Evaluation of a new gradient-diffusion system for MIC determination with Gram-negative pathogens

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Amended Abstract

Objectives: Minimum Inhibitory Concentration (MIC) remains the cornerstone parameter for evaluation of antimicrobial susceptibility. MIC determination is crucial with some antimicrobial agents – pathogens combinations, when disk diffusion testing is not reliable or when the MIC value correlates with outcome. MIC determination has become important also when dealing with multiresistant pathogens and treatment options are seriously limited. However, most clinical laboratories cannot afford the expensive and labor-intensive reference MIC testing or complex automated systems for precise MIC determination, and a number of products based on gradient-diffusion has been developed for easy determination of MIC values. In this work we evaluated the performance in MIC determination of a new gradient-diffusion system against a panel of Gram-negative pathogens including several multiresistant strains with emerging resistance mechanisms.

Methods: 100 bacterial isolates were studied, (70% *Enterobacteriaceae* and 30% Gram negative non fermenters), including 30 reference strains with known resistance mechanisms (acquired ampC, MBL, KPC and ESBL producing *Enterobacteriaceae*, OXA-carbapenemase producing *Acinetobacter baumannii*, MBL producing *Pseudomonas aeruginosa*), determined by molecular methods, and 70 routine isolates. MIC determinations were performed in parallel by MIC Test Strips (Liofilchem srl, Italy) and Etest (Biomerieux, France) on MHA (Becton-Dickinson, USA) medium, according to EUCAST methods. The following strips were evaluated: Ceftazime(CAZ), Cefotaxime(CTX), Colistin(COL), Piperacillin/Tazobactam (PTZ), Imipenem(IMI), Meropenem(MEM). Paper-strips containing CAZ or CTX, alone and in combination with clavulanic acid, were used for the detection of ESBL. Comparator MICs were obtained by broth microdilution (BMD), following EUCAST guidelines.

Results: MIC determinations were considered concordant when obtained results fell in the experimental error (±1 log2 dilution). Overall agreement of MIC values between MIC test strips and BDM was 91,30% (n=467), with a specific agreement ranging from 90.09% of IMI to 92,07% of CAZ. Combination strips correctly detected ESBL in 24 cases without any false positive. Similar values were obtained using the Etest method.

Conclusions: The evaluated product gave overall good agreement with BMD method, similar to that obtained with Etest. MIC test strips appear therefore to be a valid alternatives to the Etest.

Introduction

Antimicrobial Susceptibility Testing (AST) should represent the basis for the establishment of a correct antimicrobial therapy. Methods used in AST are based either on antimicrobial dilution techniques (quantitative) or diffusion procedures (qualitative) (1, 2). While the latter techniques employ paper discs containing a defined quantity of a given antibiotic, dilution methods are generally based on two-fold serial dilutions of antibiotics in solid or liquid standardized media. Dilution techniques give a MIC value of a certain antibiotic that corresponds to the concentration that inhibits the growth of a particular bacterium under defined experimental conditions. Unfortunately, results obtained from the two techniques does not always well correlate to each other and to the clinical efficacy of a particular antimicrobial regimen. For this reason clinicians has the need to have MIC values, at least for a number of pathogen - drug combinations (e.g. colistin and *Enterobacteriaceae* or Gram negative non fermenters, carbapenems in MDR isolates of *Pseudomonas aeruginosa*). A wide number of automated methods have been developed for determining MICs but very often these require expensive systems that cannot be adopted by all microbiology laboratories. On the other hand, a number of cheap and easy to use products able to give an MIC value, are present in the market.
In this work we evaluated the performance in MIC determination of a new paper-based gradient-diffusion system (MIC test strips) against a panel of Gram-negative pathogens including several multiresistant strains with different resistance mechanisms.

