RESEARCH STUDY

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Vancomycin Resistance among Methicillin Resistant Staphylococcus aureus Isolates from General Hospitals

ARTICLE INFORMATION

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ABSTRACT

Background: Multidrug resistant methicillin-resistant Staphylococcus aureus (MRSA) is a major cause of nosocomial and community acquired infections. The glycopeptides vancomycin has been proposed as the drug of choice for treating such infections; this lead to the emergence of vancomycin intermediate sensitive S. aureus (VISA) and vancomycin resistant S.aureus (VRSA).

Objectives: To identify the vancomycin resistance both phenotypically and genotypically among MRSA isolates from different hospitals and to determine the sensitivity of these isolates to different antimicrobial agents

Methods: A total of 204 S. aureus isolates were obtained randomly from various clinical specimens including (wound swab, burn swab, ear swab, urine, sputum, blood and other body fluids) from different inpatient and outpatient who were attending different hospitals in Baghdad. The susceptibility pattern of the S. aureus isolates to different antibiotics was determined by disk diffusion method and vancomycin minimum inhibitory concentration (MIC) for MRSA isolates were determined using broth dilution method following clinical laboratory standard institution (CLSI) guidelines. Van A gene was amplified by PCR using standard primers.

Results: All VRSA isolates were MRSA. Twelve VRSA isolates were positive for van A gene, while the remaining ten isolates were negative. All VRSA had a vancomycin MIC of 16µg/ml or more. In the present study, VRSA showed resistance to a wide range of antimicrobial agents (Ampicillin, Cefalothin, Cefoxitin, Erythromycin, Gentamycin, Oxacillin, Penicillin, Rifampin, Tetracycline and Trimethoprim).

Conclusions: There were high incidences of resistance to the commonly used antibiotics among VRSA isolates compared to VISA and VSSA. Further molecular studies such as PCR technique to identify genes rather than van A (e.g van HAX analogue) might be suitable to predict VRSA lacking the van A gene

Introduction:

Multidrug resistant *S.aureus* is a common cause of nosocomial infection. Measures to control *S.aureus* infections are challenged by a large and continuing increase in the prevalence of methicillin-resistant *Staphylococcus aureus* (MRSA) worldwide, the spread of highly virulent community-associated MRSA and the emergence of *S.aureus* with reduced susceptibility to vancomycin and other glycopeptides [(1).]

The condition has been further worsened by the emergence of vancomycin intermediate sensitive *S.aureus* (VISA) and Vancomycin resistant *S.aureus* (VRSA). Strains with upper levels of Minimum inhibitory concentration (MIC) of vancomycin, induce high morbidity and mortality rates compared to those which have lower levels of vancomycin MIC [(2).] As per latest clinical and laboratory standard institute guidelines staphylococci with MIC of vancomycin ≤ 2 µg/ml is susceptible (VSSA), while for which MIC is 4-8 µg/ml are intermediate (VISA) and those with MIC ≥ 16 µg/ml are resistant (VRSA) [(3).] The glycopeptide vancomycin was considered to be the best alternative for the treatment of multidrug resistant MRSA. However, there are increasing numbers of reports indicating the emergence of VRSA strains exhibiting two different resistance mechanisms. Initially VISA noted in Japan in 1996 and subsequently in United States in 1997, was believed to be due to the thickened cell wall [(4),] where many vancomycin molecules were trapped within the cell wall. The trapped molecules clog the peptidoglycan meshwork and finally form a physical barrier towards further incoming vancomycin molecules [(5).] The second, noted in

United States in 2002 among *S.aureus*, was identical to the mechanism seen in vancomycin-resistant Enterococcus. vancomycin-resistant *Enterococcus faecium* harbours the van A operon, which contains five genes, *Van*S, -*R*, -*H*, -*A* and -*X* [4].

The genetic exchange of antimicrobial resistance determinants among enterococci and staphylococci is well documented. The resistance genes are typically found on conjugative plasmids or transposons [6]. However, Tiwari and Sen have reported a VRSA which is van gene negative [7]. Subsequent isolation of VISA and VRSA isolates from other countries including Brazil, France, United Kingdom, Germany [4], India [8], and Belgium has confirmed that the emergence of these strains is a global issue.

The aim of the present study is to identify the vancomycin resistance both phenotypically and genotypically among MRSA isolates from different hospitals and to determine the sensitivity of these isolates to different antimicrobial agents.

Methods:

Bacterial isolates: A total of 204 *S.aureus* isolates were obtained randomly from various clinical specimens including (wound swab, burn swab, ear swab, urine, sputum, blood and other body fluids) from different inpatient and outpatient who were attending Baghdad Teaching Hospital, Burns Hospital, Al-Karama Hospital, and Al-Imam Ali Hospital, for the period from November 2012 to May 2013.

