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Original article

Development of a national EUCAST challenge panel for antimicrobial susceptibility testing

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ABSTRACT

A challenge panel of bacterial strains useful for clinical laboratories to validate their European Committee on Antimicrobial Susceptibility Testing (EUCAST) antimicrobial susceptibility test (AST) system was established. A total of 117 strains, obtained from Belgian Reference Centres ($n = 57$) and from routine clinical samples ($n = 60$) was selected based on resistance pattern. These strains were analysed in seven different laboratories by three different automated AST systems (Vitek ($n = 2$), Phoenix ($n = 2$) and Microscan ($n = 2$)) and by disc diffusion from five different manufacturers (Rosco ($n = 2$), Becton-Dickinson ($n = 2$), Biomérieux ($n = 1$), Bio-rad ($n = 1$) and i2a ($n = 1$)). To select the challenge panel, selection criteria were set for categorical agreement between the different systems and the number of very major errors, major errors and minor errors. Very major and major errors for at least two antibiotics were observed in 43% of all strains, leading to the exclusion of these strains from the selected panel. In only 10% of all tested strains was there 100% categorical agreement for all antibiotics. Finally, 28 strains (14 Gram-positive and 14 Gram-negative) covering a wide spectrum of resistance mechanisms were selected. Pilot-testing of this challenge panel in 20 laboratories mainly confirmed the results of the validation study. Only six strains withheld for the pilot study could not be used as challenge strain due to an overall (very) major error rate of >5% for a particular antibiotic ($n = 5$) or for two antibiotics ($n = 1$). To conclude, this challenge panel should facilitate the implementation and use of EUCAST breakpoints in laboratories. **S. Desmet, CMI 2016;22:704**

Introduction

The use of common clinical breakpoints for antimicrobial susceptibility testing (AST) is important for both consistent clinical reporting of antimicrobial susceptibility and epidemiological surveillance purposes. The goal of the European Committee on Antimicrobial Susceptibility Testing (EUCAST) is to harmonize antimicrobial breakpoints in Europe and to define breakpoints for new agents in collaboration with the European Medicines Agency. EUCAST breakpoints are set following a defined procedure including clinical results from various types of infections, wild-type

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MIC distributions for relevant species of organisms, knowledge about resistance mechanisms, antimicrobial dosing and pharmacokinetic and pharmacodynamic aspects [1–4]. A shift from national and CLSI breakpoints to EUCAST breakpoints in European laboratories is gradually observed [5]. In April 2015, 55% of all Belgian laboratories had implemented EUCAST breakpoints (personal communication Kris Vernelen, Belgian Scientific Institute for Public Health). To facilitate the implementation of EUCAST breakpoints, EUCAST promoted the establishment of National Antimicrobial susceptibility testing Committees. In 2012, the Belgian National Antimicrobial susceptibility testing Committee decided to prepare a panel of challenge strains with different resistance mechanisms, which could be made freely available to laboratories for validating their AST system with EUCAST breakpoints. Development of a challenge panel is important because routinely used quality control strains frequently have very high or low MICs, without being challenging for the AST systems and these do not always reflect local circulating resistance mechanisms. To be eligible as a challenge strain, the strain should harbour a stable resistance mechanism and should show reproducible results with different AST systems, both automated AST and disc diffusion (DD) methods. Moreover each strain should be suitable for testing all relevant antibiotics. In this study, we describe the establishment of such an AST challenge panel.

Materials and methods

Bacterial strains

Six of seven validation laboratories selected ten strains prospectively from clinical samples in 2013. Five Belgian Reference Centres provided 57 strains with a known and, for most of the strains, genetically defined resistance mechanism. A total of 117 strains consisting of 61 *Enterobacteriaceae*, 11 non-fermenters, 20 *Staphylococcus* spp., nine β-haemolytic streptococci, eight *Enterococcus* spp., six *Streptococcus pneumoniae* and two viridans group streptococci were included in the study (Table 1). Strains were subcultured and distributed among the seven validation laboratories.

