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Staphylococcus aureus with reduced vancomycin susceptibility among clinical isolates in Erbil City

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Abstract

Background and objective: Staphylococcus aureus (S. aureus) is responsible for a wide range of diseases and increased number of the strains that acquired resistance to antibiotics. The emergence of Vancomycin resistance of S. aureus has been a significant impact on human health. The distribution of Vancomycin minimum inhibitory concentrations (MIC) and minimum bactericidal concentrations (MBC) in S. aureus isolates, and compared antibiotic susceptibility to non-glycopeptideantibiotics in different Vancomycin MIC value groupswere assessed in this study.

Methods: S. aureuswere isolated by standard method and subjected to MIC tested by broth microdilution method for Vancomycin and eight non-glycopeptideantibiotics, alsoVancomycin MBCs were determined.

Results: Approximately 56% of S. aureus with a 0.5 µg/ml Vancomycin MIC were accounted, whereas 1.77% of S. aureushadan 8 µg/ml Vancomycin MIC. In other hand, most S. aureus had 1 and 2 µg/ml Vancomycin MBC.

Conclusion: About half of the S. aureus isolates had 0.5 µg/ml of Vancomycin MIC. Relationship between Vancomycin MIC and resistance to non-glycopeptideantibiotics were observed, with increased Vancomycin MIC, the resistance to others antibiotics also elevated, and vice versa.

Keywords: Vancomycin creep; Staphylococcus aureus; MRSA; MIC creep; Resistance

Introduction

Clinicians are continually being challenged by infections caused by *S. aureus*, and remained a serious threat to human health[1,2]. The strain of *S. aureus* had been major cause of both community-acquired and health care-associated infections, and causes a severe financial burden for health systems[3,4].*S. aureus*has been a continuing threat because of the emergence of antibiotic resistance and particularly efficient at developing resistance to antibiotics[5,6]. In addition, the treatment of suspected *S. aureus*infections is becoming increasingly more complicated[1]. An important distinctive feature of *S. aureus*strains is the susceptibility to methicillin, strains are categorised intothe methicillinsensitive *S. aureus* (MSSA) and methicillinresistant *S. aureus* (MRSA)[7,8]. Therefore, most MRSA strains are multidrug resistant[9,10]. Currently, measures to control *S. aureus* infections are challenged by a large and continuing increase in the prevalence of MRSA worldwide[11-13]. The glycopeptide antibiotic Vancomycinis believed to be the most effective antibiotic against *S. aureus*, and it has been widely used for the treatment of MRSA infections for a long time[14-16]. Vancomycin has been the most reliable therapy for serious *S. aureus* and MRSA infections[17-19]. Consequently, the widespread occurrence of *S. aureus*made the increased use of Vancomycin inevitable, and this has resulted in a selective pressure and the emergence of *S. aureus* with reduced susceptibility to Vancomycin[5,11,20]. Nevertheless, intermediate or full resistance to Vancomycin has emerged recently in *S. aureus* and MRSA[21,22]. Definition of

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Vancomycin resistance is controversial of which confusion over the definitions of Vancomycin resistance has been generated by recent literature. The source of this confusion seems to be the different breakpoints in Vancomycin susceptibilities used in various countries[23-28]. In United States, the Clinical and Laboratory Standards Institute (CLSI) guidelines define *S. aureus*for which the MIC of Vancomycin is 4 µg/ml to be susceptible, while isolates for which the MIC is 8 to 16 µg/ml are intermediate and those for which the MIC is 32 µg/ml are resistant. However, in Japan, the same isolates for which the MIC is 8 µg/ml to be resistant[27,29,30] . *S. aureus* strains with Vancomycin MICs of ≤2 µg/ml were considered as fully Vancomycinsusceptible[31- 34]. Susceptibility information is typically provided as the percentage of susceptible[22, 35]. Rising MICs of Vancomycin among Vancomycin susceptible *S. aureus*, referred to the 'Vancomycin MIC creep' that has caused a re-evaluation of Vancomycin susceptibility criteria in cases of complicated infections[23,24,36,37]. Furthermore, breakpoints may allow for shifts in MIC populations to go unrecognized unless there is a change in the categorical interpretation[22,38]. Vancomycinhas traditionally been considered a bactericidal agent, and its potency is reduced to bacteriostatic levels in the setting of high-inoculum infections[37]. Bactericidal activity of Vancomycinis probably essential for effective treatment of highbacterialdensity infections and serious infections in immunocompromised patients[6]. Tolerance describes the ability of a small minority of strains of a bacterial species to exhibit a bacteriostatic response to an antibacterial challenge that is bactericidal for the majority[39].

