

# Antimicrobial susceptibility of Pseudomonas aeruginosa isolated from cystic fibrosis patients through Northern Europe

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#### 1 AAC01046-16 – revised version

2 Antimicrobial susceptibility of Pseudomonas aeruginosa isolated from cystic fibrosis

## 3 patients through Northern Europe

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- 27

## 28 ABSTRACT

29	Pseudomonas aeruginosa is a major cause of morbidity and mortality in cystic fibrosis
30	patients. This study compares the antimicrobial susceptibility of 153 P. aeruginosa isolates
31	from the United Kingdom (UK) (n=58), Belgium (n=44), and Germany (n=51) collected from
32	120 patients during routine visits over the 2006-2012 period. MICs were measured by broth
33	microdilution. Genes encoding extended spectrum $\beta$ -lactamases (ESBL), metallo- $\beta$ -
34	lactamases and carbapenemases were detected by PCR. Pulsed Field Gel Electrophoresis
35	and Multi-Locus Sequence Typing were performed on isolates resistant to $\geq$ 3 antibiotic
36	classes among penicillins/cephalosporins, carbapenems, fluoroquinolones, aminoglycosides,
37	polymyxins. Based on EUCAST/CLSI breakpoints, susceptibility was $\leq 30\%/\leq 40\%$
38	(penicillins, ceftazidime, amikacin, ciprofloxacin), 44-48%/48-63% (carbapenems), 72%/72%
39	(tobramycin), and 92%/78% (colistin) independently of patient's age. Sixty percent of strains
40	were multidrug resistant (MDR; European Centre for Disease prevention and Control
41	criteria). Genes encoding ESBL (most prevalent BEL, PER, GES, VEB, CTX-M, TEM, SHV,
42	and OXA), metallo $\beta$ -lactamases (VIM, IMP, NDM), or carbapenemases (OXA-48, KPC)
43	were not detected. The Liverpool Epidemic Strain (LES) was prevalent in UK isolates only
44	(75% of MDR isolates). Four MDR ST958 isolates were found spread over the three
45	countries. The other MDR clones were evidenced in $\leq$ 3 isolates and localized in a single
46	country. A new sequence type (ST2254) was discovered in one MDR isolate in Germany.
47	Clonal and non-clonal isolates with different susceptibility profiles were found in 21 patients.
48	Thus, resistance and MDR are highly prevalent in routine isolates from 3 countries, with
49	carbapenem (meropenem), tobramycin and colistin remaining the most active drugs.
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## 52 Introduction

53	Pulmonary infection represents a major cause of morbidity and mortality among cystic
54	fibrosis (CF) patients (1). These patients are therefore regularly exposed to antibiotics for the
55	treatment of infectious exacerbations as well as for the prevention of chronic colonization.
56	Pseudomonas aeruginosa is one of the most prevalent bacterial species, especially in the
57	adult population (2). It is well known for its genetic plasticity and capacity to accumulate
58	resistance mechanisms, including acquisition of foreign genetic material (3). The
59	percentage of patients colonized by <i>P. aeruginosa</i> has decreased in recent years (2) but,
60	with improved life expectancy, the absolute number of colonized patients has increased. It
61	has also been proposed that multidrug resistant (MDR) strains are more frequent in older
62	patients, primarily due to cumulative exposure to antibiotics (2). A further reason for the
63	spread of antibiotic resistance in CF patients is the dissemination of MDR clones. The
64	Liverpool Epidemic Strain (LES), first described in 1996 (4), has proven particularly
65	successful for acquiring resistance mechanisms over the years (5,6) and for spreading from
66	the UK to other countries such as Canada, Spain and Australia (7).
67	In this study, we compared the antimicrobial susceptibility of <i>P. aeruginosa</i> isolated from CF
68	patients in the United Kingdom (UK), where the MDR LES clone is known to be highly
69	prevalent (5), with an equivalent number of strains collected in Germany and Belgium, where
70	no specific survey has been published over the last years. We determined the presence of
71	co-resistance to unrelated antibiotic classes and its possible association with MDR clones.
72	We found that resistance was high in the three countries, but not related to the dissemination
73	of a specific MDR clone in Germany or Belgium. Carbapenems, tobramycin, and colistin
74	remain the most active drugs against <i>P. aeruginosa</i> respiratory isolates. Importantly, no
75	carbapenemases were detected in these strains.

