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Short Communication

Multicentre interlaboratory analysis of routine susceptibility testing with a challenge panel of resistant strains

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ABSTRACT

Objectives: In order to elaborate a new national challenge panel of resistant Gram-negative bacilli and Gram-positive cocci strains for the validation of routine antimicrobial susceptibility testing (AST) methods, an interlaboratory evaluation was organised.
Methods: The results of 12 well-characterised multidrug-resistant strains tested by nine laboratories using local disk diffusion (DD) and automated AST (AUST) methods were compared with the reference broth microdilution method.
Results: Overall categorical agreement ranged from 70% to 100% both for DD and AUST and was >90% for all but one strain for all antibiotics.
Conclusion: Our multicentre AST study showed good reproducibility and the panel can be used as national resistant reference strains for routine AST validation.

1. Introduction

Reliable antimicrobial susceptibility testing (AST) results among multidrug-resistant (MDR) Gram-negative bacilli (GNB) and Gram-positive cocci (GPC) are critical to avoid the use of inactive antibiotics and to define active drugs for the treatment of infections [1,2]. Appropriate quality control GNB/GPC strains with specific resistance mechanisms to challenge AST methods additional to the recommended ATCC susceptible strains are often lacking. In 2015, the Belgian National Antimicrobial Susceptibility Testing Committee (NAC) initiated the development of a first national collection of GNB/GPC strains that could serve as a validation panel (NACP1) for new AST systems and for the implementation of European Committee on Antimicrobial Susceptibility Testing (EUCAST) breakpoints [3]. However, some resistance determinants of relevance were not covered. Here we aimed to elaborate a second national challenge panel (NACP2) of GNB/GPC strains including relevant resistance traits not covered in NACP1, based on the agreement results of a multicentre evaluation of routine AST methods.

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2. Materials and methods

Nine GNB and three GPC clinical strains were selected based on their specific resistant determinants as previously characterised by three national reference centres (NRCs) for MDR organisms (Table 1). Strains were subcultured and were provided to nine proficient clinical microbiology laboratories selected based on their geographical distribution, broad coverage of various routine AST methods used, and their experience in performing AST studies (see Acknowledgments section).

Isolates were tested by the nine laboratories in 2020 using their routine AST methods including disk diffusion (DD) from three different disk manufacturers [Bio-Rad (Hercules, CA, USA) (n = 4), Becton Dickinson (BD, Franklin Lakes, NJ, USA) (n = 2) and ROSCO (Taastrup, Denmark) (n = 2)] and by two different automated AST (AUST) systems, namely VITEK®2 (bioMérieux, Marcy-l'Étoile, France) [n = 4: AST-N366 (n = 3), AST-N367 (n = 1), AST-N353 (n = 1), AST-P652 (n = 3), AST-P655 (n = 2) and AST-P650 (n = 1)] and BD Phoenix Automated Microbiology System (BD) [n = 3: NMIC-417 (n =2), NMIC408 (n = 1), NMIC-502 (n = 1), PMIC-90 (n =2) and PMIC-96 (n =1)] according to each manufacturers' instructions following EUCAST methodology. Recorded raw results were interpreted according to the EUCAST 2021 clinical breakpoints, except for tigecycline that was interpreted using pharmacokinetic/pharmacodynamic (PK/PD) breakpoints [4]. Reference results were obtained by broth microdilution (BMD) using Sensititre (Thermo Fisher Scientific, Waltham, MA, USA) customised panels (BEGN5A for GNB, B0101B for *Staphylococcus aureus* and BENRC2 for *Enterococcus faecium*) at the NRCs. Categorical agreement (CA; agreement of category results), very major errors (VME; susceptible by the evaluated routine method and resistant by the reference method), major errors (ME; resistant by evaluated routine method and susceptible by the reference method) and minor errors (mE; susceptible or resistant by the evaluated routine method versus intermediate by the reference method, or vice versa) rates comparing the results of DD/AUST and the reference BMD were calculated [5]. We set 90.0% agreement as the threshold to accept/reject strains [5].

3. Results

In total, 2117 (1817 GNB and 300 GPC) organism–drug results were obtained. The resistance rates per antibiotic tested against GNB was between 44% and 100% and against GPC was between 33% and 100%. All results of agreement and error rates are detailed in Tables 1 and 2.

