

# MRSA and MRSE: Its prevalence, antibiotic sensitivity and correlation with biofilm formation observed in various clinical isolates

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## Abstract

Biofilm formation has become a major factor in enhancing the antibiotic resistance of staphylococci. In the present study, we observed methicillin resistance and further biofilm production by two methods – Congo red agar method and Christenson's test tube method and compared the antibiotic resistance pattern between biofilm producers and non biofilm producers. 102 *S. aureus* and 168 *S. epidermidis* were isolated from various clinical samples with 70.5% and 79.7% methicillin resistance resp. 51.4% MRSA and 61.2% MRSE were biofilm producers. Antibiotic sensitivity was observed in biofilm producing *S. epidermidis* and *S. aureus* for Vancomycin (63%/85%), Doxycycline (48%/61%), Tetracycline (41%/52%), Cephalexin (19%/15%), Cefotaxime (41%/43%), Ceftriaxone (31%/37%), Cotrimoxazole (10%/11%), Ciprofloxacin (45%/52%), Amoxicillin (34%/35%), Ampicillin (4%/2%), Amoxycylave (26%/17%), Erythromycin (6%/14%), Amikacin (71%/76%), Gentamycin (66%/65%) and for urinary isolates Norfloxacin (9%/11%) and Nitrofurantoin (57%/78%) which was significantly less as compared to non biofilm producers.

**Keywords:** Biofilm, methicillin resistance, staphylococci, antibiotic sensitivity.

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## INTRODUCTION

Biofilm forming organisms are a great cause of many nosocomial infections<sup>1</sup>. According to some reports, over 65% of hospital-acquired infections occur by the infecting organisms that are capable of producing biofilms<sup>2</sup>. Certain surface protein i.e. extracellular proteins, capsular polysaccharides, adhesins (PS/A) are involved in regulation of biofilm production. The *ica* gene codes for intracellular adhesion (ICA) and may also code for TS/A and is required for biofilm production<sup>2,3,4</sup>. It includes processes like initial bacterial adhesion to the surface and bacterial communication known as quorum sensing.<sup>5</sup> *Staphylococcus aureus* is a known agent forming biofilms

on different surfaces<sup>6</sup>. The chronic infections that are caused by *S. aureus*, persist and increase the rate of morbidity and mortality in human population due to the development of biofilm produced<sup>7</sup>. Slime (mucooid exopolysaccharide) plays a major role as a virulence factor in *Staphylococcus epidermidis* as well.<sup>8</sup> Microbial biofilms are seen to be associated with lot of persistent infections which respond poorly to antibiotic therapy and can withstand host immune response<sup>9,10</sup>. This mechanism also helps the spread of antibiotic resistant strains in nosocomial infection<sup>10</sup>. Methicillin resistant staphylococci that have the ability of biofilm formation can become resistant to the most currently use antibiotics<sup>6</sup>. Infections by methicillin resistant strains are life-threatening due to emergence of multidrug resistance strains along with occurrence of isolates capable of forming strong biofilms<sup>11</sup>. In this study we screened 736 total isolates of various clinical samples at Department of Microbiology, RNT Medical College and Hospital, Udaipur out of which we observed 270 staphylococcal isolates and studied them for methicillin resistance and further biofilm production was observed by two different methods- Congo red agar method and Christensons test tube method and their antibiotic susceptibility pattern was tested for various antibiotics.

## AIMS AND OBJECTIVE

To evaluate methicillin resistance in staphylococcal isolates and correlate with biofilm formation and further antibiotic sensitivity pattern to other antibiotics.

### Inclusion Criteria

Pure culture isolates with single type of bacterial growth

### Exclusion Criteria

Mixed growth

## MATERIAL AND METHOD

270 isolates of staphylococci were isolated from various clinical samples from January 2014 to September 2014. All the isolates were observed for methicillin resistance and biofilm formation and further antibiotic sensitivity pattern.

Methods of biofilm formation.

### Christensons Test Tube method (CTTM)

A qualitative assessment of biofilm formation was determined as described by Christensen *et al.*<sup>12</sup> TSBglu (10mL) was inoculated with loopful of microorganism from overnight culture plates and incubated for 24 hours at 37°C. The tubes were decanted, washed with saline (pH 7.3) and dried. Dried tubes were stained with crystal violet (0.1%). Excess stain was removed and tubes are washed with deionized water. Tubes were then dried in inverted position and observed for biofilm formation<sup>13</sup>. A visible film lined the wall and bottom of the tube is considered as positive biofilm formation. Ring formation at the liquid interface is not indicative of biofilm formation. Tubes were examined and the amount of biofilm formation is scored as positive or negative. Experiments are performed in triplicate and repeated three times<sup>14</sup>.

