

# การทดสอบความไวในหลอดทดลองของยา Vancomycin, Fosfomycin, Fusidic Acid และ Linezolid ต่อเชื้อ Methicillin-Resistant *Staphylococcus aureus* ในโรงพยาบาลสงขลานครินทร์ ปี 2554-2555

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## In Vitro Activities of Vancomycin, Fosfomycin, Fusidic Acid and Linezolid Against Methicillin-Resistant *Staphylococcus aureus*, Year 2011-2012.

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### บทคัดย่อ:

**วัตถุประสงค์:** เพื่อทดสอบฤทธิ์ของยา vancomycin, fosfomycin, fusidic acid และ linezolid ต่อเชื้อ methicillin-resistant *Staphylococcus aureus* (MRSA, 96 สายพันธุ์) ที่แยกได้จากผู้ป่วยในโรงพยาบาล สงขลานครินทร์ ปี พ.ศ. 2555-2556

**วัสดุและวิธีการ:** ทำการทดสอบ minimum inhibitory concentration (MIC) โดยวิธี Epsilon (E) test และทดสอบการตี้อย่า hVISA โดยวิธี one point population analysis (OPA)

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**ผลการศึกษา:** จากการทดสอบเชื้อ MRSA จำนวน 96 สายพันธุ์ พบว่า ยา vancomycin, linezolid และ fusidic acid มีความไวร้อยละ 100 สำหรับ fosfomycin มีความไวร้อยละ 46 ผลการทดสอบหาเชื้อดื้อยา hVISA ในเชื้อที่ไวต่อยา ยังไม่ปรากฏ

**สรุป:** เชื้อ MRSA โรงพยาบาลสงขลานครินทร์ ยังมีความไวต่อยามาตรฐานที่ใช้ในรักษาโรคติดเชื้อ MRSA ยกเว้น ยา fosfomycin การเฝ้าระวังเชื้อดื้อยา hVISA ในเชื้อที่ไวต่อยาควรทำต่อไป

**คำสำคัญ:** เชื้อดื้อยาแวนโคมัยซินแอบแฝงในเชื้อที่ไว, เชื้อ *Staphylococcus aureus* ดื้อยาเมทธิซิลลิน, ฟอสโฟมัยซิน, แวนโคมัยซิน

## Abstract:

**Objective:** To determine in vitro activities of vancomycin, fosfomycin, fusidic acid and linezolid against methicillin-resistant *Staphylococcus aureus* (MRSA) isolated in Songklanagarind Hospital, collected during the period July 2011 to December 2012.

**Material and Method:** The minimum inhibitory concentrations (MICs) of these antibiotics were determined by the Epsilon-test method, and heterogeneous vancomycin-intermediate *Staphylococcus aureus* (hVISA) screening was done by one point population analysis (OPA).

**Results:** Of the 96 MRSA isolates tested, the results showed 100 percent susceptibility to vancomycin, linezolid and fusidic acid, but only 46 percent susceptibility to fosfomycin. No hVISA was found among MRSA screened by one point population analysis.

**Conclusion:** MRSA isolates in Songklanagarind Hospital are still susceptible to standard anti-MRSA except for fosfomycin, and a surveillance for hVISA screening should be continued.

**Keywords:** fosfomycin, heterogeneous vancomycin- intermediate *Staphylococcus aureus*, MRSA, vancomycin

## Introduction

*Staphylococcus aureus* is a gram positive coccus, forming grape-like clusters indicative of ability to divide in more than one plane. They produce catalase, coagulase and extracellular cell clumping factor. In the past, methicillin was used for treatment, and is used for routine susceptibility testing. After methicillin resistant isolates had been described, these isolates were termed as methicillin resistant *S. aureus* (MRSA).<sup>1</sup>

MRSA is a major problem around the world, causing hospital-acquired infections and, more recently, infections in the community.<sup>2</sup> The reliable anti gram positive agents are vancomycin, fosfomycin, linezolid, glycopeptide and fusidic acid. Vancomycin is recommended for serious MRSA infection treatment. However, since 1997, infection caused by MRSA isolates with vancomycin intermediate *S. aureus* (VISA) have been reported from Japan, USA, France, Korea and

