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Development of a national EUCAST challenge panel for antimicrobial susceptibility testing

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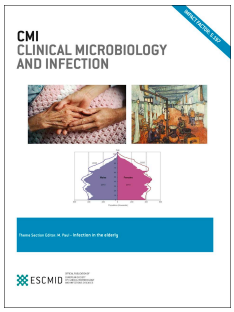

Development of a national EUCAST challenge panel for antimicrobial susceptibility testing

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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 Centers (n=57) and from routine clinical samples (n=60) was selected based on resistance pattern. These strains were analyzed in 7 different laboratories by 3 different automated AST systems (Vitek (n=2), Phoenix (n=2) and Microscan (n=2)) and by disk diffusion from 5 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 (CA) between the different systems and the number of very major errors (VME), major errors (ME) and minor errors (MI). VMEs or MEs for at least 2 antibiotics were observed in 43% of all strains, leading to the exclusion of these strains to be selected in the panel. In only 10% of all tested strains there was a 100% CA 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 6 strains withheld for the pilot-study could not be used as challenge strain due to an overall (very) major error rate of more than 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.

Keywords: challenge panel, antimicrobial susceptibility testing, EUCAST, MIC breakpoints, zone diameter breakpoints, antibiotic, automated susceptibility testing.

Introduction

The use of common clinical breakpoints for antimicrobial susceptibility testing (AST) is important both for 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 MIC distributions for relevant species of organisms, knowledge about resistance mechanisms, antimicrobial dosing and pharmacokinetic and pharmacodynamic aspects [1-4]. A shift from

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national and Clinical and Laboratory Standards Institute (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 (NACs). Therefore, the Belgian NAC decided in 2012 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 as 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 harbor a stable resistance mechanism and should show reproducible results with different AST systems, both automated AST and disk 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 10 strains prospectively from clinical samples in 2013. Five Belgian Reference Centers 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., 9 beta-hemolytic streptococci, 8 *Enterococcus* spp., 6 *Streptococcus pneumoniae* and 2 viridans group streptococci was included in the study (Table 1). Strains were subcultured and distributed among the 7 validation laboratories.

Validation study

Antimicrobial susceptibility testing and categorisation of strains

Six validation laboratories determined antimicrobial susceptibility of the 117 strains with automated AST systems according to manufacturer's instructions: MicroScan WalkAway (Siemens Healthcare Diagnostics, West Sacramento, CA) (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 3 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) disks.

Antibiotics with at least 4 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 micro-organisms to be included in the challenge panel was set up [7-9] (Supplementary material). 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 4 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 group 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 candidate 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 (very) major error 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.

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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 disks (n=7) and Rosco Neo-Sensitab disks (n=3) according to EUCAST. Raw results of MICs and zone diameters were collected in one center for interpretation according to EUCAST 2015 breakpoints.

Defining the susceptibility categorisation

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 equal or less than 5%. In case of higher error rate, no susceptibility category was set. When a strain had a MI rate of more than 10% for an antibiotic, both interpretation categories were accepted (S/I or R/I). When the MI rate was less than 10%, the interpretation category of the majority of the test systems was chosen.

Results

Validation study

Antimicrobial susceptibility testing and categorisation of strains

For 10 percent 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 percent 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 the supplementary material. Concerning the Enterobacteriaceae, (V)MEs occurred for all antibiotics, ranging from 2 strains with (V)MEs for colistin to 15 strains with (V)MEs for amoxicillin-clavulanate. Other antibiotics with (V)MEs in more than 10 strains were piperacillin-tazobactam, ceftazidime, trimethoprim-sulfamethoxazole and cefotaxime. For

the non-fermenters, amikacin was the antibiotic with the highest number of strains with (V)MEs (6 strains), followed by gentamicin (4 strains) and trimethoprim-sulfamethoxazole, cefepime and piperacillin-tazobactam (each 2 strains). Concerning the Gram-positives, clindamycin had the highest number of (V)MEs.

