International Journal of Microbiology Research
ISSN: 0975-5276 & E-ISSN: 0975-9174, Volume 10, Issue 7, 2018, pp.-1295-1298.
Available online at https://www.bioinfopublication.org/jouarchive.php?opt=&jouid=BPJ0000234

Research Article

DETECTION AND EFFICACY OF DAPTOMYCIN VERSUS VANCOMYCIN IN BLOOD STREAM INFECTIONS BY METHICILLIN – RESISTANT *Staphylococcus aureus* WITH HIGH VANCOMYCIN MINIMUM INHIBITORY CONCENTRATION

DEEPALI DESAI, SAVITA JADHAV*, CHANDA VYAWAHARE, SHAHZAD BEG MIRZA AND R.N. MISRA
Department of Microbiology, Dr D.Y. Patil Medical College, Hospital and Research Centre, Dr D. Y. Patil Vidyapeeth, Pimpri, Pune, 411018, India
*Corresponding Author: Email - patilsv78@gmail.com

Received: June 27, 2018; Revised: July 12, 2018; Accepted: July 15, 2018; Published: July 30, 2018

Abstract- Blood stream infections (BSI) caused by Methicillin-resistance *S. aureus* (MRSA) is a common disease with high risk of mortality and complications. Prevalence of MRSA with high vancomycin Minimum inhibitory concentrations (MICs) have been increased and usually associated with prolonged bacteraemia and increased mortality. Recent consensus guidelines recommend that clinicians consider using alternative agents for MRSA infection when the vancomycin MIC is greater than 1 mcg/ml. Recent reports comparing daptomycin and vancomycin in treatment of BSI by MRSA with high vancomycin MIC demonstrated that daptomycin presented improved outcome, both in terms of rates of clinical success and mortality, compared to vancomycin. The aim of our study to compare the in-vitro susceptibility of daptomycin verses vancomycin susceptibility by E-test in MRSA BSIs isolates. Materials and Methods: In this pilot study, 100 MRSA BSIs isolates were collected over a period of one year. Antimicrobial susceptibility testing was performed by Kirby Bauer disc diffusion method. The inducible Clindamycin resistance was performed by D- zone test. Dual MRSA Detection EZY MIC Strip EM063 [Hi - media] for detection of Oxacillin and vancomycin by E-test. Daptomycin E test Daptomycin was performed by Ezy MIC TM Strip (DAP) (0.016-256 mcg/ml) EM088 [Hi- Media]. Results and Observations: all strains were susceptible for vancomycin and tigecycline by Kirby- Bauer Disc diffusion method. 96% MRSA strains were susceptible with linezolid, 86% MRSA strains were susceptible to TMP-SMX, 70% susceptibility to gentamycin while 56 % MRSA strains were resistant to erythromycin and subjected for detection of inducible clindamycin resistant [ICR] test. Of which 20 % strains were positive for ICR. All 100 MRSA BSIs strains were shown susceptibility by Daptomycin Ezy MIC strip test. Conclusion: These results supports the practice of switching early from vancomycin to daptomycin for the treatment of BSIs by MRSA when the vancomycin MIC is >1 mcg/ml.

Keywords- *Daptomycin, Vancomycin*

Citation: Deepali Desai, *et al.,* (2018) Detection and Efficacy of Daptomycin Versus Vancomycin in Blood Stream Infections by Methicillin -Resistant Staphylococcus *aureus* with High Vancomycin Minimum Inhibitory Concentration. International Journal of Microbiology Research, ISSN: 0975-5276 & E-ISSN: 0975-9174, Volume 10, Issue 7, pp.-1295-1298.

Introduction

Blood stream infections (BSI) caused by Methicillin-resistance *S. aureus* (MRSA) is a common disease with high risk of mortality and complications. Vancomycin is the standard first-line treatment for the MRSA BSIs recommended by current guidelines by the Infectious Diseases Society of America (2001) [1-2]. Prevalence of MRSA with high vancomycin Minimum inhibitory concentrations (MICs) have been increased and usually associated with prolonged bacteraemia and increased mortality. There are concerns about the efficacy of vancomycin. Recent consensus guidelines recommend that clinicians consider using alternative agents for MRSA infection when the vancomycin MIC is greater than 1 mcg/ml [1-3].

