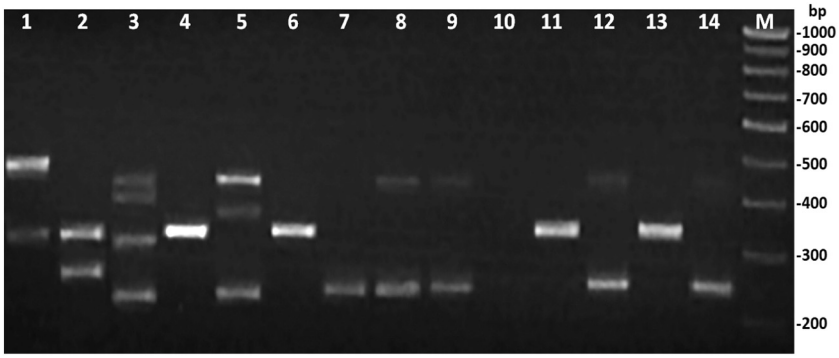

Fig. 4. SCCmec typing multiplex PCR amplified product banding pattern in 1.5% agarose gel. Reference strains Lane 1–5; Lane 1- SCCmec type I - NCTC 10442 (495 bp and 342 bp), Lane 2 - SCCmec type II-N315 (342 bp and 284 bp), Lane 3- SCCmec type III 85/2082 (449 bp, 414 bp, 303 bp and 243 bp), Lane 4- SCCmec type IV- JCSC 4744 (342 bp), Lane 5- SCCmec type V - WIS-JCSC 3624 (243 bp and 449 bp) had an additional band at 377 bp which is an amplicon of the J1 region, Lane 6–9 and 11–14 - Clinical MRSA isolates, Lane 10- No template control, M- 100 bp DNA marker.

moxifloxacin, ofloxacin and levofloxacin)was found to increase significantly (Fig. 3).

3.3. Genotyping of methicillin-resistant Staphylococcus aureus

Molecular characterization was done for 90 MRSA isolates that were randomly selected from a total of 274 ocular isolates. All of the 90 MRSA strains tested were positive for *mecA* by PCR.

3.3.1. SCCmec typing

Molecular characterization of SCC*mec* type was done by multiplex PCR for the selected strains. All of the five SCC*mec* types were identified based on the banding pattern of amplicons obtained following agarose gel electrophoeresis, when compared with the banding pattern of reference strains (Fig. 4).The type V reference strain (WIS (JCSC 3624)) used in this study had an additional band at 377 bp which is an amplicon of the J1 region. The clinical strains of this study that belonged to type V did not have this band (Fig. 4).

Among the 90 isolates that were typed,57 were identified to have SCC*mec* type V (63%) followed by 26 have SCC*mec* type IV (29%). Only 2 isolates (2%) were found to beHA-MRSA, which harbored SCC*mec* type III. The banding pattern for 5 isolates (6%) did not matched with that of any of the known reference strains and it could not be typed (Table 4).

3.3.2. Multilocus sequence typing (MLST)

MLST sequencing was performed for seven housekeeping genes which revealed 55 isolates were ST772 (61%) belonging to SCCmec V, followed by 12 isolates of ST22 and 8 isolates of ST1037 of SCCmec type IV, accounting for 13% and 9% of all isolates, respectively. As mentioned earlier, only 2 strains were identified as HA-MRSA (*SCCmec* III), typed as ST368 and ST239. The other 5 non-typeable strains belonged to the ST672 (2), ST2066 (2) and ST121 (1) (Table 4).

Table 4
Genotypic characterization of MRSA isolates (n = 90). Numbers in parentheses denotes number of isolates.

SCC*mec* type	MLST	*agr* type	*spa* type
Type V (57)	ST772 (55)	II (55)	t657(54), t3387 (1)
	ST72 (1)	I (1)	t8317 (1)
	ST1 (1)	III (1)	t386 (1)
Type IV (26)	ST22 (12)	II (7), I (5)	t902 (5), t852 (5), t005 (2)
	ST1037 (8)	II (7), I (1)	t852 (7), t11714 (1)
	ST30 (4)	III (4)	t021 (3), t363 (1)
	ST2124 (1)	I (1)	t902 (1)
	ST8 (1)	III (1)	t064 (1)
Type III (2)	ST368 (1)	I (2)	t425 (1)
	ST239 (1)		t037 (1)
Non typeable (5)	ST672 (2)	I (2)	t7191 (2)
	ST2066 (2)	III (2)	t1598 (2)
	ST121 (1)	IV (1)	t2700 (1)

3.3.3. Agr typing

Agr typing revealed that all of the ocular MRSA isolates could be classified into one of four *agr* groups. Most of the isolates (69) were found to belong to *agr* type II (77%) followed by *agr* type I (13%) and *agr* type III (9%). Only one isolate had *agr* type IV with ST121 sequence type (Table 4).

