Emergence of vancomycin-intermediate resistant *Staphylococcus aureus* in north of Palestine

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**Abstract**

**Objective:** This study was conducted to update the prevalence of methicillin-resistant *Staphylococcus aureus* (MRSA) isolates among human clinical S. aureus isolates recovered from Northern Palestine, to evaluate the possible presence of vancomycin-Resistant S. aureus (VRSA) and vancomycin- intermediate resistant S. aureus strains (VISA) and to determine the antimicrobial susceptibilities of these clinical isolates. **Methods:** The *in vitro* activities of 11 antibiotics against 204 non-duplicate S. aureus isolates from clinical samples in North of Palestine were determined by the disk-diffusion method. These samples were isolated between June 2006 and December 2007. The minimum inhibitory concentration (MIC) of vancomycin for 115 methicillin resistant *Staphylococcus aureus* (MRSA) strains was carried out using the agar dilution method. **Results:** One hundred and fifteen (56.4%) of these isolates were MRSA and according to their antibiotic profile these are multidrug resistant (resistant to three or more non-β-lactam antibiotics). Ninety nine (43.6%) isolates were methicillin sensitive S. aureus (MSSA), forty four of MSSA isolates (44.4%) were multidrug resistant, while forty five (45.6%) were non multidrug resistant. Our results showed that the most common resistance (95.6%) was to penicillin. Two strains of MRSA have shown to be vancomycin- intermediate resistant, had MIC of 4 μg/mL and 8 μg/mL and these vancomycin- intermediate resistant S. aureus strains (VISA) are resistant to all antibiotics tested. **Conclusion:** According to our information this is the first study report about VISA in Palestine.

**Keywords:** Methicillin resistant *Staphylococcus aureus* (MRSA); Vancomycin- intermediate resistant *Staphylococcus aureus* (VISA); *Staphylococcus aureus*; Multidrug resistant *Staphylococcus aureus*; Palestine

**INTRODUCTION**

*Staphylococcus aureus* is a major human pathogen responsible for serious community-acquired and nosocomial infections worldwide. It is considered as one of the most important pathogen because of both the diversity and the severity of the infections it causes, including endocarditis, and bacteremia, as well as a variety of toxin-mediated diseases such as gastroenteritis, staphylococcal scalded-skin syndrome, and toxic shock syndrome\(^1\).

Indeed, over 90% *S. aureus* strains are resistant to penicillin\(^2\). Currently one of the most serious aspects is concerned, resistance to methicillin, and essentially resistance to all other beta-lactam antibiotics. Epidemic strains are more prevalent and can spread within or be-
tween hospitals and between countries. Methicillin-resistant *S. aureus* (MRSA) isolates are genetically heterogeneous [3], and conferred resistance by carriage of the *mecA* gene encoding an alternate penicillin binding protein (PBP2). This gene is located on a genetic element called the staphylococcal cassette chromosome mec (SCCmec) [4]. Once *mecA* gene introduced into a microbial population, it may be transferred horizontally and recombined among methicillin-sensitive *S. aureus* (MSSA) cells. This has led to the global spread of MRSA in association with increasing geographic mobility of infected patients and carriers.

Infections caused by MRSA are associated with significant adverse outcomes and higher health care costs than infections caused by MSSA [5]. In addition to that, MRSA infections are associated with significant morbidity and mortality, especially in patients with bacteremia [5,6].

The increased incidence of MRSA has led to more frequent use of vancomycin in early 1990s, the drug commonly relied on for treating MRSA infections. As a consequence, selective pressure was established that eventually led to the emergence of strains of *S. aureus* with decreased susceptibility to vancomycin. The first report on an infection with *S. aureus* exhibiting reduced susceptibility to glycopeptides came from Japan in 1997 [7], and that decreased susceptibility to vancomycin became a clinical reality. The first two clinical Vancomycin-Resistant *S. aureus* (VRSA) isolates were reported in 2002 from USA [8,9].

Microbiological and epidemiological studies are of crucial importance due to the growing incidence of MRSA infections worldwide, their multidrug resistance, several reservoirs of resistant strains, facility to spread outside hospitals and to cause outbreaks requires efficacious infection control measures. This study was designed to update the prevalence of MRSA isolates among human clinical *S. aureus* isolates recovered from Northern Palestine, to evaluate the possible presence of VRSA and vancomycin-intermediate resistant *S. aureus* strains (VISA) and to determine the antimicrobial susceptibilities of these clinical isolates.

**MATERIALS AND METHODS**

**Bacterial strains and identification**

A total of 204 *S. aureus* were investigated for the period between June 2006 and December 2007. The strains were collected from various clinical specimens including pus, urine, wound, surgical infection, ear, diabetic foot, vaginal swabs, blood, sputum, and semen from the patients of different inpatient and outpatient of hospitals and from some private medical laboratories in the North of Palestine. All isolates were identified by routine laboratory procedures in microbiology laboratories of An-Najah National University, Palestine, using Gram stain, culture properties on nutrient agar and mannitol salt agar, detection of hemolysis on 5% sheep blood agar, and coagulase reaction.