Regarding GNB strains, all except *Enterobacter cloacae* NAC2-2 had CA ranging from 88.3% to 98.6%. *Enterobacter cloacae* NAC2-2 yielded the lowest CA of 73.3% with a high number of ME for amikacin and tigecycline, and of mE for aztreonam, meropenem and ciprofloxacin/levofloxacin. Considering antibiotics individually, the highest CA was observed for piperacillin/tazobactam and extended-spectrum cephalosporins (<2% error rates). Fosfomycin showed the highest unacceptable major discrepancy rates among all antibiotics for DD/AUST methods with VME of 0/21% and ME of 13/6.4%, respectively. Meropenem showed the highest mE rate at 17.1/18.3% (DD/AUST). Aztreonam and ciprofloxacin also had high mE at 8.2/13.5% (DD/AUST) and 8.6/10.4% (DD/AUST), but all observed with strain *E. cloacae* NAC2-2.

Among the MDR-GPC strains, the CA for both methods was >90%. For *S. aureus* NAC2-1, excellent agreement was observed for all antibiotics tested except one VME for linezolid using ROSCO tablet, one ME for rifampicin by VITEK®2 and eight mE discrepancies for trimethoprim/sulfamethoxazole using DD methods. For the two MDR *E. faecium* strains, one false-susceptible ampicillin result was obtained by ROSCO tablet with strain NAC2-4 and one false-resistant tigecycline result was given by VITEK®2 with strain NAC2-5 (Table 2).

4. Discussion

In 2016, a first study supported by the Belgian NAC developed a EUCAST challenge panel for AST based on the susceptibility results of a collection of strains evaluated in 20 laboratories. The pilot testing study resulted in a selection of 28 GPC and GNB strains that can be used both for AUST and DD testing. Use of that panel aimed to facilitate the implementation of new AST methods and the switch to EUCAST breakpoints in clinical laboratories [3]. While this first panel covered a wide spectrum of susceptibility profiles, several emerging resistance mechanisms were not included [6,7]. Therefore, we compiled a second challenge panel of 12 MDR strains reflecting resistance mechanisms among GNB and GPC recently documented in Belgium, such as acquired colistin resistance (including a plasmid-mediated *mcr-1*-positive isolate), OXA-48 carbapenemase-producing Enterobacterales, OXA-23 carbapenemase-producing *Acinetobacter baumannii*, and linezolid resistance (including a *cfr*-positive methicillin-resistant *S. aureus* and *optrA*-positive vancomycin-resistant *E. faecium*) to test clinical laboratory routine methods.

Our multicentric study in nine proficient clinical laboratories showed reproducible routine AST results (CA > 90% for 10/12 strains) between laboratories and methods despite expected variation in inhibition zone reading by DD and difference in AUST systems and cards used.

Our data obtained from GNB susceptibility testing showed that fosfomycin, tigecycline, meropenem and aztreonam were more prone to discrepancies. For fosfomycin, we observed numerous discrepant results that could not be considered as true errors since we did not perform agar dilution as the reference method [8,9]. Therefore, the reliability of our challenge panel against fosfomycin could not be certified based on our observations. When fosfomycin was excluded from the analysis, the overall CA increased above the 90% acceptance cut-off for all strains except NAC2-2 (Table 1). High ME observed for tigecycline by AUST could be in part explained by the minimum inhibitory concentrations (MICs) that were close to the EUCAST PK/PD breakpoints for three of these strains (NAC2-2, NAC2-6 and NAC2-7). Interestingly, all tigecycline-resistant strains were correctly identified. These data are in line with other studies showing the trends of AUST to overcall tigecycline resistance especially in species other than *Escherichia coli* and we would suggest to confirm AUST tigecycline-resistant results by BMD for MDR-GNB strains [10,11]. As several GNB strains were included for their resistance to carbapenems (Table 1), we observed discrepancies for meropenem between AST methods that were mainly mE (17.1/18.3% for DD/AUST), while VME remained low (1.4%) for both routine methodologies. The disagreements were detected mostly for strains showing low levels of meropenem resistance including NAC2-2, NAC2-3, NAC2-9 and NAC2-6, an OXA-48 carbapenemase-producing *E. coli* known to be frequently meropenem susceptible [6]. The variability of meropenem AST results in MDR-GNB strains was previously reported [12,13] and our data highly support verification of meropenem susceptibility by determination of the MIC using BMD especially when it is considered as a therapeutic option for infections by these MDR organisms. More specifically regarding the DD method, we observed a higher number of errors for ROSCO tablets compared with paper disks, mainly for meropenem (data not shown), similar to another previous study [14]. Interestingly, we did not observe any discrepancy for piperacillin/tazobactam, ceftazidime and cefepime, which have been highlighted in previous studies [4,15–17]. For temocillin, the CA (>96%) was excellent as the majority of tested strains were highly resistant. The

