Original article:

Biofilm formation and antibiotic susceptibility pattern in MRSA strains in a tertiary care rural hospital.

Dardi CharanKaur, Khare A.S

Department of Microbiology, MIMER Medical College, Talegaon Dabhade, Pune , Maharashtra , India
Corresponding author: DardiCharanKaur

Abstract

Introduction: Methicillin-resistant *Staphylococcus aureus*(MRSA) is one of the most important nosocomial pathogens and has emerged as a serious threat to public health worldwide.Biofilms have an enormous impact on healthcare. Antimicrobial resistance is an innate feature of bacterial biofilms and Biofilm formation is higher in MRSA.The present study was undertaken with the aim to find the prevalenceof biofilm& their antimicrobial resistantpattern of MRSA strains

Materials & Methods: Total of 231 MRSAisolated from clinical samples were identified by standard microbiological techniques& the isolates were further tested for biofilm formation & Antibiotic susceptibility testing.

Results: Of 231 MRSA, biofilm formationwas observed in 182(78.78%).Strong biofilm formationin 121 isolates(52.38%)weak biofilm formationin 61(26.40%), biofilm non-producer in 49(21.21%).Highest prevalence of biofilm formation was noted in miscellaneous(86.11%),followed byurine(81.81%), sputum(81.25%), pus(80.82%),blood(64.28%).Prevalence of MRSA in females was higher(57.2%) as compared to males(42.8%).

Conclusion: The threat of MRSA infections results from not only the occurrence of multidrug resistance but also the emergence of bacteria that form strong biofilms. Strains of MRSA should be routinely screened for biofilm formation.

Keywords: Antibiotic resistance

Introduction:

Methicillin -resistant staphylococcus aureus (MRSA) is associated with serious infections. Having the ability of biofilm-formation decrease their susceptibility to antibiotics. Staphylococcus aureus is known to form biofilms on different surfaces. [1]The chronic infections caused by S. aureus, persist and increase the rate of morbidity and mortality in human population due to the development of biofilm. [2] Biofilms have an enormous impact on healthcare, and are estimated to be associated with 65% of nosocomial infections [3].Biofilms are the population of bacteria growing on the biotic and abiotic surfaces and embed themselves in a self-produced extracellular matrix of exopolysaccharide (EPS), proteins and some micro molecules such as DNA. [2]

The formation of biofilm is an example of phenotypic change. Formation of a biofilm is the hallmark characteristic of S. aureus infection which consists of multiple layers of bacteria encased within an exopolysaccharideglycocalyx. Presence of glycocalyx prote-cts the enclosed bacteria from host defences and impedes delivery of antibiotics. [4]Infact biofilms can resist antibiotic concentration 10-10,000 folds higher than those required to inhibit the growth of free floating bacteria. [5] Biofilm formation in S. aureus is regulated by expression of Polysaccharide Intracellular Adhesion (PIA) which mediates cell to cell adhesion and is the gene product of ica ABDC [6]

www.ijbamr.com P ISSN: 2250-284X E ISSN :2250-2858

Adaptation to surface attached growth within a biofilm is accompanied by significant changes in gene and protein expression, as well as metabolic activity. [7, 8] which confers resistance to antimicrobial therapy [9] and host mechanisms of clearance [10] MRSA infections are life-threatening due to emergence of multidrug resistance strains and also occurrence of isolates that are able to form strong biofilms. [11]Early identification and adopting efficient control protocol against biofilm forming MRSA can be one of the essential steps towards the prevention of the most serious nosocomial infections. The present study was planned to find the prevalence of biofilm formation in various specimen and to know the antimicrobial resistantpattern of MRSA strains.

Materials & Methods:

The study was carried out in the department of Microbiology, MIMER Medical College, TalegaonDabhade, Pune from the period of July 2012 to August 2013. Methicillin resistant Staphylococcusaureus isolated from various clinical specimens like Pus, Blood, indwelling urinary catheter, Urine, sputum, sterile fluids were identified by standard microbiological techniques.All MRSA isolates were included & Repeat Isolates were excluded.