**Antimicrobial susceptibility testing**

*S. aureus* strains were tested for antibiotic resistance using the disc diffusion method [10]. Antibiotic disks (Oxoid) used were penicillin G (10 μg), Gentamicin (10 μg), Tetracycline (15 μg), Norfloxacine(10μg), sulfonamides compound (300 μg), kanamycin(30μg), erythromycin (15 μg), nalidixic acid (30 μg), oxacillin (1 μg), amoxicillin-clavulanic acid (30μg) and methicillin (5 μg). Inhibition zones were determined in accordance with procedures of the Clinical and Laboratory Standards Institute (formerly, the NCCLS) [11], isolates were categorized as susceptible and resistant. According to methicillin and oxacillin, *S. aureus* isolates considered susceptible if inhibition zones are ≥14 mm and ≥13 mm, respectively. MRSA ATTC 43300 and MSSA ATCC29213 were included in this assessment as references.

**Assessment of VISA by agar dilution method**

All MRSA strains isolated between June 2006 and December 2007 were screened for VISA using Mueller-Hinton agar containing 4 μg of vancomycin per mL (MH-V4). MRSA strains grew on MH-V4 were subjected to determine minimal inhibitory concentration (MIC) testing against vancomycin. MIC of vancomycin was determined by agar dilution method recommended by the Clinical and Laboratory Standards Institute [12]. Briefly, Vancomycin (Sigma) was incorporated into Mueller-Hinton agar plates in serial dilution from 32 μg/mL to a final concentration 4 μg/mL using twofold dilutions. By direct colony suspension method 0.5 McFarland equivalent inoculum were prepared in normal saline from 18-24 h agar plate culture. 10 μL of bacterial suspension (inoculum size of 10³ CFU/spot) were...
inoculated on Mueller-Hinton agar containing vancomycin. The inoculated plates were incubated overnight at 35 °C for any visible growth. Strains that showed MIC 4-8 μg/mL were considered VISA [13].

**RESULTS**

One hundred and fifteen (56.4 %) S. aureus isolates were MRSA and according to their antibiotic profile these were multidrug resistant isolates (resistant to three or more non-β-lactam antibiotics). Ninety nine (43.6 %) isolates were MSSA, forty four of MSSA isolates (44.4 %) were multidrug resistant MSSA, while forty five (45.6 %) were non multidrug resistant MSSA. Using the disk diffusion method, our results showed that most isolates (95.6 %) were resistance to penicillin. Two strains of MRSA have shown to be vancomycin-intermediate resistant, had MIC 4 and 8 μg/mL and these VISA are resistant to all antibiotics tested. However, none of these two VISA strains could demonstrate the presence of vanA and or vanB gene by PCR. These strains were isolated from diabetic foot swab and urine sample of patients visiting outpatient departments.

**DISCUSSION**

The results of this study indicated that methicillin resistance has become a serious problem in Palestine as well as in other countries. MRSA is currently the most commonly identified antibiotic-resistant pathogen in hospitals in many parts of the world, including Europe, the Americas, North Africa, and the Middle and Far East [26]. The prevalence of MRSA in human infections reported for countries such as USA, southern Europe, northern Europe, Iran, Korea, Japan, Pakistan and Israel has ranged from <1 % - 60 % [16-25]. In 1998/1999 the prevalence of MRSA among S. aureus isolated from clinical isolates from North Palestine was 8.7 % [26]. Our results showed high prevalence of MRSA, which is higher than previous report 6.5 folds. The prevalence of MRSA is believed to be increasing internationally. In the United States, MRSA prevalence among all hospital S. aureus isolates has increased from 2.4 % in 1975 to 29 % in 1991 [27]. Between 1992 and 2003, the proportion of S. aureus isolates from patients in intensive care units that were methicillin-resistant rose from 35.9 % to 64.4 % [28]. In England and Wales, the proportion of S. aureus bacteraemia due to MRSA increased from 1 % to 2 % in 1990-1992 to ap-
proximately 40% in 2000\(^{29}\).

MRSA is typically resistant to multiple classes of antibiotics including aminoglycosides, Macrolides, chloramphenicol, tetracycline, and fluoroquinolones\(^{30}\). Therefore, treatment options for the management of serious MRSA infections are limited. Glycopeptides have approximately 40% in 2000sistantstrainsemerged. The absence of monthere.Nationwidesurveillanceprogramshouldbe
tibioticsincludingaminoglycosides

Inconclusion,our study confirms the high prevalence of MRSA in the North of Palestine and this is the first study report about VISA in North of Palestine. The emergence of VISA might also be prevalent in other parts of Palestine as antibiotic misuse is equally common there. Nationwide surveillance program should be carried out to map the vancomycin susceptibility pattern in this country. The current vancomycin resistant staphylococci in hospitals as well as in community are alarming situation to the clinicians. Hence, there should be an immediate response from the concerned authorities to check further emergence and spreading of these strains. A strict regulation on irrational antibiotic usages might be an appropriate and effective approach in this direction and enhancing the specificity of the routine laboratory identification of MRSA, VRSA, and VISA is important in hospitals with a high prevalence of this organism.

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