Comparison of disk diffusion, Etest and VITEK2 for detection of carbapenemase-producing *Klebsiella pneumoniae* with the EUCAST and CLSI breakpoint systems

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Abstract

The aim of this study was to compare CLSI and EUCAST MIC and disk diffusion carbapenem breakpoints for the detection of carbapenemase-producing *Klebsiella pneumoniae*. *K. pneumoniae* strains with known KPC (n = 31) or VIM (n = 20) carbapenemases were characterized by disk diffusion (Oxoid) and Etest (bioMérieux) vs. imipenem, meropenem and ertapenem, and with VITEK2 (bioMérieux, five different cards). Extended-spectrum β-lactamase (ESBL) testing was performed with VITEK2 (bioMérieux), ESBL combination disks (Becton Dickinson) and the ESBL Etest (bioMérieux). With CLSI and EUCAST MIC breakpoints, respectively, 11 and seven of the strains were susceptible to imipenem, 12 and eight to meropenem, and seven and none to ertapenem. The EUCAST epidemiological cut-off (ECOFF) values for meropenem and ertapenem identified all carbapenemase producers, whereas the imipenem ECOFF failed in five strains. All carbapenemase producers were detected with EUCAST disk diffusion breakpoints for ertapenem and meropenem, and four strains were susceptible to imipenem. CLSI disk diffusion breakpoints characterized 18 (imipenem), 14 (meropenem) and three (ertapenem) isolates as susceptible. When cards with a single carbapenem were used, detection failures with VITEK2 were four for imipenem, none for meropenem and one for ertapenem. Cards containing all three carbapenems had one to two failures. With ESBL combination disks, 21/31 KPC producers and 2/20 VIM producers were positive. With VITEK2, no VIM producers and between none and seven KPC producers were ESBL-positive. All carbapenemase producers were detected with the meropenem MIC ECOFF, or the clinical EUCAST breakpoint for ertapenem. EUCAST disk diffusion breakpoints for meropenem and ertapenem detected all carbapenemase producers. VITEK2 had between none and four failures in detecting carbapenemase producers, depending on the antibiotic card.

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Introduction

In recent years, carbapenem resistance resulting from β-lactamase production among *Enterobacteriaceae* (especially *Klebsiella pneumoniae*) has increased [1]. KPC is the most common class A carbapenemase and VIM is the most common class B β-lactamase among *K. pneumoniae* strains. Both enzymes effectively inactivate most β-lactam antibiotics, including carbapenems, restricting treatment options [2]. Recently, it has been proposed that carbapenemases of all molecular classes should be designated as extended-spectrum β-lactamases $(ESBLs)_{CARBA}$ [3].

In some countries, carbapenemase-producing *Enterobacteriaceae* have become a public health problem. In Greece, an increase in imipenem-resistant *K. pneumoniae* from 1% in 2001 to 20–50% (hospital wards/intensive-care units) in 2006 was observed [4]. Other countries, such as Israel and the USA, have experienced similar problems [1,5–7]. Whereas Israel and the USA have experienced mainly a problem with KPC-producing *K. pneumoniae*, the carbapenemase-producing strains in Greece have included both VIM and KPC producers [4,8], and, lately, also strains with the simultaneous presence

of VIM and KPC enzymes [9]. This results in a multidisciplinary challenge spanning diagnostic microbiology, infection control and antimicrobial treatment [10,11]. Few antimicrobial alternatives exist, and infections with carbapenemase-producing *K. pneumoniae* are associated with a high mortality rate [5,7,12]. The MICs of carbapenemase-producing strains differ between strains, and some strains have MICs for carbapenems below the current clinical susceptibility breakpoints [1,11]. The current clinical breakpoints used in Europe and the USA are, at present, not set to detect all carbapenamase-producing *Enterobacteriaceae*.

The aim of this study was to compare the performance of disk diffusion, MIC testing with Etest and VITEK2 in detecting carbapenemase (KPC or VIM)-producing *K. pneumoniae*, using the CLSI and EUCAST breakpoint systems. Furthermore, the performance of ESBL tests among carbapenemase producers was examined with ESBL combination disk testing (CDT), ESBL Etest and VITEK2. The results were interpreted with both CLSI and EUCAST breakpoints, as well as EUCAST epidemiological cut-off (ECOFF) values.