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## 77 Materials and methods

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#### 79 Bacterial isolates

A total of 153 clinical P. aeruginosa isolates were selected at random among those collected 80 81 between 2006 and 2012 in 3 CF centers from Belgium (Hôpital des enfants malades Reine Fabiola/Erasme Hospital, n = 44); Germany (University Hospital of Münster, n = 51) and UK 82 83 (Queen's University of Belfast, n = 58) during routine visits. The details on the collection are 84 shown in Table 1. When successive strains were collected from a single patient, only those collected at the first occasion were considered. Nevertheless, more than one isolate were 85 86 analyzed for some patients based on differences in their phenotypic appearance (see Figure 87 S1 in supplemental material).

88

#### 89 Antibiotics

90 The following antibiotics were obtained as microbiological standards (with abbreviations and

91 potencies shown in parentheses): amikacin disulfate (AMK; 74.80%), colistin sulfate (CST;

92 79.64%); piperacillin sodium (PIP; 94.20%), and ticarcillin disodium salt (TIC; 85.25%) from

93 Sigma-Aldrich, St. Louis, MO; ciprofloxacin (CIP; 85.00%) from Bayer, Leverkusen,

94 Germany; and tobramycin (TOB; 100%) from Teva, Wilrijk, Belgium. The remaining

95 antibiotics were obtained as the corresponding branded product in Belgium for intravenous

- 96 use and complying with the provisions of the European Pharmacopoeia with respect to
- 97 content in active agent: ceftazidime as Glazidim® (CAZ; 88.20%) from GlaxoSmithKline,
- 98 Genval, Belgium; imipenem as Tienam® [also containing cilastatin which does not have any
- 99 antibacterial activity] (IPM; 45.60%) from MSD, Brussels, Belgium; meropenem as
- 100 Meronem® (MEM; 74.00%) from AstraZeneca, Brussels, Belgium; piperacillin-tazobactam
- 101 as Tazocin® (TZP; 97.00%) from Wyeth, Louvain-La-Neuve, Belgium [now part of Pfizer].

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#### 103 Susceptibility testing

- 104 Minimal Inhibitory Concentrations (MIC) were determined by microdilution in cation-adjusted
- 105 Mueller-Hinton broth following CLSI (Clinical and Laboratory Standards Institute)
- 106 recommendations, using *P. aeruginosa* ATCC 27853 as quality control strain (8).
- 107 Susceptibility was assessed according to the interpretive criteria of both the European
- 108 Committee on Antimicrobial Susceptibility Testing (EUCAST) (9) and the CLSI (8). Isolates
- 109 were considered as multi-drug resistant (MDR) if resistant to at least three antibiotic classes
- among those tested (penicillins/cephalosporins, carbapenems, fluoroquinolones,
- 111 aminoglycosides and polymyxins), according to ECDC (European Centre for Disease
- 112 Prevention and Control) criteria (10).
- 113

#### 114 Screening for extended-spectrum $\beta$ -lactamases (ESBL) and carbapenemases

- 115 For all isolates (n=51) showing MICs > 8 mg/L for ceftazidime and meropenem, *bla*<sub>TEM</sub>,
- 116 bla<sub>SHV</sub>, bla<sub>CTX-M</sub> (groups 1, 2 and 9), bla<sub>VIM</sub>, bla<sub>IMP</sub>, bla<sub>KPC</sub>, and bla<sub>NDM</sub> gene families were
- 117 detected by real-time multiplex PCR, using group-specific primers ([11-13] and references
- 118 cited therein). Other genes encoding OXA (1,2,9,10,18,20,23,24,30,48, 58,198), BEL (1 to
- 119 3), PER (1 to 5, and 7), GES (1 to 18), and VEB (1 to 7) enzymes were also detected by 120 multiplex PCR.
- 121

