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Brief Original Article

Vancomycin susceptibility trends of methicillin-resistant *Staphylococcus aureus* isolated from burn wounds: a time for action

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Abstract

Introduction: Methicillin-resistant *Staphylococcus aureus* (MRSA) with reduced susceptibility to vancomycin poses a threat for patients in burn units throughout the world. This study aimed to investigate the reduced susceptibility to vancomycin of MRSA isolated from wounds of patients admitted to the Burns and Plastic Surgery Centre in Tripoli, Libya.

Methodology: All isolates were initially identified by chromagen medium then confirmed by PCR. The minimum inhibition concentration (MIC) was determined by E-test glycopeptide resistance detection (GRD).

Results: During the study, 210 isolates were obtained from 560 patients representing 132 (62.9%) and 78 (37.1%) of total samples received during years 2009 and 2010, respectively. MIC levels for vancomycin ranged from 0.5 to 2 µg/ml during the study, 13% of isolates displayed MIC of 1.5 µg/ml and 9% of the isolates displayed 2 µg/ml. Although MRSA isolates decreased dramatically during 2010 (37.1%) compared to 2009 (62.9%), overall, there was a significant increase in the proportion of MRSA isolates exhibiting higher vancomycin MICs during 2010 compared to 2009 (P = 0.0155). There was a significant increase of MICs at 1 µg/ml during 2010 compared with 2009 (P = 0.36). No vancomycin intermediate or resistant strains were found.

Conclusion: There was a significant increase in the proportion of MRSA isolates exhibiting higher vancomycin MICs. We recommend that MRSA isolates should be monitored. Furthermore, implementation of infection control measures is urgently needed to prevent the spread of MRSA.

Key words: MRSA; vancomycin; MIC; burn; Libya.

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Introduction

Infection remains the leading cause of morbidity and mortality among patients with burns in developing and conflict-affected countries [1]. The treatment and prevention of infection among burns patients presents a particularly difficult challenge in these contexts. Methicillin resistant *Staphylococcus aureus* (MRSA) strains exhibit resistance to β-lactams and other antimicrobials. Vancomycin, a glycopeptide, is the drug of choice for the treatment of MRSA infections. Although the prevalence of *S. aureus* strains with reduced vancomycin susceptibility remains low, such strains have been associated with vancomycin treatment failure, limiting the treatment options for patients with such infections and necessitating that clinicians consider the use of other antimicrobials [2]. Many health care facilities have reported an increasing prevalence of MRSA strains with vancomycin MICs of 2 µg/ml, which is at the upper limit of the Clinical and Laboratory Standards Institute (CLSI) susceptibility range [3,4], and some have detected an association of these isolates with prolonged bacteremia, greater rates of complications, and vancomycin therapeutic failures [5]. In addition, MRSA with vancomycin MICs of 2 µg/ml are more likely than MRSA with vancomycin MICs of ≤1 µg/ml, to represent a heterogeneous population that may include subpopulations with intermediate resistance to vancomycin [6]. The objective of this study was to determine the minimum inhibitory concentration (MIC) values of vancomycin for the MRSA isolated from wounds of burn patients at the Burns and Plastic Surgery Centre (BPSC) in Tripoli using E-test glycopeptide resistance detection (GRD).

Methodology

This was a retrospective study conducted at the BPSC, Tripoli, Libya. The BPSC is a 120-bed hospital that provides medical care for burns patients in separate male, female and pediatric burns and plastic surgery units. Routine cultures performed in the microbiology laboratory from wound and abscess swabs recovered from burns patients treated at the centre in the period between January 2009 and June 2010 were evaluated. Initially, isolates were identified as staphylococcal isolates by the standard microbiological procedures such as coagulase test (bioMérieux, Marcy l'Etoile, France) and DNAase test (Oxoid, Basingstoke,UK).