Materials and Methods

Selection of strains

A total of 51 isolates of carbapenem-non-susceptible *K. pneumoniae* with known KPC production (n = 31) or VIM production (n = 20) were tested. The clinical isolates were collected from microbiological laboratories in Sweden, Greece, the USA and Norway, and had earlier been genotypically characterized [13–15]. Previously conducted epidemiological typing had shown that the majority of the KPC producers belonged to sequence type 258, but a certain level of diversity was observed within this clone [14]. Among the VIM producers, the diversity was greater [13].

Disk diffusion susceptibility testing

Antibiotic susceptibility testing was performed by the disk diffusion method, with Mueller–Hinton II agar (Becton Dickinson, Franklin Lakes, NJ, USA), according to EUCAST (http://www.eucast.org/eucast_disk_diffusion_test/disk_diffusion_methodology/; last accessed 25 March 2010) and CLSI methodology [16]. The plates were inoculated with samples of each strain adjusted to a turbidity of 0.5 McFarland. Disks containing 10 μg of imipenem, meropenem or ertapenem (Oxoid, Basingstoke, UK) were applied to the surface of the inoculated agar, and the plates were incubated for 20 h at 35°C. The strains were interpreted according to current CLSI and EUCAST breakpoints. The CLSI breakpoints for susceptibility are ≥16 mm for imipenem, ≥16 mm for meropenem and ≥19 mm for ertapenem [16]. The EUCAST disk diffusion breakpoints for susceptibility are ≥21 mm for imipenem, ≥22 mm for meropenem and ≥25 mm for ertapenem (http://www.eucast.org/clinical_breakpoints; last accessed 25 May 2010). Furthermore, ECOFFs, the lower borders of the wild-type populations, were applied. The carbapenem ECOFFs for *K. pneumoniae* are 23 mm for imipenem, 24 mm for meropenem and 25 mm for ertapenem (G. Kahlmeter, personal communication).

Etest

The MICs for imipenem, meropenem and ertapenem were determined with Etest (bioMérieux, Marcy l'Etoile, France) carried out on Mueller–Hinton II agar (Becton Dickinson), according to the manufacturer's instructions, and interpreted according to clinical breakpoints from the CLSI and EUCAST. The CLSI breakpoints are ≤4 mg/L for imipenem-susceptible, ≥16 mg/L for imipenem-resistant, ≤4 mg/L for meropenem-susceptible, ≥16 mg/L for meropenem-resistant, ≤2 mg/L for ertapenem-susceptible, and ≥8 mg/L for ertapenem-resistant [16]. The EUCAST clinical breakpoints are ≤2 mg/L for imipenem-susceptible, >8 mg/L for imipenem-resistant, ≤2 mg/L for meropenem-susceptible, >8 mg/L for meropenem-resistant, ≤0.5 mg/L for ertapenem-susceptible and >1 mg/L for ertapenem-resistant (http://www.eucast.org/clinical_breakpoints; last accessed 25 March 2010). The strains were also analysed according to carbapenem ECOFFs, which, in *K. pneumoniae*, are ≤1mg/L for imipenem, ≤0.125 mg/L for meropenem and ≤0.064 mg/L for ertapenem (http://www.eucast.org/mic_distributions/; last accessed 25 March 2010).

VITEK2

Colonies from an overnight agar plate culture of each isolate were suspended in 3 mL of 0.45% saline and adjusted to a turbidity of 0.5–0.63 McFarland standard with VITEK Densicheck (bioMérieux). Antimicrobial susceptibility testing of the isolates was performed with VITEK2 (bioMérieux), using five different cards containing imipenem, meropenem or ertapenem, and combinations of these: AST N025 (imipenem, meropenem and ertapenem), AST N027 (imipenem), AST N029 (meropenem), AST N106 (ertapenem) and AST N107 (imipenem, meropenem and ertapenem). In the VITEK2 report, the MIC correlates are reported and the advanced expert system interprets the results. For all cards except AST N029, an ESBL test was included in the card.

ESBL CDT

ESBL CDT was performed with commercial disks (Becton Dickinson), according to the manufacturer's instructions,