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Biofilm formation and antimicrobial susceptibility Pattern of Methicillin Resistant *Staphylococcus aureus* from wound infection

Dardi Charan Kaur*, Sachin Wankhede

Department of Microbiology, MIMER Medical College, Talegaon Dabhade, Pune, India
Department of Microbiology, S.K.N medical college and General Hospital, Pune, India

ABSTRACT

Objective: MRSA poses a great risk to wound patients with potential to cause significant mortality and morbidity in human population due to the development of biofilm. So the present study was undertaken with the aim to know the prevalence of different microorganism from wound, to know the biofilm formation and the antimicrobial resistant pattern of MRSA strains. **Materials & Methods:** The prospective study was carried out in the department of Microbiology during the period of July 2012 to December 2013.Total of 85 MRSA isolated from clinical samples were identified by standard microbiological techniques and the isolates were further tested for biofilm formation and Antimicrobial susceptibility testing by Kirby-Bauer disc-diffusion method as per CLSI guidelines. **Result:** Among 290 wound sample received, culture was positive in 230 (79.31%). The common pathogens were MRSA 85(32.44%), MSSA 60(22.90%), *E.coli* 25(9.54%) and *Pseudomonas aeruginosa* 24(9.16%). Higher prevalence was noted in Obgy (38.82%) followed by surgery (17.65%), Skin (16.47%), ICU(9.41%), Orthopedics(7.06%), Medicine(4.71%), OPD(3.53%), Paediatric (2.35%),Of 85 MRSA, biofilm formation was observed in 70 (82.35%). Strong biofilm formation in 42(49.41%), weak biofilm formation in 28(32.94%) and negative biofilm formation in 15(17.65%). In our study the Antimicrobial resistance pattern in strong biofilm forming MRSA isolates when compared to biofilm non producers was for Amikacin 57.14%/ 26.67%, Erythromycin 73.80%/ 26.67%, Clindamycin 66.67%/ 20%, Ciprofloxacin 90.47%/46.67%, Gentamycin 88.09%/46.67%, Fusidic acid 35.71%/6.67%, Mupirocin 14.28%/6.67%.No resistance was noted to Linezolid and Teicoplanin **Conclusions:** 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.

Keywords: Antimicrobial resistance, Biofilm formation,MRSA, Wound.

Introduction

A wound is the result of physical disruption of the skin, one of the major obstacles to the establishment of infections by bacterial pathogens in internal tissues. When bacteria breach this barrier, infection can result[1, 2] The most common underlying event for all wounds is trauma. Trauma may be accidental or intentionally induced. The latter category includes hospital-acquired wounds, which can be grouped according to how they are acquired, such as surgically and by use of intravenous medical devices.

**Correspondence*
Dr. Dardi Charan Kaur
Department of Microbiology, MIMER Medical College, Talegaon Dabhade, Pune, India
Email: charan13@rediffmail.com

Although not intentionally induced, hospital-acquired wounds can be the pressure sores caused by local ischemia, too. They are also referred as decubitus ulcers, and when such wounds become infected, they are often colonized by multiple bacterial species.[2]Most wound infections can be classified into two major categories: skin and soft tissue infections, although they often overlap as a consequence of disease progression**.** [2, 3]Wound infections can be caused by different groups of microorganisms, most commonly isolated aerobic microorganisms includes *S.aureus*, CoNS, Enterococci, *E.coli, P.aeurginosa, Klebsiella pneumoniae, Enterobacter, Pr.mirabilis, Streptococci, Candida, Acinetobacter.* [4]The overall incidence of wound sepsis in India is from 10-33 %. [5]Among the Gram-positive pathogens, *S. aureus* continues to cause skin and soft tissue infections (SSTI) in the community as well as

invasive infections in the hospitalized patients and is a frequent cause of bacterial infections in both developed and developing countries. [6-8]It is a highly versatile and adaptable pathogen, causing a range of infections of varying severity affecting the skin, soft tissue, respiratory system, bone, joints and endovascular tissues. [6]MRSA poses a great risk to wound patients with potential to cause significant mortality and morbidity in human population due to the development of biofilm.[9]Biofilms have an enormous impact on healthcare, and are estimated to be associated with 65% of nosocomial infections.[10]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.[11]Adaptation to surface attached growth within a biofilm is accompanied by significant changes in gene and protein expression, as well as metabolic activity which confers resistance to antimicrobial therapy.[12]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 [13].MRSA infections are life-threatening due to emergence of multidrug resistance strains and also occurrence of isolates that are able to form strong biofilms.

