Clinical Microbiology and Infection 28 (2022) 1290.e1–1290.e4

Contents lists available at ScienceDirect

Clinical Microbiology and Infection

journal homepage: www.clinicalmicrobiologyandinfection.com

Research note

Clinical consequences of very major errors with semi-automated testing systems for antimicrobial susceptibility of carbapenem-resistant Enterobacterales

Michele Bartoletti [1, 2, *], Alberto Antonelli [3, 4], Linda Bussini [1], Silvia Corcione [5, 6], Daniele Roberto Giacobbe [7, 8], Lorenzo Marconi [1], Renato Pascale [1], Silvia Dettori [7, 8], Nour Shbaklo [5], Simone Ambretti [9], Paolo Gaibani [9], Tommaso Giani [3, 4], Marco Coppi [3, 4], Matteo Bassetti [7, 8], Francesco Giuseppe De Rosa [5, 10], Anna Marchese [11, 12], Rossana Cavallo [13], Russell Lewis [2], Gian Maria Rossolini [3, 4], Pierluigi Viale [1, 2], Maddalena Giannella [1, 2]

[1] *Infectious Diseases Unit, IRCCS Azienda Ospedaliero-Universitaria di Bologna, Policlinico di Sant'Orsola, Bologna, Italy*
[2] *Department of Medical and Surgical Sciences, Alma Mater Studiorum University of Bologna, Bologna, Italy*
[3] *Department of Experimental and Clinical Medicine, University of Florence, Florence, Italy*
[4] *Microbiology and Virology Unit, Careggi University Hospital, Florence, Italy*
[5] *Department of Medical Sciences, University of Turin, Turin, Italy*
[6] *Tufts University School of Medicine, Boston, MA, USA*
[7] *Department of Health Sciences, University of Genoa, Genoa, Italy*
[8] *Infectious Diseases Unit, San Martino Policlinico Hospital-IRCCS, Genoa, Italy*
[9] *Operative Unit of Clinical Microbiology, IRCCS Azienda Ospedaliero-Universitaria di Bologna, Bologna, Italy*
[10] *Infectious Diseases Unit, Cardinal Massaia Hospital, Asti, Italy*
[11] *Department of Surgical Sciences and Integrated Diagnostics, University of Genoa, Genoa, Italy*
[12] *Clinical Microbiology Unit, San Martino Policlinico Hospital-IRCCS, Genoa, Italy*
[13] *Unit of Microbiology and Virology, Department of Public Health and Pediatric Sciences, University of Turin, A.O.U. "Città della Salute e della Scienza di Torino", Turin, Italy*

ARTICLE INFO

Article history:
Received 7 October 2021
Received in revised form
7 March 2022
Accepted 10 March 2022
Available online 17 March 2022

Editor: Panayotis T. Tassios

Keywords:
Category agreement
Essential agreement
Major errors
Mortality
Very major errors

ABSTRACT

Objectives: In this study we investigated the rate of susceptibility testing discrepancies between semi-automated and reference systems with carbapenem-resistant Enterobacterales (CRE) and the impact of alleged errors by semi-automated systems on guiding targeted therapy for CRE bloodstream infection (BSI).

Methods: This was a multicentre, retrospective study enrolling patients with monomicrobial BSI caused by CRE from January 2013 to December 2016. Nonduplicate isolates from index blood cultures tested locally with semi-automated systems were centralized at a referral laboratory and retested with a reference broth microdilution or agar dilution method.

Results: We enrolled 366 patients with CRE-BSI; 220 (60%) were male, and the median age was 67 years (interquartile range, 54–76 years). When compared with the results of the reference methods, those of the semi-automated systems exhibited variable rates of very major errors (VMEs; i.e. false susceptibilities) and major errors (MEs; i.e. false resistances). The highest rates of VMEs were observed with fosfomycin (14%) and colistin (13.9%), and the highest rates of MEs were observed with gentamicin (21%), fosfomycin (7.7%), and tigecycline (34%). Overall, VMEs and MEs led clinicians to prescribe or confirm ineffective therapy in 25 of 341 patients (7%). Receipt of ineffective therapy supported by a misleading susceptibility test was associated with higher 30-day mortality rates by Kaplan–Meier survival curves rates compared with receipt of active therapy (56% vs. 26%; $p = 0.002$), and the difference was confirmed after adjustment for confounders in a Cox regression model (adjusted hazard ratio: 2.91; 95% CI, 1.62–5.22; $p < 0.001$).

