Screening for Vancomycin-Resistant and Intermediate *S. aureus* (VRSA & VISA) by the E-test from selected hospitals of Bethlehem and Jerusalem Districts

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Abstract

The glycopeptide antibiotic vancomycin is frequently used as the drug of choice for the treatment of infections caused by methicillin-resistant *Staphylococcus aureus* (MRSA). MRSA has been reported to cause severe life threatening infections that mandate the utilization of aggressive antimicrobial therapy such as the glycopeptides to clear these infections. However, the increase utilization of glycopeptides and in particular vancomycin has lead to the emergence of vancomycin-intermediate *S. aureus* (VISA) and vancomycin-resistant *S. aureus* (VRSA). The present study was carried out to screen for the presence of VISA and VRSA in the hospitals of Bethlehem and East Jerusalem districts. A total of 190 *S. aureus* isolates from blood culture and abscesses were subjected to MIC testing using the E-test for vancomycin with concentration 0.016-256 µg/ml. All of the 190 *S. aureus* isolates were found to be sensitive to vancomycin according to the Clinical and Laboratory Standard Institute (CLSI) M40-A antimicrobial guidelines where vancomycin MIC ≤ 2µg/ml is considered as susceptible, MIC = 4-8 µg/ml is intermediate and MIC ≥ 16 µg/ml is resistant. Of the 190 *S. aureus,* 4.2% (8/190) were sensitive to vancomycin at a concentration ≤ 2 µg/ml, 40.5% (77/190) with a concentration ≤ 1.5 µg/ml, 46.8% (89/190) with a concentration ≤ 1 µg/ml, and 8.4% (16/190) with a concentration ≤ 0.5 µg/ml. The present study reveals that vancomycin still remains the drug of choice as no isolates in both districts were found to be either VISA or VRSA; However, the physicians must be aware that the misuse of prescribing vancomycin might lead to the emergence of VISA strains as 4.2% (8/190) of the tested isolates had the highest vancomycin MIC of ≤ 2 µg/ml.

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Introduction

Staphylococcus aureus is facultative anaerobic, Gram-positive cocci that occur singly, in pairs, and in irregular clusters. *S. aureus* is non-motile, non-spore forming and coagulase positive. There are two different tests that can be performed to detect the presence of coagulase: a tube test to detect free coagulase and a slide test to detect bound coagulase. Typical colonies are yellow to golden yellow in color, smooth, entire, slightly raised, and hemolytic on 5% sheep blood agar. It also gives a positive mannitol fermentation and deoxyribonuclease test.

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S. aureus is part of the skin flora and nasal flora. About 20% of the human populations are long-term carriers of *S. aureus* (Turnidge, 2008). *S. aureus* is the most common cause of staphylococcal infections. It can cause a range of illnesses from minor skin infections such as cellulitis, folliculitis, to more severe infections that cause life threatening conditions such as meningitis and pneumonia. This pathogen frequently causes community-acquired infections and is the one of the most common causes of hospital acquired infections. *S. aureus* infections were a common cause of death in the pre-antibiotic period (Sievert et al., 2008).

Since the 1970s, *S .aureus* strains have emerged as resistant to the penicillinase-stable penicillins (cloxacillin, dicloxacillin, methicillin, nafcillin and oxacillin). The resistance is the result of a supplemental penicillin binding protein (PBP 2a) encoded by the chromosomal *mec*Agene (Turnidge, 2008). These strains historically are termed as methicillin resistant *S. aureus* (MRSA) and are resistant to all β-lactam agents. Over time, the wide-spread use of antibiotics has led some *S. aureus* to become more resistant to several antimicrobial agents. In many U.S. hospitals, strains of staphylococci such as *S. aureus* or coagulase-negative staphylococci are resistant to all available antimicrobials except vancomycin (Hiramatsu, 1997).

While most *S. aureus* are still susceptible to the antibiotic vancomycin, some called "Vancomycin-Resistant *Staphylococcus aureus* VRSA" others "Vancomycin-Intermediate *Staphylococcus aureus* VISA" or "heteroresistant VISA" have emerged. These resistant bacterial strains represent a thickening of the cell wall, which is believed to deplete the vancomycin available to kill the bacteria. More specifically the reduced susceptibility has been attributed to unusually thickened cell walls containing D-alanyl-D-alanine targets capable of binding vancomycin (Tenover et al., 2004).This worries many physicians and microbiologists because it leads to high-level resistance to vancomycin in *S. aureus.*