#### 122 Molecular typing

- 123 All MDR isolates in the collection showing co-resistance to penicillins and/or cephalosporins
- 124 and two other classes (n=56) were characterized by Pulsed-Field Gel Electrophoresis
- 125 (PFGE) analysis (14). In addition, 42 pairs of isolates collected simultaneously and in the
- 126 same sample from 21 patients (see Figure S1) but differing in their susceptibility profile to at
- 127 least one class of antibiotics were also genotyped by PFGE to determine their genetic
- 128 relatedness. The pulsotype classification criteria designated a pulsotype by one or two
- 129 letters including patterns showing zero to six DNA fragments differences (14). An epidemic

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- 130 pulsotype was defined as a pulsotype recovered from ≥ 2 patients while a sporadic
- 131 pulsotype was recovered only once.
- 132 Multilocus sequence typing (MLST) was performed on a representative strain of epidemic
- 133 pulsotypes detected in ≥ 3 strains, as previously described (15). The reference LES B58
- 134 strain (4) was used as control. MLST data were uploaded to the *P. aeruginosa* MLST
- 135 Database (<u>http://pubmlst.org/paeruginosa</u>) for allele type and sequence type (ST)
- 136 assignments (16).

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#### 137 Results

#### 138 MIC distributions

Table 2 shows the MIC distribution for 9 antipseudomonal drugs against 153 isolates 139 140 collected from 120 CF patients originating from three different countries over the 2006-2012 141 period, together with the percentage susceptible and resistant based on both EUCAST and CLSI interpretive criteria. The corresponding MIC cumulative distributions are illustrated in 142 143 Figure S2. Resistance was high in this collection. Using the EUCAST or the CLSI "R" breakpoints, respectively, full resistant isolates were ≥71% or ≥54% for penicillins (ticarcillin, 144 piperacillin, piperacillin-tazobactam), 69% or 59% for ceftazidime, 61% or 46% for amikacin, 145 56% or 27% for ciprofloxacin, ≥20% for carbapenems, and 28 or 16% for tobramycin. Full 146 resistance to colistin was noted for only 8% of the isolates. Strains resistant to ceftazidime 147 148 and meropenem were screened for the expression of frequent ESBLs, metallo  $\beta$ -149 lactamases, and carbapenemases, which returned negative results.

150

#### 151 Cross- or co-resistance

Cross- or co-resistance was examined among pairs of antibiotics. Cross-resistance is 152 153 defined as a single resistance mechanism that confers resistance to antimicrobial molecules with a similar mechanism(s) of action. It thus describes resistance to an entire class of 154 155 antibiotics, or to different classes of agents with overlapping drug targets, or to different classes of antibiotics that are substrates for the same broad-spectrum efflux system. Co-156 resistance rather refers to the presence of different mechanisms of resistance in the same 157 158 bacterial isolate, and is thus necessarily confers resistance to unrelated antibiotic classes (17). Ninety-four strains were considered as MDR according the ECDC (10). The right upper 159 part of Table 3 shows the percentage of strains showing cross- or co-resistance to pairs of 160 161 antibiotics according to EUCAST criteria. About 2/3 of the strains were resistant to both penicillins and ceftazidime and more than 40%, to penicillins and ceftazidime together with 162 amikacin or ciprofloxacin. Co-resistance between any studied drug and tobramycin, 163

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164	meropenem, and colistin was lower than 28%, 20% and 8%, respectively. Of note, only 4 $$
165	strains in the whole collection were co-resistant to meropenem, tobramycin, and colistin
166	(Figure S3).
167	The left lower part of Table 3 shows the correlation coefficient between the individual MIC for
168	each pair of antibiotics, with the corresponding multivariate analysis presented in details as
169	supplementary Figure S4. The highest degrees of correlation (> 0.75) between individual
170	MICs were observed for ticarcillin vs. ceftazidime, piperacillin vs. piperacillin-tazobactam,
171	ceftazidime vs. piperacillin-(tazobactam), imipenem vs. meropenem, and amikacin vs.
172	tobramycin, suggesting common mechanisms of resistance between these pairs of
173	antibiotics. Yet, differences in the intrinsic potency were nevertheless observed between
174	these pairs of drugs throughout the collection; they are illustrated in Figure S4 and
175	associated Table B: tazobactam reduced the MIC of piperacillin by a factor of 1.5 dilution,
176	while ceftazidime MICs were 0.5 and 1 dilution lower than those of ticarcillin and piperacillin
177	respectively, and similar to those of piperacillin-tazobactam. Meropenem MICs were 1
178	dilution lower than those of imipenem, and tobramycin MICs were 3 dilutions lower than
179	those of amikacin.
180	
181	Typing of MDR isolates
182	Among the 94 MDR isolates, most were resistant to penicillins and/or cephalosporins. Only
183	those showing resistance to at least 2 other classes (n = 56) were characterized by PFGE