* Corresponding author. Michele Bartoletti, Infectious Diseases Unit, Sant'Orsola Malpighi Hospital, Via Massarenti 11, 40137, Bologna, Italy.
E-mail address: m.bartoletti@unibo.it (M. Bartoletti).

https://doi.org/10.1016/j.cmi.2022.03.013

Discussion: MEs and VMEs were relatively common with semi-automated susceptibility testing systems. VMEs were associated with inappropriate use of antibiotics and poorer outcomes. **Michele Bartoletti, Clin Microbiol Infect 2022;28:1290.e1–1290.e4**

Introduction

Carbapenem-resistant Enterobacterales (CRE) are considered a global health emergency. Infections caused by CRE have been associated with high rates of mortality, relapse, and microbiological failure [1–3].

The reliability of antimicrobial susceptibility tests (ASTs) performed by semi-automated systems for several drugs commonly used for the treatment of CRE was shown to be variable [4–7]. Both the Clinical and Laboratory Standards Institute and European Committee on Antimicrobial Susceptibility Testing (EUCAST) have recommended the use of reference broth microdilution for susceptibility testing of some last-resort antibiotics, such as colistin and tigecycline or agar dilution for fosfomycin, to circumvent this problem [8,9]. Moreover, reference methods are not always used for routine susceptibility testing by diagnostic laboratories because of the additional workload required compared with semi-automated systems.

The objectives of this study were (a) to investigate the rate of discrepancies between semi-automated and reference systems testing susceptibility of CRE isolates obtained from a multicentre cohort of 366 patients diagnosed with monomicrobial CRE bloodstream infection (BSI) between 2013 and 2016 and (b) to analyze the impact of errors by the semi-automated systems on the selection of antimicrobial therapy and the outcome of patients.

Methods

Study design and population

This was a multicentre, retrospective study conducted in three Italian tertiary teaching hospitals. All consecutive adult patients with BSI caused by CRE (defined as resistant to at least one of the following carbapenems: ertapenem, meropenem, and imipenem) between January 2013 and December 2016 were enrolled in the study. Only the first episode of BSI was included. The study was approved by the Ethical Committee of the Area Vasta Emilia Centrale (ref 79/2017/O/OssN, approved March 14, 2017).

Antimicrobial susceptibility testing

At each participating hospital, either the Vitek-2 system (bioMérieux, Marcy l'Etoile, France) or MicroScan 96 plus Walkaway System (Beckman Coulter) was used by the local laboratory for ASTs. Nonduplicate isolates of CRE from index blood cultures of the enrolled patients were stored and subsequently transferred to the Microbiology and Virology Unit of the Careggi University Hospital in Florence, Italy, for retesting. For meropenem, amikacin, gentamicin, colistin, and tigecycline, retesting was carried out with reference broth microdilution using custom lyophilized plates (Sensititre, TREK Diagnostic Systems, Cleveland, OH). This system was among the most reliable commercial broth microdilution systems for colistin susceptibility testing by EUCAST [8,10], and is validated by the U.S. Food and Drug Administration for tigecycline susceptibility testing. For fosfomycin, reference agar dilution (ISO 20776:1–2019) was used.

Researchers doing the retesting were blinded to the original results from the local microbiology laboratories. AST results were interpreted using the EUCAST clinical breakpoints valid at the time when the cases were diagnosed (https://www.eucast.org/clinical_breakpoints; versions 4.0, 5.0, and 6.0). *Escherichia coli* ATCC 25922 and *Pseudomonas aeruginosa* ATCC 27853 were always included as quality control standards, as recommended by EUCAST (https://www.eucast.org/ast_of_bacteria/quality_control/). For all retested isolates, identification was also confirmed by MALDI-ToF.

Molecular testing

The presence of carbapenemase determinants was assessed by real-time PCR as previously described [11].

Variables and definitions

The primary microbiologic endpoints were category agreement, major errors (MEs; i.e. false resistances), and very major errors (VMEs; i.e. false susceptibilities) of automated tests compared with reference tests according to the ISO 20776-2:2007 guidelines. The primary clinical endpoint was all-cause mortality assessed at day 30 after the collection of index blood cultures.

The main exposure variable was targeted inappropriate therapy, defined as receipt of *in vitro* inactive drugs according to reference test results due to MEs/VMEs of semi-automated systems. We collected data on demographics, comorbidities, immunosuppression, source of BSI [12], and severity of BSI using Sepsis-3 criteria [13]. Patients were followed up to 30 days from BSI onset.

Statistical analysis

Continuous variables were presented as mean and standard deviation, and median and interquartile range where appropriate, and categorical variables as numbers and percentages. Variables were compared with parametric or nonparametric tests, according to data distribution, for continuous variables and with Pearson's χ^2 test (Fisher exact test where appropriate) for categorical variables.

Descriptive statistics were used to evaluate the primary objective. The clinical outcomes of patients who received targeted inappropriate versus appropriate treatment were compared using Kaplan–Meier survival analysis. Additionally, survivors and nonsurvivors after 30 days from blood-culture collection were compared. Variables associated with 30-day mortality in the univariable analysis ($p < 0.1$) were included in a Cox regression model to identify independent predictors of 30-day mortality.