Methods

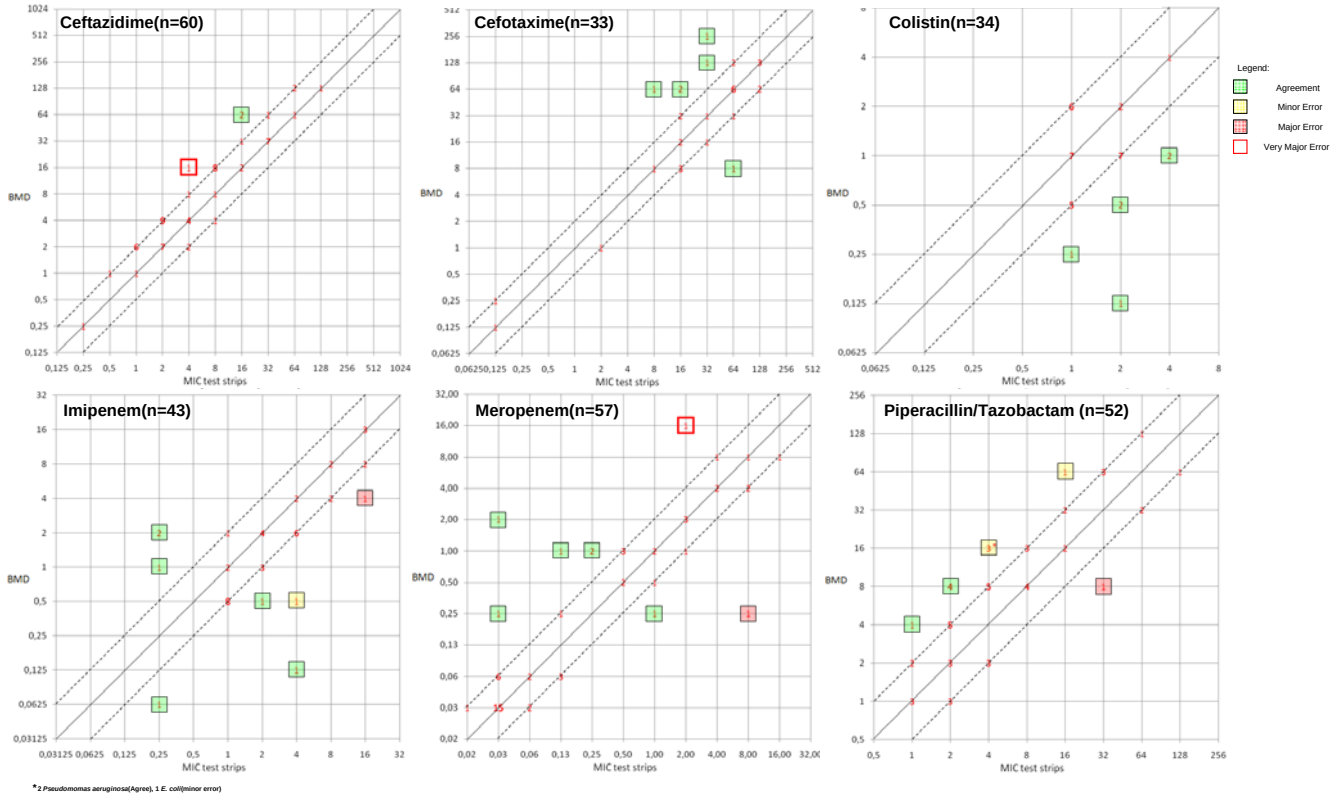
Bacterial Isolates. 100 bacterial isolates, including reference and routine isolates, were included in the study (Table 1). Reference isolates were selected to represent relevant national and international clones and phenotypes (Table 1). Routine isolates were reflecting what normally processed in a microbiology laboratory for routine purposes. Multiple isolates of the same species from the same patient has been excluded. All the investigated isolates were frozen at -80° C and subcultured on Mueller Hinton Agar plates for two consecutive days prior using to ensure the purity of the colture.
MICs Determination: Reference MICs were obtained using the BMD method, as recommended by international guidelines (2). Both Etest (BioMérieux, Marcy-l'Etoile, France) and MIC test strips (Liofilchem srl, Teramo, Italy) were used following manufacture suggestions, using the same inoculum obtained from freshly grown plates (aged < 24h). Evaluated antibiotics were: Ceftazime(CAZ), Cefotaxime(CTX), Colistin(COL), Piperacillin/Tazobactam (PTZ), Imipenem(IMI) and Meropenem(MEM). Paper-strips containing CAZ or CTX, alone and in combination with clavulanic acid, were employed for the detection of ESBL. Mueller Hinton Agar (MHA) medium (BioMérieux) were used. Results were recorded after a 16-20 hours of incubation at 37° C.
Quality Control (QC) Check: During each experimental session, appropriate reference strains (*Escherichia coli* ATCC25922 and *Pseudomonas aeruginosa* ATCC 27853) were used for quality control (QC) assessment. Results from experimental sessions where QC strains didn't give the expected MIC value, where discarded.
Data analysis: MIC data obtained with MIC test strips or Etest were compared to those deriving from BMD and considered in agreement when results were within one doubling dilution. In all cases where results obtained using MIC test strips were not in agreement with reference data, the test has been performed again. If the second test was in agreement with the reference data, the originally obtained discrepant result were not considered. On the contrary, if discrepancy persisted the data were included in the final dataset.