Antibiotic susceptibility testing: The antibiotic resistance profile was determined by the disc agar diffusion method using different antimicrobial agents; Ampicillin (10µg), Cefalothin (30µg), Cefoxitin (30µg), Erythromycin (15µg), Gentamicin (10µg), Oxacillin (1µg), Penicillin (10 units), Rifampin (5µg), Tetracycline (30µg), Trimethoprim (5µg), Vancomycin (30µg). The procedure which was accepted by the National Committee for Clinical Laboratory Standard (NCCLS) was employed as described by Bauer et al (10).

Determination of MIC: Minimal inhibitory concentration (MIC) of vancomycin was determined by broth dilution method. Gradient tubes of nutrient broth were prepared with vancomycin (2 - 32 µg / ml) and inoculated with bacterial suspension of moderate turbidity (compared to that of MacFarland 0.5 barium sulfate standard). The broth was incubated at 37°C for about 24 hours. The turbidity of each tube was compared with the turbidity of control tube; the MIC is defined as the lowest concentration of antibiotic at which there is no visible growth of organism [11].

PCR amplification for *van A* gene: *Van A* gene was detected in VRSA strains by conventional Polymerase chain reaction (PCR) technique using primers of *van A* gene with the following sequence [4]:

Forward: 5´-ATGAATAGAATAAAAGTTGC-3´ and
Reverse: 5´- TCACCCCTTTAACGCTAATA-3´.

The amplification conditions were initial denaturation at 95°C for 3min, followed by 35 cycles of denaturation at 95 °C for 15 seconds; primer annealing at 55 °C for 15 seconds; extension at 72 °C for 15 seconds and one cycle of final extension at 72 °C for 5 minutes, then cooled at 4 °C.

Statistical Analysis: The collected data were analyzed statistically using Chi-square test to find significant difference between 2 variables. P value less than 0.05 is considered as significant. Statistical analyses were performed using MINITAB [12] and SPSS (Statistical Package for social Sciences) version 17.0 computer software [13].

Results:

Among the 204 isolates of *S.aureus*, 72 were identified as methicillin MRSA by disc diffusion method, 22 (30.5%) of these MRSA isolates were identified as VRSA and 12(16.7%) were VISA and the remaining 38(52.8%) of the isolates were VSSA by determination of MIC (table 1). There were no significant differences (p =0.466) between the number of MRSA (72 out of 204 *S.aureus* isolates) and VRSA (22 out of 72 MRSA) isolates, while the differences between the number of MRSA and each of VISA (12 out of 72 MRSA) and VSSA (38 out of 72 MRSA) were significant (p= 0.003) and (p= 0.009) respectively as shown in (table 2)

In the present study, VRSA showed resistance to a wide range of antimicrobial agents. There were no significant differences in resistance rates to all antibiotics used between VRSA and VISA (P > 0.05). There were also highly significant differences in resistance rate to all antibiotics between VRSA and VSSA (p < 0.001). On the other hand, the differences in the resistance rate between VRSA and MRSA were also significant to some antibiotics (P < 0.05 and P < 0.01) and non significant to others (P > 0.05) as shown in (table 3).

In PCR method, out of 22 VRSA isolates, 12(55%) showed a band in the region of 1200 bp compared with the ladder used; indicating that these isolates have *van A* gene as shown in (Figure 1).

Table 1 Distribution of 72 MRSA isolates according to the MICs of vancomycin

MIC (µg/ml)	No. of strains	%
≥ 16*	22	30.6
8**	5	6.9
4**	7	9.7
2***	38	52.8
Total	**72**	**100**

*MIC ≥16 µg/ml = VRSA, **4-8 µg/ml = VISA, *** ≤ 2 µg/ml =VSSA

Table 2 Distrbution of *S.aureus* isolates according to its methicillin and vancomycin resistance

MRSA* No. (%)	VRSA** No. (%)	p-value	VISA** No. (%)	p-value	VSSA** No. (%)	p-value
72(100)	22(30.5)	0.466	12(16.7)	0.003	38(52.8)	0.009

Chi-sequer: MRSA vs VRSA, VISA and VSSA. *MRSA; according to disk diffusion method
**VRSA, VISA and VSSA according to vancomycin MIC

Table 3 Comparison of antimicrobial resistance rates of VRSA, VISA, VSSA and MRSA

Antibiotics	VRSA strains resistance Total: 22 No. (%)	VISA strains resistance Total: 12 No. (%)	VSSA strains resistance Total: 38 No. (%)	MRSA strains resistance Total: 72 No. (%)
Ampicillin	22(100)	12(100) ^{NS}	19(50) ***	55(76.4) *
Tetracycline	22(100)	12(100) ^{NS}	18(47.3) ***	52(72.2) **
Oxacillin	22(100)	12(100) ^{NS}	2(5.2) ***	72(100) ^{NS}
Penicillin	21(95.5)	12(100) ^{NS}	22(57.8)**	68(94.4) ^{NS}
Cefoxitin	22(100)	12(100) ^{NS}	1(2.6) ***	72(100) ^{NS}
Gentamicin	19(86.4)	10(83.3) ^{NS}	7(18.4) ***	36(50) **
Cefalothin	17(77.3)	8(66.7) ^{NS}	4(10.5) ***	52(72.2) ^{NS}
Erythromycin	17(77.3)	7(58.3) ^{NS}	11(28.9) ***	35 (48.6) *
Rifampin	17(77.3)	6(50) ^{NS}	7(18.4) ***	30(41.7) **
Trimethoprim	16(72.7)	8(66.7) ^{NS}	6(15.7) ***	30(41.7) **
Vancomycin	6(27.3)	4(33.3) ^{NS}	0(0) ***	10(13.9) **

