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The need to assay the real MIC when making the decision to eradicate *Staphylococcus aureus* with vancomycin

Konieczność oceny MIC rzeczywistego przy podjęciu decyzji o eradykacji *Staphylococcus aureus* wankomycyną

Authors' Contribution:
A Study Design
B Data Collection
C Statistical Analysis
D Data Interpretation
E Manuscript Preparation
F Literature Search
G Funds Collection

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Summary

Purpose: The aim of the study was a comparison of the MIC (minimal inhibitory concentration) evaluated in the automatic system Vitek 2 and the real MIC of vancomycin by the Etest method for *S. aureus* strains isolated from clinical materials.

Material and Methods: Over a twelve-month study period we compared the results obtained with two commercial methods – the automatic system VITEK 2 and the real MIC by Etest – for 359 strains of *S. aureus* isolated from clinical materials.

Results: Most of the strains of *S. aureus* were cultured from wounds (84), the ear (60) and nose (42). MSSA (methicillin-sensitive *Staphylococcus aureus*) was isolated in 342 cases and MRSA (methicillin-resistant *Staphylococcus aureus*) in 17 cases. The test with the Vitek automatic method showed that vancomycin had MIC values of ≤1.0 μg/ml in more than 96% and 2.0 μg/ml in over 3% of cases. Using the Etest technique MIC ≤ 1.0 μg/ml was obtained in only 16.4% of cases and values of >1.0 μg/ml in 83.6% of cases.

Discussion: In view of such big differences between the MIC values obtained with the two methods the authors suggest that the Etest method of assaying the real MIC is more useful than the automatic method.

Keywords: **MIC, vancomycin, *S. aureus***

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Introduction

Vancomycin is a glycopeptide antibiotic with the molecular mass of about 1500 Daltons. Due to poor drug absorption from the alimentary tract it is administered intravenously [25]. Vancomycin is a nephrotoxic antibiotic. Therefore, its concentrations in the serum need to be monitored, permitting one to modify the dose in order to obtain desirable concentrations [5,8,10,25]. The increase of *S. aureus* strains with reduced susceptibility to vancomycin forces doctors to administer high doses in order to obtain trough levels of 15 to 20 mg/L [9]. In severe sepsis it is necessary to increase the dose of vancomycin in order to achieve the steady-state concentration of C_{trough} 15-20 mg/l [3]. The generally accepted regimen comprises the loading dose of 15 mg/kg with subsequent continuous infusion of 15-40 mg/kg in order to obtain higher concentrations. Continuous infusions do not increase toxicity, cause smaller fluctuations of concentrations in the blood and reduce costs [1,4,12].

Among most clinical strains of Gram-positive microbes, resistance to vancomycin is very rare [25]. However, recently the phenomenon of gradual increase in the value of minimal inhibitory concentration (MIC) of glycopeptides for *S. aureus* has been observed, which is defined in the literature as MIC creep [21].

The study is a comparison of the MIC assayed with the automatic system Vitek 2 from bioMerieux company and the real MIC of vancomycin by the Etest method for *S. aureus* strains isolated from clinical materials in the Central Microbiological Laboratory, H. Święcicki Clinical Hospital, Poznań University of Medical Sciences. Both of the procedures applied in the studies are consistent with the recommendations related to test choice in estimation of bacterial sensitivity to antibiotics and chemotherapeutics of agents edited by the National Reference Centre for Antimicrobial Susceptibility, where the Etest is an obligatory technique when *S. aureus* is isolated from the clinical material [14].

Material and methods

The research material was collected from November 2010 to December 2011. *S aureus* was cultured 359 times during the period. The pathogen under analysis was mostly cultured from wounds (84), from the ear (60), nose (carried in the nasal atrium) (42), blood (35), pus (29), BAL (bronchoalveolar lavage) (25), throat (23), skin lesions (16) and fistulas (9). The other materials were individual cases.