Selection of strains for the challenge panel

Twenty-eight strains, 14 Gram-positives and 14 Gram-negatives, were selected based on the group categorisation and the list of resistance profiles to be included in the panel. Some strains were used for different resistance profiles. On the other hand, for 6 profiles no strain could be withheld: multidrug-resistant *Acinetobacter* spp., extended-spectrum cephalosporin-resistant *Citrobacter freundii* and *Serratia marcescens*, high-level aminoglycoside-resistant Enterococcus, penicillin-resistant and -intermediate Streptococcus viridans.

Pilot-testing of the challenge panel and defining susceptibility categorisation

For the Gram-negatives, a mean of 21 measurements (range: 11-26) per antibiotic per strain were retrieved in the pilot-testing. The categorisation results based on the pilot-testing (PC) are indicated in table 3. To define the DC, a mean of 34 AST measurements (range: 16-41) was included per antibiotic per strain. For 11 of the 143 defined categories, a discordance was seen between the validation study categorization (VC) and the DC. Compared to the validation study, 4 strains could not be used as challenge strains each for a particular antibiotic (n=3) or two antibiotics (n=1) due to an overall (V)ME rate of more than 5%. Two AmpC-producing strains could not be used for cefepime testing. Moreover one of this AmpC-producing strains, one *P. aeruginosa* and one *Klebsiella pneumoniae* were not suitable for respectively ciprofloxacin, cefepime and tobramycin testing. The other 6 discordant results were only minor discrepancies.

For the Gram-positives, pilot-testing and definite categorisation included respectively a mean of 14 (range: 7-24) and 22 (range: 7-35) AST measurements per antibiotic per strain. Only for 2 of the 143 defined categories, a discordance was seen between VC and DC (Table 4). One *S. aureus* strain and one *S. pneumoniae* strain could not be used for respectively tetracycline and trimethoprim-sulfamethoxazole testing. Two other strains, one *S. pneumoniae* and one *S. agalactiae* also had a (V)ME rate above 5% for levofloxacin. However these strains could still be used for levofloxacin testing as they had only one discordant measurement on a total of 15 and 16 AST measurements respectively. Moreover moxifloxacin showed a 100% CA for these strains.

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157 More antibiotics were tested in the pilot-study than in the validation study. Concerning the staphylococci,
158 levofloxacin, rifampicin and tigecycline were only tested in the pilot-study. For these antibiotics the DC was
159 exceptionally based on the pilot-testing results only. Likewise, for the streptococci of group A, B, C and G
160 tetracycline and trimethoprim-sulfamethoxazole were added. Oxacillin DD in pneumococci and gentamicin
161 high-dose testing in enterococci were not included as they were not tested in the pilot-study and are only
162 screening tools for resistance.

163 Discussion

164 In this study a challenge panel was developed covering epidemiologically relevant resistance profiles based on
165 the susceptibility results of a starting panel of 117 strains. A pilot-study performed in 20 laboratories mainly
166 confirmed the categorisation results encountered in the validation study. Moreover, the pilot-testing took
167 place 2 years after the validation and confirmed the stability of the strains' resistance mechanisms.

168 Since the introduction of EUCAST breakpoints, more and more European laboratories are changing from
169 national or CLSI guidelines to EUCAST guidelines for AST. In the era of accreditation, it is important for clinical
170 laboratories to properly validate these modifications and to ensure good performance of their AST systems.
171 Due to the lack of well described criteria to select a challenge panel, criteria were set up based on CA and the
172 number of (V)MEs and MIs. Essential agreement was not a criterion as this is only applicable for systems
173 measuring MICs. Finding a good balance between showing the same categorical result with the different AST
174 systems and being challenging enough for the different systems proved to be difficult. One of the reasons was
175 the decision to include both broth dilution and DD methods and preferentially all tested antibiotics. The latter
176 criterion was defined considering that many laboratories use automated AST systems which test all relevant
177 antibiotics in one panel at the same time. We are aware that this selection criterion, only including strains
178 useful for testing a majority of the antibiotics, compromised the number of suitable strains.