- Identification of MRSA isolates byCefoxitin disc (30µg) using disk diffusion method according to Clinical and Laboratory Standards Institute guidelines. [12]
- The Isolates were further tested for Antibiotic susceptibility testing by Kirby-Bauer disc diffusion method on Mueller Hinton agar as per CLSI Approved Standard M100-S17)**.**[12] The antibiotics tested were Amikacin (Ak) 30µg ,Ciprofloxacin (CIP)5µg, Gentamicin (G)30µg, Calithromycin (CLR)15 µg, Cefotaxime(CF) 30µg, Sparfloxacin (SF)5µg, Cefuroxime(CR)30µg, Cefoperazone(CFP)30µg, Ampiclox(ACX) µg, Azithromycin(AZ) µg,Cefadroxil (CD) µg, Roxythromycin(RX) µg,Vancomycin (VA) µg.Antibiotic disc was obtained from Hi-media Laboratories Pvt. Ltd, Mumbai, India.
- The MRSA isolates were tested for biofilm formation by Tube Method (TM): A qualitative assessment of bifilm formation was determined as described by Christensen et al.[13]TSBglu (10mL) were inoculated with the loopful of microorganism from overnight culture plates and incubated for 24 hours at 37°C. The tubes were decanted and washed with PBS (pH 7.3) and dried. Dried tubes were stained with crystal violet (0.1%). Excess stain was removed and tubes were washed with deionized water. Tubes were than dried in inverted position and observed for biofilm formation. Assays were performed in triplicate at three different times. The data obtained was recorded and analysed by using appropriate statistical methods.
- A special rule has been applied in defining antimicrobial resistance in S. aureus. Once a S.aureusisolate is characterized as an MRSA it is instantly classified as an MDR, because resistance to oxacillin or cefoxitin infers non-susceptibility to all categories of β-lactam 8 antimicrobials listed in this document (i.e. all categories of penicillins, cephalosporins, β lactamase inhibitors and carbapenems currently approved up until July 22, 2010). Table d [14]

www.ijbamr.com P ISSN: 2250-284X E ISSN :2250-2858

Results:

Of 231 MRSA, biofilm formation was observed in 182 (78.78%). Strong biofilm formation in 121 isolates (52.38%) weak biofilm formation in 61(26.40%) & negative for biofilm formation in 49(21.21%).(Fig 1)

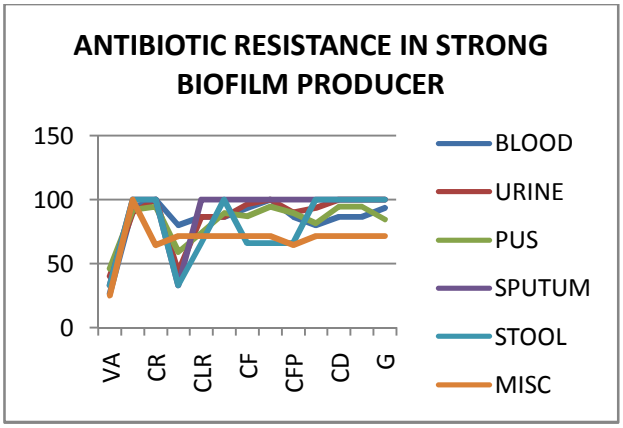
Table No 1: Specimen -wise distribution of biofilm formation