Daptomycin, a lipopeptide highly bactericidal antibiotic, is approved by US Food and Drug Administration for the treatment of *S. aureus* bacteraemia is considered a reasonable alternative to vancomycin. Recent reports comparing daptomycin and vancomycin in treatment of blood stream infections (BSI) by MRSA with high vancomycin MIC demonstrated that daptomycin presented improved outcome, both in terms of rates of clinical success and mortality, compared to vancomycin [3-5]. Daptomycin is also recommended for MRSA infection in patients who are allergic to vancomycin or linezolid, thrombocytopenia thought to be secondary to linezolid. Daptomycin should not be used for treatment of pneumonia, surgical prophylaxis or empirical coverage [7]. Daptomycin is a promising therapeutic option for the treatment of paediatric diseases due to MDR Gram-positive bacilli. These pathogens are increasingly common among children, particularly in the first days and months of life, and in children with a chronic underlying disease [8].

Therapeutic use of daptomycin in treating BSIs caused by MRSA remained under investigation.Hence, we have investigated incidence of prevalent MRSA infection in relevance of BSIs with associated risk factors and vancomycin MIC was greater than 1 mcg/ml. Antimicrobial susceptibility profiles were also analysed to improve antibiotic policy in support to reduce frequency of MRSA infection. The aim of our study to compare the in-vitro susceptibility of daptomycin verses vancomycin susceptibility by E-test in MRSA BSIs isolates

Materials and Methods

In this pilot study, 100 MRSA BSIs isolates were collected over a period of one year. Samples were received in the department of microbiology of tertiary care teaching hospital. Isolation and identification were done by standard conventional methods [9-10]. The study was conducted from January 2014 to December 2014. The study was approved by the Institutional ethics committee. Medical records for the source patients were reviewed for the demographic information, history of prior hospitalization, presence of major comorbid conditions. MRSA isolates were designated as HA - MRSA if the source patient had any of the following risk factors: a history of hospitalization, residence in a long-term care facility (e.g. nursing home), dialysis, or surgery within one year to the date of specimen collection; growth of MRSA within 48 h or more after admission to a hospital, presence of permanent indwelling catheter or percutaneous device at the time of culture; or prior positive MRSA culture report. If none of the above risk factors were present, the isolates were considered CA – MRSA [10-11].

Antimicrobial susceptibility testing

Antimicrobial susceptibility testing was performed by Kirby Bauer disc diffusion method for co-trimoxazole (25µg), Gentamycin(30µg), erythromycin(15µg), linezolid (30µg), tetracycline(30µg), and vancomycin (30µg) as per guidelines from Clinical and Laboratory Standards Institute (CLSI). Screening for Oxacillin resistance using Oxacillin (1µg) on (Muller-Hinton) M-H agar supplemented with 2% NaCl followed by overnight incubation at 35°C [12].

Phenotypic detection of inducible resistance to Clindamycin by D-zone test

The inducible Clindamycin resistance was performed by D- zone test using erythromycin (15µg) and clindamycin (2 µg) discs as per CLSI (Clinical Laboratory Standard Institute) guidelines. Three different phenotypes were interpreted as MS phenotype, Inducible MLSB phenotype and Constitutive MLSB phenotype [10,13].

Quality control: S. aureus ATCC 25923 were used as the quality control strain.

Dual MRSA Detection EZY MIC Strip EM063 [Hi - media] for detection of Oxacillin and vancomycin by E-test: [14]

It is a unique MIC determination paper strip which is coated with two different antibiotics on a single strip in a concentration gradient manner. The upper half has Oxacillin with a highest concentration tapering downwards and capable of showing MIC in the range of 0.064 – 8.0 mcg/ml, whereas lower half is similarly coated with Vancomycin concentration gradient in reverse direction to give MIC in the range of 0.19 – 16.0 mcg/ml.

CLSI Recommendation for Vancomycin Sensitivity Test

High molecular weight antibiotics such as Vancomycin do not diffuse in concentration gradient manner while diffusing through the agar medium when the disc susceptibility test is employed. The Antimicrobial Susceptibility Testing using disc diffusion test does not differentiate vancomycin-susceptible isolates of *S. aureus* from Vancomycin intermediate isolates, nor does the test differentiates among Vancomycin–susceptible, intermediate, and resistant isolates of coagulase-negative staphylococci, all of which may give similar size zones of inhibition. CLSI therefore recommends that MIC test should be performed to determine the susceptibility of all isolates of staphylococci to Vancomycin.