So the present study was undertaken with the aim

- To know the prevalence of different microorganism from wound
- To know the biofilm formation of MRSA in wound
- To know the antimicrobial resistant pattern of MRSA strains

Materials & Methods:

The prospective study was carried out in the department of Microbiology from the period of July 2012 to December 2013. Pus sample were collected with sterile disposable cotton swabs and isolates were identified by standard microbiological techniques.

All the confirmed *S. aureus* strains were subsequently tested for methicillin resistance using cefoxitin disc (30µg). The Isolates were considered methicillin-resistant if the zone of inhibition was 21mm or less.[14]All MRSA isolates was included and Repeat Isolates were excluded.

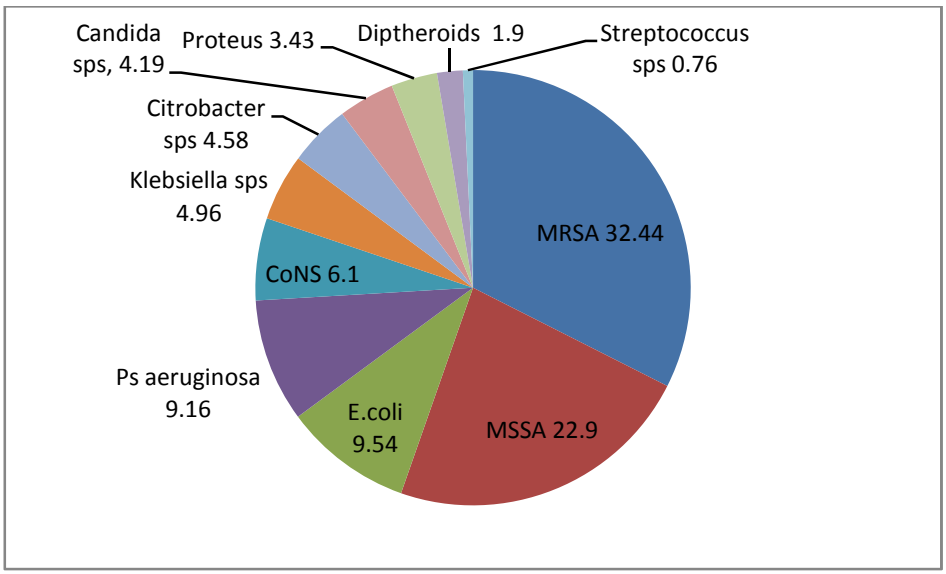
- The MRSA isolates were tested for biofilm formation by Tube Method (TM): A qualitative assessment of biofilm formation was determined as described by Christensen *et al*[15]TSBglu (10mL) were inoculated with the loopful of microorganism from overnight culture plates and incubated for 24 hours at 37°C. 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 analyzed by using appropriate statistical methods.
- The Isolates were further tested for Antimicrobial susceptibility testing by Kirby-Bauer disc diffusion method on Mueller Hinton agar as per CLSI Approved Standard M100-S17)[14]except for Fusidic acid where the French Society of Microbiology recommendations were used.
- The following antimicrobial agents were tested amikacin (30µg), erythromycin (15µg), clindamycin (2µg), Ciprofloxacin (CIP) 5µg, Linezolid (30µg), gentamycin (30µg), fusidic acid (10µg), teicoplanin (30 µg) and mupirocin (5µg). (Discs were procured from Hi-media Laboratories, Mumbai, India &Oxoid)

Result:

