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Prevalence of Reduced Vancomycin Susceptibility measured by minimum inhibition concentration (MIC) among Methicillin Resistant *Staphylococcus aureus* (MRSA) isolates from Surgical Site Infected Patients at Clinical Training Centre, Sungai Buloh, Malaysia

Abstract— Methicillin Resistant *Staphylococcus aureus* (MRSA) causing surgical site infections is one of the most common nosocomial infections affecting post-surgery patients. Vancomycin is the recommended treatment with MRSA-resistance breakpoint for minimum inhibition concentration (MIC) of ≤2 ug/mL where the pathogen can be considered as susceptible. Here, we describe the MIC of vancomycin against our MRSA isolates. Retrospective data of MRSA positive cultures from post-surgical patients who were admitted to the Clinical Training Centre Sungai Buloh public section from 2016-2017 with documented MIC to vancomycin were analyzed. The specimens consist of pus swabs, mediastinal fluid, sternal bone, and tissue. A total of 29 MRSA were isolated from 11 patients. There were 19, 3, 3 and 4 MRSA with vancomycin's MIC (ug/mL) of ≤0.5, 1, 1.5 and 2 respectively. The MRSA with MIC of 2 ug/mL were observed from two different patients with one of them showing MIC of 0.5 ug/mL which grew from wound swabs to 2.0 ug/mL which grew from sternal bone and mediastinal fluid isolates. Vancomycin reduced susceptibility MRSA has been observed in our clinical training centre with a 1.1% incidence. Identification of possible risk factors and follow up of outcomes is required to fully elucidate the importance of this occurrence.

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1 INTROD UCTION

Staphylococcus aureus is a major pathogen both within hospitals and in the community [1, 2] . For surgical site infections, *S. aureus* is one of the top three most common causative agent worldwide [3-5]. The significant impact of this pathogen in healthcare industries has been translated into manifestation of many guidelines that outlined steps to prevent SSI with screening, decolonizing and prophylaxis towards *S. aureus* [6, 7].

Methicillin resistant *S. aureus* (MRSA) are S*. aureus* that become resistant to methicillin and other β-lactam antibiotics through the expression of a foreign penicillin binding protein (PBP) called PBP2a. PBP 2a that is encoded by *mecA* gene (which is acquired from another species) makes MRSA differ genetically from methicillin-sensitive *S. aureus* isolates [8]. Hence, MRSA infection if not treated with appropriate antibiotics may result in morbidity and mortality to patients.

Vancomycin has been used as a front-line drug of choice for treating infections caused by MRSA for the past 60 years. Vancomycin acts by binding to the D-Ala-D-Ala moiety of the monomers, which inhibits the later stage of the growing peptidoglycan of *S. aureus* with consequent interruption of its cell wall synthesis [9].

Apart from vancomycin, there are many other antibiotics available that can be used to treat MRSA infections such as trimethoprim-sulfamethoxazole, clindamycin, linezolid, quinupristin-dalfopristin, daptomycin, telavancin and the latest available in the market being ceftaroline. Therefore, the decision to use vancomycin among clinicians is guided by the susceptibility value which is reflected by the minimum inhibition concentration (MIC), type of infection, and adverse reactions towards vancomycin [7, 10].

There has been some observation which showed high MIC values (reduced in susceptibility) towards vancomycin among MRSA isolates. This has been a concern since some studies have shown data of poor clinical outcomes being associated with this phenomenon [11, 12]. Thus our objective is to determine the MIC values of vancomycin against MRSA isolated from our surgical site infected patients.

2 METHODS

Our study started with data collection of consecutive post-surgical patients admitted to the UiTM Clinical Training Centre, Sungai Buloh from 2016-2017 with MRSA infections. The MIC value of these MRSA were recorded and analysed.

The clinical specimens consist of 18 pus swabs, 7 tissues, 1 blood, 1 sternal bone, 1 mediastinal fluid and 1 peritoneal fluid. All clinical specimens received were processed according to standard microbiological method and the bacterial colonies were identified by using VITEK2 Compact (Biomerieux, France).

The susceptibility testing of all the isolated *S. aureus* were determined against penicillin (P, 10ug), cefoxitin (FOX, 30 ug), erythromycin (E, 15 ug), clindamycin (CC, 2ug), trimethoprim-sulfamethoxazole (SXT, 1,25/23, 75 ug), gentamicin (GN, 10ug), rifampicin (RA, 5ug) and fusidic acid (FD, 10ug) by using Kirby-Bauer disk diffusion technique according to Clinical Laboratory Standard Institute (CLSI) guidelines. *S. aureus* samples which showed resistant to antibiotic cefoxitin (FOX, 30 ug), were regarded as MRSA and was subjected to further test for MIC against vancomycin. The method used for vancomycin MIC was agar gradient method by E test (Biomerieux, France).