Non-duplicate *S. aureus* isolates were inoculated on BBL CHROMagar MRSA (BD Diagnostic, Sparks, MD, USA), incubated at 37°C for 48 hours and MRSA was suspected if mauve-colored colonies were observed. All suspected MRSA isolates detected by chromogenic medium were confirmed by a real-time PCR technique using the BD GeneOhmTM MRSA assay (BD Diagnostics, Franklin Lakes, USA) following the manufacturer's instructions. The BD GeneOhm MRSA PCR assay has a sensitivity and specificity of 100% and 97%, respectively, compared to culture by use of BBL CHROMagar MRSA [7]. Quality control for antimicrobial susceptibility testing was performed with *S. aureus* ATCC 25923.

The E-test glycopeptide resistance detection (GRD) was performed according to the manufacturer's instructions (AB Biodisk, Solna, Sweden) using a double-sided predefined gradient of vancomycin and teicoplanin for the detection of vancomycin-intermediate *S. aureus* (VISA) or hetero-VISA (hVISA). A 0.5 McFarland standard inoculum was prepared and swabbed onto Mueller-Hinton agar with 5% sheep blood (Oxoid, UK). The zone of inhibition was read at 24 and 48 hours after incubation at 35°C (endpoints were read according to the manufacturer's recommendations). The test isolate was considered positive for hVISA if the E-test GRD strip result was ≥8 μg/ml for either vancomycin or teicoplanin according to the manufacturer's instructions and as previously described [8]. Methicillin resistance was defined as a MIC of ≥4μg/ml for a strain with the ability to grow in agar screening medium containing 4% NaCl plus 6 μg/ml oxacillin, whereas vancomycin resistance was defined as a MIC of ≥16 μg/ml for a strain with the ability to grow in agar screening medium supplemented with 6 μg/ml vancomycin and, like MRSA with defined VISA as a MIC of 4-8μg/ml [9].

All statistical analysis was performed using the statistical package software SPSS 16 (SPSS inc, Chicago, IL). The Kolrogorov-Smiernov test was used to test the normality of the distribution of continuous measures; for discrete variables, the differences between two groups were examined using the single-tailed T-test with a P-value of < 0.05 considered to indicate a statistical significant difference.

Results

During the study period, 210 isolates obtained from a total of 560 patients were identified as MRSA by PCR, representing 132 (62.9%) and 78 (37.1%) of total samples received during the years 2009 and 2010, respectively (Table 1). Patients were aged between 2 months to 90 years and the mean total burn surface area was 56% (range 25-80%). GRD E-test were performed on all isolates for MICs detection, MICs ranged from 0.5 to 2μg/ml (mode = 0.75 μg/ml) for vancomycin and 0.5 to 3μg/ml (mode = 0.5 μg/ml) for teicoplanin during 2009 compared with 0.5 to 2 μg/ml (mode = 0.5 μg/ml) for vancomycin and 0.5 to 4 μg/ml (mode = 0.5 μg/ml) for teicoplanin during 2010. During the study period we found that 13% of isolates displayed a MIC of 1.5 μg/ml and 9% of the isolates displayed 2 μg/ml. There was a significant increase of MICs at 1 μg/ml during 2010 compared with 2009 (P = 0.36, odd ratio [OR] = 2.2). Although MRSA isolates dramatically decreased during 2010 (37.1%) compared to 2009 (62.9%), there was evidence of

Table 1. MIC values for MRSA isolates

MIC (μg/ml)	2009	2010	
	Total 132 No (%)	Total 78 No (%)	P value (Odds ratio)
<1	68 (51.5)	50 (64.1)	NS
1	35 (26.5)	11 (14.1)	0.036 (2.2)
1.5	18 (13.6)	10 (12.8)	NS
2	11 (8.3)	7 (9.0)	NS

anoverall increase in the proportion of MRSA isolates with higher vancomycin MICs during 2010 compared to 2009 (P = 0.0155). No vancomycin intermediate or resistant strains were found.