Among 290 wound sample received, culture was positive in 230 (79.31%). A single etiologic agent was identified in 198(68.27%) patients, mixed etiologic agents were found in 32 (11.03%) and no etiologic agent was identified in 60(20.68%). Among the common pathogens were *Staphylococcus aureus* accounted for 145(50%)

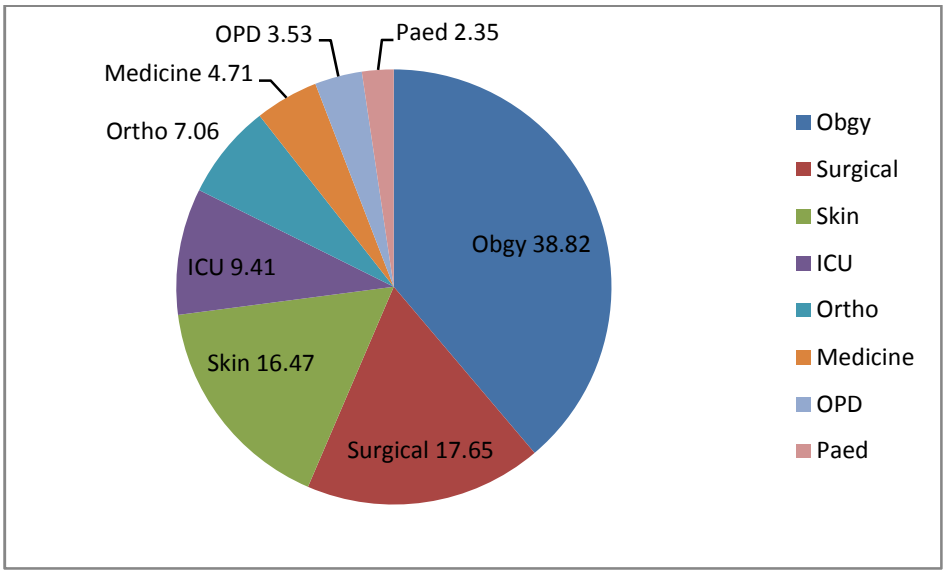
Chart No 1: Distribution of microorganism in wound
Chart No 2: Ward-wise distribution of microorganism in wound
Table No 1: Biofilm formation in MRSA isolates
Table No 2: Biofilm formation and Antimicrobial resistant Pattern of MRSA among wound
Figure No 1: Biofilm producer

Chart No 1: Distribution of microorganism in wound



The above chart depicts *Staphylococcus aureus*; *E.coli* and *Ps aeruginosa* is the predominant organism from wound

Chart No 2: Ward-wise distribution of microorganism in wound



The above chart depicts higher prevalence of MRSA from Obgy(38.82%) followed by Surgical (17.65%)

Table No 1: Biofilm formation in MRSA isolates

Total No of MRSA isolates tested for Biofilm	Total No of isolates forming Biofilm N=70 (82.35%)		
	Strong	Weak	Negative
85	42(49.41%)	28(32.94%)	15(17.65%)

The above table depicts the higher percentage of biofim formation by strong biofilm former

Table No 2: Biofilm formation and Antimicrobial resistant Pattern of MRSA among wound

Antimicrobial agent	Antibiotic Resistance pattern in		
	Strong biofilm n=42 (%)	Weak biofilm n= 28 (%)	Negative biofilm n=15 (%)
Amikacin	24(57.14)	8(28.57)	4(26.67)
Erythromycin	31(73.80)	15(53.57)	4(26.67)
Clindamycin	28(66.67)	13(46.42)	3(20)
Ciprofloxacin	38(90.47)	14(67.85)	7(46.67)
Linezolid	0	0	0
Gentamycin	37(88.09)	20(71.42)	6(46.67)
Fusidic acid	15(35.71)	5(17.86)	1(6.67)
Teicoplanin	0	0	0
Mupirocin 5	6(14.28)	3(10.71)	1(6.67)

The above table depicts the higher percentage of Antimicrobial resistant by strong biofilm former compared to negative biofilm former. No resistance was noted to Linezolid and Teicoplanin