### Congo red Agar method (CRA)

Freeman *et al.*<sup>8</sup> had described an alternative method of screening biofilm formation; which requires the use of a specially prepared solid medium -brain heart infusion broth (BHI) supplemented with 5% sucrose and Congo red. The medium was composed of BHI (37 gms/L), sucrose (50 gms/L), agar no.1 (10 gms/L) and congo red stain (0.8 gms/L). Congo red was prepared as concentrated aqueous solution and autoclaved at 121°C for 15 minutes, separately from other medium constituents and is then added when the agar had cooled to 55°C. Plates are inoculated and incubated aerobically for 24 to 48 hours at 37°C. Positive result is indicated by black colonies with a dry crystalline consistency. A darkening of the colonies with the absence of a dry crystalline colonial morphology is considered an

indeterminate result. Red or pink colored colonies indicate negative result<sup>13</sup>. Controls included are one uninoculated media as negative and a biofilm producing strain *S.epidermidis* ATCC 35984 as a positive control. Strain giving both test positive are considered positive. In case of discrepancy in results isolate is not included as it cannot be confirmed by PCR for *ica* gene production

### Antibiotic susceptibility testing

It is done by Kirby Baur disk diffusion method using the reference strain *S. aureus* ATCC 25923 using commercially available discs from Himedia, Mumbai. Colonies are inoculated into peptone water and turbidity is adjusted at 0.5 McFarland standard. Broth culture is spread on the plate to make lawn culture on Mueller Hilton agar. Discs are applied on surface of agar and plates are incubated overnight at 30-35°C in ambient air. Results are interpreted using CLSI guidelines<sup>15</sup>. Antibiotic sensitivity pattern was observed by following antibiotics Amikacin, Gentamycin, Cephelaxin, Cefotaxime, Ceftriaxone, Erythromycin, Amoxicillin, Ampicillin, Amoxycylave, Ciprofloxacin, Cotrimoxazole, Doxycycline, Tetracycline, Vancomycin, Norfloxacin, Nitrofurantoin. Methicillin resistance was observed by using Oxacillin disc (1µg) by above method.

## RESULTS AND OBSERVATIONS

Total 270 samples of staphylococci were studied out of which 102 were *S. aureus* and 168 were *S. epidermidis*. Of these 134 (79.7%) *S. epidermidis* and 74 (70.5%) *S. aureus* were found to be methicillin resistant as shown in graph 1.

