Estimation of vancomycin MIC for *Staphylococcus aureus* in patients treated in the Chair and Department of Dermatology, Poznan University of Medical Sciences

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Abstract

Introduction: *Staphylococcus aureus* (*S. aureus*) represents a significant aetiological factor in infections of skin and soft tissues. Resistance to vancomycin is very rare. In recent years the *MIC creep* phenomenon has been noted. At present, it is suggested that *S. aureus* strains with vancomycin minimal inhibitory concentration (MIC) at a level > 1 µg/ml poorly respond to treatment.

Aim: The study was aimed to compare MIC evaluated in the automatic system of Vitek with the real MIC of vancomycin for *S. aureus* strains isolated from patients treated in the Department of Dermatology, Poznan University of Medical Sciences.

Material and methods: Material for the studies involved 80 smears sampled from dermal lesions in patients diagnosed and treated in the Department of Dermatology, Poznan University of Medical Sciences in Poznan between May 2008 and December 2010.

Results: Methicillin-sensitive *S. aureus* strains (MSSA) were isolated in 76 cases (95%), while methicillin-resistant *S. aureus* (MRSA) – in 4 cases (5%). Using the automatic Vitek technique in 72 cases (90%), the obtained vancomycin MIC for *S. aureus* amounted to ≤ 0.5 µg/ml, in 5 cases to 1 µg/ml and in 3 cases to 2 µg/ml. Using Etest technique, the real MIC amounted in one case to 0.5 µg/ml, in 1 case to 0.75 µg/ml, in 1 case to 1 µg/ml, in 19 cases to 1.5 µg/ml and in 58 cases to 2.0 µg/ml.

Conclusions: Due to the *MIC creep* phenomenon and extensive diversities obtained in estimates of *S. aureus* resistance to vancomycin, the authors suggest estimating the real MIC.

Key words: infections of skin, *Staphylococcus aureus*, vancomycin.

Introduction

Infections in skin and soft tissues are induced most frequently by Gram-positive flora [1]. *Staphylococcus aureus* (*S. aureus*) is a very important aetiological factor in this group of infections [2, 3]. The microbe is responsible for several primary dermal infections. Most frequently it induces folliculitis, furunculosis and bullous impetigo [4-7]. The bacteria produce many enzymes and toxins this way increasing its invasiveness [8]. An example involves its exotoxin of epidermolytic activity, which may cause staphylococcal scalded skin syndrome (SSSS) [9, 10].

Apart from the generally recognized involvement of *S. aureus* in primary dermal infections, it is worth mentioning that the microbe represents one of the principal pathogens isolated from superinfected lesions, primarily developing in the course of other dermatoses (impetiginization). The examples are provided by secondary bacterial superinfections of exematous dermatoses, ulcerations of shanks or lesions of primarily viral aetiology [6, 7]. The investigators focused their attention also on involvement of *S. aureus* in pathogenesis of non-infectious diseases, such as atopic dermatitis or Kawasaki disease

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(a postulated role of superantigens) [6, 11-13]. In the clinical aspect, the problem of carriers of *S. aureus* seems also significant, in whom the main reservoir of infection involves nasopharynx and, more exactly, nasal vestibule [8].

Taking into account the broad spectrum of potential clinical manifestations induced by the discussed pathogen, the choice of an appropriate therapy is extremely important. In cases of several dermatological morbid units, such as e.g. folliculitis, a few days' application of locally acting antibiotics (fusidic acid, mupirocin, retapamulin) is sufficient. On the other hand, in cases of severe infections, undoubtedly oral or parenteral antibiotics should be introduced [5]. In the treatment, penicillins in association with β-lactamase inhibitor and second generation cephalosporins continue to play a principal role. In cases of infections of skin and soft tissues induced by *S. aureus* strains resistant to methicillin (methicillin-resistant *S. aureus* – MRSA), vancomycin remains to be the antibiotic of choice [14-16].

Vancomycin manifests molecular weight of around 1500 Da and belongs to glycopeptides. It is poorly absorbable from the alimentary tract and, therefore, the appropriate way of administration involves an intravenous pathway [14]. Shortly after introduction of vancomycin to therapy it was found to be an antibiotic of a relatively significant toxicity, particularly as regards kidneys. Therefore, its concentration in serum needs to be monitored, which would allow the dose to be modified in order to obtain desirable concentrations [14, 16, 17].