In 1997, the first case of vancomycin-intermediate *S.aureus* infection was reported from Japan (Sievert et al., 2008). Then within a short period of time two cases were reported from the United States. The first clinical isolate of vancomycin resistant *S.*

aureus (VRSA) was reported from the United States in 2002 (Sievert et al., 2008). Vancomycin resistant *S. aureus* have been reported recently in Brazil and in Jordan (Tiwari and Sen, 2006). For the last seven years incidence of Vancomycin-Intermediate *S. aureus* and Vancomycin-Resistant *S. aureus* (VISA and VRSA respectively) has been increasing in various parts of the world (Tiwari and Sen, 2006). *S. haemolyticus* and *S. epidermidis* (coagulase negative staphylococci CNS) with reduced susceptibility to vancomycin have been recognized (Hiramatsu et al., 1997). More clinical and laboratory studies had shown that both CNS and *S. aureus* isolates have been exposed to increasing levels of vancomycin which have demonstrated the ability of these agents to select for resistant subpopulations (Hiramatsu et al., 1997). Reduced vancomycin susceptibility may signal the onset of an increase in the MICs (minimum inhibitory concentration) of vancomycin against *S. aureus*. The clinical importance of reduced susceptibility may become most evident for treatment of infections at sites where achievable drug concentrations are lower than those commonly achieved in the bloodstream. Patients with infections caused by *S. aureus* with reduced susceptibility to vancomycin and have not responded to appropriate therapy may be candidates for treatment with an investigational drug (Hiramatsu et al., 1997). Vancomycin MIC's interpretations for *S. aureus* strains with reduced vancomycin susceptibility include vancomycin-resistant *S. aureus* strains (VRSA; MIC $\geq$ 16 µg/ml), vancomycin-intermediate *S. aureus* strains (VISA; MIC = 4-8 µg/ml) (Maor et al., 2007), and *S. aureus* strains with MIC $\leq$ 2 µg/ml are considered as susceptible.

The purpose of our study is to screen for the presence of *S. aureus* strains with decreased susceptibility to vancomycin (heteroresistant VISA and VRSA) in the hospitals of Bethlehem and East Jerusalem districts.

Methodology

A total of 190 isolates, were collected from hospitals in Bethlehem and Jerusalem Districts, 160 from Caritas Baby Hospital in Bethlehem and 30 from Al Makassed Hospital in Jerusalem. According to the recommendations by the Clinical and Laboratory Standard Institute (CLSI) (Wikler, 2009) vancomycin MICs were determined by the E-test (Biomérieux, France Lot 430525508 and REF 525508) with a concentration range of 0.016-256 µg/ml. 0.5 Mcfarland Standard *S. aureus* strains were prepared in saline and streaked on 90 mm Mueller Hinton Agar plate (Biomérieux, France). The 0.5 Mcfarland turbidity was determined by measuring the absorbance at λ= 625 nm (Secomam spectrophotometer RS232, France). The measured O.D of 0.08 was according to the Clinical Microbiology Procedures Handbook of Henry Isenberg (2004). MIC results were read after plate incubation at 37°C for complete 24 hours. *S. aureus*, ATCC 25923, was used as quality control for the E-test. MIC $\leq$ 2 µg/ml was obtained for this isolate throughout the study period (Behera, 2009).

Results and Discussion

The 190 isolates were sensitive to vancomycin with MIC $\leq$ 2 µg/ml as shown in Table 1 and Figure 1. The S, I and R in the table refers to sensitive, intermediate, and resistant to vancomycin respectively.

Table (1): E-Test results of 190 *S. aureus* isolates from hospitals in Bethlehem and Jerusalem Districts

			Vancomycin MIC (µg/ml)					
			S ≤ 2 µg/ml				I 4-8 µg/ml	R ≥ 16 µg/ml
Area	**Number of samples**	**Sample Site**	**2 µg/ml**	**1.5 µg/ml**	**1.0 µg/ml**	**0.75 µg/ml**	—	—
Jerusalem	30	Blood	1	12	11	6	—	—
Bethlehem	24	Blood	1	8	14	1	—	—
Bethlehem	136	Skin Abscess	6	57	64	9	—	—

Table 1 indicates that 28.43% (54 /190) of our isolates were from blood culture while 71.57 % (136/190) were from skin abscesses from both Bethlehem and Jerusalem districts. All of the isolates were sensitive to vancomycin with MIC ≤ 2µg/ml and were distributed according to the following percentages in Table 2.

