Int.J.Curr.Microbiol.App.Sci (2019) *8*(6): 1784-1791

International Journal of Current Microbiology and Applied Sciences
***ISSN: 2319-7706* Volume 8 Number 06 (2019)**
Journal homepage: http://www.ijcmas.com

Original Research Article https://doi.org/10.20546/ijcmas.2019.806.213

In-Vitro Susceptibility of Daptomycin in MRSA by E-Test

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Keywords

MRSA, Daptomycin, E test, MIC

Article Info

Accepted:
15 May 2019
Available Online:
10 June 2019

A B S T R A C T

Methicillin-resistant *Staphylococcus aureus* (MRSA) is one of the leading pathogens for hospital and community-acquired infections. Increased use of Vancomycin has resulted in the emergence of MRSA with reduced susceptibility to Vancomycin. The emergence of Vancomycin-intermediate or resistant *S. aureus* has created the need for other anti- MRSA antibiotics. To isolate the Staphylococcus aureus from various clinical samples, determine the prevalence of MRSA and to assess the in-vitro susceptibility of Daptomycin against MRSA isolates. A total of 418 clinical samples like pus, blood, urine, sputum, body fluids; received from various departments in the department of microbiology were included in the study. All samples were processed for *Staphylococcus aureus* isolates. Methicillin resistance was identified in all isolates by using Cefoxitin disc diffusion method. Susceptibility and MIC of Daptomycin against MRSA was determined by E (Epsilometer) test method. Out of 418 clinical samples, 126 (30.1%) were *Staphylococcus aureus*. Out of 126 *S.aureus*, 50(39.7%) were MRSA. Susceptibility and MIC of Daptomycin were determined by E-Test method in all MRSA isolates. All (100%) MRSA isolated were susceptible to Daptomycin with MIC range 0.25µg/ml to 0.75 µg /ml. Daptomycin showed 100% sensitivity against all MRSA isolates in our study. Suggesting Daptomycin can be used as alternative agents for the treatment of infections caused by MRSA.

Introduction

Staphylococcus is the most common bacterial pathogen causing complicated skin and soft tissue infections [9]. The grave concern is the growing incidence of drug-resistant pathogens, such as Methicillin-resistant Staphylococcus aureus (MRSA), for which therapeutic options are limited [19]. MRSA are resistant to beta-lactams and to almost all available antibiotics like macrolides, quinolones, aminoglycosides, cephalosporins. Methicillin-resistant *Staphylococcus aureus* (MRSA) is an important nosocomial pathogen[1] and accounts for 60% of *S.aureus* infections[17]. Infections caused by MRSA strains are associated with a longer hospital stay, more days of antibiotic administration, and higher costs than infections caused by methicillin-susceptible *Staphylococcus aureus* (MSSA) strains[1,5]. There is a growing concern about MRSA with reduced susceptibility to

vancomycin, which is the most extensively used antibiotic for its treatment [16,13]. This has made treatment of MRSA infections more difficult and there is a need for evaluating newer agents alternative to Vancomycin.

Daptomycin is a cyclic lipopeptide antibiotic that is rapidly bactericidal in vitro against a broad spectrum of gram-positive bacteria. Its unique mechanism of action involves calcium-dependent binding to the bacterial plasma membrane and disruption of membrane function[6]. In 2003, Daptomycin has been approved by Food and Drug Administration (FDA) for the treatment of complicated skin and skin structure infections (cSSTI) and later for the treatment of *S. aureus* bacteremia and right-sided endocarditis [14]. This drug causes myotoxicity and cannot be used in respiratory infections. Various studies demonstrated that Daptomycin proved to be more effective than vancomycin against MRSA[15,16].

We conducted this study to determine the *in vitro* activity of Daptomycin against clinical isolates of MRSA obtained in the Department of Microbiology, Andhra medical college, Visakhapatnam

The aim and objectives includes, isolating the Staphylococcus aureus from various clinical samples and determining the prevalence of MRSA among *S.aureus* isolates. And also to determine the in-vitro susceptibility of Daptomycin against MRSA isolates by E test

Materials and Methods

The present study was conducted in the Department of Microbiology, Andhra Medical College, Visakhapatnam from September 2016 to November 2016. A total of 418 clinical samples like pus, blood, urine, sputum, body fluids; received from various departments were included in the study.