Table 1
Characteristics and results (rates in %) of interlaboratory testing for the challenge panel NAP2.

Disk diffusion method

Number	Species	Resistance mechanisms, susceptibility profile	CA	CA*
NAC2-2	*E. cloacae*	VIM-1 carbapenemase, CTX-M group 9	73.3	72.4
NAC2-3	*K. pneumoniae*	CTX-M group 1 tigecycline-R	94.7	94.5
NAC2-6	*E. coli*	OXA-48 carbapenemase , colistin-R MCR-1	97.3	97.2
NAC2-7	*K. pneumoniae*	KPC-3 carbapenemase colistin-R	88.3	90.7
NAC2-8	*K. pneumoniae*	NDM-1 carbapenemase, CTX-M group 1, Plasmidic cephalosporinases DHA-1 and CMY-2, 16S RNA methylase ArmA	97.3	98.6
NAC2-9	*K. pneumoniae*	IMP carbapenemase tigecycline-R	95.5	97.7
NAC2-10	*K. pneumoniae*	Plasmidic cephalosporinase DHA-1	93.7	95.2
NAC2-11	*A. baumannii*	OXA-23 carbapenemase, 16S RNA methylase ArmA	98.4	98.4
NAC2-12	*K. pneumoniae*	NDM-1+ OXA-48 carbapenemase, SHV-12 ESBL, 16S RNA methylase RmtC	98.6	99.5

Number	Temocillin					Piperacillin-tazobactam					Aztreonam					Cefuroxime					Ceftazidime				
	Ref.	CA	VME	ME	MinE	Ref.	CA	VME	ME	MinE	Ref.	CA	VME	ME	MinE	Ref.	CA	VME	ME	MinE	Ref.	CA	VME	ME	MinE
NAC2-2	R	100	0	0	0	R	100	0	0	0	I	0	0	0	100	R	100	0	0	0	R	100	0	0	0
NAC2-3	R	88	0	0	12	R	100	0	0	0	R	100	0	0	0	R	100	0	0	0	R	100	0	0	0
NAC2-6	R	100	0	0	0	R	100	0	0	0	S	100	0	0	0	I	100	0	0	0	S	100	0	0	0
NAC2-7	R	88	0	0	12	R	100	0	0	0	R	100	0	0	0	R	100	0	0	0	R	100	0	0	0
NAC2-8	R	100	0	0	0	R	100	0	0	0	R	100	0	0	0	R	100	0	0	0	R	100	0	0	0
NAC2-9	R	100	0	0	0	R	100	0	0	0	S	100	0	0	0	R	100	0	0	0	R	100	0	0	0
NAC2-10	I	100	0	0	0	R	100	0	0	0	R	60	20	0	20	R	100	0	0	0	R	86	14	0	0
NAC2-11	R	NR	NR	NR	NR	R	NR	NR	NR	NR	R	NR	NR	NR	NR	R	NR	NR	NR	NR	R	NR	NR	NR	NR
NAC2-12	R	100	0	0	0	R	100	0	0	0	R	100	0	0	0	R	100	0	0	0	R	100	0	0	0

