Emergence of vanA and vanB in Methicillin Resistant Staphylococcus aureus in Baghdad

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**ABSTRACT**

The aim of this study was to determine the distribution of vanA and vanB gene in Methicillin-resistant *Staphylococcus aureus* (MRSA) isolates in Baghdad. A total of 105 *Staphylococcus* isolates were recovered from hospitalized patients 67(63%) were *S. aureus*. The highest number of *S. aureus* were isolated from pus 26(38.8%) followed by blood 18(26.86%), ear 15(22.38%) and urine 8(11.94%). The resistance patterns of MRSA isolates (n = 40) to 10 antimicrobial agents are showed that the highest resistance was observed to ampicillin (95%) followed by cloxacillin (92.5%) and ceftriaxone (90%). The least resistance was shown to enorfloxacin (20%). The detection of plasmid DNA by gel electrophoresis showed that some *S. aureus* isolates carried a high molecular weight plasmid. PCR assay after gel electrophoresis analysis showed vanB gene in all vancomycin resistant MRSA isolates, but none of these isolates could demonstrate the presence of vanA.

**Keywords:** Methicillin-resistant, *Staphylococcus aureus*, PCR

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**INTRODUCTION**

Staphylococci are common commensals and opportunistic pathogens mainly found on the skin and in the nose of humans and also in domestic and companion animal (Pantosti, 2012). *Staphylococcus aureus* is being carried by 30% of humans in developed countries (Julia et al., 2011) is a leading cause of healthcare-associated (HA) infections and is increasingly responsible for life-threatening community-acquired (CA) infections in otherwise healthy persons (Plata et al. 2009). *S. aureus* is the number one cause of bloodstream, skin and lower respiratory infections (Goethgebeur et al., 2007).

Analyses of *S. aureus* genomes revealed that most virulence factors and antibiotic resistance genes are carried on mobile genetic elements (MGE) (Feng et al., 2008) such as pathogenicity islands, chromosomal cassettes, transposable elements, bacteriophages, and plasmids (Highlander et al., 2007). Thus, understanding the MGEs in staphylococci is critical to controlling dissemination of these virulence factors that markedly increase the hazard of these pathogens. In 1988, when the first *Enterococcus faecium* isolate with transmissible vancomycin resistance was reported in France (Malachowa and Deleo, 2010). Public health officials and infection control specialists were concerned that the vanA determinant, which mediated high-level vancomycin resistance in the enterococcal isolate, would be transferred to *S. aureus* (Leclerq et al., 1988). Further heightening the concern about the potential spread of vanA to *S. aureus*.

Thus, it surprised many scientists that the first *S. aureus* isolate reported to manifest reduced susceptibility to vancomycin did not contain vanA or any of the other known vancomycin resistance determinants. Instead, the reduced susceptibility has been attributed to unusually thickened cell walls containing D-alanyl-D-alanine targets capable of binding Vancomycin (Cui et al., 2000). The appearance of similar vancomycin-intermediate *S. aureus* strains in the United States (Smith et al., 1999), France, Brazil, Korea (Tenover et al., 2004) fueled speculation that the transfer of vanA from Enterococci to Staphylococci may not occur in nature. Such speculations were discarded in June 2002 when a vanA containing vancomycin-resistant *S. aureus* (VRSA) isolate was obtained from a dialysis patient in Michigan (Chang et al., 2003).

There are limited reports evaluating susceptibility patterns and molecular characteristics of VRSA in Baghdad. The aim of this study was to determine the distribution of vanA and vanB gene in Methicillin-resistant *Staphylococcus aureus* (MRSA) isolates in Baghdad.

**MATERIALS AND METHODS**

**Bacterial isolates**

Samples were collected from four big hospitals in Baghdad (Iraq), from October to December 2011. The isolates were initially characterized as *Staphylococcus aureus*, based on biochemical tests and Gram staining, according to the criteria established by Forbes et al. (2002).

**Antimicrobial susceptibility test**

Susceptibility of *S. aureus* isolates was tested by the disk diffusion test for Methicillin (5µg) and Vancomycin...
The MRSA and VRSA isolates were subjected to antimicrobial susceptibility testing using Kirby-Bauer disk diffusion method following CLSI guidelines (CLSI, 2009), using commercially available 6 mm discs (Bioanalyse, Turkey). The susceptibility of the isolates was determined against 10 antibacterial agents by disc diffusion method. They include Ampicillin (AMP), Cloxacillin (CX), Ceftriaxone (CRO), Erythromycin (E), Ceftixime (FEP), Azithromycin (AZM), Lincomycin (L), Fusidic acid (FA), Neomycin (N), Enofloxacin (ENR).

**Minimal inhibitory concentrations**

The MICs of Vancomycin were determined by a broth dilution method. We used Mueller-Hinton broth (Oxoid, England) with vancomycin concentrations (2-512) µg/ml according to the guidelines recommended by the CLSI document.

**Plasmid isolation**

Plasmid DNA were isolated using plasmid extraction kit (Promega,USA), and analyzed on 0.8% agarose gel.

