

Special Report

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Reporting elevated vancomycin minimum inhibitory concentration in methicillin-resistant *Staphylococcus aureus*: consensus by an International Working Group

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Methicillin-resistant *Staphylococcus aureus* (MRSA) remains an important cause of serious infection, for which vancomycin is often recommended as the first-choice antibiotic treatment. Appropriate vancomycin prescribing requires accurate measurement of minimum inhibitory concentrations (MICs) to avoid treatment failure, and yet determination can be challenging due to methodological difficulties associated with susceptibility testing. An International Working Group of infectious disease specialists and clinical/medical microbiologists reached a consensus that empirical MRSA infection therapies should be chosen regardless of the suspected origin of the infecting strain (e.g., community or hospital) due to the complex intermingling epidemiology of MRSA clones in these settings. Also, if an elevated vancomycin MIC in the susceptible range is obtained in routine testing, an alternative second method should be used for confirmation and to aid antibiotic therapy recommendations. There is no absolutely dependable method for the accurate determination of vancomycin MIC, but broth microdilution appears to be the most reliable.

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Methicillin-resistant *Staphylococcus aureus* (MRSA) remains one of the most important causes of serious infection, particularly among hospitalized patients. MRSA infections are also associated with poorer outcomes than those caused by methicillin-sensitive *S. aureus* infections [1,2]. Therefore, understanding and immediately recognizing risk factors for hospital-acquired/healthcare-associated (HA) MRSA [3] are considered vital to improve management of the patients at risk of MRSA infection until culture confirmation [4]. Nevertheless, the epidemiology of MRSA has changed over the past decade, with infections also now being observed in community settings [5]. However, the value of differentiating between the origin/place of onset of the infection for disease diagnosis and treatment management is uncertain.

Guidelines recommend the use of vancomycin, linezolid, daptomycin, telavancin and clindamycin to treat serious MRSA infections, with minocycline, doxycycline and clindamycin used for less severe infections [6]. In recent years, several new antibiotics with high potency against MRSA have been approved for the treatment of acute bacterial skin and skin structure infections, including ceftaroline, dalbavancin, tedizolid, oritavancin and delafloxacin [7–9]. While

these newer agents provide useful alternative therapies for multidrug-resistant pathogens and treatment benefits for specific patient groups, vancomycin still remains the first-choice antibiotic in most countries for suspected or confirmed MRSA infections. Indeed, vancomycin is recommended by guidelines for both HA-MRSA and community-associated (CA) MRSA infection, despite reports of treatment failures for infection caused by some strains with elevated vancomycin minimum inhibitory concentrations (MICs) [6,7,10]. Appropriate prescribing of vancomycin requires measurement and reporting of elevated MICs, yet vancomycin MIC determination can be challenging due to the limitations associated with available susceptibility testing methods in real medical practice. Consequently, a working group of infectious disease specialists and clinical/medical microbiologists from Brazil, Canada, Mexico, Saudi Arabia, Russia and the UK was convened to discuss elevated vancomycin MIC in both CA-MRSA and HA-MRSA, the importance of differentiating between strains, and issues surrounding the discordance between various vancomycin susceptibility testing methods. This article summarizes their recommendations on these matters and suggests the most suitable methodology to adopt, which could enable hospitals to monitor local trends and accurately guide antibiotic therapy for the management of MRSA infections.

Differentiation between CA-MRSA & HA-MRSA

MRSA isolates were originally restricted to hospital and other healthcare settings; however, over the past few decades, an increasing proportion of younger patients without known HA-MRSA risk factors have acquired MRSA in community settings (i.e., CA-MRSA). CA-MRSA, at least at the outset, had markedly different antimicrobial susceptibility patterns compared with conventional HA-MRSA [5,11]. Differentiation between CA-MRSA and HA-MRSA infections was considered relevant initially since it was recognized that the former were susceptible to more antibiotic classes than HA-MRSA; consequently, there were more choices of empiric antibiotic therapy for CA-MRSA infections [5]. However, CA-MRSA strains are now acquiring resistance to additional antibiotics, blurring the distinction between the two types [12]. Furthermore, investigations of the genetic background of CA-MRSA, from an epidemiological point of view, also highlight a tendency toward a broader mixing among MRSA clones, with infections acquired in the community setting now being categorized as community-onset (CO) MRSA infections. The distribution of their clonal origin has also shown the emergence of clones that would typically be categorized as HA-MRSA (e.g., CO-HA-MRSA based on *SCC_{mec}* typing) [5]. Similarly, some studies have demonstrated the emergence of 'typically' CA-MRSA clones in hospitals/healthcare settings (HO) [12–14]. This blurring of the distribution of clones in both settings therefore makes appropriate antibiotic selection more challenging. Mixing of CA-MRSA and HA-MRSA clones has been described globally [12] and contributes to the constant evolution and change of regional and/or local susceptibility patterns [13,14].