Results

During the study period, 366 CRE-BSI episodes were analyzed. *Klebsiella pneumoniae* was identified in 364 cases (99%), and the remaining two BSI cases were caused by *Enterobacter cloacae* complex. After genotyping, 355 of the CRE isolates (97%) were

Table 1
MIC, MIC_{50}, MIC_{90}, and interpretation according to European Committee on Antimicrobial Susceptibility Testing breakpoints[a] of meropenem, fosfomycin, amikacin, gentamycin, colistin, and tigecycline, performed on 366 strains of carbapenem-resistant Enterobacterales collected from three large tertiary teaching hospitals

Drug	MIC value (mg/L)												MIC_{50}	MIC_{90}	Interpretation of referral test			Interpretation of automated test				Essential agreement, *n* (%)	Category agreement, *n* (%)	Very major/major errors, *n* (%)
	0.12, *n* (%)	0.25, *n* (%)	0.5, *n* (%)	1, *n* (%)	2, *n* (%)	4, *n* (%)	8, *n* (%)	16, *n* (%)	32, *n* (%)	64, *n* (%)	128, *n* (%)				S	I	R	S	I	R	Not tested/data not available			
Meropenem	1 (0)	2 (0)	2 (0)	1 (0)	1 (0)	10 (3)	14 (4)	42 (11)	41 (11)	252 (69)	—	64	64	128	7 (2)	23 (6)	336 (92)	10 (3)	3 (1)	353 (96)	0 (0)	354 (97)	330 (90)	5 (1)/3 (1)
Fosfomycin	—	—	—	—	—	8 (2)	55 (14)	62 (17)	90 (25)	48 (13)	103 (28)	32	128	216 (59)	—	150 (41)	27 (33)	—	55 (67)	284 (78)	87 (24)	144 (39)	15 (4)/28 (7)	
Amikacin	—	—	—	—	65 (18)	20 (5)	3 (1)	83 (23)	195 (53)	—	—	16	32	88 (24)	83 (23)	195 (53)	75 (20)	16 (4)	254 (69)	21 (6)	256 (69)	259 (71)	8 (2)/14 (4)	
Gentamicin	—	—	37 (10)	78 (21)	160 (43)	22 (6)	69 (18)	—	—	—	—	2	8	275 (75)	22 (6)	69 (19)	92 (25)	117 (32)	155 (43)	2 (0)	266 (73)	190 (52)	6 (2)/77 (21)	
Colistin	—	—	115 (31)	116 (32)	8 (2)	10 (3)	117 (32)	—	—	—	—	1	8	239 (65)	—	127 (35)	243 (81)	—	59 (19)	64 (17)	247 (67)	263 (72)	42 (11)/1 (0)	
Tigecycline	—	13 (4)	111 (30)	217 (59)	18 (5)	7 (2)	—	—	—	—	—	1	1	341 (93)	7 (2)	18 (5)	76 (27)	52 (19)	145 (53)	93 (25)	101 (27)	99 (27)	1 (0)/126 (34)	

MIC_{50} and MIC_{90} refer to MICs required to inhibit 50% and 90% of bacterial strains, respectively. S, susceptible; I, intermediate; R, resistant.
[a] Breakpoint established and valid during the period of study was used (https://www.eucast.org/clinical_breakpoints; versions 4.0, 5.0, and 6.0).

found to be carbapenemase producers (*Klebsiella pneumoniae* carbapenemase: 95.6%; Verona Integron-mediated Metallo-β-lactamase: 1.1%, and oxacillinase-48: 0.3%) and 11 (3%) were non−carbapenemase-producing CRE.

Susceptibility of the CRE isolates to the study drugs performed with reference tests, described as MIC distribution, MIC_{50}, MIC_{90}, and interpretation according to EUCAST clinical breakpoints, as well as categorical agreement of results obtained with semi-automated systems and corresponding error rates, are described in Tables 1 and S1.

Clinical data

The clinical characteristics of the 366 patients with CRE-BSI are summarized in Table S1. The crude 30-day mortality rate was 30%, and a comparison of survivors and nonsurvivors after 30 days from BSI onset is shown in Table S2.

A targeted therapy was administered to 341 patients (92%). In this group, considering the reference susceptibility test as the reference standard, the targeted therapy chosen according to the results of the semi-automated systems was labelled as inappropriate in 25 of 341 patients (6%; Tables S3 and S4). Notably, all patients received a high dose of meropenem (i.e. 2 g every 8 hours by extended infusion). The median meropenem MIC was 64 mg/L (32−64 mg/L) and was considered inactive. According to Kaplan−Meier analysis (Fig. 1), patients receiving inappropriate targeted therapy due to MEs/VMEs had a significantly higher 30-day mortality rates than patients receiving active drugs (56% vs. 26%; $p = 0.002$; hazard ratio: 2.36; 95% CI, 1.33−4.17). After adjustment for confounders, the impact of targeted inappropriate therapy was still significant (adjusted hazard ratio: 2.91; 95% CI, 1.62−5.22; $p < 0.001$; Table S5).

