

In vitro efficacy of Daptomycin against clinical isolates of Methicillin-resistant *Staphylococcus aureus* (MRSA)

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Abstract

This descriptive cross-sectional study was performed in the Department of Microbiology, Fauji Foundation Hospital, Rawalpindi, from March 2019 to September 2019 to determine the in vitro efficacy of Daptomycin against clinical isolates of Methicillin-Resistant *Staphylococcus aureus* (MRSA). Consecutive non-probability sampling technique was used and a total number of 270 patients' Pan Cultures having MRSA growth on Cefoxatin Disc with size less than 22 mm zone size were included in the study. Cultures were inoculated on MacConkey, Chocolate and Blood agar and then incubated for 24 hours at 37 degree Celsius. After incubation, Coagulase test, Catalase test and Gram staining technique were used for further identification. Minimum Inhibitory Concentration (MIC) of the isolates for Daptomycin was obtained by using E strips (Oxoid UK) according to Clinical & Laboratory Standards Institute (CLSI) guidelines. The mean age of the patients was 46.73 ± 12.22 years, and the study included 147 (54.44%) males and 123 (45.56%) females. Regarding the type of specimen, there were 154 (57.04%) pus specimens, 54 (20.00%) blood specimens, 27 (10.00%) fluid specimens, 18 (6.67%) urine specimens, 10 (3.70%) high vaginal swabs (HVS) specimens and 7 (2.59%) sputum specimens. Daptomycin was effective in 264 (97.78%) patients with MIC range from .015 to 1 µg/ml on E strip.

Keywords: Methicillin Resistance *Staphylococcus aureus*, Daptomycin, In vitro efficacy.

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Introduction

Methicillin-resistant *Staphylococcus aureus* (MRSA) is a gram positive bacterium which causes serious infections in people who have weak immune system or reside in various health care units and hospitals. It shows resistance to different antibiotics and the infection can spread from person to person or from skin to skin contact.¹

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Bacteraemia caused by MRSA is the major cause of high mortality rate, even with appropriate antimicrobial treatment.² Daptomycin (DAP) antibiotic is produced by the soil bacterium *Streptomyces roseosporus* often used to treat severe Gram-positive bacterial infections and those bacteria which are resistant to different drugs such as Vancomycin-resistant *Staphylococcus aureus*.³ It is active against *S. pyogenes*, *S. agalactiae*, linezolid-resistant *S. aureus* and *E. faecium* and anaerobic bacteria such as *Clostridium perfringens*, *Propionibacterium acnes* and *Clostridium difficile*.⁴ DAP inhibits the biosynthesis of lipoteichoic acid of Gram positive organisms and thus dissolve the cell membrane of the bacteria.⁵ Vancomycin is used worldwide to treat skin and soft tissue infections of Methicillin-resistant *Staphylococcus aureus*. Resistance and mortality rates increase due to overuse of Vancomycin. To overcome this issue, the Food and Drug Administration approved new drugs including Linezolid, Tedizolid and Daptomycin to treat infections caused by MRSA.⁶ The frequency of Methicillin-resistant *Staphylococcus aureus* infections is high in India and Pakistan and low in northern Europe. In Pakistan, the frequency of Methicillin-resistant *S. aureus* has been observed to be up to 51%.⁷ Minimal inhibitory concentration less than or equal to 1 µg/ml was considered the susceptibility breakpoint by the European Committee on Antimicrobial Susceptibility Testing.⁸ Daptomycin having MIC ≤ 1.0 µg/ml is very effective in inhibiting 99% MRSA growth.⁹ The current study was designed to determine the efficacy of Daptomycin against MRSA cultures because anti-microbial susceptibility of drugs is prone to change over time. The results of this study will help us recognise whether the susceptibility of Daptomycin is still the same or has it reduced over the period of time, because knowledge regarding efficacy of drugs for treatment of patients having MRSA is essential for prescribing appropriate antibiotics to the affected patients.

Methods and Results

A descriptive cross-sectional study was conducted in the Microbiology Department of Fauji Foundation Hospital, Rawalpindi, from March 2019 to September 2019 after the approval of the ethical review board. Consecutive non-

Table-1: Stratification of age to determine the association of age with efficacy.

Age Group	Efficacy		P-value
	Effective	Non Effective	
20-45 Years	121 (97.60%)	03 (2.42%)	0.840
46-70 Years	143 (97.95%)	03 (2.05%)	

Table-2: Stratification of type of specimen to determine the association of type of specimen with efficacy.

Type of Specimen	Efficacy		P-value
	Effective	Non Effective	
Pus	151	3	0.592
Blood	53	1	
Fluids	26	1	
Urine	18	0	
HVS	9	1	
Sputum	7	0	