While the majority of Working Group members differentiate between CA-MRSA and HA-MRSA, using a variety of methods including clinical findings, time of diagnosis of MRSA infection (e.g., <48 h or >48 h after hospital admission), antibiogram (local susceptibility pattern) and genetic testing, such differentiation does not play a critical role in antibiotic stewardship programs. Although differentiation between strains remains of scientific interest, particularly from an epidemiological point of view for clinical microbiologists, the group highlight that prescribing physicians generally only require confirmation of the pathogen being an MRSA strain and the antibiotic susceptibility pattern in order to make an informed decision on the most appropriate antibiotic. Furthermore, they stress that reporting to the treating physician of the actual antibiotic susceptibility of culture-confirmed MRSA strains by the microbiologist is more relevant than information on the origin of the clone (e.g., CA-MRSA or HA-MRSA clone).

Reporting susceptibility based on elevated vancomycin MIC

Vancomycin has been the mainstay antibiotic for the treatment of MRSA infections globally for many years [6,11,15,16], although it has several well-known limitations, including renal toxicity associated with high trough levels, and within the susceptible range it may also be associated with treatment failure and/or resistance emergence during prolonged treatment (e.g., heterogeneous vancomycin-intermediate *S. aureus*) [17–19]. Analyses suggest a correlation between vancomycin MIC and outcome, with a meta-analysis of 22 studies conducted in *S. aureus* bacteremia patients from different locations finding a threefold increase in the risk of treatment failure and increased mortality when MRSA had an elevated vancomycin MIC (i.e., ≥ 1.5 $\mu\text{g/ml}$) irrespective of the source of infection or MIC methodology [10]. While the decision to change to an alternative antibiotic should be based on individual circumstances and the clinical response to vancomycin rather than MIC alone, this link between vancomycin MIC and outcome clearly influences antibiotic selection and indeed underlies the recommendation that an