Results

- A total of 100 isolates and 8 MIC test strip types were tested, with 467 antibiotic/organism combinations represented (Tab. 1)
- Agreement between MIC test strips and BMD ranged from 90,09% for imipenem to 92,07% obtained with ceftazidime and meropenem
- When off-scale results were not taken into account, agreement ranged from 81,40%(n=43) obtained with imipenem to 95,00%(n=60) obtained with ceftazidime (Fig. 1)
- Categorization of MICs value, considering the EUCAST breakpoints, resulted in 2 very major-, 3 major- and 3 minor-errors, while the other experimental discordances didn't gave any difference in categorization (Fig. 1)
- Combination strips were able to detect ESBL in all cases and never gave a false positive result (data not shown)

Species	Number	Routine isolates	Reference isolates	beta-lactam resistance genes/mechanism
Escherichia coli	42	18	24	$bla_{CTX-M-15}$, $bla_{CTX-M-1}$, $bla_{CTX-M-32}$, bla_{TEM-1},bla_{CMY-2}
Acinetobacter baumannii	13	1	12	bla_{IMP-2}, bla_{OXA-23}, $bla_{OXA-139}$,bla_{OXA-58}, resident bla_{OXA-51}-like hyperproducer
Enterobacter spp	2	1	1	ampC hyperproducer
Klebsiella oxytoca	3	3	0	
Klebsiella pneumoniae	9	3	6	bla_{VIM-2}, bla_{FOX-7}, bla_{TEM-1}, bla_{KPC-3}, bla_{TEM-1}, bla_{OXA-9}, bla_{SHV-12}
Morganella morganii	1	1	0	
Pseudomonas aeruginosa	26	19	7	bla_{VIM-1}, bla_{IMP-13}, bla_{VIM-2}, bla_{PER-1}
Proteus mirabilis	6	0	6	bla_{CMY-16}, bla_{TEM-1}

Table 1. Distribution of isolates included in the study.



*2 *Pseudomonas aeruginosa*(Agree), 1 *E. coli*(minor error)

Figure 1. Scattergrams showing results of MICs determined by the evaluated product versus those determined by the BMD. Off-scale results were excluded. The diagonal black line represents complete agreement, while numbers represent the occurrences observed at each point. The broken lines represent the experimental error (±1-$\log_2$ MIC).

Conclusions

- MIC test strips gave essentially equivalent results to other analogous on the market and also acceptable agreement with BMD results
- MIC test strips appears to be a valid alternative to analogous products present on the market

References

1. European Committee on Antimicrobial Susceptibility Testing. Data from the EUCAST MIC distribution website, last accessed 02 05 2011. http://www.eucast.org
2. Clinical and Laboratory Standards Institute. 2010. Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically. Eight edition, document M07-A8. Clinical and Laboratory Standards Institute, Wayne, PA.