179 The low mean CA in our validation study can be explained by the use of different AST systems, different AST
180 cards/panels and testing in 7 different laboratories. Although the aim of the study was not to compare
181 performance of different AST methods, some interesting observations were made. Our results indicate that
182 amoxicillin-clavulanate, piperacillin-tazobactam, amikacin, trimethoprim-sulfamethoxazole and clindamycin are
183 most prone for discrepancies. The different concentrations of clavulanate in the different AST systems could

not explain the discrepancies for amoxicillin-clavulanate, as only results of methods with a fixed concentration of clavulanate were included. However, half of the (V)MEs occurred with DD methods which may be caused by instability of the disks, lack of standardization of the disk contents or reading problems. The method-dependent variation in results for piperacillin-tazobactam susceptibility testing is another known problem (53rd Interscience Conference of Antimicrobial Agents and Chemotherapy, Poster D-596). Apart from method-dependent differences, differences within the same method have also been described (23rd European Congress of Clinical Microbiology and Infectious Diseases; abstract R2780). Moreover the concentration of tazobactam in the Vitek cards was 8 mg/l instead of 4 mg/l for the highest piperacillin concentration. Concerning clindamycin and trimethoprim-sulfamethoxazole, higher resistance rates were measured with DD compared with broth dilution methods.

The pilot-study showed a good CA between the different methods for the majority of the tested antibiotics, including amoxicillin-clavulanate and piperacillin-tazobactam. On the other hand, the CA for cefepime in Gram-negatives was low, resulting in the exclusion of 2 AmpC-producing Enterobacteriaceae and 1 *P. aeruginosa* from the challenge panel. This high error rate is previously described for Vitek and Microscan systems in ESBL-producing *E. coli* and *K. pneumoniae* compared to reference methods. [10, 11]

This study has some limitations. Not all proposed relevant resistance mechanisms could be covered by the panel due to a lack of strains with reproducible results over the different susceptibility testing systems. Moreover three antibiotics were not tested by disk-diffusion in the validation study, which was resolved by the inclusion of the data from the pilot-study. Thirdly, due to the different composition of the used cards/panels of the automated AST systems, not all antibiotics were equally tested and validated. Finally, we have not used EUCAST broth dilution method as reference method. In contrast, our reference categorisation was the result of the majority of the results of the different automated AST and DD methods, which may have biased the results. On the other hand our own reference reflects the results of the methods that are routinely used in Belgian clinical laboratories. Moreover a comparison of the reference results with the results of the Reference Centers showed no (V)MEs.

To our knowledge, this is the first description of the development of a national challenge panel which will serve as validation panel for new AST systems, for the implementation of EUCAST breakpoints or for benchmarking between labs. Moreover these strains might serve as internal quality control strains, covering more MIC ranges

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and more resistant phenotypes than the proposed ATCC strains. Other NACs could use this strategy to select additional challenge strains harboring the local circulating resistance mechanisms.

Conclusion

A EUCAST challenge panel for AST was developed based on the susceptibility results of a panel of 117 strains. Pilot-testing in 20 laboratories confirmed that the strains can both be used for automated AST testing and for DD testing. Moreover this panel covers a wide spectrum of resistance mechanisms, particularly of interest for validation studies and to cover the lack in quality control materials provided by other institutions. The use of this panel should facilitate the implementation of new AST methods, the switch to EUCAST breakpoints in clinical laboratories and it may be used for benchmarking between laboratories.

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Conflicts of interest

None to declare

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Tables: see uploaded files

Table 1 Overview of the 117 strains included in the study to select a EUCAST challenge panel.

Table 2 Categorisation of the 117 strains in four groups based on the categorical agreement (CA) and the number of (very) major errors ((V)ME) in the validation study.

Table 3 Results of the validation-testing and pilot-testing of the Gram-negatives of the challenge panel. Validation-testing categorisation (VC), pilot-testing categorisation (PC) and categorisation based on all measurements (DC) are indicated per strain per antibiotic: sensitive (S), resistant (R) and intermediair (I). Categorical agreement (CAt), percentage (very) major errors ((V)ME) and percentage minor errors (ME) were calculated for all measurements together (VC+PC). (in bold: discordant results between validation-testing categorisation and definite categorisation; ESBL: extended-spectrum beta-lactamase; WT: wild type; '-': no categorisation; **: intrinsic resistance; §: strain not useful for one antibiotic after pilot-testing; #: strain not useful for two antibiotics after pilot-testing).

