Dear Editor,

Increasing antibiotic resistance in *Staphylococcus aureus*, a leading cause of ulcerative keratitis in the developing world, is of great concern.[1] Its ability to form biofilms on ocular surfaces enhances antibiotic resistance through several mechanisms.[2] Understanding of the resistance patterns amongst clinical isolates is a prerequisite for devising better treatment strategies and measures to mitigate emerging antibiotic resistance.

A total of 42 independent *Staphylococcus* isolates from cases of ulcerative keratitis around Kanpur were evaluated for antibiotic resistance using antibiotic discs (Hi Media, Mumbai, India) as per CLSI guidelines.[3] The ability of the isolates to form biofilms was characterised using the static microtitre plate assay.[4] Microbiological and biochemical characterisation of the isolates was performed as per Bergey’s determinative bacteriology.[5] Of these, 75% (30/40) isolates were *S. aureus* and 23.8% (10/42) were coagulase-negative *Staphylococcus epidermis* and 4.7% (2/42) were *Micrococcus* sp.

85.72% (36/42) of the isolates were found to be high biofilm formers and 83% (35/42) were biofilm forming, multiple drug resistant (resistant to three or more classes of antibiotics). Pearson’s correlation between biofilm formation and antibiotic resistance was found for *S. aureus* isolates of 0.6. Table 1 details the percentage resistance of total and biofilm forming isolates to the various antibiotics. Of the total isolates, 83.3% (35/42) were found to be oxacillin resistant, 57.14% (24/42) were ceftriazone resistant, 54.7% (23/42) were vancomycin resistant and 47.6% (20/42) were tobramycin resistant. It is alarming to note the high percentage of resistance to a number of antibiotics preferentially used for treatment of ocular infections, such as fluoroquinolones. Frequent usage of moxifloxacin in the treatment of ocular infections may be the cause of 76.2% (32/42) resistance to the fourth-generation fluoroquinolone moxifloxacin over ofloxacin (30.9%; 13/42) and levofloxacin (40.4%; 17/42). Low resistance is reported to gentamicin (26.1%; 11/42) which is less frequently used in ocular infections due to problems of poor ocular penetration. Low resistance to extended β lactamase antibiotic imipenem (4.7%; 2/42) is likely a consequence of drug usage only in emergency situations. Judicious use of emerging drugs is advisable as high antibiotic resistance is being measured in biofilm forming, methicillin-resistant *S. aureus* (MRSA) ocular infections to the most commonly used ophthalmic drugs.

**Acknowledgement**

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**Table 1: Antibiotic resistance and biofilm formation in ulcerative keratitis isolates**

| Isolates     | CTR | GEN | IPM | LE  | MO  | OF  | OX  | TB  | VA  |
|--------------|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| *S. aureus*  |     |     |     |     |     |     |     |     |     |
| N = 30 Total| 17(56.5) | 10(33.3) | 1(3.3) | 13(43.3) | 23(76.6) | 10(33.3) | 26(86.6) | 17(56.5) | 17(56.5) |
| Biofilm formers N = 27 | 16(53.3) | 9(30) | 1(3.3) | 12(40) | 21(70) | 9(30) | 24(80) | 16(53.3) | 13(43.3) |
| *S. epidermis* |     |     |     |     |     |     |     |     |     |
| N = 10 Total | 6(60) | 0(0) | 0(0) | 3(30) | 8(80) | 2(20) | 8(80) | 2(20) | 5(50) |
| Biofilm formers N = 8 | 4(40) | 0(0) | 0(0) | 2(20) | 4(40) | 1(10) | 4(40) | 0(0) | 2(20) |
| *Micrococcus* sp. |     |     |     |     |     |     |     |     |     |
| N = 2 Total | 1(50) | 1(50) | 1(50) | 1(50) | 1(50) | 1(50) | 1(50) | 1(50) | 1(50) |
| Biofilm formers N = 2 | 1(50) | 1(50) | 1(50) | 1(50) | 1(50) | 1(50) | 1(50) | 1(50) | 1(50) |
| **Total N = 42** | 32(76.2) | 11(26.1) | 2(4.7) | 17(40.4) | 32(76.2) | 13(30.9) | 35(83.3) | 20(47.6) | 23(54.7) |
| **Biofilm formers** | 21(50) | 10(23.8) | 2(4.7) | 15(35.7) | 26(61.9) | 11(26.1) | 29(69) | 17(40.4) | 16(38) |

CTR, Ceftriazone (30 mcg); GEN, Gentamicin (10 mcg); IPM, Imipenem (10 mcg); LE, Levofloxacin (5 mcg); MO, Moxifloxacin (5 mcg); OF, Ofloxacin (5 mcg); OX, Oxacillin (1 mcg); TB, Tobramycin (10 mcg); VA, Vancomycin (30 mcg) Figure within parentheses indicates percentage resistance.
Dear Editor,

The emergence of multidrug-resistant methicillin-resistant *Staphylococcus aureus* (MDR MRSA) resulted in the establishment of selective pressure that lead to development of vancomycin intermediate and vancomycin-resistant *Staphylococcus aureus* (VISA and VRSA). The increasing levels of MIC of vancomycin in MRSA must act as an alarm for vancomycin abusers.

Vancomycin resistance is difficult to detect in the clinical microbiology laboratory as it is not the homogenous characteristic of the majority of staphylococci. Disc diffusion sensitivity testing using the standard 30 µg vancomycin disc frequently misclassifies intermediately susceptible isolates as fully susceptible. This study composed of 250 consecutive coagulase positive staphylococci isolated from various clinical specimens of indoor patients to know the presence of VISA/VRSA. All the isolates were subjected to susceptibility testing by Kirby–Bauer disc diffusion method and brain heart infusion vancomycin screen agar (BHI-VSA) test (6 µg vancomycin/ml). Minimum inhibitory concentration (MIC) for vancomycin and oxacillin was calculated by broth macrodilution method.

Tests were performed according to CLSI criteria. ATCC 29213 strain was used as a reference strain. The 30 µg cefoxitin disc and MIC testing revealed 115 (46%) MRSA strains. All isolates including MRSA were sensitive to vancomycin by all the three methods used. A total of 115 (46%) strains showed MIC of 0.5 µg/ml, 128 (51.2%) of 1 µg/ml, and 7 (2.8%) of 2 µg/ml against vancomycin. The strains that showed MIC of 2 µg/ml were cross-checked by E-test and the results matched. Resistance to ciprofloxacin, erythromycin, amikacin, and linezolid among MRSA was 67.8%, 61.7%, 37.4%, and 1.7% respectively.

In contrast to the recent reports of vancomycin intermediate and resistant strains from various parts of the country, our study revealed 100% sensitivity of MRSA to vancomycin. This might be because of less usage of glycopeptides as first line of drug. However, 2.8% strains showed MIC on higher side of the susceptible range, suggesting prudent use of glycopeptides.

References

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