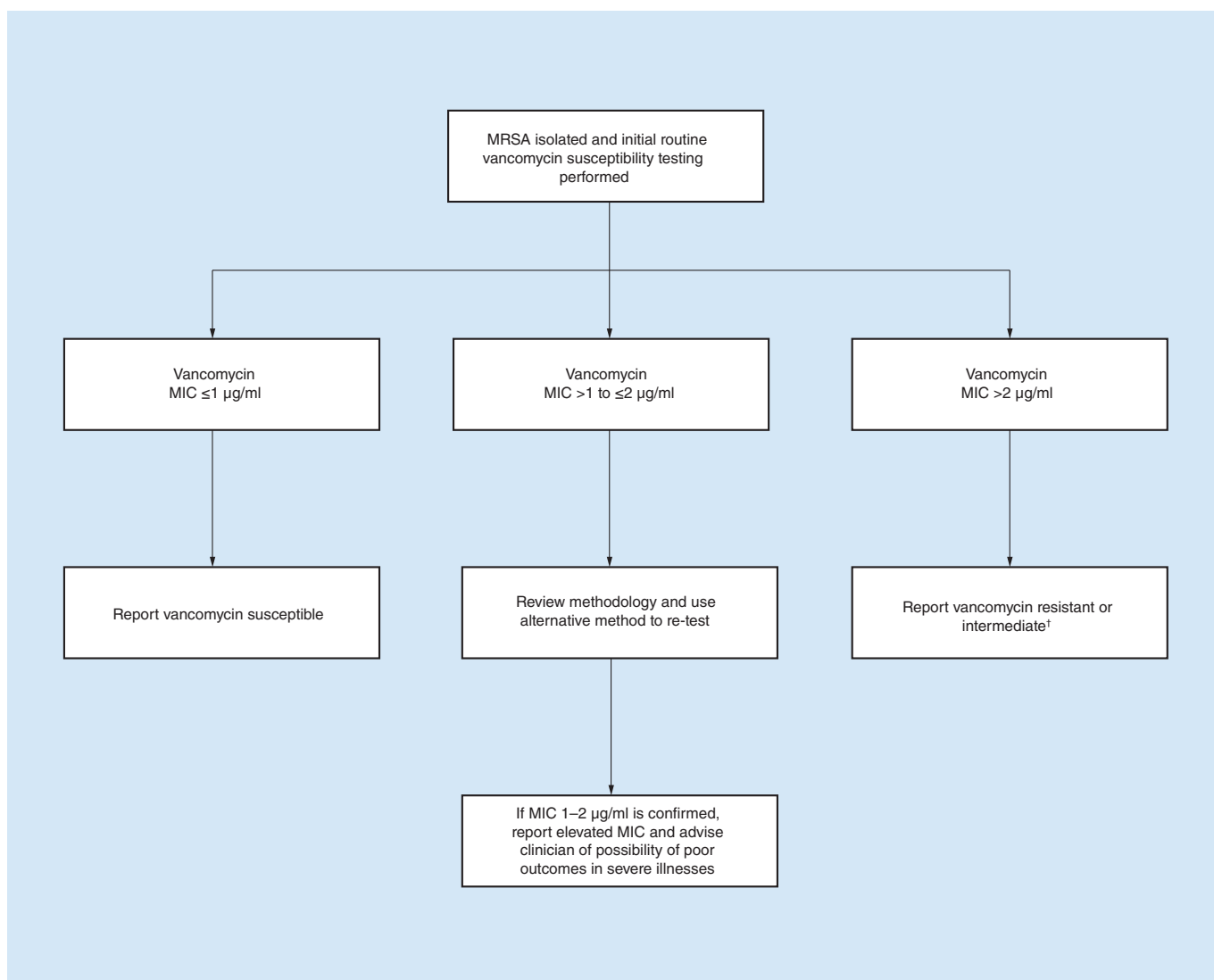


Figure 1. Recommended testing methodology for reporting elevated vancomycin minimum inhibitory concentration in methicillin-resistant *Staphylococcus aureus*.

†EUCAST susceptibility breakpoints: Susceptible: ≤ 2 $\mu\text{g/ml}$, Resistant: > 2 $\mu\text{g/ml}$ [39]; CLSI susceptibility breakpoints: Susceptible: ≤ 2 $\mu\text{g/ml}$, Intermediate: 4–8 $\mu\text{g/ml}$, Resistant: ≥ 16 $\mu\text{g/ml}$ [37,38].

MIC: Minimum inhibitory concentration; MRSA: Methicillin-resistant *Staphylococcus aureus*.

alternative second-line antibiotic, such as linezolid, tedizolid, daptomycin, telavancin, dalbavancin or clindamycin, to which the infecting isolate is susceptible should be used for isolates with a vancomycin MIC > 2 $\mu\text{g/ml}$ [6,9,15]. Indeed, it may be reasonable to consider more potent alternatives for empiric therapy in areas where prevalence of MRSA is high. Nevertheless, accurate measurement of vancomycin MIC is essential in order to ensure correct management of MRSA, reduce inappropriate antibiotic use and lessen the emergence of resistant strains.

The precise determination of vancomycin MIC is complex, due to the limitations of current methods for testing susceptibility [15], some of which may be inherent of the method itself and not linked to the conditions used [20]. A number of methods are currently used for antibiotic susceptibility testing and can be automated (i.e., Vitek[®] 2, BD Phoenix[™], MicroScan, Sensititre[™]) or manual (i.e., disk diffusion, broth microdilution and Etest) [21], although broth microdilution is the standard method used to define vancomycin MIC [15,20]. Importantly, however, discrepancies in MIC values have been reported, with a one to two dilution difference in the obtained MIC values apparent when the results of various methods are compared [20,22,23]. The lack of agreement in vancomycin