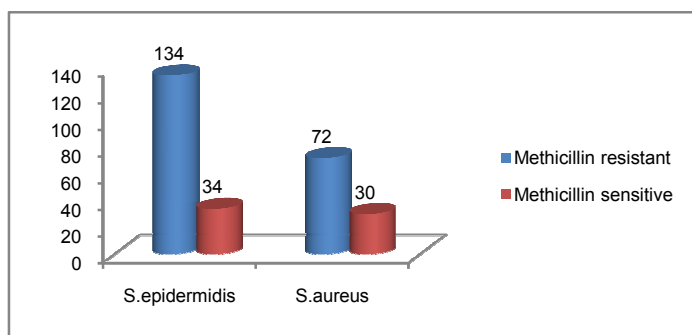


Figure 1: Methicillin resistance among staphylococcal isolates

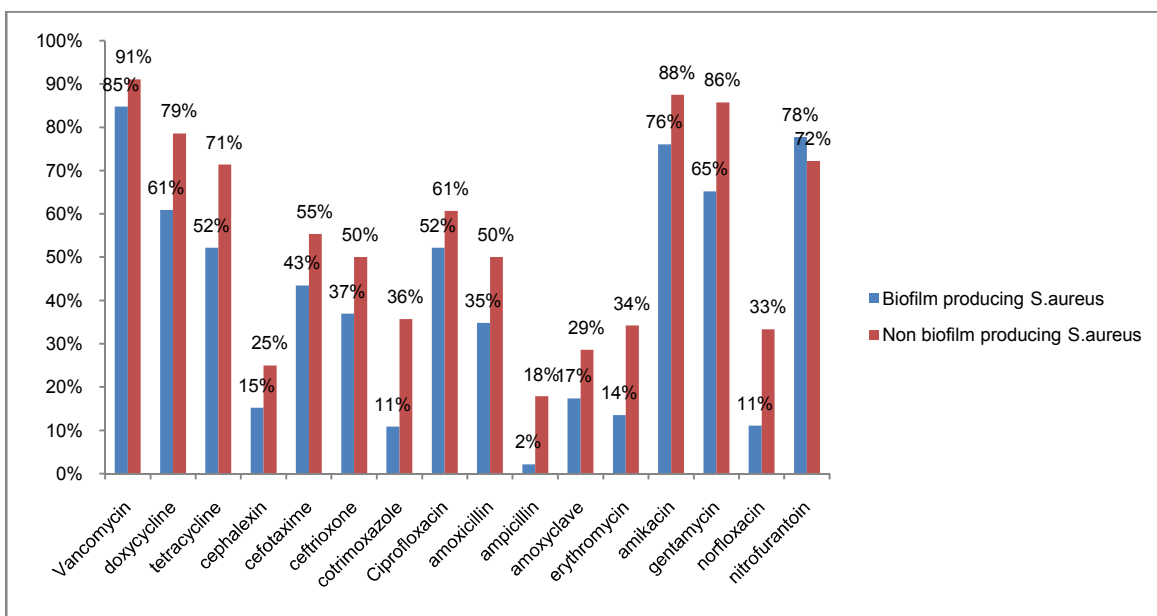
Out of 270 samples, 140 (51.9%) were biofilm producers (*S.epidermidis*=94, *S.aureus*=46) Distribution of biofilm production among methicillin resistant strains is shown in table 1:

Table 1: Biofilm production in relation to methicillin resistance

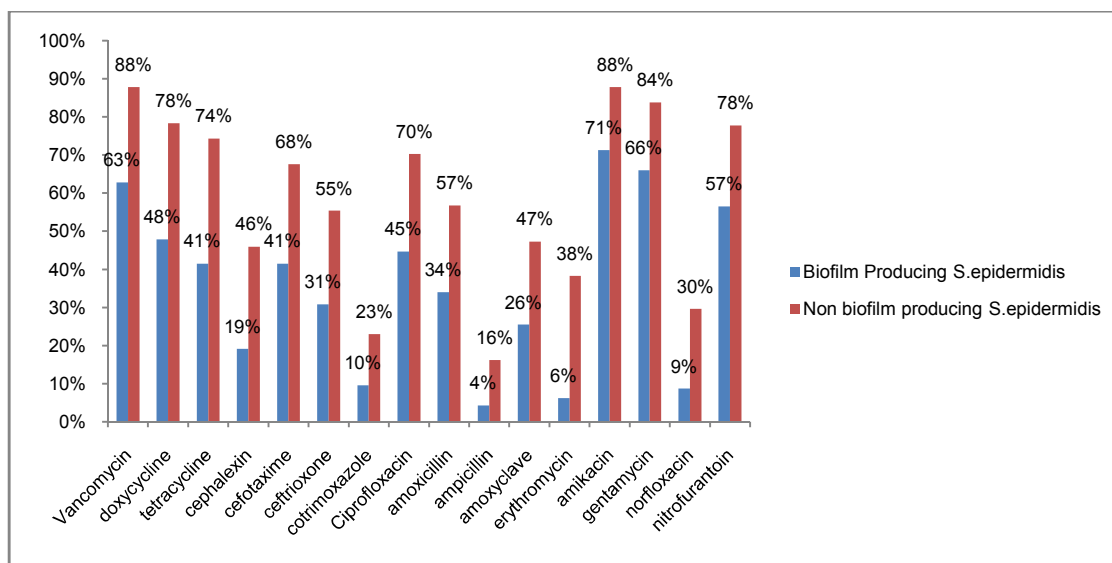
| Biofilm production    | MRSA      | MSSA    | MRSE      | MSSE      |
|-----------------------|-----------|---------|-----------|-----------|
| Biofilm producers     | 37(51.4%) | 9(30%)  | 82(61.2%) | 12(35.4%) |
| Non biofilm producers | 35(48.6%) | 21(70%) | 52(38.8%) | 22(64.7%) |