All the samples were inoculated on nutrient agar (Figure 1), blood agar (Figure 2), Mannitol salt agar and incubated at 35°C for 18 – 24 hours. *Staphylococcus aureus* isolates were identified by Gram Stain (Figure 3) and Standard laboratory tests (Figure 4)[3]. Methicillin resistance was identified by Cefoxitin disc diffusion method as per CLSI guidelines [7]

The susceptibility and MIC of Daptomycin were determined by E-test system (Hi-Media, Mumbai) with a concentration range of 0.016 to 256 µg/ml. The Daptomycin E-test contained a concentration gradient of Daptomycin with a standard amount of calcium throughout the strip. E-test strips were applied to the surface of 150-mm Mueller-Hinton agar plates. Plates were incubated at 35°C for 18 to 24 hr prior to reading the MIC results (Figure 5). For disc diffusion,

Quality control strains of *S. aureus* ATCC 25923 and for MIC, Quality control strains of *S. aureus* ATCC 29213 was used with every set of test. The isolates were categorized as susceptible or resistant to Daptomycin according to breakpoints published by the CLSI. Susceptibility breakpoint for Daptomycin was considered as <1 mcg/ml for *Staphylococcus aureus* ATCC 29213.

Results and Discussion

A total of 418 clinical samples like pus, blood, urine, sputum, body fluids; received from various departments in the Department of Microbiology were included in the study (Figure 6).

Out of 418 samples, 126 (30.1%) were Staphylococcus aureus isolates. Out of 126 *Staphylococcus aureus* isolates, 50 (39.7%) were MRSA (Figure 7).

Out of 50 MRSA isolates, the majority (52%) were from pus samples followed by 22% from sputum samples (Figure 8). All MRSA (100%) were susceptible to Daptomycin with MIC range 0.25 µg to 0.75ug

MRSA is one of the leading pathogens for hospital and community-acquired infections. For decades, vancomycin was the mainstay in the treatment of infections caused by MRSA. Increased use of vancomycin has resulted in the emergence of MRSA with reduced susceptibility to vancomycin.[8,10,18].

The emergence of vancomycin-intermediate or resistant *S. aureus* has created the need for other anti- MRSA antibiotics. Many alternatives for treatment of MRSA infection including Linezolid and Daptomycin are currently approved by the Food and Drug Administration.

Fig.1 Nutrient agar showing golden yellow pigment colonies of *Staphylococcus aureus*