A- Strong Biofilm B- Negative Biofilm C- Weak Biofilm

Figure 1:Biofilm Producer

Discussion

Wound patients have been shown the potential to become colonized and infected more readily than other patients due to deprivation of mechanical barrier provided by the skin and mucous membrane as well as the depression of immunological response. [9]Pathogens that infect wounds can be part of normal flora or acquired from the hospital environment or other infected patients. *Staphylococcus aureus*, being the normal microbial flora of the skin, is one of the commonest causes of wound infections. Its increasing incidence is a growing concern with emergence of virulent, antibiotic resistant strains in the community settings.[16]

The important reservoirs of MRSA in hospitals/ institutions are infected or colonized patients and transient hand carriage is the predominant mode for patient to patient transmission. In India, the significance of MRSA has been recognized relatively late and epidemic strains of these MRSA strains are usually resistant to several antibiotics. During the last 15 years, the appearance and world wide spread of many such clones have caused major therapeutic problems in many hospitals, as well as diversion of considerable resources to attempts at controlling their spread.[17]A considerable increase in the prevalence of MRSA has been observed globally during the last decade. [18]
In our study the predominant organisms were *Staphylococcus aureus* (50%), *E.coli* (9.54%) and *Ps aeruginosa* (9.16%). Similar were the findings of Mohanty *et al* who reported *S. aureus*, *E. coli* and *Pseudomonas spp*. are the top 3 pathogens isolated from skin and soft tissue infections in hospitalized patients. [19]In the present study the prevalence of MRSA in wound was 85 (32.44%). Similar were the findings of Mohanty 38.56%, Singh 45%. [19, 20]Sangeeta Joshi *et al*, INSAR observed the prevalence of MRSA isolated from skin and soft tissue infections (36% in 2008 and 40% in 2009) [21].The prevalence varies considerably from one region to another and among hospitals in the same city. Methicillin resistance in *S. aureus* isolates (mostly health-care-associated MRSA) varies from less than 1 % in Norway, Sweden and Denmark, less than 5 % in the Netherlands, 5 - 10 % in Canada, 40 % in Greece and the United Kingdom of Great Britain and Northern Ireland, 25 - 50 % in the USA, 37.5 % in India, to more than 50 % in China, Hong Kong SAR and Singapore.[22, 23]In Japan, the percentage of MRSA isolated from skin infections have been shown to vary from 10 to 20%.[24]About 51.6 % of *S. aureus* isolates among patients admitted to burns and orthopedics units in India were reported to be MRSA. [25]
Maximum MRSA isolates 27(31.76%) were observed in the age group of 21-30 years followed by 14(16.47%) in 31-40 yrs age group. the predominance of the MRSA was higher among females 50(58.82%) than males 35 (41.17%).

Having the ability of biofilm-formation decrease their susceptibility to antibiotics. *Staphylococcus aureus* is known to form biofilms on different surfaces. In fact biofilms can resist antibiotic concentration 10-10,000 folds higher than those required to inhibit the growth of free floating bacteria.[26]

Of 85 MRSA, biofilm formation was observed in 70 (82.35%). Strong biofilm formation in 42(49.41%), weak biofilm formation in 28(32.94%) and negative biofilm formation in 15(17.65%).S Singh reported 85.72% (36/42) of the isolates were found to be high biofilm formers.[27]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. [28]Similar were the observation of Fatima Khan *et al*.,[29]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. In our study the antibiotic resistance pattern in strong biofilm forming MRSA isolates when compared to biofilm non producers in percentage was for Amikacin57.14/ 26.67, Erythromycin73.80/ 26.67, Clindamycin66.67/ 20, Ciprofloxacin90.47/46.67, Gentamycin 88.09/46.67, Fusidic acid 35.71/6.67, Mupirocin 14.28/6.67.Fatima Khan *et al*., 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.[29]In our study we observed the isolates were sensitive to Linezolid and teicoplanin (100%).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. aureus* restricts 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. 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.Routine surveillance for hospital-acquired wound infections is recommended by both the Centers for Disease Control and Prevention and the Surgical Infection Society.

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