Table 4 Results of the validation-testing and pilot-testing of the Gram-positives of the challenge panel. Validation-testing categorisation (VC), pilot-testing categorisation (PC) and categorisation based on all measurements (DC) are indicated per strain per antibiotic: sensitive (S), resistant (R) and intermediair (I). Categorical agreement (CAt), percentage (very) major errors ((V)ME) and percentage minor errors (ME) were calculated for all measurements together (VC+PC). (in bold: discordant results between validation-testing categorisation and definite categorisation; '-': no categorisation result; NT: not-tested; MRSA: methicillin-resistant *Staphylococcus aureus*; WT: wild type; ** intrinsic resistance; §: strain not useful for one antibiotic after pilot-testing).

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EUCAST interpretation group	species	n	resistance mechanism/profile	n
Fermentative Gram-negative bacilli (n=61)	*Aeromonas hydrophila*	1	extended-spectrum beta-lactamase-producing	8
	Citrobacter braakii	1	AmpC-producing	3
	Citrobacter freundii	2	carbapenemase-producing	8
	Citrobacter koseri	2	OXA-48	4
	Enterobacter aerogenes	5	VIM	2
	Enterobacter cloacae complex	6	KPC	1
	Escherichia coli	13	NDM	1
	Klebsiella oxytoca	3	AmpC-producing with carbapenem porine deficiency	1
	Klebsiella pneumoniae	9	wild type	3
	Morganella morganii	6	colistin-resistant (non-intrinsic)	1
	Proteus mirabilis	6	combined or not genetically determined resistance	37
	Proteus penneri	1		
	Proteus vulgaris	1		
	Providencia rettgeri	1		
	Serratia marcescens	4		
***Acinetobacter* spp.** (n=2)	*Acinetobacter baumannii*	1	AmpC-producing	1
	Acinetobacter haemolyticus	1	carbapenemase-producing: OXA-58	1
***Pseudomonas* spp.** (n=9)	*Pseudomonas aeruginosa*	9	wild type	2
			carbapenemase-producing: VIM-2	2
			carbapenem-impermeability	2
			AmpC-producing	1
			combined or not genetically determined resistance	2
***Enterococcus* spp.** (n=8)	*Enterococcus faecium*	4	VanA	2
	Enterococcus faecalis	4	VanB	2
			other	4
***Staphylococcus* spp.** (n=20)	Coagulase-negative *Staphylococcus*	7	methicillin-resistant *Staphylococcus aureus*	4
	Staphylococcus saprophyticus	1	wild type	2
	Staphylococcus aureus	11	other	14
	Staphylococcus lugdunensis	1		
Streptococcus groups A, B, C and G (n=9)	*Streptococcus agalactiae*	5	macrolide-resistant (4 MLSB, 2 macrolide-efflux pump, 1 phenotype L)	7
	Streptococcus dysgalactiae	1	fluoroquinole-resistant	1
	Streptococcus pyogenes	3	wild type	1
Streptococcus pneumoniae (n=6)			only fluoroquinole-resistant	1
			macrolide-resistant	1
			different combined resistance	4
Viridans group streptococci (n=2)	*Streptococcus anginosus* groep	1	wild type	2
	Streptococcus mitis groep	1		

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Eucast interpretation group	number of strains per categorisation group				TOTAL
	group 1 100% CA	group 2 no (V)ME	group 3 1 (V)ME	group 4 >1 (V)ME	
Enterobacteriaceae	4	9	16	32	61
Acinetobacter spp.	0	0	0	2	2
Pseudomonas spp.	0	1	4	4	9
Enterococcus spp.	1	3	3	1	8
Staphylococcus spp.	2	3	7	8	20
Streptococcus pneumoniae	1	2	2	1	6
Streptococcus groups A, B, C and G	3	3	2	1	9
Viridans group streptococci	0	0	1	1	2
TOTAL	12 (10%)	20 (17%)	35 (30%)	50 (43%)	117