Number	Cefotaxime/ Ceftriaxone					Cefepime					Ertapenem					Meropenem				
	Ref.	CA	VME	ME	MinE	Ref.	CA	VME	ME	MinE	Ref.	CA	VME	ME	MinE	Ref.	CA	VME	ME	MinE
NAC2-2	R	88	12	0	0	R	100	0	0	0	R	100	0	0	0	I	38	0	0	62
NAC2-3	R	100	0	0	0	R	100	0	0	0	R	100	0	0	0	R	75	12	0	13
NAC2-6	S	100	0	0	0	S	100	0	0	0	R	100	0	0	0	S	50	0	0	50
NAC2-7	R	100	0	0	0	R	100	0	0	0	R	100	0	0	0	R	100	0	0	0
NAC2-8	R	100	0	0	0	R	100	0	0	0	R	100	0	0	0	R	100	0	0	0
NAC2-9	R	100	0	0	0	R	100	0	0	0	R	100	0	0	0	R	75	0	0	25
NAC2-10	R	100	0	0	0	S	100	0	0	0	R	60	40	0	0	S	100	0	0	0
NAC2-11	R	NR	NR	NR	NR	R	NR	NR	NR	NR	R	NR	NR	NR	NR	R	100	0	0	0
NAC2-12	R	100	0	0	0	R	100	0	0	0	R	100	0	0	0	R	100	0	0	0

Number	Amikacin					Gentamicin					Ciprofloxacin/ Levofloxacin					Fosfomycin				
	Ref.	CA	VME	ME	MinE	Ref.	CA	VME	ME	MinE	Ref.	CA	VME	ME	MinE	Ref.	CA	VME	ME	MinE
NAC2-2	S	25	0	75	0	R	83	17	0	0	I	25	0	0	75	R	100	0	0	0
NAC2-3	S	100	0	0	0	S	100	0	0	0	R	100	0	0	0	R	100	0	0	0
NAC2-6	S	100	0	0	0	R	100	0	0	0	R	100	0	0	0	S	100	0	0	0
NAC2-7	R	100	0	0	0	S	67	0	33	0	R	100	0	0	0	S	0	0	100	0
NAC2-8	R	100	0	0	0	R	100	0	0	0	R	100	0	0	0	R	100	0	0	0
NAC2-9	R	100	0	0	0	R	100	0	0	0	R	100	0	0	0	R	100	0	0	0
NAC2-10	S	100	0	0	0	S	100	0	0	0	R	86	14	0	0	R	100	0	0	0
NAC2-11	R	100	0	0	0	R	100	0	0	0	R	100	0	0	0	R	/	/	/	/
NAC2-12	R	100	0	0	0	R	100	0	0	0	R	100	0	0	0	R	100	0	0	0

Automated antibiotic susceptibility testing method

Number	Species	Resistance mechanisms, susceptibility profile
NAC2-2	*E. cloacae*	VIM-1 carbapenemase, CTX-M group 9
NAC2-3	*K. pneumoniae*	CTX-M group 1 tigecycline-R
NAC2-6	*E. coli*	OXA-48 carbapenemase , colistin-R MCR-1
NAC2-7	*K. pneumoniae*	KPC-3 carbapenemase colistin-R
NAC2-8	*K. pneumoniae*	NDM-1 carbapenemase, CTX-M group 1, Plasmidic cephalosporinases DHA-1 and CMY-2, 16S RNA methylase ArmA
NAC2-9	*K. pneumoniae*	IMP carbapenemase tigecycline-R
NAC2-10	*K. pneumoniae*	Plasmidic cephalosporinase DHA-1
NAC2-11	*A. baumannii*	OXA-23 carbapenemase, 16S RNA methylase ArmA
NAC2-12	*K. pneumoniae*	NDM-1+ OXA-48 carbapenemase, SHV-12 ESBL, 16S RNA methylase RmtC

Number	Temocillin					Piperacillin-tazobactam					Aztreonam					Cefuroxime					Ceftazidime				
	Ref.	CA	VME	ME	MinE	Ref.	CA	VME	ME	MinE	Ref.	CA	VME	ME	MinE	Ref.	CA	VME	ME	MinE	Ref.	CA	VME	ME	MinE
NAC2-2	R	100	0	0	0	R	100	0	0	0	I	38	0	0	62	R	100	0	0	0	R	100	0	0	0
NAC2-3	R	100	0	0	0	R	100	0	0	0	R	100	0	0	0	R	100	0	0	0	R	100	0	0	0
NAC2-6	R	100	0	0	0	R	100	0	0	0	S	100	0	0	0	I	100	0	0	0	S	100	0	0	0
NAC2-7	R	75	0	0	25	R	100	0	0	0	R	100	0	0	0	R	100	0	0	0	R	100	0	0	0
NAC2-8	R	100	0	0	0	R	100	0	0	0	R	100	0	0	0	R	100	0	0	0	R	100	0	0	0
NAC2-9	R	100	0	0	0	R	100	0	0	0	S	88	0	12	0	R	100	0	0	0	R	100	0	0	0
NAC2-10	I	86	0	0	14	R	100	0	0	0	R	100	0	0	0	R	100	0	0	0	R	100	0	0	0
NAC2-11	R	NR	NR	NR	NR	R	NR	NR	NR	NR	R	NR	NR	NR	NR	R	NR	NR	NR	NR	R	NR	NR	NR	NR
NAC2-12	R	100	0	0	0	R	100	0	0	0	R	100	0	0	0	R	100	0	0	0	R	100	0	0	0