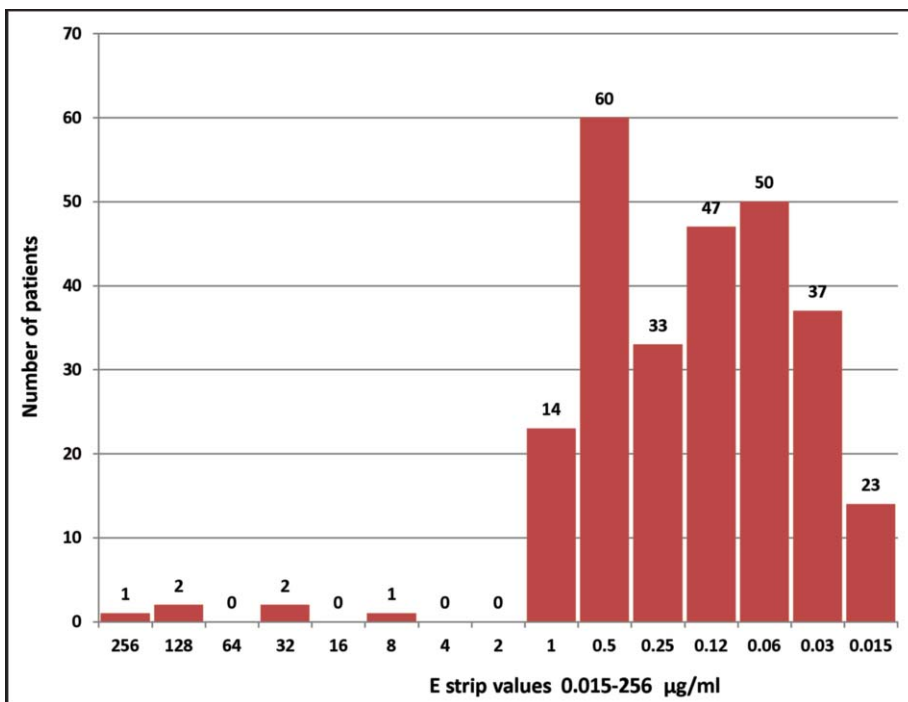
HVS= High Vaginal Swab.

probability sampling technique was used. All indoor patients' having Pan Cultures with MRSA growth on Cefoxitin Disc with size less than 22 mm zone size and patients aged between 12-60 years from both genders (male and female) were included in the study. Patients on antibiotics, cultures with MRSA growth with other organisms, cultures showing growth of Methicillin-

resistant *Staph epidermidis* and cultures taken from multiple sites were excluded from the study.

A total number of 270 sample cultures were included in this analysis. We calculated this sample size using the previous study results of Qureshi et al by taking expected efficacy of Daptomycin 98.2% and margin of error 1.6%.¹⁰ Cultures that fulfilled the inclusion and exclusion criteria were inoculated on Chocolate, MacConkey and Blood agar and were incubated for 24 hours at 37 degree Celsius. Species confirmation was done after Catalase, Coagulase test and Gram staining technique. MRSA was identified if the inoculated *Staph* species from cultures by the above given procedure has less than 21 mm zone size growth on Muller Hilton agar plate. MIC of the isolates for the Daptomycin was obtained by using E strips (Oxoid UK). IBM SPSS statistics 21 was used for statistical analysis and P-value <0.05 was considered as significant.

The mean age of patients in the current study was 46.73±12.22 years including 147 (54.44%) males and 123 (45.56%) females. Regarding the type of specimen, there were 154 (57.04%) pus specimens, 54 (20.00%) blood specimens, 27 (10.00%) fluid specimens, 18 (6.67%) urine specimens, 10 (3.70%) high vaginal swab (HVS) specimens and 7 (2.59%) sputum specimens. Daptomycin was effective in 264 (97.78%) patients with MIC range from .015 to 1 µg/ml and it was non-effective in just 6 (2.22%) patients (Figure). Daptomycin was found effective in 121

**Figure:** MIC on E strip of Daptomycin.

(97.60%) patients between the ages 20 and 45 years, and in 143 (97.95%) patients between the ages 46 and 70 years. This difference was statistically insignificant with p-value of 0.840 (Table-1). Stratification of type of specimen was also performed; Daptomycin was effective in 151 patients of pus specimen, in 53 patients of blood specimen, in 26 patients of fluid specimen, in 18 patients of urine specimen, in 9 patients of high vaginal swab (HVS) specimen and in 7 patients of sputum specimen. This difference was statistically insignificant (p= 0.592). (Table-2).

Discussion

Daptomycin has proved an to be an effective drug for MRSA infection. A study conducted in south India to find out the MIC of Daptomycin for MRSA isolated

from different clinical samples showed that Daptomycin MIC was ≤ 1 $\mu\text{g/ml}$ for all isolates correlated with our study as in our study most strains of MIC were ≤ 1 $\mu\text{g/ml}$.¹¹ In the present study Daptomycin was non-effective in just 6 (2.22%) patients which was also reported in the study conducted in south India i.e. in 2 (6.7%) patients.¹² Another study conducted from 2012 to 2014 in a south Indian hospital reported the Daptomycin MIC, which was from 0.016 to 0.5 mg/L, close to the current study.¹³ MIC of Daptomycin was documented in between 0.75 - 0.5 $\mu\text{g/ml}$ and 0.50 - 0.36 $\mu\text{g/ml}$ in a study conducted in 2015 and 2016 which also supports our study.¹⁴ The study conducted in Andhra Medical College, India, showed Daptomycin 100% sensitivity having MIC less than 1 $\mu\text{g/ml}$ i.e. 0.25 - 0.75 $\mu\text{g/ml}$ by E-test suggesting that Daptomycin can be used as alternative drug while dealing with MRSA infections.¹⁵ In vitro activity of Daptomycin was determined by E-test in another study showed the MIC less than or equal to 1 $\mu\text{g/ml}$.¹⁶ The Daptomycin was highly effective against MRSA but its resistance was very low in MRSA infections.¹⁷ Daptomycin was more effective against MRSA infection and chance of acute kidney injury was low when it was compared with Vancomycin, in which chances were higher.¹⁸ The current study was performed only in one hospital with a limited numbers of samples. Molecular based study is required at a broader scale including different hospitals or health care units with larger number of patients.

Conclusion

Daptomycin is very effective against the treatment of Methicillin-resistant *Staphylococcus aureus* infections with very low resistance.

Disclaimer: None.

Conflict of Interest: The person who signed the ethical review statement is also a co-author of this article.

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