SPECIMEN	No of Samples	Total isolates forming Biofilm	Strong Biofilm formation	Weak Biofilm formation	Negative Biofilm formation
Blood	42	27 (64.28%)	15 (35.71%)	12 (28.57%)	15 (35.71%)
Urine	55	45 (81.81%)	30 (54.54%)	15 (27.27%)	10 (18.18 %)
Pus	73	59 (80.82%)	39 (53.42 %)	20 (27.39%)	14 (19.17 %)
Stool	9	7 (77.77%)	3 (33.33 %)	4 (44.44%)	2 (22.22 %)
Sputum	16	13 (81.25%)	10 (62.50 %)	3 (18.75%)	3 (18.75%)
Misc	36	31 (86.11%)	24 (66.67 %)	7 (19.44%)	5 (13.89 %)
Total	231	182 (78.78%)	121 (52.38 %)	61 (26.40%)	49 (21.21 %)

Table No 2: Biofilm formation& antibiotic resistant pattern of the isolates

Antibiotic tested	Biofilm formation		
	Strong %	Weak %	Negative %
Vancomycin(VA)	38.01	11.47	10.20
Ampiclox (ACX)	94.21	70.49	46.93
Cefuroxime (CR)	93.38	59.01	46.93
Amikacin (AN)	63.63	47.54	20.4
Calithromycin (CLR)	84.29	54.09	48.97
Ciprofloxacin (CIP)	90.08	70.49	46.93
Cefotaxime (CF)	93.38	62.29	34.69
Sparfloxacin (SF)	97.52	80.32	61.22
Cefoperazone (CFP)	90.08	57.37	20.4
Azithromycin (AZ)	87.6	60.65	40.81
Cefadroxil (CD)	96.69	77.04	55.1
Roxythromycin (RX)	95.04	63.93	22.1
Gentamicin (G)	90.9	67.21	46.93

www.ijbamr.com P ISSN: 2250-284X E ISSN :2250-2858



Discussion:

The hallmark of biofilm-related infections is the dramatic resistance to antimicrobials and to host defenses. Patients with chronic infectious diseases, such as otitis media and osteomyelitis, experience cycles of acute exacerbation and remission. Many chronic infections result in treatment failure, suppression of infection followed by reoccurrence, or the inability to culture micro-organisms despite obvious clinical symptoms. [15]Infectious processes in which biofilms have been implicated include common problems such as urinary tract infections, catheter infections, middle ear infections, formation of dental plaque, gingivitis, coating contact lenses and less common but more lethal processes such as infective endocarditis, cystic fibrosis and infections of permanent indwelling devices such as joint prosthesis and heart valves. [16, 17]

MRSA infections range from those of the skin and surgical sites, to infections relating to catheters and prosthetic implants, to pneumonia [18]In India, the significance of MRSA had been recognized relatively late and epidemic strains of these MRSA are usually resistant to several antibiotics. During the past 15 years, the appearance and world-wide spread of many such clones have caused major therapeutic problems in many hospitals. [19] A considerable increase in the prevalence of MRSA has been observed globally during the last decade. [20]

Antimicrobial resistance is an innate feature of bacterial biofilms that, in addition to the increasing rates of reported antimicrobial resistance amongst clinical strains, may further complicate patient treatment [21]Of 231 MRSA, biofilm formation was observed in 182 (78.78%). Strong biofilm formation in 121 isolates (52.38%) weak biofilm formation in 61(26.40%) & negative biofilm formation in 49(21.21%). S Singh reported 85.72% (36/42) of the isolates were found to be high biofilm formers. [22]Fatima Khan etal in their studyobservedbiofilm formation by tissue culture plate method in 64.89% and by tube method in 63.74% & 47.79% by Congo red Agar method (CRA) method. [23]

However other studies have reported a slightly less number of biofilm productions by staphylococcal species. (Mathuret al 2006; Boseet al 2009). [24, 25]

Sasirekha B reported 61.90% of MRSA isolates have the potential to make biofilm and in their study biofilm producing MRSA showed high resistance to almost all the groups of antibiotics compared to the biofilm non- producer. [26]

www.ijbamr.com P ISSN: 2250-284X E ISSN :2250-2858

Highest prevalence of biofilm formation was noted in miscellaneous (86.11%), urine (81.81%), sputum (81.25%), pus (80.82%). Lower prevalence of (64.28%) biofilm formation from MRSA isolates from blood (Table 1)