those showing resistance to at least 2 other classes (n = 56) were characterized by PFGE
analysis. A high genetic diversity was observed, with 19 sporadic pulsotypes and 9 epidemic
pulsotypes (Table 4). With the exception of pulsotype YY recovered for 1 or 2 isolates in the
three countries, each epidemic pulsotype remained localized in a single country. The CA
epidemic pulsotype found in 3/4 of the UK isolates corresponded to the pulsotype of the LES
clone. MLST analysis of epidemic pulsotypes CA, H and YY showed ST146, ST2254 (new
ST) and ST958, respectively (data not shown).

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- 190 PFGE analysis was also performed on 42 isolates collected as pairs from 21 patients and
- 191 displaying different susceptibility profiles (Table S1). In twelve patients, the pair of
- 192 *P. aeruginosa* isolates had the same pulsotype, while the 9 other patients had isolates with
- 193 different pulsotypes.

194

#### 195 Analysis per country or age group

- 196 Because of the genetic diversity observed between countries, we then examined the
- 197 distribution of susceptible, intermediate (when applicable) and resistant isolates classified
- 198 based on the country where they were collected (Figure 1). Susceptibility rates differed
- 199 among countries, with lower resistance in Belgium (significant for all antibiotics except
- 200 ticarcillin and ciprofloxacin) and higher resistance in Germany and UK (significant for
- 201 piperacillin-tazobactam in Germany and for imipenem, ciprofloxacin, and colistin in UK) as
- 202 compared to the mean value for the whole collection. There was no significant correlation
- 203 between the patient's age when the isolate was collected and the number of antibiotic
- classes to which the isolate was resistant (Figure S5).

205

### 206 Discussion

207	In this study, we examined antibiotic susceptibility of a collection of P. aeruginosa isolated
208	from CF patients in three Northern European countries during routine examination, which
209	provides a broader view than the majority of previous surveys that have focused on a single
210	country (18-20) or a single center (21-23). A key observation is that resistance rates were
211	high in this population, confirming previous studies with CF patients (2), and notably
212	A.9.much higher than that which has been reported for isolates collected in Northern
213	European from intensive care units (24-26). Resistance rates were also higher than those
214	previously reported for strains from CF patients in a German survey from the University of
215	Würzburg except for tobramycin (27; collection in 2006), or in a multicentric study in the UK,
216	except for meropenem and ciprofloxacin (28; collection in 2000). Moreover, a high degree of
217	cross- or co-resistance among antibiotics was observed, which is important from both a
218	pharmacological and clinical perspective.
219	
220	From a pharmacological perspective, we noticed, as expected, significant correlations
220 221	From a pharmacological perspective, we noticed, as expected, significant correlations between MIC values for antibiotics belonging to the same or similar classes (penicillins and
221	between MIC values for antibiotics belonging to the same or similar classes (penicillins and
221 222	between MIC values for antibiotics belonging to the same or similar classes (penicillins and ceftazidime or other penicillins, imipenem and meropenem, and amikacin and tobramycin),
221 222 223	between MIC values for antibiotics belonging to the same or similar classes (penicillins and ceftazidime or other penicillins, imipenem and meropenem, and amikacin and tobramycin), but with systematic differences in the potency of each antibiotic within these pairs (see
221 222 223 224	between MIC values for antibiotics belonging to the same or similar classes (penicillins and ceftazidime or other penicillins, imipenem and meropenem, and amikacin and tobramycin), but with systematic differences in the potency of each antibiotic within these pairs (see Figure S3 and related Table B). Focusing on $\beta$ -lactams, the impact of tazobactam on
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221 222 223 224 225 226	between MIC values for antibiotics belonging to the same or similar classes (penicillins and ceftazidime or other penicillins, imipenem and meropenem, and amikacin and tobramycin), but with systematic differences in the potency of each antibiotic within these pairs (see Figure S3 and related Table B). Focusing on $\beta$ -lactams, the impact of tazobactam on piperacillin activity was modest, but of the same order of magnitude as that observed on MIC distribution for wild-type strains reported by EUCAST (29), probably denoting the inhibition