Validation study

Antimicrobial susceptibility testing and categorization of strains. Six validation laboratories determined antimicrobial susceptibility of the 117 strains with automated AST systems according to the manufacturer's instructions: MicroScan WalkAway (Siemens Healthcare Diagnostics, West Sacramento, CA, USA) ($n = 2$; panels: NBC46 ($n = 2$), PBC33 ($n = 2$)), Phoenix Automated Microbiology System (Becton-Dickinson, Sparks, MD, USA) ($n = 2$; panels: NMIC-84 ($n = 2$), UNMIC-85 ($n = 1$), PMIC-72 ($n = 2$), SMIC-11 ($n = 2$)), Vitek 2 (Biomérieux, Marcy l' Etoile, France) ($n = 2$; cards: N205 ($n = 1$), N236 ($n = 1$), N256 ($n = 1$), N237 ($n = 1$), P610 ($n = 2$), P633 ($n = 1$), ST01 ($n = 1$), P586 ($n = 1$)). DD testing according to EUCAST was performed in three validation laboratories by means of Rosco Neo-Sensitab (Taastrup, Denmark) ($n = 2$), Becton Dickinson (Sparks, MD, USA) ($n = 2$), Bio-rad (Marnes-la-Coquette, France) ($n = 1$), Biomérieux (Marcy l' Etoile, France) ($n = 1$) and i2a (Montpellier, France) ($n = 1$) discs.

Antibiotics with at least four measurements per strain were included in the analysis. Interpretation of MICs and zone diameters was performed using EUCAST breakpoints 2015 [6]. Categorical agreement (CA) was calculated between the results of all automated AST methods and all DD methods considered together [7]. For each strain, very major errors (VME), major errors (ME) and minor errors (MI) were calculated per antibiotic [8]. The result of more than 50% of the methods was considered as the reference result.

Selection of strains for the challenge panel. Based on clinical microbiology guidelines to evaluate AST systems, a list of microorganisms to be included in the challenge panel was set up [7–9] (see Supplementary material 3). Additional resistant phenotypes, such as colistin resistance, not (yet) included in these guidelines, were added. Selection of the challenge strains was based on the mean percentage CA between all systems for all antibiotics and the number of VMEs, MEs and MIs. All strains were divided into four groups. Group 1 showing 100% CA for all antibiotics, group 2 not having 100% CA, but with only MI(s), group 3 with (very) major errors ((V)ME) for one antibiotic and group 4 with (V)MEs for more than one antibiotic. Strains belonging to the last group were excluded for selection into the challenge panel. In case different strains from groups 1, 2 or 3 were eligible as challenge strain, priority was given to strains from group 1 and 2 respectively. When several strains from the same group were candidates to be included in the panel, the most 'challenging' strain was selected. 'Challenging' was defined as having a high number of MICs in the measurable range of the testing system and showing results close to the susceptibility breakpoints. A strain could only serve as a challenge strain in the pilot study for an antibiotic for which it had not more than one (V)ME in the validation study. To exclude interference of a malfunctioning test system in a laboratory, not more than two (V)MEs of one system were accepted for the same strain. In case more than two (V)MEs occurred, the particular systems' results for that strain were excluded from analysis.

Pilot-testing of the challenge panel

In May 2015, the selected strains of the challenge panel were sent to 20 Belgian pilot-testing laboratories. Susceptibility testing was performed with Vitek 2 ($n = 8$; cards: ST01 ($n = 2$), P633 ($n = 3$), P586 ($n = 6$), P610 ($n = 4$), GP-74 ($n = 1$)), Phoenix ($n = 7$; panels: PMIC-75 ($n = 1$), PMIC-72 ($n = 5$), SMIC-11 ($n = 2$), NMIC-93 ($n = 1$), NMIC-205 ($n = 4$), NMIC206 ($n = 2$)), Microscan ($n = 2$; panels: PM28 ($n = 1$), PBC33 ($n = 1$), MM37 ($n = 1$), MBC46 ($n = 1$)), Bio-Rad discs ($n = 7$) and Rosco Neo-Sensitab discs ($n = 3$) according to EUCAST. Raw results of MICs and zone diameters were collected in one centre for interpretation according to EUCAST 2015 breakpoints.

Defining the susceptibility categorization

Taking all results of the validation study and pilot-testing into account, categorical agreement, (V)ME rate and MI rate were again calculated per antibiotic per strain. Based on these results, a definite susceptibility category (DC) per antibiotic was defined. A strain could only serve as a challenge strain for an antibiotic for which it had a (V)ME rate $\leq$5%. In case of a higher error rate, no susceptibility category was set. When a strain had an MI rate of >10% for an antibiotic, both interpretation categories were accepted (S/I or R/I). When the MI rate was <10%, the interpretation category of the majority of the test systems was chosen.

Results

Validation study

Antimicrobial susceptibility testing and categorization of strains. For 10% of all strains (12/117) there was 100% CA for all antibiotics. No (V)ME was observed for 17% of strains (Table 2). In the remaining 73% there was a (V)ME for at least one antibiotic. In 43% of the strains (V)MEs were observed for more than one antibiotic, and accordingly these strains were excluded. Details on (V)MEs and MIs per antibiotic per EUCAST interpretation group are available in