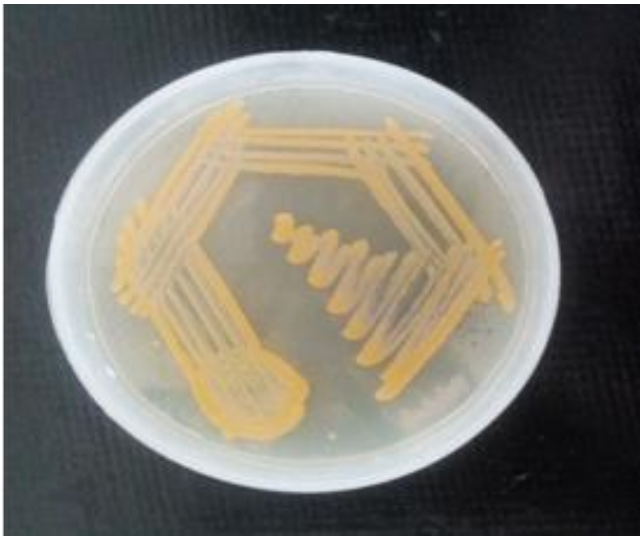


Fig.2 Blood agar showing hemolysis around *Staphylococcus aureus* colonies

Fig.3 Gram stained smear showing Gram positive cocci in clusters

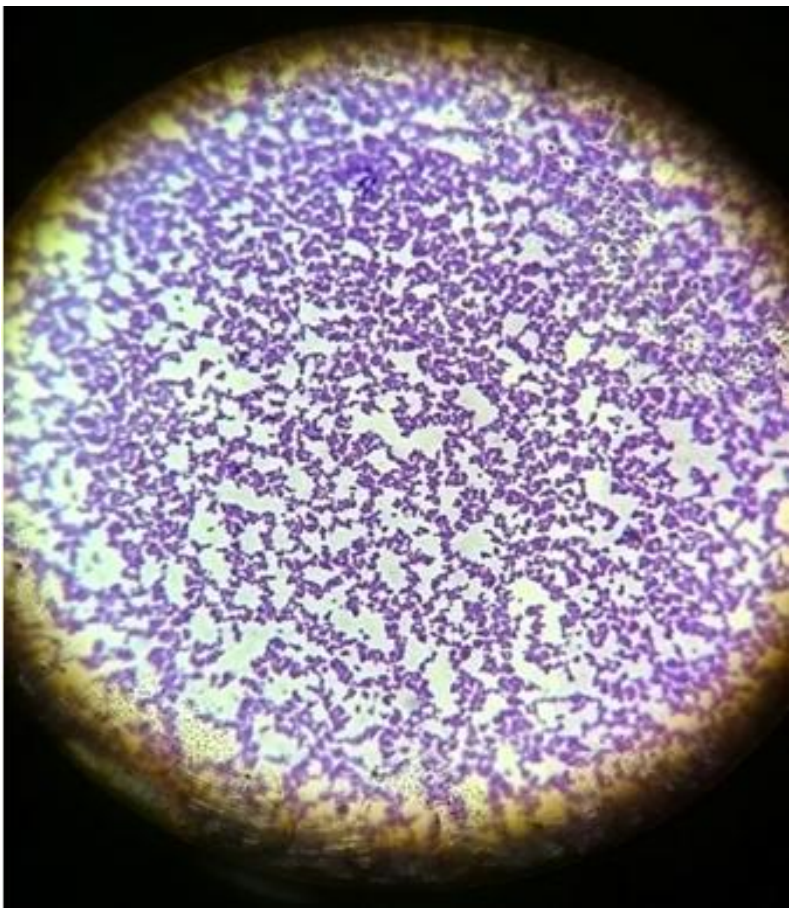


Fig.4 Coagulase test

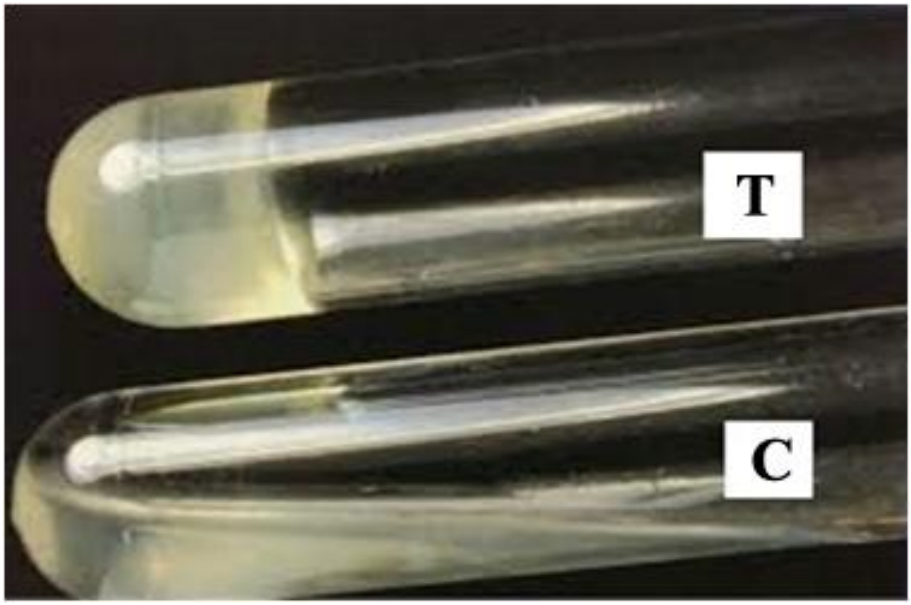


Fig.5 E-test showing MIC value as 0.38µg/ml

Fig.6 Prevalence of *S.aureus* and MRSA isolates among various clinical samples (no 418)