Discussion

Clinical isolates exhibiting decreased susceptibility to glycopeptides are reported in various countries and represent a crucial challenge for antimicrobial therapy, antimicrobial susceptibility testing, and hospital infection control [10-12]. The prevalence of hVISA ranges widely from 0 to 74%, depending on the geographic location, study population, and methodology used [4,13,14]. Causes for treatment failures are not fully understood: clinical failures have been reported in patients infected with VISA or hVISA strains [15]. Treatment failure has also been reported with strains displaying MICs that were within the susceptibility range (≤2.0 mg/liter) [16]. In a previous study, 66 patients with vancomycin MICs of ≥1.5 mg/liter had a 2.4-fold increase in failure compared to patients with MICs of ≤1.0 mg/liter [17]. We found that 13% of isolates displayed MICs of 1.5 µg/ml and 9% of the isolates displayed 2 µg/ml, which is considered the upper limit of the sensitive zone that calls for prompt preventative measures. During 2010 there was a remarkable decrease of MRSA isolates (37.1%) compared with 2009 (62.9%), but overall there was a significant increase in the proportion of MRSA isolates exhibiting higher vancomycin MICs during 2010 compared with 2009 (P = 0.0155). In addition, there was a significant increase of MICs at 1 µg/ml during 2010 compared with 2009. This result might indicate the presence of the vancomycin creep phenomenon. "Vancomycin creep" is a term that has been used to describe a gradual increase in vancomycin MICs over time [18]. Most hospitals report estimated vancomycin MICs through automated methods. However, different authors showed evidence that MIC creeps are not accurately detected by automated systems [19,20].

Our isolates were screened using E-test GRD strips. This is a highly sensitive and specific method for hVISA detection, that can be easily implemented in the clinical microbiology laboratory [8,21]. It has been found that this method has higher agreement with broth microdilution, presenting the most homogeneous performance of different MIC values [22]. Reiderer and colleagues suggested that the high sensitivity of E-test GRD strips may make it a useful screening procedure for detection of VISA or hVISA, but confirmatory testing would be required [23]. No vancomycin intermediate or resistant strains were found in the current study.

All our isolates were obtained from wounds of burn patients reflecting the predominant site of infection at BPSC. Most previous studies have focused on hVISA and VISA strains isolated from blood [24,25]. Recently, Hu and colleagues found that hVISA and VISA strains were identified from diverse infection sites, predominantly from sputum (56.3%), followed by pus (18.8%), blood (8.8%), secretions (6.3%), drainage (3.8%), and other (6.3%) [26]. Tiwari and co-workers found that the emergence of strains from North India are beginning to develop resistance to vancomycin and has increased the morbidity and mortality associated with wound infections [27]. Previous studies conducted at the BPSC indicated high prevalence of multi-drug resistant MRSA patients (46.7%-54%) among burn patients [28-30]. With high rates of MRSA infections and increases in the upper zone of MIC level to vancomycin comes an urgent need to treat severe life threatening MRSA infections using alternative therapy for burn patients. Based on previous results, we found that both tigecycline and linezolid showed excellent *in vitro* activity against MRSA isolated from burn patients; as a result linezolid and tigecycline were introduced at BPSC to treat serious infections [30]. Therefore, with the effort to prevent the emergence and spread of vancomycin resistance, we recommend that MRSA isolates from diverse clinical sites should be included when testing for reduced vancomycin susceptibility, especially from burn patients, to avoid therapy failures when treating MRSA infections with a vancomycin MIC 2 µg/ml.

Conclusions

A significant increase in the proportion of MRSA isolates exhibiting higher vancomycin MICs was found during our study. These results may provide an incentive for a larger-scale investigation of hVISA and VISA detection using recommended methods. However, continued surveillance to follow the changing patterns of reduced vancomycin MICs among clinical MRSA isolates would be prudent to evaluate the clinical outcomes of serious MRSA infections.

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