

**IDENTIFICATION OF BIOFILM PRODUCING
STAPHYLOCOCCUS SPECIES FROM CLINICAL SAMPLES BY
THREE DIFFERENT PHENOTYPIC METHODS AND
COMPARISON OF THEIR ANTIBIOGRAM**

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ABSTRACT

Background:

Staphylococci are significant nosocomial as well community acquired pathogens responsible for a wide spectrum of diseases ranging from mild cutaneous infections to severe blood stream infections. Undoubtedly *S. aureus* is an important pathogen by virtue of its number of virulence factors. From the past two-three decades CoNS have been gaining importance as significant opportunistic, nosocomial pathogens in immunocompromised patients. Biofilm forming ability is a major attribute for their virulence. They are the main cause of chronic polymer associated infections, dental plaque, contact lens colonization, infective endocarditis etc. Their antimicrobial resistance has made biofilm producers important in infection control. Here, we have tried to detect biofilm production in staphylococci isolated from clinical specimens.

Objectives:

- To speciate staphylococci isolated from clinical samples.
- To detect biofilm production in clinical Staphylococcal isolates by three different methods and compare the efficacy of the methods.
- To compare antimicrobial susceptibility pattern of biofilm producers with that of biofilm non – producers.

Methodology:

Study was conducted in a tertiary care hospital in North Karnataka. A total of 202 samples sent for routine culture and sensitivity to the microbiology laboratory which yielded *Staphylococcus spp.* were subjected for speciation, biofilm detection by Congo red agar method (CRA), Tube method (TM) and Tissue culture plate

method (TCP). Statistical analysis was done using McNemar's chi square test. TCP was considered as gold standard method and other two tests were compared with TCP. Antimicrobial susceptibility testing was done against Penicillin, ceftiofur, erythromycin, gentamycin, tetracycline, linezolid, vancomycin, amikacin, norfloxacin, ciprofloxacin, nitrofurantoin and cotrimoxazole using disk diffusion method for 120 isolates and VITEK 2 system for 80 isolates. Antibiotic susceptibility pattern of biofilm producers were compared with that of biofilm non producers.

Results:

Out of 202 samples 99 were *S. aureus* and 103 were CoNS. Methicillin resistance was exhibited by a total of 83 isolates out of 202 (41.1%). The predominant species isolated was *S. epidermidis* (43.7%) followed by *S. hemolyticus* (14.6%) and *S. caprae* (11.7%). A total of 117 isolates were true biofilm producers which had given positive result in TCP. In comparison with TCP, TM was more sensitive (88.9%) but was less specific (81.2%). TM correlated well with TCP. The discordance was statistically not significant (p value >0.05). When CRA was compared with TCP, it showed high specificity (96.55%) but least sensitivity (45.4%). The discordance between CRA and TCP was very high (P <0.0001). *S. epidermidis* were found to be having a more biofilm forming ability (57.8%) and were isolated from blood sample (51.0%). The biofilm producers were more resistant to routine antibiotics than biofilm non producers. Marginally higher methicillin resistance was exhibited by biofilm producers in comparison with biofilm non producers.

Conclusion:

S. epidermidis, isolated from blood sample were more biofilm producers. Biofilm producers were found to be more resistant to antibiotics in comparison with biofilm non producers and the same was observed among coagulase negative staphylococci. This makes them clinically even more important and makes the common norm of considering CoNS as contaminants questionable. Though TCP is the gold standard method for detection of biofilm, TM can be used as an alternative method in low economic laboratories where spectrophotometer is not available. The only drawback is that it is highly subjective and needs expertise.

Key words

Staphylococci; CoNS speciation; Staphylococcus aureus; Biofilm; Methicillin resistance; Antibiotic resistance