3.3.4. Spa typing

A total of 16 *spa* types were identified and 60% of the isolates had *spa* type t657, which mainly seen in ST772. The *spa* type t852 (13%) and t902 (7%), were seen in SCC *mec* IV CA-MRSA strains and the remaining *spa* types were present at very low percentages (Table 4).

3.3.5. Phylogenetic analysis

The genetic relatedness of methicillin-resistant *Staphylococcus aureus* isolates under investigation in this study has been represented as a maximum likelihood tree constructed using the sequences of seven housekeeping genes, obtained from MLST profiles. Four major clusters were revealed using these sequences (Fig. 5). The phylogenetic analysis indicates that ST30 and ST121 are likely to be the ancestral clones from which other strains could have emerged. ST772, the clone causing the majority of ocular infections showed close similarity to ST1, which is

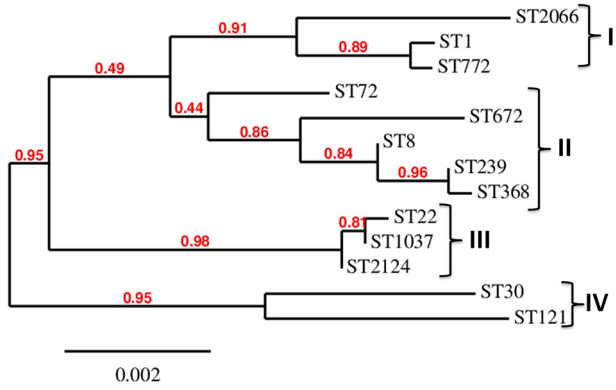

Fig. 5. The phylogenetic tree was constructed using the seven housekeeping gene sequences, which were aligned using MUSCLE, followed by phylogenetic trees construction using the PhyML 3.0 implementing a fast aLRT for branches (Anisimova and Gascuel, 2006).

mainly because of a single change in the allelic profile of the *pta* gene.

4. Discussion

MRSA is a major concern globally and in recent years have revealed an increasing incidence of MRSA in ocular infections in areas of particular concerns are vision-threatening post-operative endophthalmitis and keratitis (Asbell et al., 2008). The current study hence sheds light on the prevalence, antimicrobial susceptibility pattern and molecular type of MRSA in ocular infections, assayed over a decade, spanning the years 2007 to 2017.

A report from India had shown an increase in the proportion of ophthalmic MRSA infections from 26% to 38% in the period from 2006 to 2008 (Bagga et al., 2010).A similar surveillance study from New York also demonstrated an increasing prevalence of ocular MRSA infections from 29.5% in 2000, to 41.6% in 2005, with MRSA being more commonly associated with serious ocular infections than MSSA (Asbell et al., 2008). Another ten-year study from Taiwan has shown that ocular MRSA infections displayed a stable average prevalence of 52.8% between 1999 and 2008 (Hsiao et al., 2012). In our study conducted in South India, a significant increasing trend of ocular MRSA infections was observed over the time period of study, from 9% in 2007 to 38% in 2017 (Fig. 1). This raises future concerns over the limited choice of antibiotics that are currently available to treat such conditions.

With reference to ocular disorders most commonly associated with MRSA infections, Elsahn et al. have demonstrated that the majority (52.5%) of their MRSA isolates were obtained from *S. aureus* caused keratitis. Another study showed that catastrophic eye infections caused by MRSA include orbital cellulitis, panophthalmitis and corneal flap melt after LASIK, and the incidence of these ocular infections was found to increase from 4.1% in 1999 to 16.7% in 2006 (Freidlin et al., 2007). In our study, however, the most common manifestation of ophthalmic MRSA infection was lacrimal sac disorder followed by orbital infections (Table 2). Similar non-sight threatening infections have been reported from London and also from California (Shanmuganathan et al., 2005; Asbell et al., 2008).

Multidrug resistance of MRSA isolates is a major world-wide concern. In our study, there is an increased resistance to fluoroquinolones. Cephalosporins and cefotaxime resistance was observed among both MRSA and MSSA isolates, with MRSA showing higher resistance to both, when compared with MSSA (Fig. 3).The MSSA strains showed a significant increase in resistance to fluoroquinolones when compared with the MRSA isolates (Fig. 3). Among all fluoroquinolones, gatifloxacin has been previously proposed to be the most effective in treating ocular MRSA infections (Bagga et al., 2010) but in our study, there is an increase in resistance to this antibiotic among the MSSA strains, but not among the MRSA strains (Fig. 3). Both the MRSA and MSSA isolates showed 100% sensitivity to vancomycin (Table 3), eventhough the current recommendations to not use vancomycin as the first drug of choice to treat multi-drug resistant MRSA (Sakoulas et al., 2004). Abundant use of broad spectrum antibiotics like fluroquinolones have slowly replaced the usage of chloramphenicol over a period of time, this might be the reason for reduction in the pattern of resistance shown by MRSA and MSSA against chloramphenicol.