Germany.<sup>3-5</sup> In Thailand, MRSA isolate with reduced susceptibility have been reported from Siriraj Hospital and Srinagarind Hospital.<sup>6,7</sup> This study purposed to determine in vitro activities of vancomycin, fosfomicin, linezolid and fusidic acid against MRSA strains in Songklanagarind Hospital. Since 2006, Clinical and Laboratory Standards Institute (CLSI) has changed vancomycin breakpoint from 4 mg/L to 2 mg/L.<sup>8</sup> We also screened the MRSA strains with minimum inhibitory concentration (MIC) of vancomycin 2 mg/L for heterogeneous vancomycin-intermediate *Staphylococcus aureus* (hVISA) finding.

## Material and Method

### Bacterial isolates

All MRSA isolates were obtained from infectious patients admitted at Songklanagarind Hospital, during July 2011 to December 2012. Each isolate represented a single isolation from each patient. The identification of MRSA isolates was confirmed by colony morphology, coagulase test and oxacillin disc diffusion test as described by the Clinical and Laboratory Standards Institute (CLSI) 2009.

### Susceptibility test

The minimum inhibitory concentration (MIC) of four anti-MRSA agents, vancomycin, fosfomicin, fusidic acid and linezolid were determined by E test method. E test of fosfomicin was performed on Mueller-Hinton Agar containing  $\alpha$ -D-glucose-6-phosphate (Sigma) at a final concentration of 25 mg/L, following the manufacturer's instructions. *S. aureus* ATCC 29213 was used as the control strain for MIC determination.

The MIC of each drug were reported as an MIC range, MIC<sub>50</sub> and MIC<sub>90</sub>. The MIC<sub>50</sub> and MIC<sub>90</sub> were expressed as the nearest log 2 concentration of antibiotic that inhibit 50 and 90 percent of the strains.

The percentage of susceptibility to MRSA was obtained by using the following breakpoint concentration: vancomycin <2 mg/L<sup>8</sup>, fusidic acid <0.5 mg/L<sup>9</sup>, fosfomicin <64 mg/L, and linezolid <4 mg/L.<sup>8,11</sup>

### Heterogeneous vancomycin- intermediate *Staphylococcus aureus* (hVISA) screening by one-point population analysis (OPA)

All of the studied isolates were screened for hVISA by OPA required a 100- $\mu$ L aliquot of MRSA suspension, adjusted to 0.5. McFarland standard (absorbance at 625 nm is ~ 0.08-0.10), was spread on a BHIA plate with 2 mg/L of vancomycin. The plates were then incubated at 37 °C, at 24 or 48 hours (h) were recorded.

### Interpretation

Any isolate yielding growth within 24 h was considered VISA, while any that grew during the 24-48-h incubation, hVISA. If there are any isolates that grew were subjected to be confirmed by the population analysis profile (PAP).

## Results

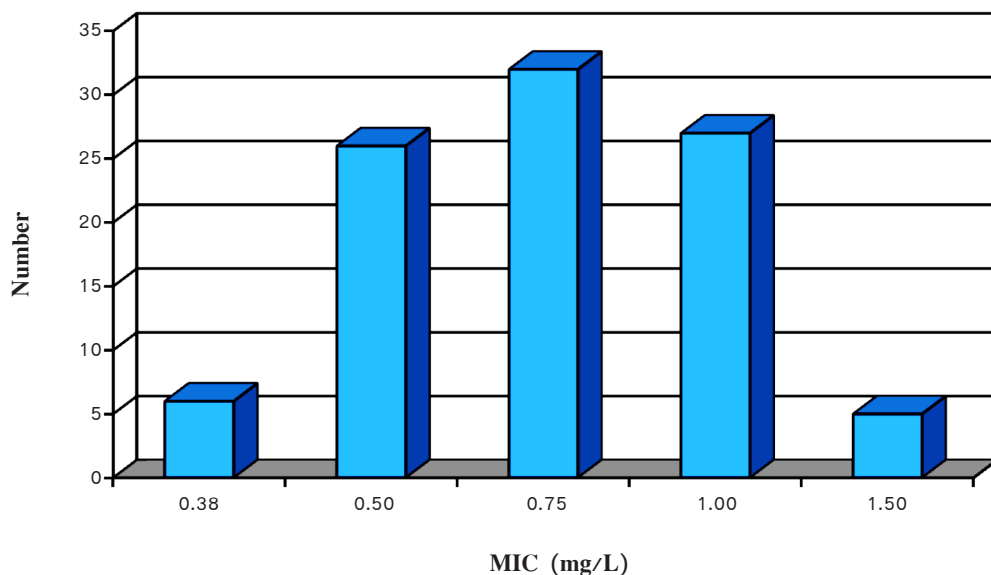
The specimens of 96 MRSA isolates were blood (16), sputum (48), pus (13), body fluid and tissue (13), and urine (6). The MIC<sub>50</sub> and MIC<sub>90</sub> of vancomycin, fosfomicin, fusidic acid and linezolid are shown in Table 1. Based on the CLSI breakpoint of vancomycin MIC <2 mg/L, the