MIC values has been observed in several regions, including Australia [24], India [25], Taiwan [26], the USA [22,27] and Brazil [28]. Based on these investigations, it appears that results from different testing methodologies are consistent for the lower range MICs ($<1 \mu\text{g/ml}$) although Etest, MicroScan and Phoenix are likely to report higher values (compared with broth microdilution) for MICs $\geq 1 \mu\text{g/ml}$ [15,22]. The broth microdilution method provides the lowest MIC values, but may fail to identify small changes in vancomycin MIC values because the method uses twofold dilutions of vancomycin concentration by convention [15]. Of note, storage may alter the susceptibility readings of MRSA [20,29]. Edwards *et al.* have found that the Etest method results in higher vancomycin MIC values for freshly isolated MRSA strains compared with those of stored and subsequently tested MRSA strains (within 6 months); this tendency was found to be significant [29]. In contrast, storage does not seem to have a large impact on vancomycin MIC values when measured with broth microdilution up to 6 months but decreased vancomycin MICs were obtained after storing the samples for at least 12 months [20]. Such findings may have implications in retrospective susceptibility measurements or in large surveillance studies with frozen samples being transported to central laboratories.

Lack of consistency in MIC readings obtained by different methodologies may also account for conflicting reports of increases in vancomycin MIC over time in recent years. The existence of MIC creep was reported by some laboratories [30,31], while other studies could not confirm any increase in vancomycin MIC over the years [29,32–34]. Lack of observed creep was also echoed by many Working Group members, who have not found increased incidence of elevated MIC over a 5-year period (data not shown). The discrepancies between published reports of MIC creep may be partly explained by the MIC methodologies used in the different studies, with creep generally observed in smaller single-center studies that employed Etest assays, while negative findings reported by larger multicenter studies may be due to the use of broth microdilution [15]. Such issues also complicate recent reports of elevated vancomycin MICs in both CA-MRSA and HA-MRSA isolates in Russia [35] and Taiwan [36]. In particular, the Taiwanese study, which compared Vitek 2 and Etest with standard broth microdilution methodology in the measurement of vancomycin MIC, found that agreement rates at $2 \mu\text{g/ml}$ were only 53.6% for Vitek 2 versus 86.7% for Etest, with discordance rates being higher in HA isolates than in CA isolates [36]. Thus, the limitations of the susceptibility testing methods in obtaining accurate and reproducible vancomycin MIC for MRSA make comparison between studies implausible to explain the presence or absence of vancomycin MIC creep. This controversial issue may be resolved in a prospective, large multicenter study across various regions and countries which use identical laboratory methods, standardized to a high degree, to measure vancomycin susceptibility on freshly collected MRSA isolates. Furthermore, it is plausible that MIC creep could be associated with some but not all MRSA clones, and so local strain distribution/epidemiology will likely determine whether this phenomenon is measurable and/or observed.

Conclusion & recommendations

The Working Group recommends that microbiology laboratories should report the isolation of MRSA and recount the full susceptibility profile of the organism without any differentiation between CA-MRSA and HA-MRSA; it also discourages the use of these terms. The Group further stresses that methodological difficulties associated with susceptibility testing described above create a real challenge for clinicians when selecting the most appropriate antibiotic for the treatment of MRSA infections. While elevated vancomycin MIC poses a risk of treatment failure, the agent remains the standard of care for most MRSA infections, necessitating ongoing monitoring of MIC to ensure effective therapy. Either Clinical Laboratory Standards Institute (CLSI) [37,38] or European Committee on Antimicrobial Susceptibility Testing (EUCAST) [39] breakpoints can be followed for susceptibility testing, although there is no clear guidance currently on the methods to be used to determine MIC. Indeed, the discrepancy among the susceptibility testing methods used at present suggests that the current methodologies may not be optimized to determine vancomycin susceptibility results [29]. Nevertheless, recommendations on the precise method to use to test for vancomycin susceptibility are problematic due to variations in the cost and availability of the various methods in different regions. Consequently, until better susceptibility testing methods are available, hospitals must use the methods currently available to them.