In most of clinical strains of Gram-positive microbes, resistance to vancomycin represents a very seldom phenomenon [14]. Nevertheless, in recent years it has been noted to gradually increase in values of minimal inhibitory concentration (MIC) of glycopeptides for *S. aureus,* termed in the literature as the *MIC creep* [15].

Aim

This study aimed at comparison of MIC evaluated in the automatic Vitek system with the real MIC for vancomycin estimated using Etest for *S. aureus* strains isolated from patients treated in the Department of Dermatology, Poznan University of Medical Sciences. Both of the procedures applied in the studies are consistent with recommendations related to test choice in estimation of bacterial sensitivity to antibiotics and chemotherapeutic agents edited by the National Reference Centre for Susceptibility Testing (*Krajowy Ośrodek Referencyjny ds. Lekowrażliwości Drobnoustrojów* – KORLD), with the reservation that the Etest represents the obligatory technique when *S. aureus* becomes isolated from the plated material [18].

Material and methods

Material for the studies involved smears taken from dermal lesions in patients diagnosed and treated in the Dermatological Clinic (14 samples) and from patients hospitalized in the Department of Dermatology, Poznan University of Medical Sciences (66 samples) in the period between May 2008 and December 2010.

The sampled material was microbiologically processed in line with the binding, generally accepted procedures of clinical microbiology, recommended by KORLD [18]. Every sample was plated and routinely evaluated for presence of bacteria and fungi. Identification of microbes was conducted using the Vitek system (bioMerieux) and ATB (bioMerieux). In cases when staphylococci were isolated from the material, it was determined if they represented coagulase-positive (*S. aureus*) or coagulase-negative staphylococci using Slidex Staph Plus (test of rapid agglutination, bioMerieux). On every occasion, vancomycin MIC for *S. aureus* was estimated both for methicillin-sensitive *S. aureus* strains (MSSA), and for MRSA, using two techniques, including the fully automated Vitek system (bioMerieux), used also for identification of the microbes, and Etest (bioMerieux), providing real MIC values for vancomycin. The latter technique is based on the quantitative gradient of concentrations, used for estimation of real MIC value of a studied drug against the tested microbes, in μg/ml. The scope of 15 consecutive double dilutions allows for a very precise estimation of MIC in between the conventional dilutions. The gradient of drug on plastic strips remains stable for 18-24 h, which covers the critical times of microbial growth. In contrast to Etest, in the automatic Vitek system, the result can be obtained already within 6 h to 8 h.

Results

Staphylococcus aureus was isolated from 80 samples originating from 70 patients suffering from dermal lesions due to a primary infection (10 samples) or a secondary superinfection (70 materials). Methicillin-sensitive *S. aureus* strains was isolated in 76 cases (95%), while MRSA – in 4 cases (5%). In evaluation using the automatic Vitek technique, in 72 cases (90%) the obtained result of MIC of vancomycin for *S. aureus* was ≤ 0.5 μg/ml, in 5 cases it was 1 μg/ml and in 3 cases – 2 μg/ml (Table 1). The real MIC value, evaluated by Etest technique, in 1 case amounted to 0.5 μg/ml, in 1 case – 0.75 μg/ml, in 1 case – 1 μg/ml, in 19 cases – 1.5 μg/ml and in 58 cases –

Table 1. MIC of vancomycin for *S. aureus* evaluated using the automatic Vitek technique

MIC of vancomycin [μg/ml]	*S. aureus* *n* (%)	MSSA *n* (%)	MRSA *n* (%)
≤ 0.5	72 (90.0)	69 (90.8)	3 (75.0)
1	5 (5.6)	4 (5.3)	1 (25.0)
2	3 (3.8)	3 (4.0)	0 (0)

MIC – minimum inhibitory concentration, MRSA – methicillin resistant strains, MSSA – methicillin-sensitive strains, n – number of strains