Prevalence of MRSA in females were higher (57.2%) as compared to males (42.8%)

Fatima Khan etal in their studyfound that biofilm producing strains were more resistant when compared to the biofilm non producers .All the strong biofilm producer thirtyeight were found to be methicllin resistant. Out of the remaining 47 MRSA strains 40 were moderate biofilm producers and just 7 (8.23%) strains did not produced any biofilm. Amongst the 177 MSSA strains 92 strains (51.98%) were found to be moderate biofilm producers and none was strong producer of biofilm. [23]

Many studies have shown that biofilm formation is higher in MDR strains [21, 23, 26]

In our study the antibiotic resistance pattern in strong biofilm forming MRSA isolates when compared to biofilm non producers was for Amikacin 63.63/ 20.4, Ampiclox 94.21/ 46.93, Azithromycin 87.6/ 40.81, Ciprofloxacin 90.08/46.93, vancomycin 38.01/10.20 . Fatima Khan etal observed for Amikacin 73.53/55.43%, Ciprofloxacin 83.53/76.09%, clindamycin 87.79/78.26%, cotrimoxazole 93.60/79.35%, erythromycin 65.29/53.26%, gatifloxacin 48.23/40.22%, gentamycin 70.00/67.39%, levofloxacin 12.35/6.42%, ofloxacin 24.71/21.74%, sparfloxacin 43.53/33.69%. However they found all the strains were sensitive to Linezolid and vancomycin. [23] Whereas in our study we observed Vancomycin resistant of 38.01/10.20 similar were the finding of S Singh, who reported vancomycin resistant 54.7% (23/42) Sasirekha B et al reported vancomycin resistance of 7.14% [22, 26]

S Singh et al found the Pearson′s correlation between biofilm formation and antibiotic resistance was found for S. aureus isolates of 0.6. [22]Jeong-Ok cha in their studies reported 51.2 % strains formed biofilms and demonstrated that strong biofilms producing may cause problems in hospital setting and daptomycin, gentamicin, and tigecycline may be choice therapeutics against biofilm-mediated S. aureus infections. [27] Fatima Khan etal in their study found Ciprofloxacin was effective against biofilm producers. [23] Keli et al reported Vancomycin was not able to inhibit adherent cells or eradicate mature biofilms at the same concentration necessary for killing planktonic cells. [28] Rifampicin has putative antibiofilm properties, ability to penetrate staphylococcal biofilm. [29] The age of the biofilm also affects its susceptibility to antibiotics. Older (10-day-old) biofilms are significantly more resistant than 2-day-old biofilms.This emphasizes the need for prompt diagnosis and treatment. [30]

Conclusion:

Methicillin resistance in S. aureusrestricts therapeutic options for clinical isolates and the incidence of MRSA is escalating in India.The threat of MRSA infections results from not only the occurrence of multidrug resistance but also the emergence of bacteria that form strong biofilms. (Biofilm–forming capacity increases the resistance to common use antibiotics). Isolating biofilm-formation MRSA is an alarming for public health.Treatment of MRSA is one of the most challenging task for the clinicians and the microbiologists. With the emergence of Vancomycin resistance in role of antimicrobials is becoming limited. Strains of MRSA should be routinely screened for biofilm formation.

www.ijbamr.com P ISSN: 2250-284X E ISSN :2250-2858

Acknowledgements:

We are grateful to the Management of MIMER Medical College, TalegaonDabhade ,Pune for their support and encouragement. Thanks to Mrs.Hemvati Satone for technical support.