Number	Cefotaxime/ Ceftriaxone					Cefepime					Ertapenem					Meropenem					Amikacin				
	Ref.	CA	VME	ME	MinE	Ref.	CA	VME	ME	MinE	Ref.	CA	VME	ME	MinE	Ref.	CA	VME	ME	MinE	Ref.	CA	VME	ME	MinE
NAC2-2	R	100	0	0	0	R	100	0	0	0	R	100	0	0	0	I	25	0	0	75	S	38	0	62	0
NAC2-3	R	100	0	0	0	R	100	0	0	0	R	100	0	0	0	R	13	0	0	87	S	100	0	0	0
NAC2-6	S	86	0	14	0	S	100	0	0	0	R	100	0	0	0	S	100	0	0	0	S	100	0	0	0
NAC2-7	R	100	0	0	0	R	100	0	0	0	R	100	0	0	0	R	100	0	0	0	R	100	0	0	0
NAC2-8	R	100	0	0	0	R	100	0	0	0	R	100	0	0	0	R	88	12	0	0	R	88	12	0	0
NAC2-9	R	100	0	0	0	R	100	0	0	0	R	100	0	0	0	R	100	0	0	0	R	100	0	0	0
NAC2-10	R	100	0	0	0	S	100	0	0	0	R	83	17	0	0	S	100	0	0	0	S	100	0	0	0
NAC2-11	R	NR	NR	NR	NR	R	NR	NR	NR	NR	R	NR	NR	NR	NR	R	100	0	0	0	R	100	0	0	0
NAC2-12	R	100	0	0	0	R	100	0	0	0	R	100	0	0	0	R	100	0	0	0	R	100	0	0	0

Number	Gentamicin					Ciprofloxacin/ Levofloxacin					Fosfomycin					Tigecycline					Colistin				
	Ref.	CA	VME	ME	MinE	Ref.	CA	VME	ME	MinE	Ref.	CA	VME	ME	MinE	Ref.	CA	VME	ME	MinE	Ref.	CA	VME	ME	MinE
NAC2-2	R	100	0	0	0	I	0	0	0	100	R	100	0	0	0	S	38	0	62	0	S	100	0	0	0
NAC2-3	S	100	0	0	0	R	100	0	0	0	R	100	0	0	0	R	100	0	0	0	S	100	0	0	0
NAC2-6	R	100	0	0	0	R	100	0	0	0	S	100	0	0	0	S	88	0	12	0	R	100	0	0	0
NAC2-7	S	0	0	100	0	R	100	0	0	0	S	50	0	50	0	S	25	0	75	0	R	100	0	0	0
NAC2-8	R	100	0	0	0	R	100	0	0	0	R	50	50	0	0	R	100	0	0	0	S	100	0	0	0
NAC2-9	R	100	0	0	0	R	100	0	0	0	R	17	83	0	0	R	100	0	0	0	S	71	0	29	0
NAC2-10	S	100	0	0	0	R	100	0	0	0	R	100	0	0	0	S	86	0	14	0	S	100	0	0	0
NAC2-11	R	100	0	0	0	R	100	0	0	0	R	NR	NR	NR	NR	R	NR	NR	NR	NR	S	88	0	12	0
NAC2-12	R	100	0	0	0	R	100	0	0	0	R	67	33	0	0	R	100	0	0	0	R	100	0	0	0

Disk diffusion method

Number	Species	Resistance mechanisms, susceptibility profile	CA
NAC2-1	*S. aureus*	mecA, CFR	91.9
NAC2-4	*E. faecium*	VanA, OptrA	98.6
NAC2-5	*E. faecium*	VanB	96.9