Table 2. Real MIC of vancomycin for *S. aureus* evaluated by Etest technique

MIC of vancomycin [µg/ml]	*S. aureus* *n* (%)	MSSA *n* (%)	MRSA *n* (%)
0.5	1 (1.2)	1 (1.3)	0 (0)
0.75	1 (1.2)	1 (1.3)	0 (0)
1	1 (1.2)	1 (1.3)	0 (0)
1.5	19 (23.8)	16 (21.0)	3 (0)
2	58 (72.5)	57 (75.0)	1 (0)

MIC – minimum inhibitory concentration, MRSA – methicillin resistant strains, MSSA – methicillin-sensitive strains, n – number of strains

2.0 µg/ml (Table 2). Out of 72 strains of *S. aureus*, for which MIC of vancomycin was estimated using the automatic Vitek technique at ≤ 0.5 µg/ml, only in a single case the same result (0.5 µg/ml) was obtained using Etest, in 1 case the value amounted to 0.75 µg/ml, in 1 case – 1 µg/ml, in 18 cases – 1.5 µg/ml and in 51 cases it was as high as 2 µg/ml. In turn, out of the group of 5 strains in which MIC of vancomycin, using the automatic Vitek technique, was evaluated at 1 µg/ml, use of Etest disclosed in one case the value of 1.5 µg/ml and in 4 cases – 2 µg/ml (Table 3, Figure 1). In a group of MRSA strains, MIC of vancomycin evaluated by the automatic technique in 3 cases amounted to ≤ 0.5 µg/ml and in 1 case it was 1 µg/ml, while using Etest, in 3 cases the value of 1.5 µg/ml was obtained and in 1 case it was 2 µg/ml (Tables 1 and 2).

Discussion

In recent years, year by year the proportion of *S. aureus* strains with a decreased sensitivity to vancomycin has been dramatically increasing. In 2006, due to the decrease in clinical effectiveness of vancomycin toward strains of *S. aureus*, CLSI (Clinical and Laboratory Standards Institute, the USA), for MIC > 4 µg/ml, decreased the breakpoint of the bacteria susceptibility from 4 µg/ml to 2 µg/ml and the breakpoint of resistance from 32 µg/ml to 16 µg/ml. Nevertheless, the tendency continues to be observed for an increasing proportion of strains with a decreased sensitivity to glycopeptides, which provides grounds for discussion about a further decrease of the established breakpoint values [16]. Wang *et al*. observed a marked increase in a proportion of *S. aureus* strains with MIC of vancomycin of 1 µg/ml from 19.9% to 70.4% between the years of 2000 and 2004. An increase was also noted in the number of strains with MIC ≥ 2 µg/ml [19]. The main cause is supposed to involve bacterial exposure to antibiotic doses in concentrations defined as sub-MIC ones [16]. Subsequently, it was demonstrated that even a slight increase in MIC in the range of values below the susceptibility breakpoint may affect clinical effectiveness of glycopeptides [16]. Currently, it is suggested that *S. aureus* strains with MIC of vancomycin at the level of > 1 µg/ml poorly react to treatment with the antibiotic [16, 20-24]. In order to avoid therapeutic failure, in such cases introduction of an antibiotic alternative to vancomycin should be considered, e.g. linezolid or tigecycline [16, 20-24].

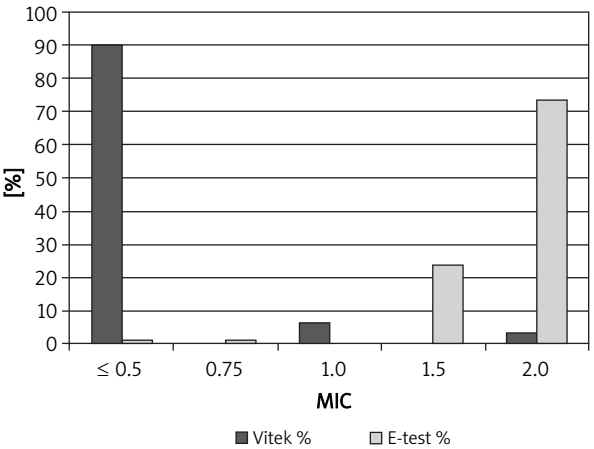

Fig. 1. MIC of vancomycin for *S. aureus* evaluated by automatic Vitek technique and real MIC estimated using Etest technique

Table 3. MIC of vancomycin for *S. aureus* evaluated by automatic Vitek technique and real MIC estimated using Etest technique