References:

1. Ando E, Monden K, Mitsuhata R, Kariyama R, Kumon H. Biofilm formation among methicillin-resistant Staphylococcus aureus isolates from patients with urinary tract infection. Acta Med Okayama. 2004;58(4):207-14.
2. Gowrishankar S, DuncunMosioma N, KaruthaPandian S. Coral-Associated Bacteria as a Promising Antibiofilm Agent against Methicillin-Resistant and Susceptible Staphylococcus aureus Biofilms. Evid Based Complement Alternat Med. 012;2012:862374.
3. Potera, C. (1999). Forging a link between biofilms and disease. Science 283, 1837–1838.
4. Stewart, P.S., 2002. Mechanisms of antibiotic resistance in bacterial biofilms. Ind. J. Med. Microbiol., 29: 107-113.
5. Jefferson, K.K., D.A. Goldman and G.B. Pier, 2005. Use of confocal microscopy to analyse the rate of vancomycinpenertration through Staphylococcus aureus biofilms. Antimicrob. Agents Chemother., 49: 2467-2473.
6. Ammendolia, M.G., R.D. Rosa, L. Montanaro, C.R. Arciola and L. Baldassarri, 1999. Slime production and expression of slime associated antigen by staphylococcal clinical isolates. J. Clin. Microbiol., 37: 3235-3238.
7. Dotsch A, Eckweiler D, Schniederjans M, Zimmermann A, Jensen V, Scharfe M, Geffers R, Haussler S: The pseudomonas aeruginosatranscriptome in planktonic cultures and static biofilms using RNA sequencing. PLoS One 2012, 7:e31092.
8. Sauer K, Camper AK, Ehrlich GD, Costerton JW, Davies DG: Pseudomonas aeruginosa displays multiple phenotypes during development as a biofilm. J Bacteriol 2002, 184:1140–1154.
9. Anderson GG, O'Toole GA: Innate and induced resistance mechanisms of bacterial biofilms. Curr Top MicrobiolImmunol 2008, 322:85–105.
10. Thurlow LR, Hanke ML, Fritz T, Angle A, Aldrich A, Williams SH, Engebretsen IL, Bayles KW, Horswill AR, Kielian T: Staphylococcus aureus biofilms prevent macrophage phagocytosis and attenuate inflammation in vivo.JImmunol 2011, 186:6585–6596.
11. Mataraci E, Dosler S. In vitro activities of antibiotics and antimicrobial cationic peptides alone and in combination against methicillin-resistant Staphylococcus aureus biofilms. Antimicrob Agents Chemother. 2012; 56(12):6366-71.
12. Performance Standards for Antimicrobial Susceptibility Testing. Twenty-First Informational Supplement. 2011; 31(1)
13. Gordon D. Christensen,W. Andrew Simpson, Alan L. Bisno, And Edwin H. Beachey. Adherence of Slime-Producing Strains of Staphylococcus epidermidis to Smooth Surfaces. Infection and Immunity, July 1982; Vol. 37(1):318-326.