MIC Reading

Read the plates only when sufficient growth is seen. Read the MIC where the ellipse intersects the MIC scale on the strip. For bactericidal drugs such Oxacillin, Vancomycin, Gentamicin and other members of β-lactams class of drugs, always read the MIC at the point of completion inhibition of all growth, including hazes, microcolonies and isolated colonies. If necessary, use magnifying glass. Isolated colonies, microcolonies and hazes appearing in the zone of inhibition are indicative of hetero nature of the culture having resistant subpopulation in it. In such cases, consider reading for MIC determination at a point on the scale above which no resistant colonies are observed close to MIC strip (within 1-3 mm distance from the strip). Since Ezy MIC™ strip has continuous gradient, MIC values "in-between" two-fold dilutions can be obtained. If the ellipse intersects the strip in between 2 dilutions, read the MIC as the value which is nearest to the intersection. 8. When growth occurs along the entire strip, report the MIC as > the highest values on the MIC strip. When the inhibition ellipse is below the strip (does not intersect the strip), report the MIC < the lowest value on the MIC scale.

Interpretation

Use following interpretive criteria for susceptibility categorization of Oxacillin and vancomycin

Test organism	Incubation	Interpretative criteria		
Staphylococcus spp. For Oxacillin	35-37^{0}C for 18-24 hrs	< S	I	>R
		2	-	4
Staphylococcus spp. For Vancomycin	35-37^{0}C for 18-24 hrs	2	4-8	16

Quality Control: Quality control of Ezy MIC™ Strips was carried out by testing the strips with standard ATCC Cultures recommended by CLSI on suitable medium incubated appropriately.

Daptomycin E test Daptomycin Ezy MIC™ Strip (DAP) (0.016-256 mcg/ml) EM088 [Hi- Media][15]

Antimicrobial Susceptibility Testing by E-test: It is a unique MIC determination paper strip which is coated with Daptomycin in a concentration gradient manner, capable of showing MICs in the range of 0.016mcg/ml to 256 mcg/ml, on testing against the test organism. Ezy MIC™ strip is useful for quantitative determination of susceptibility of bacteria to antibacterial agents. The system comprises of a predefined quantitative gradient which is used to determine the Minimum Inhibitory Concentration (MIC) in mcg/ml of different antimicrobial agents against microorganisms as tested on appropriate agar media, following overnight incubation. Preparation of inoculum-From the pure colony of MRSA ,2-3 pure isolated colonies were inoculated in the 5 ml tryptone soya broth and were incubated at 35-370C for 2-4 hrs until moderate turbidity develops. The inoculum turbidity was matched with the turbidity of 0.5 McFarland. Daptomycin MIC strips are supplemented with calcium ions therefore it can be tested on regular Muller Hinton agar (MHA). Dip a sterile nontoxic cotton swab into the standardized inoculum and rotate the soaked swab firmly against the upper inside wall of the tube to express the excess fluid. Streak the entire agar surface of the entire plate with the swab by rotating the plate. Place the strip at the desired position on the plate pre-lawned with test organism aseptically. Incubate the plates in the incubator at 35-370C for 18-24 hrs.

MIC reading

Read the MIC where the ellipse intersects the MIC scale on the strip.

Quality control- Quality control of Ezy MIC strips were carried out by testing the strips with standard *S. aureus* ATCC 29213 as per CLSI guidelines.

Interpretation –

Test organism	Incubation	Interpretative criteria		
Staphylococcus spp	35-37^{0}C for 18-24 hrs	< S	I	>R
		1	-	-
Enterococcus spp	35-37^{0}C for 18-24 hrs	4	-	-

Results and observations

During the study period, 100 consecutive MRSA isolated from BSIs with a vancomycin MIC >1mcg/ml by E-test were identified and screened for inclusion. Of which all strains were susceptible for vancomycin and tigecycline by Kirby-Bauer Disc diffusion method. 96% MRSA strains were susceptible with linezolid, 86% MRSA strains were susceptible to TMP-SMX, 70% susceptibility to gentamycin while 56 % MRSA strains were resistant to erythromycin and subjected for detection of inducible clindamycin resistant [ICR] test. Of which 20 % strains were positive for ICR [Table-1]. Of which 100 MRSA BSIs, 70% strains were HA-MRSA and 30% were CA-MRSA [Fig-1]. ICR distribution were also high in HA-MRSA *i.e.*, 18 (90%). All four linezolid resistant strains were HA- MRSA isolated from medicine intensive care unit [MICU].