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			Amikacin						Amoxicillin/ Ampicillin				Amoxicillin-clavulanate				Cefepime						Cefotaxime/ Ceftriaxone						Ceftazidime						Cefuroxime						Ciprofloxacin					
Number	Species	Resistance mechanism/susceptibility profile	VC	PC	DC	%CAt	%MI	% (V)ME	VC	PC	DC	%CAt	VC	PC	DC	%CAt	% (V)ME	VC	PC	DC	%CAt	%MI	% (V)ME	VC	PC	DC	%CAt	% (V)ME	VC	PC	DC	%CAt	%MI	% (V)ME	VC	PC	DC	%CAt	% (V)ME	VC	PC	DC	%CAt	%MI	% (V)ME
NAC11 §	*Enterobacter aerogenes*	AmpC + carbapenem porine deficiency	S	S	S	100	0	0	R	R	R	100	R	R	R	100	0	I/S	I	-	68	18	15	R	R	R	100	0	R	R	R	100	0	R	R	R	100	0	S	S	S	97	0	3
NAC14 §	*Klebsiella pneumoniae*	carbapenemase: OXA-48; ESBL: CTX-M-15	S	S	S	100	0	0	R	R	R	100	R	R	R	100	0	R/I	R/I	R/I	65	29	0	R	R	R	100	0	R	R	R	100	0	R	R	R	100	0	S	S	S	100	0	0
NAC15	*Klebsiella pneumoniae*	carbapenemase: OXA-48; ESBL: CTX-M-15, OXA-1	S	S	S	100	0	0	R	R	R	100	R	R	R	100	0	R	R/I	R/I	74	26	0	R	R	R	100	0	R	R	R	100	0	R	R	R	100	0	R/I	R/I	R/I	78	22	0
NAC20	*Klebsiella pneumoniae*	carbapenemase: KPC; ESBL: SHV-12, SHV-1, TEM-1	R	R	R	100	0	0	R	R	R	100	R	R	R	100	0	R	R	R	100	0	0	R	R	R	100	0	R	R	R	100	0	R	R	R	100	0	R	R	R	100	0	0
NAC24	*Citrobacter koseri*	WT	S	S	S	100	0	0	R	R	R	100	S	S	S	100	0	S	S	S	100	0	0	S	S	S	100	0	S	S	S	100	0	S	S	S	100	0	S	S	S	100	0	0
NAC29 #	*Morganella morganii*	AmpC hyper	S	S	S	100	0	0	R	R	R	100	R	R	R	100	0	S	-	-	88	0	12	-	-	-	84	15	R	R	R	93	0	5	R	R	R	100	0	R/I	R/I	-	76	18	6
NACA7	*Escherichia coli*	fluoroquinolone R	S	S	S	100	0	0	S	S	S	100	S	S	S	100	0	S	S	S	100	0	0	S	S	S	100	0	S	S	S	100	0	S	S	S	100	0	R	R	R	100	0	0
NACA9	*Morganella morganii*	colistin R	S	S	S	100	0	0	R	R	R	100	R	R	R	89	3	S	S	S	100	0	0	S	S	S	100	0	S	S	S	100	0	R	R	R	97	3	S	S	S	100	0	0
NACI10	*Enterobacter cloacae complex*	resistant to extended-spectrum cephalosporins	S	S	S	100	0	0	R	R	R	100	R	R	R	100	0	S	S	S	97	0	3	R	R	R	100	0	R	R	R	100	0	R	R	R	100	0	S	S	S	97	0	3
NACI4	*Escherichia coli*	ESBL	S	S/I	S/I	80	20	0	R	R	R	100	R	R	R	100	0	R/I	R	R	94	6	0	R	R	R	100	0	R/I	R	R	93	5	3	R	R	R	100	0	R	R	R	100	0	0
NACL2	*Escherichia coli*	WT	S	S	S	100	0	0	S	S	S	100	S	S	S	100	0	S	S	S	100	0	0	S	S	S	100	0	S	S	S	98	0	2	S	S	S	100	0	S	S	S	100	0	0
NAC3	*Pseudomonas aeruginosa*	AmpC	S/I	S	S/I	85	15	0										S	S	S	100	0	0						S	S	S	100	0	0						S	S	S	100	0	0
NAC4	*Pseudomonas aeruginosa*	carbapenemase: VIM-2	R	R	R	100	0	0										R	R	R	91	0	3						R	R	R	100	0	0						R	R	R	100	0	0
NAC5 §	*Pseudomonas aeruginosa*	carbapenemase: VIM-2	-	R	-	70	8	23										R	R	-	94	0	6						R	R	R	100	0	0						R	R	R	100	0	0