Initial discrimination between hospital and community-associated MRSA is important in the context of epidemiology. Based on the screening conducted in our study, 92% of the ocular MRSA isolates belonged to CA-MRSA(SCC*mec* types IV and V) (Table 4). Based on the major epidemiologic studies globally, the prevalence of CA-MRSA has substantially increased worldwide over recent decades, and successful CA-MRSA clones are usually associated with specific geographical locations. Previous study by Nadig et al., 2012 and another study by Dhawan et al., 2015 in skin and soft tissue infection, had found that the majority of MRSA strains isolated from ocular infections were CA-MRSA and belonged to ST772. In the current study, in addition to ST772, the UK EMRSA-15 clone ST22 was also found to be prevalent in ocular infections, with an incidence of 13% (Table 4). In systemic infections, these two clones have already been reported to be predominant in India (D'Souza et al., 2010; Shambat et al., 2012) and we now report their incidence in ocular infections. The study by D'Souza et al. (2010) from India had reported that in the case of systemic MRSA infections, 25% of the isolates were hospital-associated, while 75% were community-associated, it seems very clear that the incidence of CA-MRSA in ocular infections is increasing. However, another report from North India, that examined strains isolated from keratitis patients concluded that the types of their isolates conformed to HA-MRSA, rather than CA-MRSA (Khan et al., 2010). It hence appears that there might be some interesting geographical patterns to explore further here, in future clinical studies.

The predominance of CA-MRSA infections may be secondary to the persistent colonization of this bacterium in the human nasopharynx. We detected one HA-MRSA type (ST239) isolated from a patient with an infected orbital sling, who had been previously hospitalized for ptosis correction. Another HA-MRSA type, ST368 was isolated from a patient with a corneal ulcer.

In our study we found four isolates with ST30 were collected from pus samples of patients with cellulitis cases. The ST30 MRSA clone was also known as the Southwest Pacific (SWP) clone, USA1100 or West Samoan Phage Pattern (WSPP) Clone. This CA-MRSA strain was emerged in the Oceania (Adhikari et al., 2002) and become a pandemic clone, reported in many countries including Germany, Switzerland, the UK, Australia (Monecke et al., 2008), Hong Kong, Taiwan (Takano et al., 2008), Scandinavia and Latvia (Miklasevics et al., 2004).

ST22 and ST1037 are found at roughly equal incidences, next to ST772, and together they account for 22% of all MRSA isolates that were typed (Table 4). There is a single base pair difference in the *aroE* allele between these 2 sequence types, and they share *spa* type t852. A previous study by Ruimy et al. (2009) that had carried out phylogenetic analysis of several different carrier *S. aureus* isolates had already reported on the close similarity between ST22 and ST1037.

The agr typing revealed *Agr* type II was predominant and found in 77 isolates (76%) followed by 12 isolates (13%) of agr type I, 8 isolates (9%) of the agr type III and only one isolates of agr Type IV. Type IV is not much prevalent, and is found to be present in ST50 and ST121 (Robinson et al., 2005).Our studies also revealed the existence of ST121belonging to *agr* type IV, which was isolated from suture infiltrate of a patient underwent cataract surgery, that was not SCC *mec* typeable (Table 4). Earlier this ST121has been reported from Cambodia and Portugal, where strains were isolated from children with skin and soft tissue infections and other systemic illnesses (Nickerson et al., 2011;Conceição et al., 2011). Most of the isolates typed in this study belongs to ST772 which had *spa* type t657.The comprehensive typing exercise undertaken herein illuminates the inherent variation present in the MRSA isolates of this study.

Treatment of MRSA infections is a daunting challenge for clinicians due to its multi-drug resistance. The emergence of CA-MRSA is not limited to the community anymore, since these strains are progressively introduced into hospital settings as well, when CA-MRSA patients visit hospitals. Since our study demonstrates that CA-MRSA strains are more potent than HA-MRSA strains in causing ocular infections, it would be a worthwhile future exercise to investigate the environmental and geographical reasons for the preponderance of the CA-MRSA pathogens.

Declaration of interest

None.

Conflict of interest

we hereby declare no conflicting relationship exists for any of the authors.

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