Number	Oxacillin					Ampicillin					Cefoxitin					Teicoplanin					Vancomycin					Linezolid				
	Ref.	CA	VME	ME	MinE	Ref.	CA	VME	ME	MinE	Ref.	CA	VME	ME	MinE	Ref.	CA	VME	ME	MinE	Ref.	CA	VME	ME	MinE	Ref.	CA	VME	ME	MinE
NAC2-1	R	100	0	0	0	NR	NR	NR	NR	NR	R	100	0	0	0	S	NR	NR	NR	NR	S	NR	NR	NR	NR	R	83	17	0	0
NAC2-4	NR	NR	NR	NR	NR	R	86	14	0	0	NR	NR	NR	NR	NR	R	100	0	0	0	R	100	0	0	0	R	100	0	0	0
NAC2-5	NR	NR	NR	NR	NR	R	86	14	0	0	NR	NR	NR	NR	NR	S	100	0	0	0	R	100	0	0	0	S	100	0	0	0

Number	Erythromycin					Clindamycin					Tigecycline					Gentamicin					Gentamicin high dose					Cirpofloxacin/ Levofloxacin				
	Ref.	CA	VME	ME	MinE	Ref.	CA	VME	ME	MinE	Ref.	CA	VME	ME	MinE	Ref.	CA	VME	ME	MinE	Ref.	CA	VME	ME	MinE	Ref.	CA	VME	ME	MinE
NAC2-1	S	100	0	0	0	R	88	12	0	0	S	100	0	0	0	R	100	0	0	0	NR	NR	NR	NR	NR	I	100	0	0	0
NAC2-4	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	S	100	0	0	0	R	100	0	0	0	R	100	0	0	0	NR	NR	NR	NR	NR
NAC2-5	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	S	100	0	0	0	R	100	0	0	0	R	100	0	0	0	NR	NR	NR	NR	NR

Number	Minocycline					Tetracycline					Trimethoprim-sulfamethoxazole					Fusidic acid					Rifampicin				
	Ref.	CA	VME	ME	MinE	Ref.	CA	VME	ME	MinE	Ref.	CA	VME	ME	MinE	Ref.	CA	VME	ME	MinE	Ref.	CA	VME	ME	MinE
NAC2-1	R	100	0	0	0	R	100	0	0	0	I	0	0	0	100	S	83	0	17	0	S	100	0	0	0
NAC2-4	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR
NAC2-5	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR

Automated antibiotic susceptibility testing method

Number	Species	Resistance mechanisms, susceptibility profile	CA
NAC2-1	*S. aureus*	mecA, CFR	91.9
NAC2-4	*E. faecium*	VanA, OptrA	98.6
NAC2-5	*E. faecium*	VanB	96.9

Number	Oxacillin					Ampicillin					Cefoxitin					Teicoplanin					Vancomycin					Linezolid				
	Ref.	CA	VME	ME	MinE	Ref.	CA	VME	ME	MinE	Ref.	CA	VME	ME	MinE	Ref.	CA	VME	ME	MinE	Ref.	CA	VME	ME	MinE	Ref.	CA	VME	ME	MinE
NAC2-1	R	100	0	0	0	NR	NR	NR	NR	NR	R	100	0	0	0	S	100	0	0	0	S	100	0	0	0	R	86	14	0	0
NAC2-4	NR	NR	NR	NR	NR	R	100	0	0	0	NR	NR	NR	NR	NR	R	100	0	0	0	R	100	0	0	0	R	100	0	0	0
NAC2-5	NR	NR	NR	NR	NR	R	100	0	0	0	NR	NR	NR	NR	NR	S	100	0	0	0	R	100	0	0	0	S	100	0	0	0

Number	Erythromycin					Clindamycin					Tigecycline					Gentamicin					Gentamicin high dose					Cirpofloxacin/ Levofloxacin				
	Ref.	CA	VME	ME	MinE	Ref.	CA	VME	ME	MinE	Ref.	CA	VME	ME	MinE	Ref.	CA	VME	ME	MinE	Ref.	CA	VME	ME	MinE	Ref.	CA	VME	ME	MinE
NAC2-1	S	100	0	0	0	R	100	0	0	0	S	100	0	0	0	R	NR	NR	NR	NR	NR	NR	NR	NR	NR	I	100	0	0	0
NAC2-4	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	S	100	0	0	0	R	NR	NR	NR	NR	R	NR	NR	NR	NR	NR	NR	NR	NR	NR
NAC2-5	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	S	100	0	0	0	R	NR	NR	NR	NR	R	NR	NR	NR	NR	NR	NR	NR	NR	NR