			Colistin				Gentamicin					Levofloxacin					Meropenem						Piperacillin-tazobactam					Tigecycline						Tobramycin						Trimethoprim-sulfamethoxazole					
Number	Species	Resistance mechanism/susceptibility profile	VC	PC	DC	%CAt	VC	PC	DC	%CAt	%MI	% (V)ME	VC	PC	DC	%CAt	%MI	% (V)ME	VC	PC	DC	%CAt	%MI	% (V)ME	VC	PC	DC	%CAt	%MI	% (V)ME	VC	PC	DC	%CAt	%MI	% (V)ME	VC	PC	DC	%CAt	%MI	% (V)ME	VC	PC	DC	%CAt	%MI	% (V)ME
NAC11 §	*Enterobacter aerogenes*	AmpC + carbapenem porine deficiency	S	S	S	100	S	S	S	100	0	0	S	S	S	100	0	0	I/S	I	I	91	9	0	R	R	R	100	0	0	S	S	S	100	0	0	S	S	S	100	0	0	S	S	S	100	0	0
NAC14 §	*Klebsiella pneumoniae*	carbapenemase: OXA-48; ESBL: CTX-M-15	S	S	S	100	R	R	R	100	0	0	S	S	S	100	0	0	S/I	S/I	S/I	79	21	0	R	R	R	100	0	0	S	S	S	100	0	0	R/I	R/I	-	56	38	6	R	R	R	100	0	0
NAC15	*Klebsiella pneumoniae*	carbapenemase: OXA-48; ESBL: CTX-M-15, OXA-1	S	S	S	100	R	R	R	97	0	3	S/I	S/I	S/I	87	13	0	S/I	S/I	S/I	69	31	0	R	R	R	100	0	0	S/I	S/I	S/I	78	0	22	R	R	R	100	0	0	R	R	R	100	0	0
NAC20	*Klebsiella pneumoniae*	carbapenemase: KPC; ESBL: SHV-12, SHV-1, TEM-1	S	S	S	100	I/S	I/S	I/S	57	43	-	R	R	R	100	0	0	R/I	R/I	R/I	57	43	0	R	R	R	100	0	0	I/S	I/S	I/S	78	22	0	R	R	R	100	0	0	R	R	R	100	0	0
NAC24	*Citrobacter koseri*	WT	S	S	S	100	S	S	S	100	0	0	S	S	S	100	0	0	S	S	S	100	0	0	S	S	S	100	0	0	S	S	S	100	0	0	S	S	S	100	0	0	S	S	S	100	0	0
NAC29 #	*Morganella morganii*	AmpC hyper	R	R	R	100	R	R	R	100	0	0	-	-	-	73	0	36	S	S	S	100	0	0	S/I	S	S	93	8	0	**	**	**				-	S	-	81	13	6	-	R	-	93	0	7
NACA7	*Escherichia coli*	fluoroquinolone R	S	S	S	100	S	S	S	100	0	0	R	R	R	97	3	0	S	S	S	100	0	0	S	S	S	100	0	0	S	S	S	100	0	0	S	S	S	100	0	0	S	S	S	100	0	0
NACA9	*Morganella morganii*	colistin R	R	R	R	100	S	S	S	100	0	0	S	S	S	100	0	0	S	S	S	100	0	0	S	S	S	100	0	0	**	**	**				S	S	S	100	0	0	R	R	R	98	0	2
NACI10	*Enterobacter cloacae complex*	resistant to extended-spectrum cephalosporins	S	S	S	100	S	S	S	97	0	3	S	S	S	100	0	0	S	S	S	100	0	0	R/I	R/I	R/I	71	30	0	S	S	S	100	0	0	S	S	S	100	0	0	S	S	S	98	0	3
NACI4	*Escherichia coli*	ESBL	S	S	S	100	S	S	S	100	0	0	R	R	R	100	0	0	S	S	S	100	0	0	S/I	S/I	S/I	58	37	5	S	S	S	100	0	0	R	R	R	100	0	0	S	S	S	100	0	0
NACL2	*Escherichia coli*	WT	S	S	S	100	S	S	S	100	0	0	S	S	S	100	0	0	S	S	S	98	2	0	S	S	S	100	0	0	S	S	S	100	0	0	S	S	S	100	0	0	S	S	S	100	0	0
NAC3	*Pseudomonas aeruginosa*	AmpC	S	S	S	100	-	S	-	86	0	14	S	S	S	100	0	0	S	S	S	100	0	0	S	S	S	100	0	0							S	S	S	100	0	0						
NAC4	*Pseudomonas aeruginosa*	carbapenemase: VIM-2	S	S	S	100	R	R	R	100	0	0	R	R	R	100	0	0	R/I	R/I	R/I	82	18	0	R	R	R	97	0	3							R	R	R	100	0	0						
NAC5 §	*Pseudomonas aeruginosa*	carbapenemase: VIM-2	S	S	S	100	S	S	S	97	0	3	R	R	R	100	0	0	R	R	R	100	0	0	R	R	R	97	0	3							R	R	R	100	0	0						

