Minimal Inhibitory Concentration and Its Microbiological Indications

Subjects: Infectious Diseases

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Before targeting an optimal antibiotic therapy, an empirical treatment is administered, and the previous collection of a microbiological sample helps choose the most effective treatment. Among the microbiological results, antibiotic susceptibility testing (AST) is currently based on testing the ability of an antibiotic to inhibit bacterial growth in vitro under standardized experimental conditions. For most infections, classic AST e.g., critical diameter measurement, is sufficient. However, for some antibiotics and/or for some bacterial infections, the determination of the minimal inhibitory concentration (MIC) value is required. The methods that can be used, their relevance, and their microbiological indications are detailed below.

minimal inhibitory concentration infections determination antibiotic

1. MIC Determination Methods

Two main methods are used to determine minimal inhibitory concentration (MIC) [1]. Broth microdilution (BMD) is a method in which containers are filled with identical volumes of inoculated broth and identical volumes of an antibiotic solution, but incrementally (usually geometrically) increasing concentrations of the antibiotic and a defined inoculum. The results are recorded as the lowest concentration of antimicrobial agent that inhibits the visible growth of a microorganism, MIC, expressed in mg/L or µg/mL. Agar dilution involves the incorporation of an antibiotic in solid or semi-solid agar media in a geometrical progression of concentrations and the application of a defined bacterial inoculum to the surface. Its purpose is the determination of the lowest concentration that inhibits bacterial growth, namely MIC [1]. The responsible bacteria are susceptible or resistant if the antibiotic MIC is below or above the clinical breakpoint cut-off, respectively. The “European Committee on Antimicrobial Susceptibility Testing” (EUCAST) annually updates the clinical breakpoint tables for the interpretation of MICs and zone diameters [2].

2. Relevance and Microbiological Indication of MIC Determination

Classic AST raises problems in certain conditions, leading to the need to perform a specific MIC measurement. In addition, the microbiological relevance of MIC determination is summarized in **Table 1**.

Table 1. Microbiological determinants warranting MIC determination according to the EUCAST guidelines [2].

Microbiological Determinants	Bacteria of Concern	Antibiotic of Concern
Agar diffusion method as inappropriate for some antibiotics	Gram-positive bacteria	Daptomycin Dalbavancin Oritavancin Telavancin
	Staphylococcus spp.	Vancomycin Teicoplanin Fosfomycin iv
	Enterobacterales, *P. aeruginosa, A. baumannii*	Colistin
Absence of detection of the resistance level to β-lactams	*Streptococcus pneumoniae* (reduced susceptibility to penicillin strains) *Haemophilus influenzae* (BLNAR * strains)	β-Lactams
Detection of low-level antibiotic resistance	*Salmonella* sp.	Ciprofloxacin
MIC creep	*Staphylococcus aureus*	Vancomycin
Preserve broad-spectrum antibiotics	*Enterobacterales*	Piperacillin/tazobactam Cephalosporins

* Beta-lactamase negative ampicillin resistant.

2.1. Agar Diffusion Method is Inappropriate for Some Antibiotics

For some antibiotics, the agar diffusion method (disc and gradient strip) does not allow for the interpretation of the susceptibility of the tested microorganisms. This is due to their poor diffusion in a solid medium (colistin) or the need for particular chemical conditions (lipopeptides such as daptomycin or dalbavancin) [2]. Therefore, the EUCAST guidelines recommend the use of a BMD method to determine MIC [3].

2.2. Absence of Detection of the Resistance Level to β-Lactams

For some microbiological species, it is difficult in the case of resistance to the usually tested antibiotic to define the resistance mechanism and to specify the optimal treatment choices. This is the case for β-lactam resistance in *Streptococcus pneumoniae* resistant to oxacillin (e.g., suspected of reduced susceptibility to penicillin) and *Haemophilus influenzae* resistant to aminopenicillins.

In the first case, for *S. pneumoniae*, resistance is associated with alterations in penicillin-binding proteins (PBPs) that reduce the binding affinity of the antibiotic to PBPs [4]. As the *S. pneumoniae* genome encodes six PBPs and each β-lactam inhibits different PBPs, the modification of PBPs leads to an increase in the MICs of all β-lactams, but the extent of this increase varies according to the antibiotic [5].

Whereas in the second case (*H. influenzae*), two main mechanisms of amino penicillin (AMP) resistance lead to reduced susceptibility to this antibiotic class: either by the production of a β-lactamase, or by alteration of PBP3 [6].

In addition, the β-lactam MIC differs according to the degree of alteration of PBP3. It may be difficult using the disc method to distinguish β-lactamase-negative ampicillin susceptible (BLNAS) strains from β-lactamase-negative ampicillin resistance (BLNAR) strains, because most discs contain high concentrations of β-lactams [7].

Determination of the MIC of β-lactams to define the most appropriate treatment will be more justified when a practitioner is dealing with severe or invasive infections (such as bacteremia or meningitis), clinical failure, and/or an isolate suspected of reduced susceptibility to penicillin (*S. pneumoniae)* or AMP (*H. influenzae)*. EUCAST guidelines recommend testing of the β-lactams of interest, particularly in these cases [3].