The strains of *S. aureus* under analysis generally came from patients diagnosed in laryngology and dermatology clinics (88) and from hospital departments: laryngology (74), dermatology (44), nephrology (40), ICU (intensive care units) (37), surgery (27), gastroenterology (13) and maxillo-facial surgery (12).

MSSA (methicillin-sensitive *Staphylococcus aureus*) was isolated in 342 cases and MRSA (methicillin-resistant *Staphylococcus aureus*) in 17 cases.

The microbiological investigations of the materials under analysis were carried out according to the current procedures of clinical microbiology, recommended by the National Reference Centre for Antimicrobial Susceptibility. Each material was cultured and routinely assayed both for bacteria and fungi. The microorganisms were identified by means of the systems Vitek (bioMerieux) and ATB (bioMerieux). If staphylococci were obtained from the material under investigation, Slidex Staph Plus (quick agglutination test) (bioMerieux) was used to determine if they were coagulase positive (*S. aureus*) or coagulase negative. In each case the MIC of vancomycin for *S. aureus* was assayed, both for MSSA and MRSA, by means of two methods – with the automatic system Vitek 2 (bioMerieux) and the real MIC of vancomycin by Etest technique (bioMerieux). The Etest method is based on the quantitative gradient of concentration, used for estimation of the real MIC value of a studied drug against the tested microbes, in μg/ml. The range of 15 consecutive double dilutions gives a possibility to label the MIC between the conventional dilutions very precisely. The gradient of the drug on plastic strips remains stable for 18-24 hours, which covers the critical times of microbial growth. In the automatic system Vitek it is possible to obtain the result as early as within 6-8 hours, whereas it takes about 24 hours in the Etest (only after that time can mechanisms of resistance be revealed).

Results

S. aureus was cultured in 359 materials. When the automatic method Vitek was applied, in 256 cases (71.3%) the MIC of vancomycin for *S. aureus* was ≤0.5 μg/ml, in 91 cases it was 1.0 μg/ml (25.3%) and in 12 cases it was 2 μg/ml (3.3%) (Table 1). The real MIC assayed with the Etest was 0.5 μg/ml in 1 case, in 9 cases it was 0.75 μg/ml, in 49 cases 1.0 μg/ml, in 140 cases 1.5 μg/ml, in 159 cases 2.0 μg/ml, and in 1 case MIC was 4.0 μg/ml (Table 2). Out of 256 *S. aureus* strains, where the MIC of vancomycin assayed with the automatic method Vitek was ≤0.5 μg/ml, the same result, 0.5 μg/ml, was not obtained in any case when the Etest was applied. In 7 cases the value was 0.75 μg/ml, in 38 cases it was 1.0 μg/ml, in 96 cases 1.5 μg/ml, and in 115 cases it reached as much as 2.0 μg/ml. On the other hand, in the group of 91 strains where the MIC of vancomycin was assayed as 1 μg/ml with the Vitek automatic method, the Etest resulted in the following values: in 1 case the MIC was 0.5 μg/ml, in 2 cases it was 0.75 μg/ml, in 11 cases 1.0 μg/ml, in 40 cases 1.5 μg/ml, and in 38 cases 2.0 μg/ml. In the group of 12 cases where the Vitek system revealed the MIC value of 2.0 μg/ml, the Etest showed the MIC of 1.5 μg/ml in 4 cases, in 7 cases it was 2 μg/

ml, and in 1 case 4.0 µg/ml (Table 3). In the group of MRSA strains the MIC of vancomycin assayed with the automatic method was ≤0.5 µg/ml in 9 cases, in 6 cases it was 1.0 µg/ml, and in 2 cases it was 2.0 µg/ml. When the Etest was applied, the following results were obtained: in 2 cases the MIC was 1.0 µg/ml, in 2 cases it was 1.5 µg/ml, and in 5 cases it was 2.0 µg/ml (Tables 1 and 2).