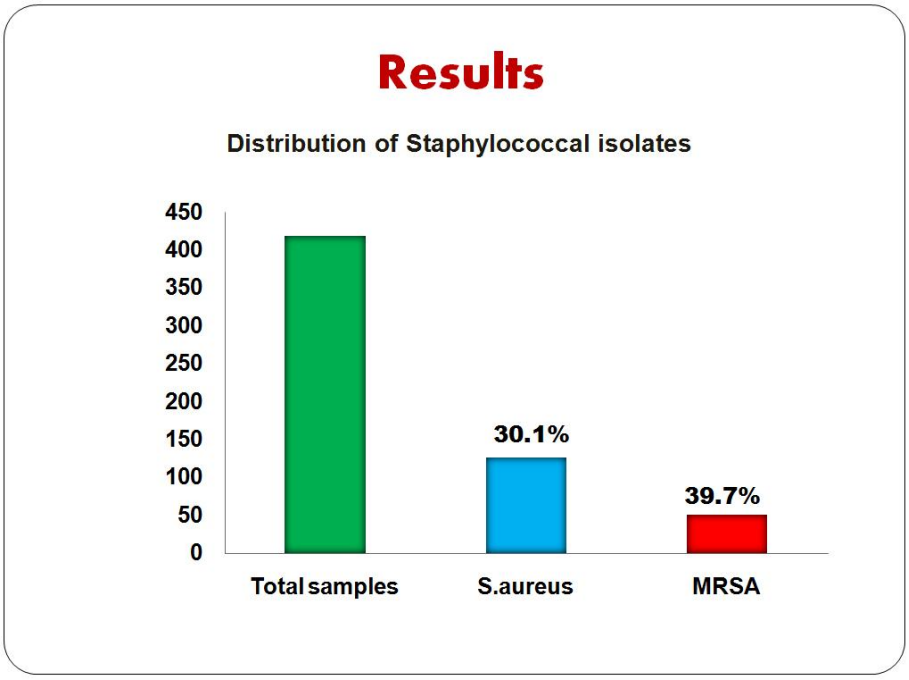


Fig.7 Isolation of MRSA from various samples

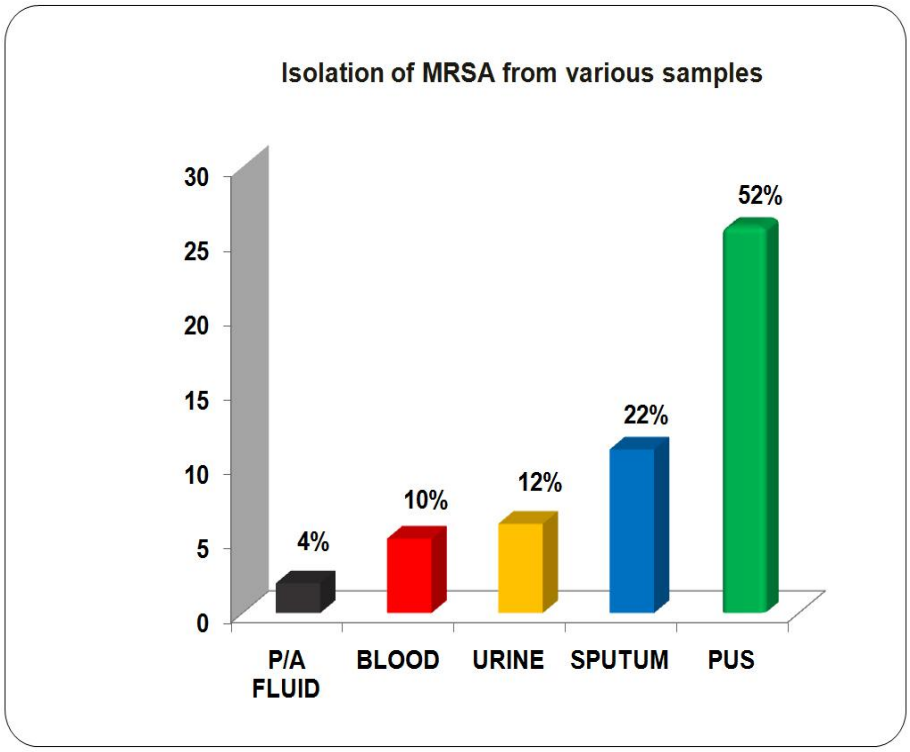
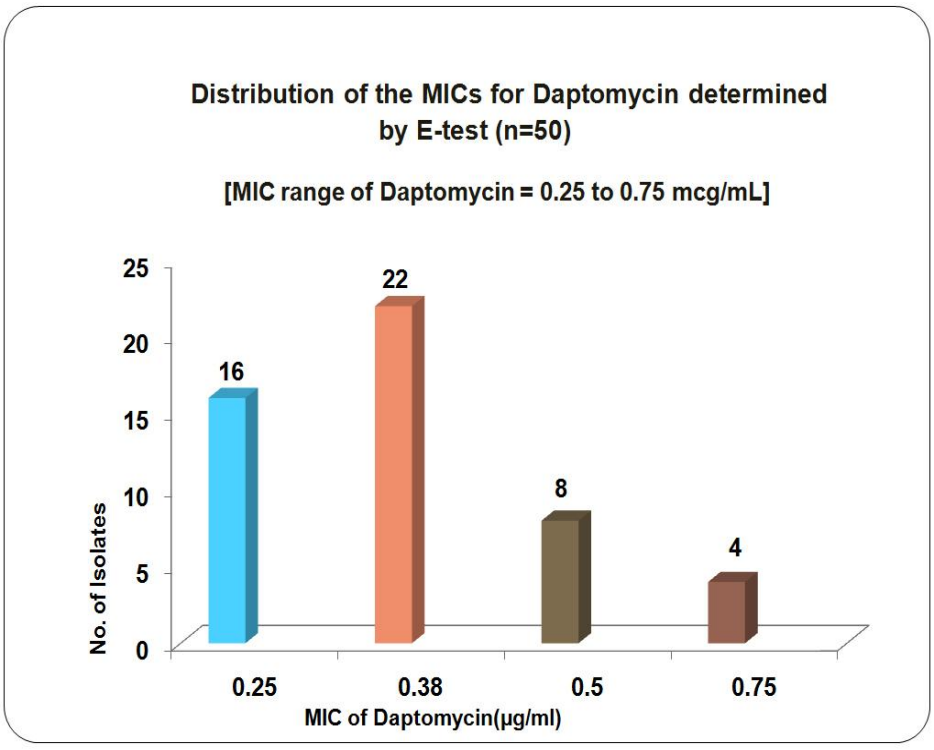