The Group recommends that for vancomycin MIC $\leq 1 \mu\text{g/ml}$, consistency between testing methods indicates that vancomycin can reliably be reported as susceptible, with MICs $> 2 \mu\text{g/ml}$ reported as intermediate/resistant in line with CLSI/EUCAST guidelines. While vancomycin MICs $\leq 2 \mu\text{g/ml}$ are susceptible according to both CLSI and EUCAST breakpoints, there is discrepancy between the guidelines regarding resistance, with EUCAST breakpoints stating MICs $> 2 \mu\text{g/ml}$ should be reported as resistant [39], whereas CLSI states that MICs 4–8 $\mu\text{g/ml}$

should be regarded as intermediate and ≥ 16 $\mu\text{g/ml}$ as resistant [37,38]. Of note, vancomycin-intermediate or vancomycin-resistant *S. aureus* strains are rare [20]. The Working Group suggests that if an anomalous MIC is found in the susceptible range (i.e., > 1 – ≤ 2 $\mu\text{g/ml}$), a retest using a different method to determine MIC values should be undertaken. This should be a manual method, either BMD or Etest, then report vancomycin MIC and the method used to test susceptibility, and warn physician of a potential poor outcome. If the value is confirmed, then elevated MIC should be reported, with the treating physician informed of the possibility of poor outcomes in severe illnesses (Figure 1). In addition, the accuracy of vancomycin MIC values obtained by Etest, broth microdilution, Vitek 2, MicroScan or BD Phoenix methods should be checked by regular quality control tests to ensure that values are genuine and are able to guide antibiotic therapy.

Future perspective

Vancomycin remains the standard of care for most MRSA infections yet methodological difficulties associated with susceptibility testing complicate the precise determination of vancomycin MIC, creating a real challenge for clinicians when selecting the most appropriate antibiotic for the treatment of MRSA infections. It is hoped that improvements in testing methodologies in the future will improve the accuracy (and ideally the timeliness) of MIC determination, enabling clinicians to make informed decisions regarding antibiotic selection with confidence. For institutions with a high prevalence of MRSA, consideration should be given to alternative choices.

Executive summary

- A working group of infectious disease specialists and clinical/medical microbiologists from Brazil, Canada, Mexico, Saudi Arabia, Russia and the UK has made recommendations for the most suitable methodology to adopt for vancomycin susceptibility testing in order to assist hospitals in monitoring local trends and accurately guide antibiotic therapy for the management of Methicillin-resistant *Staphylococcus aureus* (MRSA) infections.

Differentiation between community-associated-MRSA (CA-MRSA) & hospital-acquired/healthcare-associated-MRSA (HA-MRSA)

- Recent studies suggest a tendency toward a broader mixing among MRSA clones, making appropriate antibiotic selection more challenging.
- While differentiation between CA-MRSA and HA-MRSA strains remains of scientific interest, reporting of the actual antibiotic susceptibility of culture-confirmed MRSA strains is more relevant than information on the origin of the clone.
- Local susceptibility profiles are more critical in the selection of the most appropriate antibiotic since MRSA susceptibility patterns vary across regions.

Reporting susceptibility based on elevated vancomycin minimum inhibitory concentration (MIC)

- Analyses suggest a correlation between vancomycin MIC and outcome. Consequently, accurate measurement of vancomycin MIC is essential in order to ensure correct management of MRSA, reduce inappropriate antibiotic use and lessen the emergence of resistant strains.
- A number of methods are currently used for antibiotic susceptibility testing and can be automated (i.e., Vitek[®] 2, BD Phoenix[™], MicroScan and Sensititre[™]) or manual (i.e., disk diffusion, broth microdilution and Etest); however, results are inconsistent accounting for conflicting reports of increases in vancomycin MIC in recent years.

Conclusion & recommendations

- Microbiology laboratories should report the isolation of MRSA and recount the full susceptibility profile of the organism without any differentiation between CA-MRSA and HA-MRSA.
- Methodological difficulties associated with susceptibility testing create a challenge for clinicians when selecting the most appropriate antibiotic for the treatment of MRSA infections. Until better susceptibility testing methods are available, hospitals must use the methods currently available to them.
- For vancomycin MIC ≤ 1 $\mu\text{g/ml}$, vancomycin can reliably be reported as susceptible. If an anomalous MIC is found (i.e., 1–2 $\mu\text{g/ml}$) retesting using a different method to determine MIC values is advised.
- Susceptibility testing methods should be checked by regular quality control tests to ensure that values are genuine and are able to accurately guide antibiotic therapy.

Author contributions

All authors equally contributed in the conception, preparation and the final approval of this manuscript. All authors meet the eligibility criteria for authorship.

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