Table-1 Distribution of the antibiotic resistance pattern among the isolates

Antibiotics	n=100	
	Sensitive	Resistant
Oxacillin	00	100
Gentamycin	70	30
Tetracycline	59	41
TMP-SMX	86	14
Linezolid	96	4
Erythromycin	44	56
Tigecycline	100	00
Vancomycin	100	00
Cefotaxime	53	47
Clindamycin	17	19
Inducible Clindamycin [ICR]	20	

Total of 54 % MRSA BSIs strains were from various ICUs and 46 % MRSA BSIs strains were from various wards [Table-2]. 18% MRSA strains were from NICU , 12% from PICU, 10% from SICU and 14% strains from MICU. Maximum patients were catharised patients. All strains were susceptible to daptomycin.

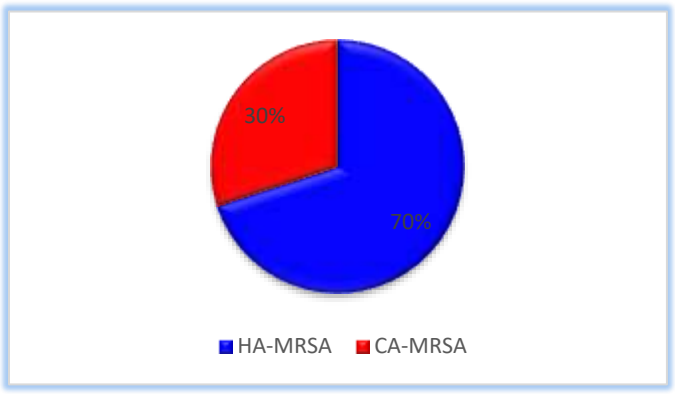

Fig-1 Distribution of HA-MRSA and CA-MRSA

Table-2 Distribution of MRSA among different wards and ICUs

Ward	No. of MRSA isolation (n=100)
NICU [Neonatal ICU]	18
PICU [Paediatric ICU]	12
SICU [Surgical ICU]	10
MICU [Medicine ICU]	14
Obstetrics and gynaecology	6
Surgery	13
Medicine	11
Orthopaedic	8
Paediatrics	6
OPD	6

Fig-2 Zone of inhibition of Oxacillin-Vancomycin Ezy MIC strip EM063 showing MIC of 2mcg/ml for Vancomycin and MIC of >8mcg/ml for Oxacillin

According to the Clinical and Laboratory Standards Institute (CLSI), daptomycin MICs of 1mcg/ml or lower in *S. aureus* isolates were considered to be susceptible.

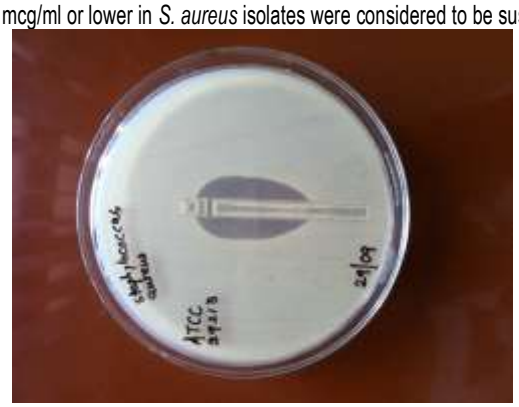

Fig-3 Zone of inhibition of Daptomycin Ezy MIC strip (EM088)for standard *S. aureus* ATCC29213 culture MIC: 10mcg/ml

All 100 MRSA BSIs strains were shown vancomycin MIC > 1 mcg/ml. Distribution of Daptomycin MIC were as follows; 0.19mcg/ml by 30% MRSA, 0.25% mcg/ml by 35% by MRSA, 0.38 mcg/ml by 21% MRSA, and 0.5% mcg/ml by 14 % MRSA.