Number	Minocycline					Tetracycline					Trimethoprim-sulfamethoxazole					Fusidic acid					Rifampicin				
	Ref.	CA	VME	ME	MinE	Ref.	CA	VME	ME	MinE	Ref.	CA	VME	ME	MinE	Ref.	CA	VME	ME	MinE	Ref.	CA	VME	ME	MinE
NAC2-1	R	100	0	0	0	R	100	0	0	0	I	86	0	0	14	S	100	0	0	0	S	86	0	14	0
NAC2-4	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR
NAC2-5	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR

categorical agreement,CA; CA*, categorical agreement excluding fosfomycin; Broth microdilution, BMD; Disk diffusion,DD; extended-spectrum b-lactamase, ESBL; susceptible increased exposure, I; not realized,NR; percentage very major error, VME; percentage major error, ME; percentage minor error, minE; reference result by broth microdilution, Ref; resistant, R; sensitive, S.

Abbreviations: categorical agreement,CA; CA*, categorical agreement excluding fosfomycin; Broth microdilution, BMD; Disk diffusion,DD; extended-spectrum b-lactamase, ESBL; susceptible increased exposure, I; not realized,NR; percentage very major error, VME; percentage major error, ME; percentage minor error, minE; reference result by broth microdilution, Ref; resistant, R; sensitive, S.

Table 2
Categorical susceptibility rates and discrepancies per antibiotic for the challenge panel NACP2

Group of strains	Antibiotic	BMD results				DD method					AUST method					Overall CA (%)
		n	%S	%I	%R	*n*	VME (%)	ME (%)	mE (%)	CA (%)	*n*	VME (%)	ME (%)	mE (%)	CA (%)	
Gram-negative bacilli	Temocillin	9	0	11	89	63	0	0	3.2	97.8	63	0	0	4.8	95.2	96.0
	Piperacillin-tazobactam	9	0	0	100	62	0	0	0	100	63	0	0	0	100	100
	Aztreonam	9	22	11	67	52	1.9	0	13.5	84.6	61	0	1.6	8.2	90.2	87.7
	Ceftazidime	9	11	0	89	61	1.6	0	0	98.4	62	0	0	0	100	99.2
	Cefotaxime/ceftriaxone	9	11	0	89	62	1.6	0	0	98.4	56	0	1.8	0	98.2	98.3
	Cefepime	9	22	0	78	55	0	0	0	100	63	0	0	0	100	100
	Ertapenem	9	0	0	100	40	5	0	0	95	46	2.2	0	0	97.8	96.5
	Meropenem	9	22	11	67	70	1.4	0	17.1	81.5	70	1.4	0	18.3	80.3	80.9
	Ciprofloxacin/levofloxacin	9	0	11	89	69	1.4	0	8.6	90	71	0	0	10.4	89.6	89.8
	Amikacin	9	44	11	56	70	0	8.6	0	91.4	70	1.4	7.2	0	91.4	91.4
	Gentamicin	9	33	0	67	54	1.8	3.7	0	94.5	69	0	11.1	0	89.9	91.3
	Tigecycline	9	44	0	56	NR	NR	NR	NR	NR	62	0	20.7	0	79.3	79.3
	Fosfomycin	9	22	0	78	23	0	13	0	87	48	21.3	6.4	0	72.3	77.1
	Colistin	9	56	0	44	NR	NR	NR	NR	NR	65	0	4.6	0	95.7	95.4
Gram-positive cocci	Oxacillin	1	100	0	0	3	0	0	0	100	7	0	0	0	100	100
	Ampicillin	2	0	0	100	14	14.3	0	0	85.7	10	0	0	0	100	91.6
	Cefoxitin	1	0	0	100	8	0	0	0	100	4	0	0	0	100	100
	Teicoplanin	3	66	0	33	8	0	0	0	100	19	0	0	0	100	100
	Vancomycin	3	33	0	66	12	0	0	0	100	24	0	4.2	0	95.8	97.2
	Linezolid	3	33	0	66	17	5.6	0	0	94.4	18	5.3	0	0	94.7	97.3
	Erythromycin	1	100	0	0	7	0	0	0	100	7	0	0	0	100	100
	Clindamycin	1	0	0	100	8	12.5	0	0	87.5	7	0	0	0	100	93.3
	Tetracycline	1	0	0	100	5	0	0	0	100	6	0	0	0	100	100
	Minocycline	1	100	0	0	5	0	0	0	100	4	0	0	0	100	100
	Tigecycline	3	100	0	0	10	0	0	0	100	9	0	0	0	100	100
	Gentamicin	3	0	0	100	9	0	0	0	100	0	0	0	0	100	100
	Gentamicin high-dose	3	0	0	100	8	0	0	0	100	10	0	0	0	100	100
	Ciprofloxacin/levofloxacin	1	0	100	0	7	0	0	0	100	4	0	0	0	100	100
	Trimethoprim-sulfamethoxazole	1	0	100	0	8	0	0	100	0	7	0	0	14.3	85.7	40.0
	Rifampicin	1	100	0	0	5	0	0	0	100	7	0	14.3	0	85.7	91.7
	Fusidic acid	1	100	0	0	8	0	14.3	0	85.7	7	0	0	0	100	92.4