MIC results revealed that all of the isolates were susceptible to vancomycin. The MIC<sub>50</sub> and MIC<sub>90</sub> of vancomycin was 0.75 and 1 mg/L, respectively. The vancomycin MIC distribution are shown

in Figure 1. For hVISA screening, there are no isolates were positive when screened by the one-point population analysis. No isolates could grow after 24 and 48-h after incubation, Table 2.

**Table 1** MIC range, MIC<sub>50</sub>, MIC<sub>90</sub> (mg/L) and percentage of susceptibility of 96 clinical isolates of MRSA

Antibiotic	MIC range	MIC <sub>50</sub>	MIC <sub>90</sub>	Sensitivity (%)	Susceptibility breakpoint
Vancomycin	0.38-1.5	0.75	1	100	≤2
Fusidic acid	<0.016-0.016	<0.016	<0.016	100	≤0.5
Fosfomycin	0.064->1,024	>1,024	>1,024	46	≤64
Linezolid	0.047-0.25	0.125	0.125	100	≤4



**Figure 1** Distribution of vancomycin MIC (n=96)

**Table 2** Result of one-point population analysis (OPA) and types of clinical specimens from infectious patients

Specimens	No. of MRSA isolates	Number of isolates that grew after		OPA test results
		24 h	48 h	
Blood	16	0	0	Negative
Body fluid, tissue	13	0	0	Negative
Pus	13	0	0	Negative
Sputum	48	0	0	Negative
Urine	6	0	0	Negative
<b>Total</b>	<b>96</b>	<b>0</b>	<b>0</b>	

## Discussion

This in vitro study demonstrated that vancomycin, linezolid and fusidic acid were active against tested MRSA with 100 percent susceptibility whereas fosfomycin susceptibility was only 46 percent. The susceptibility to fosfomycin was reduced when compared to the previous study.<sup>10</sup> Fosfomycin has been used for more than years in Songklanagarind Hospital<sup>11</sup>; routine disc susceptibility test of MRSA isolates showed 50 percent fosfomycin sensitivity in the year 2010.

Linezolid is a synthetic oxazolidinone that inhibits the initiation of protein synthesis at the 50S ribosome subunit.<sup>12</sup> The in vitro activities showed no difference in MIC of vancomycin and linezolid. Several reports have compared the clinical results of vancomycin and linezolid. They showed similar clinical efficacy.<sup>13</sup>

Vancomycin has been used to treat serious MRSA infection for many years. By the year 1997, isolates with reduced susceptibility were reported from Japan. In Thailand, the first report of MRSA

with reduced susceptibility was in 2001 from Siriraj Hospital and later in 2003 from Srinagarind Hospital, Khon Kaen. Although, this study suggested that there are no hVISA in Songklanagarind Hospital, it is noted that some isolates with MIC 1.5 mg/L should be a warning. In future, a surveillance programme for hVISA will be continued and further studied of MRSA conducted.

## Conclusion

**This study indicated that MRSA isolates in Songklanagarind Hospital have not yet developed decreasing vancomycin susceptibility.**

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