2.3. Detection of Low-Level Antibiotic Resistance

Fluoroquinolone resistance in *Salmonella* is mainly caused by chromosomal mutations in the quinolone resistance-determining regions (QRDRs) of the topoisomerase genes [8] that lead to resistance to nalidixic acid (MIC > 16 mg/L) and higher MIC values for ciprofloxacin (at least 0.12 mg/L). Moreover, resistance may be associated with other diverse mechanisms of resistance, such as plasmid-mediated quinolone resistance (PMQR) mechanisms that result in reduced susceptibility to ciprofloxacin (MIC of 0.125 to 1.0 mg/L), but only a modest or no increase in susceptibility to nalidixic acid [8]. Indeed, PMQR mechanisms are clinically relevant because patients infected with *Salmonella typhi* or non-typhoidal *Salmonella* isolates with ciprofloxacin MICs of 0.125 to 1.0 mg/L have more treatment failures and longer times to fever clearance than patients with isolates fully susceptible to ciprofloxacin (MICs < 0.06 mg/L) [9]. Thus, using the disk method, the ciprofloxacin disk fails to detect this low-level resistance [10].

2.4. MIC Creep

The first option for the treatment of invasive methicillin-resistant *Staphylococcus aureus* (MRSA) infections is vancomycin, which continues to be the reference standard approach in this context. However, an increasing number of MRSA isolates with high MICs, within the susceptible range (vancomycin MIC creep), are being reported worldwide. It has been reported that the efficacy of vancomycin therapy is contingent upon a target $AUC_{0–24}$/MIC ratio of ≥400 [11]. Nevertheless, AUC values greater than 600 mg.h/L are also associated with a higher risk of acute kidney injury, making it nearly impossible to safely and effectively treat microorganisms with vancomycin MICs > 1 mg/L [12]. Moreover, a few studies have reported poorer clinical outcomes and increased mortality associated with vancomycin MIC creep [13][14]. Divergent studies of this phenomenon have been reported in the literature [11][12] and the determination of vancomycin MIC in challenging situations will be discussed in terms of improving PK-PD target selection. Currently, EUCAST guidelines recommend the use of a reference laboratory to confirm the GISA or hetero GISA character of an *S. aureus* isolate if the vancomycin and/or teicoplanin MIC is >1 mg/L, using the BMD method [15].

2.5. Preservation of Broad-Spectrum Antibiotics

Piperacillin-Tazobactam/Cephalosporins and ESBL-Producing Strains

Carbapenems have been considered as the treatment of choice for severe infections caused by extended spectrum β-lactamase (ESBL)-producers [16][17]. The increasing worldwide incidence of ESBL-related infections has led to the increased use of carbapenems, leading to selection pressure for carbapenem resistance [18][19]. Therefore, to avoid the use of carbapenems, several authors have suggested the use of antibiotics that are active with regard to AST, despite the fact that they are hydrolyzed by the ESBL enzyme. Thus, EUCAST guidelines recommend reporting ESBL-producing strains as resistant to all penicillins, but as susceptible to BLBLI combinations or third-generation cephalosporins (3GC) when they are active on AST [20]. In addition, when susceptible, the use of β-lactam/β-lactamase inhibitor (BLBLI) combinations or cephalosporins has been proposed as an alternative to carbapenems [21]. Despite controversies [22][23], success when using these antibiotics depends on several factors, including the microbial species, the site of infection [24] and the MIC [25]. Studies show that success was more frequent in cases of urinary or biliary tract infections related to *Escherichia coli* [24] and that mortality was lower for isolates with an MIC ≤ 4 mg/L for BLBLI than for isolates with a higher MIC [22][25]. However, the use of 3CG s is more rarely possible as many ESBL-producing isolates are resistant, and such antibiotics should be limited to *Escherichia coli* strains or *Klebsiella pneumoniae*-related infections with MICs below 1 mg/L [26]. In conclusion, although alternatives have been studied, carbapenems remain the drugs of choice against ESBL-positive strains [27].

2.6. Therapy for Carbapenemase-Producing Enterobacterales (CPE)-Related Infections

The release of new antibiotics has opened up many new possibilities in the treatment of CPE-related infections [27]. Indeed, until the arrival of the new BLBLI, the cornerstone of treatment for CPE-related infections was a combination of antibiotics [28]. In addition, it has been demonstrated that these new associations (e.g., aztreonam with ceftazidime/avibactam) are effective, regardless of the mechanisms of resistance [27][29][30]. Although each molecule's MIC is independently high, the β-lactamase inhibitor will be responsible for restoring susceptibility to β-lactams. In case of associations, a simple way to determine MICs is based on the Etest strip superposition method which has been shown to be particularly effective for aztreonam/inhibitor combinations [31]. However, the lack of availability of these new antibiotics in low- and middle-income countries highlights the possibility of using carbapenems in combination or as a therapeutic option in patients with infections using CPE isolates with meropenem MIC ≤ 8 mg/L or the combination of ertapenem with meropenem in the case of infections related to *Klebsiella pneumoniae* carbapenemase (KPC)-producing bacteria [27][31].

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