14. A.-P. Magiorakos, A. Srinivasan, R. B. Carey, Y. Carmeli, M. E. Falagas, C. G. Giske, S. Harbarth, J. F. Hindler, G.Kahlmeter, B. Olsson-Liljequist, D. L. Paterson, L. B. Rice, J. Stelling, M. J. Struelens, A. Vatopoulos, J. T. Weber and D. L. Monnet: Multidrug-resistant, extensively drug-resistant and pandrug-resistantbacteria: an international expert proposal for interim standard definitions for acquired resistance: ClinMicrobiol Infect 2012; 18: 268–281.
15. Costerton W, Veeh R, Shirtliff M, Pasmore M, Post C, Ehrlich G. The application of biofilm science to the study and control of chronic bacterial infections. J Clin Invest. 2003; 112:1466-1477.
16. Lewis, K., 2001.Riddle of biofilm resistance.Antimicrob. Agents Chemother.45: 999-1007.
17. Parsek, M. and P.K. Singh, 2003. Bacterial biofilms: An emerging link to disease pathogenesis. Annu. Rev. Microbiol., 57: 677-701.
18. Diekema, D. J., Pfaller, M. A., Schmitz, F. J., Smayevsky, J., Bell, J.,Jones, R. N., Beach, M. & SENTRY Participants Group (2001).Survey of infections due to Staphylococcus species: frequency of occurrence and antimicrobial susceptibility of isolates collected in the United States, Canada, Latin America, Europe, and the Western Pacific region for the SENTRY Antimicrobial Surveillance Program,1997–1999. Clin Infect Dis 32 (Suppl. 2), S114–S132.
19. Rajaduraipandi K., Mani K.R., Panneerselvam K., Mani M., Manikandan P., Prevalence and antimicrobial susceptibility pattern of methicillin resistant S. aureus: A multicentre study. Ind. J.Med. Microbiol., 2006,26: 34- 38.
20. Boucher HW, Corey GR. Epidemiology of methicillin -resistant staphylococcus aureus. Clin Infect Dis 2008; 46 Suppl 5:S344-9.
21. Reiter KC TGDASP, CF DEO, D'Azevedo PA: High biofilm production by invasive multiresistant staphylococci. APMIS 2011, 119:776–781.
22. S Singh, R Katiyar, SD Kaistha. High oxacillin, vancomycin and fluoroquinolone resistance amongst biofilm forming Staphylococcus aureus isolates from ulcerative keratitis infections. Indian Journal of Medical Microbiology, Vol. 29, No. 3, July-September, 2011, pp. 312-313.
23. Fatima Khan, InduShukla, MeherRizvi, Tariq Mansoor and S.C. Sharma, 2011. Detection of Biofilm Formation in Staphylococcus aureus. Does it have a role in Treatment of MRSA Infections? Trends in Medical Research, 6: 116-123.
24. Mathur, T., S. Singhal, S. Khan, D.J. Upadhyay, T. Fatma and A. Rattan, 2006. Detection of biofilm formation among clinical isolates of staphylococci: An evaluation of three different screening methods. Ind. J. Med. Microbiol., 24: 25-29.
25. Bose, S., M. Khodke, S. Basak and S.K. Mallick, 2009. Detection of biofilm producing staphylococci: Need of the hour. J. Clin. Diagnostic Res., 3: 1915-1920.
26. Sasirekha B, Usha M.S, Amruta A. J, Ankit S, Brinda N, Divya R. Evaluation and Comparison of Different Phenotypic Tests to Detect Methicillin Resistant Staphylococcus aureus and their Biofilm Production. International Journal of PharmTech Research. 2012,4(2); 532-541.

27. Jeong-Ok Cha .In vitro biofilm formation and bactericidal activities of methicillin-resistant Staphylococcus aureus clones prevalent in Korea.Diagnostic Microbiology & Infectious Disease.2011. Volume 70, Issue 1, 112-118.
28. KeliCristine Reiter, Gustavo EnckSambrano, Barbara Villa, ThiagoGalvao da Silva Paim, Caio Fernando de Oliveira, Pedro Alvesd'Azevedo.Rifampicin fails to eradicate mature biofilm formed by methicillinresistant Staphylococcus aureus.Revista da SociedadeBrasileira de Medicina Tropical 45(4):471-474, Jul-Aug, 2012.
29. Zheng Z, Stewart PS. Penetration of rifampin through Staphylococcus epidermidisbiofilms. Antimicrob Agents Chemother 2002; 46:900-903.
30. Donlan R.M., Costerton W., Biofilms: Survival mechanisms of clinically relevant microorganisms. Clin. Microbiol. Rev., 2002, 15(2): 167-193.

Date of submission: 28 Aug. 2013 Date of Provisional acceptance: 22 September 2013
Date of Final acceptance: 20 October 2013 Date of Publication: 04 December 2013
Source of support: Nil; Conflict of Interest: Nil