Methods

Staphylococcus aureus isolates:

Clinical specimens were collected from various specimens taken from patients presented at Hawler, Maternity and Rizgary teaching hospitals in Erbil, Kurdistan Region, Iraq, from June 2011 to November 2012. Only one isolate per patient was included in the study. Patient age less than 18 years, pregnant woman, and patients received immune suppressive drug were excluded from the study. The study was approved by the ethics committee at College of Nursing, Hawler Medical University. The patients were asked to give informed consent before participating in the research. All *S. aureus* isolates were identified using routine bacteriological procedures, including Gram stain, colony morphology, mannitol fermentation, slide and/ or tube coagulase tests and API STAPH system (bioMérieux, France)[40].

Determination of minimum inhibitory concentration

The MIC values of Vancomycin was determined by a broth microdilution method to all *S. aureus* isolates at concentrations 0.25 to 32 µg/ml, which performed in sterile flatbottom 96-well microplates (Costar NY, USA), as described in CLSI guidelines [(41)]. In addition, the microdilution method applied on eight non-glycopeptide antibiotics, which included Gentamicin, Amikacin, Tobramycin, Azithromycin, Clarithromycin, Ciprofloxacin, Gatifloxacin, Levofloxacin, and Moxifloxacin (Sigma-Aldrich). The microplates were incubated at 35°C andthe MIC end points were read visually following 24 h of incubation. MIC defined as the lowest concentration of the antibiotic that were inhibit the growth of *S. aureus* being tested as detected by lack of visual turbidity compared with antibiotic-free growth control. The resistant of *S. aureus*to non-glycopeptidewas determined according to CLSI documents M100-S19[42].

Vancomycinminimum bactericidal concentrationdetermination

The MBC for Vancomycin were defined as 99.9% killing of the initial inoculum after 24 h of incubation[43]. Following a broth microdilution MIC assay, from each well that shows no growth, 100 µl of suspension were removed and spread onto blood agar plates. The plates were incubated overnight at 35ºC. The number of colonies growing from each of the wells was

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counted and the number of colonies corresponded to a thousand-fold reduction, as compared to the colony count of the start inoculum was recorded as the MBC[44-46]. Repeat testing was conducted on those isolates with MBCs ≥ 16 µg/ml to confirm the result.

Vancomycin tolerance

Vancomycin tolerance was defined as an MBC: MIC ratio ≥ 32[44,45,47].

Statistical method

Statistical package for social sciences (SPSS) version 18.0 was used to perform the analysis. Statistic Tables containing percentages and statistical values. A mean±SD (standard deviation) values was done to MIC and MBC. Pearson Chi-Square was used to compare non-glycopeptide antibiotic resistance among Vancomycin MICs value groups. One-Way ANOVA with Duncan test were used to compare the MICs of antibiotic among the different groups. The level of statistical significance was set at P<0.05.

Results

A total of 453 *S. aureus* isolated, which were grouped from A to E according to Vancomycin MIC values of which Vancomycin MIC of 0.5, 1, 2, 4, and 8 µg/ml are grouped into A, B, C, D and E, respectively. Three isolates (0.66%) had 0.25 µg/ml MIC to Vancomycin were non- grouped. High percentage (55.85%)of the isolates had 0.5 µg/ml MIC to Vancomycin, whereas only eight strains (1.77%) had MIC 8 µg/ml. MIC of 16, and 32 µg/ml were not recorded, Table 1. The Vancomycin MBC for *S. aureus* isolates ranged from 0.5 to 32 µg/ml, 247 (54.53%) of the strains had Vancomycin MBCs of 1 µg/ml, where as only one isolates (0.22%) had MBC of 32 µg/ml, Table 2. The susceptibility of *S. aureus* isolates to various non-glycopeptide antibiotics was likely to be associated with Vancomycin MICs value. The isolates with Vancomycin MICs of 4 and 8 µg/ml (groups D and E) revealed high resistance to other antibiotics Table 3. Statistical analysis showed that the differences to all non-glycopeptideantibiotics resistance were significant (P<0.001) among groups A to E, except Tobramycin (P= 0.105). Statistical comparisons between groups of Vancomycin MIC value (A to E groups) were done by One-Way ANOVA with Duncan test at P<0.05 for compared different groups to each non-glycopeptideantibiotic are show inTable 4. Mean MICof Gentamicin in groups E were statistically higher than groups A, B, C, and D. Furthermore, no significant differences were observed between groups C, D and E to Clarithromycin.