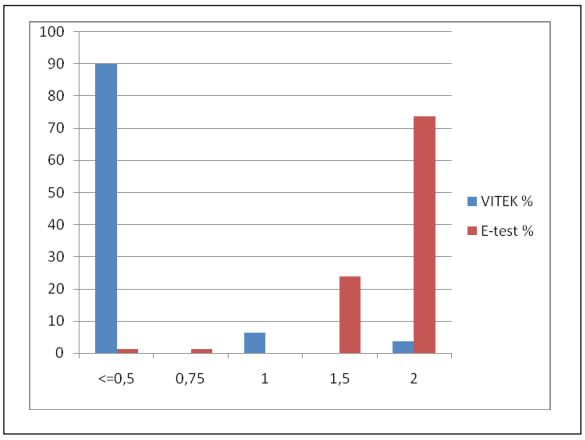

Fig. 1. MIC of vancomycin for *S. aureus* evaluated by automatic Vitek technique and real MIC estimated using Etest technique

Table 1. The MIC of vancomycin for *S. aureus* assayed with the automatic method Vitek

MIC vancomycin	*S.aureus* n/(%)	MSSA n/(%)	MRSA n/(%)
<=0,5	256 (71.3)	247 (68.8)	9 (2.5)
1	91 (25.3)	85 (23.7)	6 (1.7)
2	12 (3.3)	10 (2.8)	2 (0.6)

MIC – minimal inhibitory concentration , MRSA – methicillin-resistant *Staphylococcus aureus* , MSSA – methicillin-sensitive *Staphylococcus aureus*, n – number of strains

Table 2. The real MIC of vancomycin for *S. aureus* assayed with the Etest method

MIC vancomy cin (µg/ml) Etest	*S.aureus* n/(%)	MSSA n/(%)	MRSA n/(%)
0.5	1/(0.3)	1/(0.3)	0/(0.0)
0.75	9/(2.5)	9/(2.5)	0/(0.0)
1	49/(13.6)	46/(12.8)	3/(0.8)
1.5	140/(39.0)	137/(38.2)	3(0.8)
2	159/(44.3)	148/(41.2)	11(3.1)
4	1(0.3)	1/(0.3)	0/(0.0)

MIC – minimal inhibitory concentration, MRSA – methicillin-resistant *Staphylococcus aureus*, MSSA – methicillin-sensitive *Staphylococcus aureus* , n – number of strains

Table 3. The MIC of vancomycin for *S. aureus* – the two methods compared

Vitek		Etest	
MIC (µg/ml)	n	MIC (µg/ml)	n
		0.5	0
		0.75	7
		1	38
		1.5	96
<=0.5	256	2	115
		0.5	1
		0.75	2
		1	11
		1.5	40
1	91	2	38
		1.5	4
		2	7
2	12	4	1

The assay with the Vitek automatic method showed MIC values of vancomycin amounting to ≤1.0 µg/ml in more than 96% of cases and 2.0 µg/ml in over 3% of cases. Using the Etest technique MIC values ≤1.0 µg/ml were obtained in only 16.4% of cases and values >1.0 µg/ml in 83.6% of cases. Thus, assuming that a given strain will respond to vancomycin treatment at MIC ≤1.0 µg/ml, it should be expected that vancomycin can be administered in almost.

Discussion

In recent years the proportion of *S. aureus* strains with decreased sensitivity to vancomycin has been increasing [9]. Due to the decrease in clinical effectiveness of vancomycin towards strains of *S. aureus*, for MIC >4.0 µg/ml, in 2006 the CLSI (the Clinical and Laboratory Standards Institute, USA) decreased the breakpoint of bacteria susceptibility from 4 to 2.0 µg/ml and the breakpoint of resistance from 32.0 µg/ml to 16.0 µg/ml. The growing tendency of the percentage of strains with reduced sensitivity to glycopeptides can still be observed, which gives rise to a discussion on further reduction of the breakpoints which have been designated so far [9]. Wang et al. observed a significant increase in the percentage of *S. aureus* strains with the vancomycin MIC of 1 µg/ml from 19.9% to 70.4% between the years 2000 and 2004. The number of strains with the MIC ≥2.0 µg/ml also increased [23]. The main cause of the phenomenon is probably the exposure of bacteria to doses of antibiotics in concentrations defined as sub-MIC levels [15]. It has been proved that even a slight increase in the MIC below the sensitivity breakpoint may affect the clinical efficacy of glycopeptides