Chi-sequer: VRSA vs VISA, VSSA and MRSA
NS: P > 0.05; Not Significant,*P < 0.05; Significant at 5% ,
**P < 0.01; Significant at 1%, ***p < 0.001; Highly significant

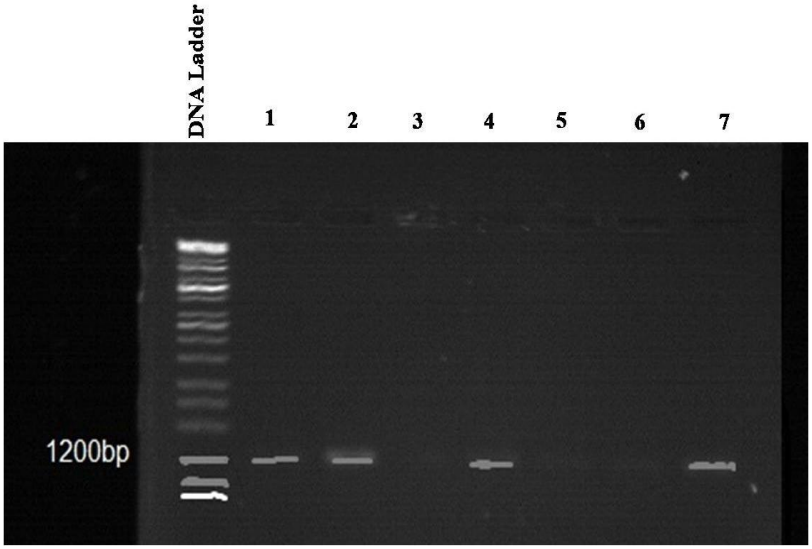

Figure 1 Electrophoresis of PCR product for *van A* gene.

Discussion:

Staphylococcus aureus is one of the important human pathogens that in last decades were a causative agent of community and hospital acquired infections and its resistance increases against β- lactams antibiotics and vancomycin. After the first report of VRSA in Japan in 1997, however prevalence of VISA and VRSA strains remained low, but in many countries is rising as heterogeneous VISA (hVISA) [14]. In present study; there was no significant difference (p =0.466) between the number of MRSA and VRSA isolates (table 1). Also, the results show that resistance to vancomycin is associated with resistance to methicillin and cefoxitin. This emergence of VRSA may also be due to the selective pressure of vancomycin, a glycopeptides which is currently the main antimicrobial agent available to treat life-threatening infections with MRSA [15].

The prevalence of the S.aureus infections vary from place to place and so also the resistance pattern which depends on the local antibiotics policy, the infection control activities, the time of the study, the number of cases which are studied and the biological characteristics of the S.aureus strains [16].

The MIC values of vancomycin for 72 MRSA isolates (table 2) indicated that different resistance rates were found; this might be related to the level of expression of van A gene in these isolates or due to other mechanisms.

Vancomycin resistant S. aureus (VRSA) tend to be multidrug resistant against a large number of currently available antimicrobial agents, compromising treatment options and increasing the likelihood of inadequate antimicrobial therapy and increase in morbidity and mortality [17]. In the present study, VRSA showed resistance to a wide range of antimicrobial agents used (table 3) this might be due to lack of sufficient knowledge on the danger of the wrong use of antibiotics, high proximity to a large number of unlicensed drug vendors, high poverty among the people which hinders them from completing the dosage regimen of the antibiotics, widespread and sometimes, the inappropriate use of broad spectrum antibiotics in the medical and the veterinary practice, antibiotic prophylaxis, high number of immune compromised patients, the increased use of invasive procedures and devices and inadequate infection control measures.

The mechanism of vancomycin resistance in VRSA is not well understood. Several genes have been proposed as being involved in certain clinical VRSA strains [18,19]. The experimental transfer of van A gene cluster from E. faecalis to S. aureus has raised fears about the occurrence of such genetic transfer in clinical isolates of methicillin resistant S. aureus [20]. In the current study a PCR amplification for van A gene among 22 VRSA isolates; showed that 12(55%) have van A gene, the remaining 10(45%) did not have van A gene.

In conclusion, results of this study showed that high incidences of resistance to the commonly used antibiotic among VRSA isolates compared to VISA and VSSA. Not all VRSA isolates have van A gene this is might be explained by the possibility of the presence of other analogous genes to van A.

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