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			Ampicillin						Benzylpenicillin					Cefotaxime					Cefoxitin					Ciprofloxacin				Clindamycin						Erythromycin					Gentamicin					Levofloxacin					
Number	Species	Resistance mechanism/susceptibility profile	VC	PC	DC	%CAt	%MI	%(V)ME	VC	PC	DC	%CAt	%MI	VC	PC	DC	%CAt	%MI	VC	PC	DC	%CAt	%(V)ME	VC	PC	DC	%CAt	VC	PC	DC	%CAt	%MI	%(V)ME	VC	PC	DC	%CAt	%(V)ME	VC	PC	DC	%CAt	%MI	%(V)ME	VC	PC	DC	%CAt	%MI	%(V)ME
NAC53 §	*Staphylococcus aureus*	MRSA, constitutive MLSb, fluoroquinolone R, rifampicin R, tetracycline R							R	R	R	100	0						R	R	R	100	0	R	R	R	100	R	R	R	97	0	3	R	R	R	100	0	R	R	R	100	0	NT	R	R	100	0	0
NACB1	*Staphylococcus aureus*	MRSA, erythromycin en clindamycin R							R	R	R	100	0						R	R	R	100	0	R	R	R	100	R	R	R	100	0	0	R	R	R	100	0	-	-	-	90	10	NT	R	R	100	0	0
NACB10	*Staphylococcus aureus*	MSSA peni R							R	R	R	100	0						S	S	S	97	3	S	S	S	100	S	S	S	95	5	0	S	S	S	100	0	S	S	S	100	0	NT	S	S	100	0	0
NACB7	*Staphylococcus warneri/Staphylococcus pasteuri*	WT																	S	S	S	100	0	S	S	S	100	S	S	S	100	0	0	S	S	S	100	0	S	S	S	100	0	NT	S	S	100	0	0
NAC44	*Enterococcus faecium*	VanA	R	R	R	91	6	3																																								
NAC45	*Enterococcus faecium*	VanB	R/I	R/I	R/I	88	13	0																																								
NAC46	*Enterococcus faecium*	VanA, ampicillin-susceptible	S	S	S	100	0	0																																								
NACL9	*Enterococcus faecalis*	WT	S	S	S	100	0	0																																								
NAC48 §	*Streptococcus pneumoniae*	penicillin intermediate	S	S	S	100	0	0	I	I	I	100	0	S	S	S	100	0										S	S	S	100	0	0	S	S	S	97	3						S	S	-	93	0	7
NAC50	*Streptococcus pneumoniae*	fluoroquinolone R	S	S	S	100	0	0	S	S	S	100	0	S	S	S	100	0										S	S	S	100	0	0	S	S	S	97	3						R	R	R	100	0	0
NAC52	*Streptococcus pneumoniae*	penicillin R, cefotaxime R, macrolide R, tetracycline R	R	R	R	100	0	0	R/I	R/I	R/I	80	20	R/I	R/I	R/I	86	14										R	R	R	100	0	0	R	R	R	97	3						S	S	S	100	0	0
NAC35	*S. agalactiae*	fluoroquinolone R							S	S	S	100	0															S	S	S	100	0	0	S	S	S	97	3						R	R	-	94	0	6
NAC38	*S. agalactiae*	macrolide effluxpump: phenotype M							S	S	S	100	0															S	S	S	100	0	0	R	R	R	97	3						S	S	S	100	0	0
NAC42	*S. pyogenes*	MLSb ermB							S	S	S	100	0															R	R	R	100	0	0	R	R	R	100	0						S/I	S/I	S/I	80	20	0