**DNA preparation and PCR**

A PCR reaction with specific primers was performed to identify van genotypes (vanA and vanB) of each MRSA isolate (Table 1). DNA template was prepared as described by Olsvik and Strockbin (1993). 25µl of PCR amplification mixture contained deionized sterile water, 12.5µl Green Go Taq Master Mix pH 8 (Promega,USA) contained [(50unit/ml) of Go Taq DNA polymerase, (400Mm) of each dNTPs and (3mM) of MgCl2], 1pmol for specific primers (Alpha DNA,Canada). The PCR cycles for van genes (vanA and vanB) were as followed: initial denaturation at 95c° for 5 min, 30 cycles of denaturation at 94c° for 45sec, annealing at 54c° for 45sec and extension at 72c° for 7min using Gradient PCR (TechNet–500, USA).

**RESULTS**

A total of 105 *Staphylococcus* isolates were recovered from hospitalized patients 67(63%) were *S. aureus*. The highest number of *S. aureus* were isolated from pus 26(38.8%) followed by blood 18(26.86), ear 15(22.38%) and urine 8(11.94%)

The resistance patterns of MRSA isolates (n = 40) to 10 antimicrobial agents are shown in Table 2. The highest resistance was observed to ampicillin (95%) followed by cloxacillin (92.5%) and ceftriaxone (90%). The least resistance was shown to neomycin (30%) followed by enofloxacin (20%).

The detection of plasmid DNA by gel electrophoresis showed that some *S. aureus* isolates carried a high molecular weight plasmid. PCR assay after gel electrophoresis analysis showed vanB gene in all vancomycin resistant MRSA isolates (Fig. 1), but none of these isolates could demonstrate the presence of vanA gene according to the expected product for each gene when they electrophoresed with DNA ladder (Promega, USA).

**DISCUSSION**

*S. aureus* has long been recognized as one of the most important bacteria that cause diseases in humans and are among the most frequently isolated bacteria in the clinical microbiology laboratories, especially as cause of hospital acquired infections (Chanda et al., 2010).

Our results generally agreed with Dar et al. (2006) who reported high isolation percentage of *S. aureus* from pus. The percentage of ear infections in the current study was 22.38% when only 15 isolates were obtained from 67 patients. Aydemir et al. (2010) illustrated that the prevalence of *S. aureus* infections in ears was 12.2% (27/221).

First recognized in 1960, methicillin-resistant *Staphylococcus aureus* (MRSA) was considered to be a medical oddity. Now, MRSA is the most common nosocomial bacterial pathogen isolated in many parts of the world (Grundmann et al., 2006). Of the 67 clinical isolates of *S. aureus* 40 (59.7%) were methicillin-resistant *S. aureus* (MRSA). These results disagreed with results of Al-Maliki (2009) who showed that the rate of MRSA was 80.3%, but in the study of Jamaluddin et al. (2008) the resistance was lower than the current study.

The Centers for Disease Control (CDC) National Nosocomial Infection Surveillance System (NNIS) reported that methicillin resistance among *S. aureus* in US hospitals increased from 2.4% in 1975 to 29% in 1991, with a higher degree of resistance in intensive care units. More recent data from 1990 through 1997 identified that the MRSA incidence rate increased 260% in hospitals that participated in the International Networks for the Study and Prevention of Emerging Antimicrobial Resistance (INSPEAR) Programme (Eileen et al., 2002).

In Europe, there is a North to South trend in the proportion of *S. aureus* that is methicillin-resistant, ranging from 0% in Northern European to > 50% in
Southern Europe (Pletz et al., 2010). Percentages of nosocomial *S. aureus* isolates resistant to methicillin ranges between 6% in Cuba to 85% in Peru (Guzman-Blanco et al., 2009).

Methicillin-resistant *Staphylococcus aureus* (MRSA) is a major nosocomial pathogen in India, and up to 70% methicillin resistance has been reported from hospitals in various parts of India (Arakere et al., 2005).

In recent years, the widespread use of antibiotics has undoubtedly accelerated the evolution of *S. aureus* and led to the emergence of strains that have systematically acquired multiple resistance genes (Hope et al., 2008).

Plasmid analysis after electrophoresis on 0.8% Agarose gel showed that some *S. aureus* isolates carried a high molecular weight plasmid. In staphylococci, the conjugative transfer of resistant determinants is usually mediated by conjugative plasmids, conjugative plasmids, usually 35 to 50 kb, spread resistance determinants between species and genera (Khan et al., 2000).

In current study, PCR assays revealed the presence of vanB in all vancomycin resistant MRSA isolates, but these isolates did not show the presence of vanA gene. Vancomycin MICs for isolates harboring vanB were 4-32μg/ml. Cui et al. (2000) suggested that cell wall thickening is responsible for the development of vancomycin resistance. The mechanism of vancomycin resistance has been extensively studied with the first clinical VRSA strain, Mu50. Biochemical and transmission electron microscopy (TEM) examination of the Mu50 cell, suggested that it produces increased amounts of peptidoglycan.

The development of antibiotic resistance in developing countries like ours seems to be very much related to the irrational antibiotic usage due to its easy availability at the drug store without prescription, injudicious use in hospitals and uncontrolled use in agriculture, animal husbandry and fisheries.

**REFERENCES**