[15]. At present there are suggestions that *S. aureus* strains with the vancomycin MIC of >1.0 μg/ml exhibit poor reaction to treatment with this antibiotic [7,8,15,16,20,24]. In such cases in order to avoid therapeutic failure it is necessary to consider inclusion of an alternative but usually more expensive antibiotic [7,8,15,16,20,24].

Before implementation of treatment with vancomycin in each case the real MIC of this antibiotic should be established (e.g. with the Etest method) for the isolated strain. According to the guidelines of the National Reference Centre for Antimicrobial Susceptibility, in each case it is necessary to label the real MIC (which can be done e.g. with the Etest method) for *S. aureus* [14]. The reliability of automatic methods applied to measure the MIC of glycopeptides for *S. aureus* must be treated with due caution. On the basis of our results we observed considerable differences between the MIC of vancomycin for *S. aureus* assayed with the automatic method and the real MIC. The investigation with the Vitek automatic method showed MIC values of vancomycin ≤0.5 μg/ml in 71%, 1 μg/ml in 25%, and 2.0 μg/ml in about 3% of cases. On the other hand, when the Etest was applied, the MIC of 0.5 μg/ml was obtained only in 0.3% of cases, 0.75 μg/ml in 2.5% of them, and 1.0 μg/ml in 13.6%. However, in as many as 39% of cases the real MIC of 1.5 μg/ml was obtained, in 44.3% it was 2.0 μg/ml, and the MIC of 4.0 μg/ml was obtained in 0.3% of cases. Furthermore, for the strains where the MIC values of ≤0.5 μg/ml were labelled with the Vitek automatic method in as many as 256 cases the real MICs were higher (i.e. 100% of the values). Thus, if we assume that a particular strain will respond to treatment with vancomycin, where the MIC is ≤1.0 μg/ml, it would be necessary to assume that the antibiotic could be implemented in nearly 96% of all cases under investigation on the basis of the results in the Vitek system. However, it could be implemented only in 16% of cases if we assume the real MIC values obtained from the Etest method. Due to such big differences in the results obtained by means of both methods and according to reports in the literature, which show that there is a higher chance for clinical success when the MIC of vancomycin does not exceed the value of 1.0 μg/ml [7,8,15,16,20], the authors underline the fact that the real MIC assayed by means of the Etest is more useful than the Vitek automatic method. It is worth noting that only the Etest enables detection of *S. aureus* heteroresistance to vancomycin (the drug gradient on the strip remains stable for 18-24 hours, which covers the microbial growth during the investigation) [25]. Furthermore, the results we present, where for more than 84% of *S. aureus* strains investigated with the Etest the MIC exceeded 1 μg/ml, may prove the global tendency of the bacteria to exhibit growing resistance to glycopeptides. However, it is impossible to draw a conclusion about the presence or absence of differences in the MIC for MSSA and MRSA due to the small number of MRSA isolates.

The application of vancomycin for eradication of MSSA is not recommended. Doing so may contribute to selection of strains with reduced sensitivity [7,8]. Clindamycin plays an important role in treating *S. aureus* infections. As a protein synthesis inhibitor it inhibits toxin production [6].

In each case when vancomycin is applied to eradicate infections with *S. aureus* aetiology, special care is recommended, especially in view of the results of sensitivity to vancomycin labelled by means of an automatic system.

Selection of the method of pathogen sensitivity labelling may significantly influence the final result [2,11,13,17,18,19,22] and thus therapeutic decisions. The antibiogram based on the real MIC assay should be an essential element when vancomycin therapy is included.

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