Table 1: Vancomycin MIC value groups in *S. Aureus*

MICvalues(groups)	No.	%
0.25 µg/ml	3	0.66
0.5 µg/ml (group A)	253	55.85
1 µg/ml (group B)	127	28.04
2 µg/ml (group C)	38	8.39
4 µg/ml (group D)	24	5.30
8 µg/ml (group E)	8	1.77
16 µg/ml	0	0.00
32 µg/ml	0	0.00
Total	453	

Table 2: Vancomycin MBC value in *S. Aureus*

MBC values	No.	%
0.25 µg/ml	0	0.00
0.5 µg/ml	8	1.77
1 µg/ml	247	54.53
2 µg/ml	129	28.48
4 µg/ml	37	8.17
8 µg/ml	25	5.52
16 µg/ml	6	1.32
32 µg/ml	1	0.22
Total	453	

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Table 3: *S. aureus* resistance to non-glycopeptide antibiotics at different Vancomycin MICs value groups

Antibiotics	Groups A		Groups B		Groups C		Groups D		Groups E		Total		P- value
	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	
Gentamicin	49	19.37	55	43.31	22	57.89	16	66.67	6	75.00	148	32.89	<0.001
Amikacin	34	13.44	38	29.92	21	55.26	9	37.50	5	62.50	107	23.78	<0.001
Tobramycin	37	14.62	27	21.26	5	13.16	7	29.17	4	50.00	97	21.56	0.105
Azithromycin	43	17.00	56	44.09	29	76.32	16	66.67	7	87.50	165	36.67	<0.001
Clarithromycin	36	14.23	37	29.13	21	55.26	14	58.33	5	62.50	154	34.22	<0.001
Ciprofloxacin	40	15.81	44	34.65	24	63.16	17	70.83	7	87.50	139	30.89	<0.001
Gatifloxacin	0	0.00	0	0.00	0	0.00	1	4.17	1	12.50	2	0.44	<0.001
Levofloxacin	7	2.77	8	6.30	2	5.26	4	16.67	3	37.50	24	5.33	<0.001
Moxifloxacin	8	3.16	10	7.87	5	13.16	6	25.00	3	37.50	32	7.11	<0.001
No. of *S. aureus*	253		127		38		24		8		450		

Groups A: Vancomycin MIC=0.5 µg/ml; groups B: Vancomycin MIC=1 µg/ml; groups C: Vancomycin MIC=2 µg/ml; groups D: Vancomycin MIC=4 µg/ml; groups E: Vancomycin MIC=8 µg/ml

Table 4: Antibiotics MIC of *S. aureus* at different Vancomycin MICs value groups

Antibiotic	Mean±SD of MIC (µg/ml)						P- value
	Groups A	Groups B	Groups C	Groups D	Groups E	Total	
Gentamicin	0.48±0.279 [a]	1.11±0.785 [b]	1.47±0.615[b]	2.49±2.178 [c]	3.88±2.937 [d]	0.91±1.029	<0.001
Amikacin	3.62±4.977 [a]	7.12±5.893 [b]	11.55±5.275[c]	9.79±7.077 [a b]	11.25±9.91 [c]	5.74±6.115	<0.001
Tobramycin	0.52±0.617 [a]	0.88±0.623 [ab]	1.36±2.509 [b]	1.35±0.961 [b]	1.38±1.246 [b]	0.75±1.003	<0.001
Azithromycin	0.75±0.884 [a]	1.16±0.761 [a]	2.38±1.382 [b]	2.92±1.577 [b c]	3.19±1.51 [c]	1.16±1.175	<0.001
Clarithromycin	0.57±0.622 [a]	0.8±0.783 [a]	1.32±0.798 [b]	1.40±0.991 [b]	1.44±1.208 [b]	0.76±0.77	<0.001
Ciprofloxacin	0.73±0.619 [a]	1.07±0.759 [a]	2.36±2.685 [b]	2.99±3.406 [b]	5.38±4.749 [c]	1.17±1.625	<0.001
Gatifloxacin	0.34±0.133 [a]	0.39±0.174 [a]	0.39±0.161 [a]	0.45±0.353 [a]	0.66±0.597 [b]	0.37±0.187	<0.001
Levofloxacin	0.62±0.592 [a]	0.69±0.763 [a b]	0.82±0.972 [a b]	1.15±1.333 [b]	1.91±1.752 [c]	0.71±0.786	<0.001
Moxifloxacin	0.43±0.314 [a]	0.44±0.473 [a]	0.63±0.551 [a b]	0.84±0.699 [b c]	1.06±0.81 [c]	0.48±0.442	<0.001