Fig.8 Distribution of the MICs for Daptomycin determined by E-test for 50 isolates of MRSA



In the present study, the prevalence rate of MRSA was 39.7 %, which correlates with the study of Hashem *et al.,* [12] who reported 40 %. Majority of MRSA isolates(52%) were from pus samples in our study which coincides with the studies of Afzal Husain *et al.,* [12] who reported majority 94.5% were from pus samples only.

Daptomycin is cyclic polypeptide semi-synthetic antimicrobial agent with activity against a broad range of Gram-positive bacteria including MRSA and vancomycin- resistant *S. aureus* [11] and also found active against strains resistant to Linezolid and quinupristin/dalfopristin [20].

As per CLSI guidelines[7], susceptibility breakpoint of Daptomycin is considered as ≤1 µg/ml for *Staphylococcus*.

In the present study, all 50 MRSA isolates were susceptible to Daptomycin with MIC range from 0.25 –0.75 µg/ml, where Husain *et al.,* [2] reported 0.19 to 1µg/ml, Rajeet Kaur *et al.,* [21] reported 0.064 to 1µg/ml and Nivedita *et al.,* [18] reported 0.016 to 0.5µg/ml, Bram M.W, Diederen *et al.,* [4] reported 0.125 to 1µg/ml.

Daptomycin showed 100% sensitivity against all MRSA isolates in our study suggesting that daptomycin can be used as alternative agents for the treatment of infections caused by MRSA. However, it should be indicated only where Daptomycin has a clear therapeutic advantage over other anti-MRSA drugs

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How to cite this article:

Lakshmi, N., T.M.S.S. Sudha Vani, B. Gowtham, C. Siva Kalyani, S. Chandra Babu Naidu and Manjula, M. 2019. *In-Vitro* Susceptibility of Daptomycin in MRSA by E-Test. *Int.J.Curr.Microbiol.App.Sci.* 8(06): 1784-1791. doi: https://doi.org/10.20546/ijcmas.2019.806.213