Discussion

In this study, we observed that MEs and VMEs occurred frequently, using semi-automated systems for several antibiotics commonly used for CRE infection at the time of the study. Despite this, discrepancies have been reported by other authors [7,14,15], but this is the first study showing that such errors were correlated with a worse outcome as a consequence of a targeted inappropriate prescription by clinicians, underscoring the importance of reliable testing when evaluating patients infected with difficult-to-treat resistance microorganisms.

It should be noted that our study refers to a period (January 2013−December 2016) that mostly antecedes the publication of formal recommendations by a joint Clinical and Laboratory Standards Institute-EUCAST Polymyxin Breakpoints Working Group to use broth microdilution for colistin susceptibility testing [9] and of other reports about the potential inaccuracy of semi-automated systems [8].

Our study has a series of limitations. First, we collected isolates in an era when novel β-lactams were not available. Therefore, the current application of our findings may be limited to the therapeutic options available at the time. Second, automated and reference tests were not performed simultaneously, which may have reduced the accuracy of the comparison. Finally, the clonality and virulence of the strains involved in the study were not assessed, even if during the study period, different Italian national surveys reported a predominance of *K pneumoniae* carbapenemase-producing *K pneumoniae* from invasive infections belonging to CG258, with a minority of other emerging high-risk clones (e.g. CG307, ST101, and ST395), increasing the clonal diversity over time [16].

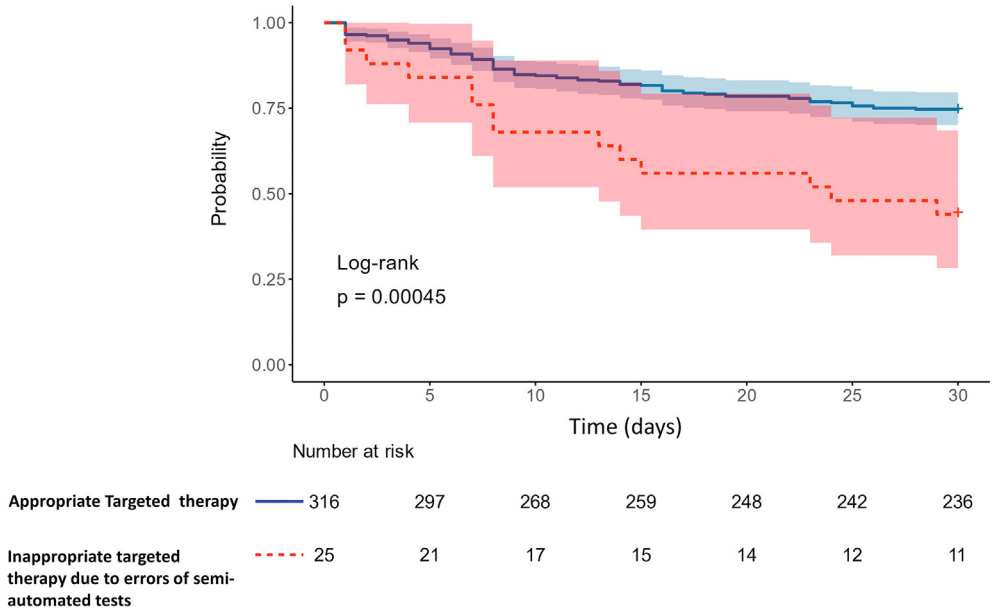

Fig. 1. Kaplan–Meier curves analyzing the impact of inappropriate targeted therapy due to misleading results of automated tests compared with active therapy according to reference tests performed retrospectively on the same strain. Comparison was performed with log-rank test.

In conclusion, our results showed that MEs and VMEs of semi-automated AST systems are common and might be associated with poor outcome due to the more frequent inappropriate use of antibiotics.

Transparency declaration

All authors declare no conflicts of interest related to this study. The study was supported by a grant from the Società Italiana Terapia Antinfettiva.

Author contributions

Conceptualization: PV, AA, MBar, MG, GMR, FGDR, TG, MBas; methodology: MBar, GMR, MG, DRG, MC; investigation: LB, SC, LM, RP, SD, NS, SA, PG, MBas, AM, RC; formal analysis: MBar, AA, MG, RL; writing- original draft: MBar, AA, MD; WRITING - REVIEW AND EDITING: MG, PV, RL, DRG; supervision: GMR, MG, MBas, FGDR, PV.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.cmi.2022.03.013.

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