AUST, automated antibiotic susceptibility testing; CA, categorical agreement; BMD, broth microdilution; DD, disk diffusion; I, susceptible, increased exposure; NR, not realised; VME, percentage very major error; ME, percentage major error; mE, percentage minor error; BMD, broth microdilution; R, resistant; S, susceptible.

two colistin-resistant strains (NAC2-6 and NAC2-7) were correctly interpreted by all methods used. Strain NAC2-2 did not attain the acceptance criterion (CA < 90%) and was not retained in the final panel. This strain yielded a high number of discrepancies for amikacin, tigecycline, meropenem, ciprofloxacin and aztreonam (Table 1) potentially explained by the reference results within the 'susceptible, increased exposure' category for most of these antibiotics.

Our evaluation on GPC AST shows excellent CA between laboratory/methods (≥90%) for most antibiotics. False susceptibility (VME) to ampicillin of the two *E. faecium* strains was observed only in one laboratory using ROSCO tablets for DD. Hence, we suggest confirming ampicillin-susceptible results in *E. faecium* strains by alternative methods [18]. For glycopeptides, no error was detected, with both vancomycin-resistant *E. faecium* correctly detected. While the *optrA*-positive linezolid-resistant *E. faecium* NAC2-4 was correctly identified by all methods, linezolid resistance in *cfr*-positive *S. aureus* NAC2-1 was missed by one laboratory using ROSCO tablets and another using BD Phoenix. We recommend using other methods (Etest or BMD) to confirm linezolid resistance [19,20]. For tigecycline, all three GPC strains were correctly categorised as susceptible.

Our study has limitations. First, the number of challenge strains selected to be complementary to the previous 2016 panel was limited. However, the new panel focused on MDR strains covering a large spectrum of emerging or prevalent resistance mechanisms that could be more challenging for the routine AST methods used by clinical laboratories. Also, a reproducibility study should be carried out to challenge intralaboratory conditions. Finally, the observations of our study performed in nine clinical laboratories in Belgium should be confirmed in a larger number of laboratories with other settings.

5. Conclusions

Using a panel of MDR strains with a wide spectrum of emerging resistance determinants, our multicentre study showed that routine DD and AUST methods are overall reliable for AST and resistance detection. However, the exact determination of resistance mechanisms still requires phenotypic and/or genotypic confirmatory tests. Discrepancies and variability of results for a few antimicrobials (especially meropenem, aztreonam, tigecycline and linezolid) raised concerns, thus we highly recommend confirmation by the BMD method. In addition, the accuracy of AST for antibiotics that can be used as rescue therapy for the treatment of MDR strains such as tigecycline, fosfomycin and colistin need to be improved, Finally, based on the global agreement between methods/laboratories (≥90%) with exclusion of fosfomycin, all strains but one (*E. cloacae* NAC2-2) could be used as reference resistant strains in a national panel for validation of routine AST methods.

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Competing interests

None declared.

Ethical approval

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