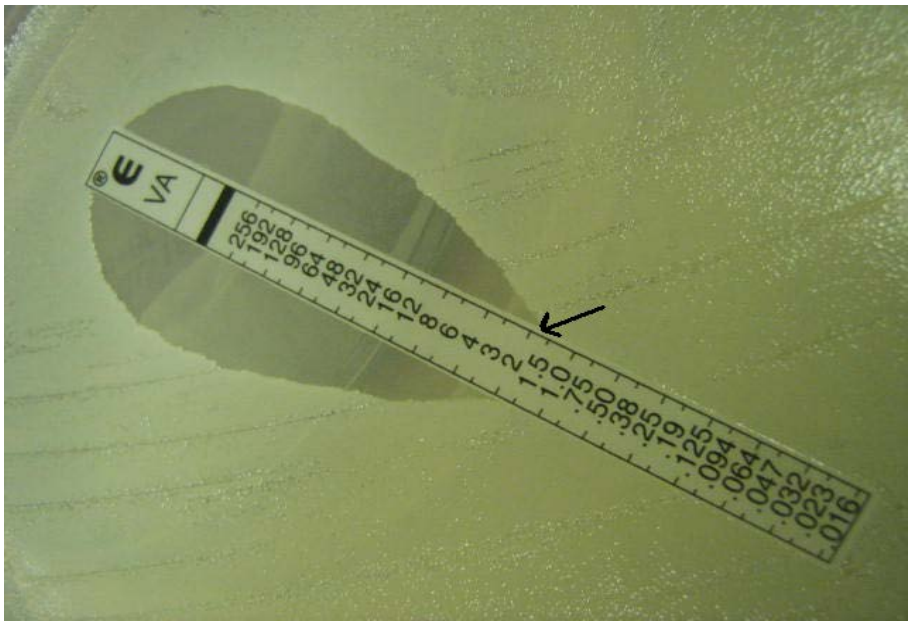

Figure (1): E-Test strip of Vancomycin sensitive *S. aureus* isolate on Mueller Hinton agar with a MIC ≤ 2µg/ml as shown by the arrow.

Table (2): Distribution of sensitive isolates of *S. aureus* according to the MIC of Vancomycin by the E-Test.

Sensitive ≤2µg/ml	Sensitive ≤1.5 µg/ml	Sensitive ≤1 µg/ml	Sensitive ≤0.5 µg/ml
4.21%	40.53%	46.84%	8.42%

Table (3) shows that the isolates were collected from the year 2003 till 2011.

Table (3): Vancomycin Concentration MIC (µg/ml) in relation with collection time.

Year	0.75 (µg/ml)	1.0 (µg/ml)	1.5 (µg/ml)	2.0 (µg/ml)	Total
2003		1			1
2004	1				1
2005		5	2		7
2006	1	9	2		12
2007	2	8	7	2	19
2008	3	13	25	3	44
2009	2	20	17	1	40
2010	6	22	22	1	51
2011	1	1	12	1	15

This study may assist physicians and microbiologists in the hospitals of Bethlehem and Jerusalem whether strains of *S. aureus* are sensitive**,** resistant or intermediate to vancomycin (heteroresistant *S. aureus*).

Recommendations

Our results reveal that vancomycin remains the drug of choice for treatment of invasive MRSA infections in Bethlehem and Jerusalem districts as no isolates were found to be either VISA or VRSA. However, the physicians must be aware that the misuse of prescribing vancomycin might lead to the emergence of VISA strains.

A future screening for VRSA and VISA should cover the whole West Bank so that the results will be more statistically significant.

Acknowledgments

- I would like to express my deep gratitude for Bethlehem University for funding this research through the Internal Research Grant 2010-2011.
- I would like to thank Medipharm Company for offering Sheep Blood Agar plates and Mueller Hinton agar for the study.

References

- Behera B, Mathur P (2009): Erroneous Reporting of Vancomycin Susceptibility for Stapyhlococcus spp. by Vitex Software Version 2.01. Jpn. J. Infect. Dis.; 62**,** 298-299.

- Isenberg D. Henry (2004): Clinical Microbiology Procedures Handbook. Second Edition.

- Hiramatsu K, MD (1997): Reduced Susceptibility of Staphylococcus aureus to Vancomycin. MMWR; 46:626-8, 635.

- Maor Y, Rahav G, Belausov N, Ben-David D, Smollan G, Keller N (2007): Prelevance and Characteristics of Heteroresistant Vancomycin-Intermediate Staphylococcus aureus Bacteremia in a Tertiary Care Center. Journal of Clinical Microbiology, p.1511-1514.

- Sievert M. Dawn, Rudrick T. James, McDonald, C., Patel, B. Jean, Wilkins, J. Melinda and Hageman, C. Jeffrey.Vancomycin-Resistant Staphylococcus aureus in the United States, 2002-2006. Clinical Infections Diseases. 2008; 46:668-74.

- Tenover C. Fred, Weigel M. Linda, Appelbaum C. Peter, McDougal K. Linda, Chaitram Jasmine, McAllister Sigrid, Clark Nancye, Killgore George, O'Hara M. Caroline, Jevitt Laure, Patel B. Jean, and Bozdogan Bülent (2004): Vancomycin-Resistant Stapylococcus aureus Isolates from Patient in Pennsylvania**.** Antimicrobial Agents and Chemotherapy, Jan. 2004, p. 275-280.

- Tiwari and Sen (2006): Emergence of vancomycin resistant Stapyhlococcus aureus (VRSA) from a tertiary care hospital from northern part of India. BMC infectious Diseases 2006, 6:156.

- Turnidge J, M.D., Rao N, MD., Chang F, M.D., Fowler Jr.V, M.D., Kellie S, M.D, MPH, Arnold, S, M.D, MS, Lee B, M.D., MBA, Tristan A, PharmaD (2008), Stapylococcus aureus .
  http://www.antimicrobe.org/sample_staphylococcus.asp retrieved (6/07/2011)

- Wikler A. Mattew, MD, MBA, FIDSA: (2009) Analysis and Presentation of Cumulative Antimicrobial Susceptibility Test Data. Third Edition.