			Linezolid					Moxifloxacin						Oxacillin				Rifampicin				Teicoplanin				Tetracycline						Tigecycline				Trimethoprim-sulfamethoxazole						Vancomycin					
Number	Species	Resistance mechanism/susceptibility profile	VC	PC	DC	%CAt	%MI	VC	PC	DC	%CAt	%MI	%(V)ME	VC	PC	DC	%CAt	VC	PC	DC	%CAt	VC	PC	DC	%CAt	VC	PC	DC	%CAt	%MI	%(V)ME	VC	PC	DC	%CAt	VC	PC	DC	%CAt	%MI	%(V)ME	VC	PC	DC	%CAt	%(V)ME
NAC53 §	*Staphylococcus aureus*	MRSA, constitutive MLSb, fluoroquinolone R, rifampicin R, tetracycline R	S	S	S	100	0	R	R	R	100	0	0	R	R	R	100	R	R	R	100	S	S	S	100	R	-	-	71	14	14	NT	S	S	100	S	S	S	100	0	0	S	S	S	100	0
NACB1	*Staphylococcus aureus*	MRSA, erythromycin en clindamycin R	S	S	S	100	0	R	R	R	96	4	0	R	R	R	100	-	S	S	100	S	S	S	100	S	S	S	100	0	0	NT	S	S	100	S	S	S	100	0	0	S	S	S	100	0
NACB10	*Staphylococcus aureus*	MSSA peni R	S	S	S	100	0	S	S	S	100	0	0	S	S	S	100	-	S	S	100	S	S	S	100	S	S	S	100	0	0	NT	S	S	100	S	S	S	100	0	0	S	S	S	100	0
NACB7	*Staphylococcus warneri/Staphylococcus pasteuri*	WT	S	S	S	100	0	S	S	S	100	0	0	S	S	S	100	-	S	S	100	S	S	S	100	S	S	S	100	0	0	NT	S	S	100	S	S	S	100	0	0	S	S	S	100	0
NAC44	*Enterococcus faecium*	VanA	S	S	S	100	0		-													R	R	R	100							S	S	S	100							R	R	R	100	0
NAC45	*Enterococcus faecium*	VanB	S	S	S	100	0		-													S	S	S	100							S	S	S	100							R	R	R	100	3
NAC46	*Enterococcus faecium*	VanA, ampicillin-susceptible	S	S	S	100	0		-													R	R	R	100							S	S	S	100							R	R	R	100	0
NACL9	*Enterococcus faecalis*	WT	S	S	S	100	0		-													S	S	S	100							S	S	S	100							S	S	S	100	0
NAC48 §	*Streptococcus pneumoniae*	penicillin intermediate	S	S	S	92	8	S	S	S	100	0	0													S	S	S	95	5	0					I	R	-	56	33	11	S	S	S	100	0
NAC50	*Streptococcus pneumoniae*	fluoroquinolone R	S	S	S	100	0	R	R	R	100	0	0													S	S	S	100	0	0					S	S	S	100	0	0	S	S	S	100	0
NAC52	*Streptococcus pneumoniae*	penicillin R, cefotaxime R, macrolide R, tetracycline R	S	S	S	100	0	S	S	S	100	0	0													R	R	R	100	0	0					R	R	R	100	0	0	S	S	S	100	0
NAC35	*S. agalactiae*	fluoroquinolone R	S	S	S	100	0	R	R	R	95	0	5													NT	R	R	100	0	0					NT	S	S	100	0	0	S	S	S	100	0
NAC38	*S. agalactiae*	macrolide effluxpump: phenotype M	S	S	S	100	0	S	S	S	100	0	0													NT	R	R	100	0	0					NT	S	S	100	0	0	S	S	S	100	0
NAC42	*S. pyogenes*	MLSb ermB	S	S	S	100	0	S/I	S/I	S/I	86	14	0													NT	R	R	100	0	0					NT	S	S	100	0	0	S	S	S	100	0