3 RESULTS

A total of 29 MRSAs were isolated from 11 patients with various type of samples. Based on the data obtained, there were 19, 3, 3, and 4 MRSA with vancomycin MICs (ug/mL) of $\leq$ 0.5 ug/mL, 1 ug/mL, 1.5 ug/mL and 2 ug/mL respectively. The MRSA with MICs of 2 ug/mL were observed from two different patients (patient 6 and 7) (Table I). Particularly, patient 6 had five specimens with MRSA positive and the respective MIC values were recorded as 1.0, 0.5, 0.5, 2.0 and 2.0 ug/mL. Whereas for patient 7, MRSA was isolated from both specimens with similar MICs value of 2.0 ug/mL. Vancomycin MIC analysis against MRSA for patient 6 showed an increased in MIC value from 0.5 ug/mL (wound swabs) to 2.0 ug/mL (sternal bone and mediastinal fluid).

4 DISCUSSION

From our observation, out of 29 MRSA isolates, we found an increased vancomycin MIC value up to 2.0 ug/mL in four (1.1%) of the MRSA isolates. This value despite being within the susceptibility range, it is thought to have a reduction in therapeutic effectiveness [13]. The detection of reduced vancomycin susceptibility is important since it may suggest that this MRSA may be a precursor to Vancomycin intermediate *S. aureus* (VISA) [14]. It is also of clinical concern because poorer treatment outcomes have been associated with higher vancomycin MICs. However, this issue is still remains controversial as there are conflicting data where patients with low MICs have poor outcomes [14]. Anyway, in our cohort, our patients were successfully treated with vancomycin [15, 16].

The gold standard test for measuring antibiotic MIC is by the broth dilution method. However, this method is laborious and has never been practiced as a routine test in many clinical diagnostic laboratories. Traditionally, agar disk diffusion has been used to measure glycopeptide susceptibility, but this method is not regarded as standard since it does not measure the MIC. Due to its large size, is not recommended for glycopeptides to be tested by using disk diffusion method [9]. Automated susceptibility testing systems are also widely used, and the performance of this methodology for measuring glycopeptide MICs is said to be reliable [17]. In our laboratory, we use E test, an agar diffusion gradient method which is also able to determine an MIC value. The E test has been shown to give greater precision than the disk diffusion method, allowing better ascertainment of the actual MIC [14]. However, with the E test, the MIC value has the tendecy to be 0.5-1.5 $\log_2$ dilutions higher than reference broth method [18]. This is a known deficiency and it is widely accepted provided each laboratory specify which MIC method are being used.

Patient 6 was the only one which had MIC values from 0.5 ug/mL to 2.0 ug/mL against MRSA which was isolated from 2 different specimens within 16 days apart.

The details on various information on the samples collected are shown in Table 1. In this study however, we did not perform any phylogenetic or finger printing studies to determine the MRSA's clone of origin. Therefore, we are not able to confirm whether the identified MRSA isolates were actually originated from the same strain which has become more resistant. The determination of clonal dissemination is important in providing some information to help in controlling and preventing the spread of this infection in future [19].

Table I: Type of surgical site infections and MIC values

Patient No.	Diagnosis	Specimen		MIC Value (um/mL)
		Date	Type	
1	Right Achilles tendon wound infection	15/1/16	swab	1.5
2	Post Right Thoracotomy chest drain infection	9/1/16	swab	1.5
		11/1	Tissue	<1.0
		29/1/16	Tissue	0.5
3	Leg wound cellulitis Post CABG	10/5/16	swab	1.5
4	Wound infection Post Parathyroidectomy	30/7/16	swab	1.0
5	Diabetic wound infection	29/8/16	Pus swab of left upper limb	0.5
			Pus swab left leg	0.5
			Pus swab right thigh	0.5
		13/9/16	Swab right inner thigh	0.075
			Swab upper left arm	0.075
			Swab (back)	0.075
6	Wound infection post CABG	23/9/16	Blood	1.0
		25/9/16	Sternal swab	0.5
			Wound swab	0.5
		11/10/16	Sternal bone	2.0
			Mediastinal fluid	2.0
7	R leg cellulitis	23/12/16	Swab of lower shin	2.0
			Swab of upper shin	2.0
8	Sternal wound breakdown post CABG	25/1/17	swab	0.5
		4/2/17	Sternal wound swab	0.25
9	Infected R AVF	24/2/17	tissue	1.0
10	Wound infection post CABG	24/2/17	Swab right leg	0.5
			Pus swab right leg	0.5
			Tissue (lower harvest wound)	0.5
			Tissue (upper harvest wound)	0.5
			Tissue (middle harvest wound)	0.5
		27/2/17	Tissue	0.38
11	Infected Empyema fistula	19/5/17	Peritoneal fluid	0.75
Total				**29 MRSA**

5 CONCLUSION

Based on this short report, MRSA with reduced Vancomycin susceptibility has been observed in our clinical training centre with 1.1% incidence. This outcome warrants further identification of possible risk factors and follow up to fully elucidate the importance of this occurrence.

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CONFLICTS OF INTEREST

The authors report no conflicts of interest. The authors are responsible for the content and writing of the paper.

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