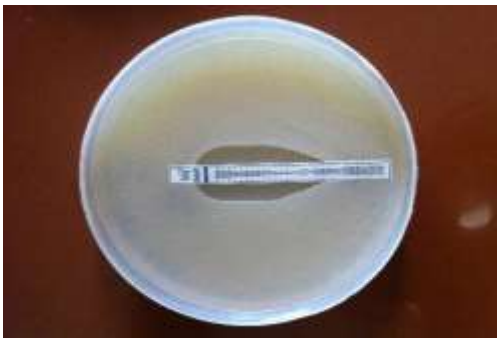

Fig-4 Zone of inhibition of Daptomycin Ezy MIC strip (EM088) for *S. aureus* culture MIC: 10mcg/ml

Discussion

The treatment of serious BSIs MRSA infections presents a great challenge to clinicians, for which bactericidal therapy is essential to improve clinical outcome. Infections caused by MRSA with higher MIC value *i.e.*, > 1mcg/ml showed significantly improved outcomes, including decreased 30- day mortality and persistent bacteraemia [1]. There are some concerns with the use of daptomycin, however. In November 2010, the Food and Drug Administration (FDA) made changes to the package insert of daptomycin warning physicians of a possible decrease in efficacy of daptomycin in patients with moderate renal impairment [4]. This was based on a subgroup analysis of the original phase 3 MRSA BSIs trial data showing a marked difference in clinical success 6 weeks after the last dose of antibiotics, in patients with a creatinine clearance (CrCl) of <50 mL/minute. In the present study 70% were HA- MRSA strains while 30 % were CA-MRSA. ICR were also detected higher *i.e.*, 90% in HA-MRSA. 54% strains were isolated from various ICUs and with vancomycin higher MIC value *i.e.* > 1mcg/ml and susceptible to daptomycin. Resistance to daptomycin in *S. aureus* has been reported in association with an increased thickness of the cell wall. Synergism between daptomycin and other antibiotics, including β- lactams and aminoglycosides, has been reported but data concerning the efficacy of combinations with other antibiotics remain rare [16]. There is limited information regarding the use of daptomycin in the neonatal population, and dosage adjustments for neonates with renal dysfunction. Kristen Gawronski *et. al*. (2015) reported on the successful use of daptomycin in a 1-month-old, former 24-week gestation neonate with persistent methicillin-resistant Staphylococcus epidermidis (MRSE) bacteraemia and impaired renal function [17]. There is limited information regarding the use of daptomycin in the neonatal population, and dosage adjustments for neonates with renal dysfunction. In the present study 18% MRSA were from BSIs of NICU patients and all were susceptible to daptomycin and showed higher MIC >1mcg/ml to vancomycin. The expanding use of intravascular catheters, prosthetic devices, invasive procedures and broad-spectrum antimicrobials use has resulted in an increased population of patients at risk for MRSA BSIs [18-19]. In the present pilot study, we have analysed in-vitro 100 MRSA from BSIs for the efficacy daptomycin in comparison with MRSA showed vancomycin MIC >1 mcg/ml and patients were not switched to daptomycin from prior therapy. Comparative and extensive evaluation with large number of patients in compliance with treatment switched to daptomycin should be studied.

Conclusion: These results supports the practice of switching early from vancomycin to daptomycin for the treatment of BSIs by MRSA when the vancomycin MIC is >1 mcg/ml.

Application of research These results supports the practice of switching early from vancomycin to daptomycin for the treatment of BSIs by MRSA when the vancomycin MIC is >1 mcg/ml.

Research Category: Blood stream infections

Abbreviations

BSI: Blood stream infections, MRSA: Methicillin-resistance *S. aureus,*

Acknowledgement / Funding Author thankful to Dr D.Y. Patil Medical College, Hospital and Research Centre, Dr D. Y. Patil Vidyapeeth, Pimpri, Pune, 411018, India

***Principle Investigator: Deepali Desai**
University: Dr D. Y. Patil Vidyapeeth, Pimpri, Pune, 411018, India
Research project name or number: NIl

Author Contributions All author equally contributed

Author statement All authors read, reviewed, agree and approved the final manuscript

Conflict of Interest None declared

Ethical approval This article does not contain any studies with human participants or animals performed by any of the authors.

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