Vitek		Etest	
MIC [µg/ml]	*n*	MIC [µg/ml]	*n*
≤ 0.5	72	0.5	1
		0.75	1
		1	1
		1.5	18
		2	51
1	5	1.5	1
		2	4
2	3	2	3

MIC – minimal inhibitory concentration, n – number of strains

Before vancomycin is introduced to treatment, on every occasion the real MIC of the antibiotic should be established (e.g. using Etest) for the isolated strain. Reliability of automatic techniques in relation to glycopeptides used against *S. aureus* should be treated with caution [25]. In accordance with KORLD recommendations, in cases of *S. aureus* every time the real MIC should be established (permitted, e.g. using the Etest technique) [18]. In this context and basing on the results obtained by us, significant differences are worth stressing, detected upon comparison of values obtained for MIC of vancomycin versus *S. aureus* obtained in the two distinct tech-

niques: the automatic Vitek technique and Etest, used for evaluation of the real MIC. Testing using the automatic Vitek technique in 72 studied cases (90%) has documented values of MIC of vancomycin ≤ 0.5 µg/ml, in 5 cases (5.6%) – 1 µg/ml and just in 3 cases (3.8%) – 2 µg/ml. In turn, using Etest, MIC of 0.5 µg/ml has been obtained in just 1 case (1.2%), similarly to the values of 0.75 µg/ml and 1 µg/ml, while in as many as 19 cases (23.8%) the real MIC has manifested the level of 1.5 µg/ml and in 58 cases (72.5%) – of 2 µg/ml. Moreover, for the strains with MIC values estimated by the automatic Vitek technique at ≤ 0.5 µg/ml in as many as 71 cases (98.6%) the value of the real MIC has proven to be higher. Thus, assuming that a given strain will respond to vancomycin treatment at MIC ≤ 1 µg/ml, it should be expected that the antibiotic can be administered in almost 96.2% of all the examined cases, basing the decision on results of Vitek test, but in only 3.8% of cases when results of the real MIC estimation are considered, using the Etest technique. In view of so drastic differences between MIC values obtained using the two techniques and taking into account literature data manifesting that vancomycin therapy brings a much higher chance for success when MIC of vancomycin for the eradicated strain did not exceed 1 µg/ml [16, 20, 21, 23, 24], the authors would like to accentuate that evaluation of the real MIC using Etest is more useful than that made using the automatic Vitek test. It should also be noted that only Etest allows to detect heteroresistance of *S. aureus* to vancomycin (gradient of the drug on a strip remains stable for 18-24 h, which covers the period of microbial growth during testing) [14]. The results presented by us and showing that for over 96% of *S. aureus* strains studied, the Etest-estimated MIC value exceeded 1 µg/ml, may also confirm the universal tendency of the growing resistance of studied bacteria to glycopeptides. However, we cannot conclude on differences or their absence in MIC for MSSA and MRSA due to the low number of isolates of MRSA. Nevertheless, it is worth noting that for all the strains of MRSA examined by us, the value of real MIC tested by Etest technique amounted to at least 1.5 µg/ml.

Obviously, it cannot be bypassed that apart from MIC evaluation, implementation of the treatment should be preceded by measurements of vancomycin concentration in serum. In therapy of most infections, attainment of high drug concentrations at the preliminary phase of therapy brings no significant benefits. The better pharmacodynamic index which allows to foresee effectiveness of therapy involves the time period in which antibiotic concentration exceeds MIC for a given pathogen. As a range of therapeutic concentrations it is accepted that the concentration of 20-40 mg/ml used to be the maximum and that of 5-10 mg/ml – the minimum before the subsequent dose of antibiotic is administered [14]. It should also be kept in mind that it is not indicated to administer vancomycin for eradication of MSSA; such attempts may promote selection of strains of lower susceptibility [16, 21]. An important role in treatment of infections induced by *S. aureus* in played also by clindamycin, which, as a protein synthesis inhibitor, restrains production of toxins [26].

In summary, an extensive caution should be suggested when taking a decision to administer vancomycin in treatment of infections induced by *S. aureus*, particularly when basing the decision on vancomycin susceptibility testing using automatic techniques. It seems much more proper to choose Etest allowing to estimate the real MIC.

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