Groups A: Vancomycin MIC=0.5 µg/ml; groups B: Vancomycin MIC=1 µg/ml; groups C: Vancomycin MIC=2 µg/ml; groups D: Vancomycin MIC=4 µg/ml; groups E: Vancomycin MIC=8 µg/ml

An antibiotic MICs marked in different groups with different letters are significant.
Antibiotics MICs value in different groups with same letters are not significant.

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Discussion

S. aureus is considered as a major human pathogen responsible for a wide range of serious acute and chronic diseases, its increasing antibiotic resistance, contribute to its success as an infective agent[48,49]. A significant relationship between decreased efficacy of Vancomycin and increased Vancomycin MIC, even within the susceptible range were documented[50]. Furthermore, it has been highlighted by the recent change in the Vancomycin MIC breakpoint for susceptibility strains[51], which suggest that Vancomycin MICs are indeed increasing amongst *S. aureus* strains over time. Clinical isolates of *S. aureus* with reduced susceptibility to Vancomycin were found in the present study, this increasing Vancomycin MICs were recorded elsewhere[11,23,52,53]. A total of 20,004 isolates of *S. aureus* were collected from 2004 to 2009 from 56 countries originating from all major regions including Africa, Asia/Pacific, Europe, the Middle East, Latin America and North America.The frequency of *S. aureus* isolates with Vancomycin MICs≥2 µg/ml increased from 4.0% in 2004 to 7.7% in 2009[20], which is in agreement with the present study. On other hand, isolates with 2µg/ml MIC accounted for 32.0% of MRSA in study done in Japan[19].In India, *S. aureus* isolates from 2004 to 2008, Vancomycin MICs ranged from 0.5to 2 µg/ml, only 1.7% of *S. aureus*had ≤0.5 µg/ml MIC, 54.9% had 1 µg/ml MIC and 43.4% had 1 µg/ml MIC[33]. In Iran, Tehran, 2.88% of *S. aureus* had ≥256 µg/ml MIC of Vancomycin[54], while in this study, theMIC of Vancomycinshowed not more than 8 µg/ml. There has been significant interest regarding the changing patterns of Vancomycin MICs within the *S. aureus* population. Therefore, changes in *S. aureus* Vancomycin MICs can occur over time[20,55]. This may raise more concerns about the potential failure of treatment of *S. aureus* infections with Vancomycin[11]. Vancomycin susceptibility and bactericidal activity may also contribute to the response to Vancomycin treatment[51,56].

Currently, measures to control *S. aureus* infections are challenged by a large and continuing increase in the prevalence of *S. aureus*, as well as prolonged exposure to glycopeptide antibiotics will increase the resistance toward Vancomycin[57]. *S. aureus* isolates with Vancomycin MICs of ≤1 µg/ml tend to be less resistant to other non-glycopeptide antibiotics than *S. aureus* with Vancomycin MICs of ≥2 µg/ml, which agrees with other studies[11,58]. This finding may raise concerns for miss use of antibiotics therapy in patients with *S. aureus* infections.

Conclusion

The prevalence of *S. aureus* with a Vancomycin MIC of 8 µg/ml was very low. This may be explained by the minimal exposure of *S. aureus* to glycopeptide antibiotics.The relationship between increased Vancomycin MIC and increased MIC of non-glycopeptide antibiotics were significantly observed. The higher Vancomycin MICs were associated with resistance to several other classes of non-glycopeptide antibiotics.

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