**Table 2:** Sample wise distribution of biofilm producers and non biofilm producers

| SAMPLES       | S.aureus          |                       | S.epidermidis     |                       |
|---------------|-------------------|-----------------------|-------------------|-----------------------|
|               | Biofilm producers | Non biofilm producers | Biofilm producers | Non biofilm producers |
| Urine         | 9                 | 18                    | 46                | 27                    |
| Pus           | 30                | 35                    | 22                | 34                    |
| Blood         | 3                 | 1                     | 12                | 7                     |
| Sputum        | 4                 | 0                     | 8                 | 3                     |
| Wound swab    | 0                 | 0                     | 1                 | 1                     |
| Vaginal swab  | 0                 | 0                     | 2                 | 1                     |
| Tracheal swab | 0                 | 0                     | 1                 | 0                     |
| Vomit         | 0                 | 2                     | 0                 | 0                     |
| Et swab       | 0                 | 0                     | 1                 | 0                     |
| Corneal swab  | 0                 | 0                     | 0                 | 1                     |
| Pleural fluid | 0                 | 0                     | 1                 | 0                     |
| <b>Total</b>  | <b>46</b>         | <b>56</b>             | <b>94</b>         | <b>74</b>             |



**Figure 2:** Comparison between antibiotic sensitivity pattern of S.aureus in relation to biofilm production



**Figure 3:** Comparison between antibiotic sensitivity pattern of S.epidermidis in relation to biofilm production

## DISCUSSIONS

Bacterial biofilm has long been considered as a virulence factor contributing to infection associated with various medical devices causing hospital acquired infection<sup>16</sup>. Antimicrobial resistance is an innate feature of bacterial biofilms that, in addition to developing antibiotic resistance amongst clinical strains, may further complicates patient treatment<sup>17</sup>. In our study, we isolated 140 (52%) biofilm producers out of 270 isolates in which 46(32.8%) were *S.aureus* and 94(67.2%) were *S.epidermidis*. Biofilm production was observed in 51% (37/72) MRSA and 30% (9/30) MSSA while 61% (82/134) MRSE and 35% (12/34) MSSE were found to be biofilm producers. Maximum biofilm production in *S.epidermidis* was observed in urine isolates (46/168) while that of *S. aureus* was in Pus samples (30/102). Similar results were observed in study conducted by Bose *et al*<sup>16</sup>. Aggrawal *et al*<sup>18</sup> reported 79% biofilm producing *S.aureus* and 43% biofilm producing *S.epidermidis* from blood samples in Lukhnow. 82% of biofilm producing *S. aureus* and 71.4% biofilm positive *S.epidermidis* were methicillin resistant which is higher in no. as compared to our study. In our study the antibiotic susceptibility pattern of biofilm producers was far more resistant as compared to the non-biofilm producers. Biofilm producer *S.aureus* showed maximum susceptibility to Vancomycin and aminoglycosides followed by tetracycline and ciprofloxacin. On the contrary, non biofilm producing *S. aureus* were comparatively much more sensitive to these antimicrobials. Similar results were seen in *S.epidermidis* isolates showing maximum sensitivity to Vancomycin. Penicillins, erythromycin and cotrimoxazole remain the drugs with least sensitivity to biofilm producers in our antibiotic panel. We observed same pattern shown by Bose *et al*<sup>16</sup> in their study with 40-50% sensitivity to all antimicrobials in biofilm producers in contrast to non biofilm producers that were 60-90% sensitive. Vancomycin was completely sensitive with only two biofilm producing strains with intermediate sensitivity. Aggrawal *et al*<sup>18</sup> also showed 30-40% sensitivity to ciprofloxacin and cephalosporins in biofilm producing *S. aureus* and 57% in biofilm producing *S.epidermidis*. Penicillins were 0-10% sensitive. Their sensitivity pattern increased to 40-80% in non biofilm producers. All the studies including ours prove that biofilm production has a major role in antibiotic resistance of staphylococci. The future expansion of this study can be done by genetic analysis of biofilm producing strains to observe the presence of *ica* gene.

## CONCLUSION

To conclude monitoring the prevalence of methicillin resistant strains and their antibiotic susceptibility along with biofilm production on biotic and abiotic surfaces is necessary to govern the treatment modalities. However Vancomycin remains the drug of choice but its emerging resistance is also a value of concern. Proper drug usage and methods to control biofilm formation should be included in routine practices.

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