- 230 in potency among these pairs of drugs in our collection are likely to reflect differences in
- 231 intrinsic activity rather than in vulnerability to resistance mechanisms. Remarkably no
- 232 carbapenemase production was apparent in this collection. A same finding was reported in

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233	two recent reports studying <i>P. aeruginosa</i> collected over the same period of time as those
234	examined here. The first of these studies was performed in Australia and examined
235	successively a collection of 662 carbapenem-resistant isolates assembled in 2007-2009
236	from diverse CF centers and of 517 isolates collected in a single CF center in 2011 (32). The
237	second study was performed in Brazil and analyzed isolates from 75 patients collected in
238	2010-2011 (19). To the opposite, carbapenemases have been detected in 63 out of 217
239	P. aeruginosa collected from CF patients in China (22). The prevalence of carbapenemase
240	genes could, however, be different in other bacteria infecting CF patients, but there is no
241	large survey published so far in other Gram-negative species (33,34).
242	Thus, carbapenem resistance in CF European isolates is probably primarily mediated by the
243	combined effect of AmpC and of a reduced accumulation (porin mutations and/or increased
244	efflux) (35; Chalhoub et al, submitted for publication]. Of note, however, carbapenem
245	resistance has previously been described in the LES clone (5) but the underlying
246	mechanism(s) have not been investigated to date. For aminoglycosides, the higher potency
247	of tobramycin over amikacin in our collection also reflects what is observed in MIC
248	distributions of wild-type strains assembled by EUCAST (29). Tobramycin has been
249	described as a poorer substrate than amikacin for the efflux pump MexXY-OprM considered
250	as responsible for natural and adaptative resistance to aminoglycosides in <i>P. aeruginosa</i>
251	(36,37).
252	Considering our findings from a clinical perspective, a high degree of cross-resistance was
253	observed between penicillins and ceftazidime, which was expected. However, a high degree
254	of co-resistance was also apparent between these antibiotics and both ciprofloxacin and
255	amikacin, resulting in 60 $\%$ of the isolates being categorized as multidrug resistant. In
256	contrast, meropenem, and colistin, and to a lesser extent, tobramycin, were active against a
257	large fraction of the isolates with few strains co-resistant to these three antibiotics.
258	Tobramycin and colistin by inhalation are often considered as first line for the eradication of
259	early P. aeruginosa infection and tobramycin, also for chronic therapies (38-40). High

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260	concentrations delivered b	this route of administration may	/ help to overcome resistance

261 (41,42).

262

We also noticed an important genetic diversity among multi-resistant isolates collected in 263 Belgium and Germany while those collected in the UK belong in majority to the Liverpool 264 265 Epidemic Strain (LES) clone. Global studies of P. aeruginosa population structure concluded 266 that CF isolates present a high genetic diversity but nevertheless belong to a 'core lineage' ubiquitous in the natural environment (43), which is highly suggestive of a direct colonization 267 268 of the patients from the environment. However, a series of epidemic clones have been described (7) among which the LES (4) representing 18 of the 24 MDR isolates collected in 269 270 the UK in our study, and the ST17 (7), which differs by only 1 nucleotide from the ST958 271 found in the three countries investigated here. The new ST2254 we described was distinct 272 from ST146 (LES clone, 5 alleles difference) and ST958 or ST17 (6 alleles difference). 273 274 We observed that a single patient can be colonized by different strains and, conversely, that

clonally-related strains isolated at the same time from a single patient can harbor diverse
susceptibility profiles. This could be a consequence of the previously described phenotypic
variability among isolates with the same colony morphotype and being part of a single clonal
lineage (44,45), as well as of recombination occurring *in vivo* and generating phenotypic and
genetic diversification (46,47).

280

Although limited, differences in resistance rates between Belgium and the other two other countries are raising questions about segmentation of clone distribution. For strains collected in the UK, higher resistance is clearly related to the high prevalence of the LES clone, which has been described as exhibiting a large proportion of MDR isolates (5). Of interest, we observed different resistance profiles within this clone, which is coherent with the previously described phenotypic variability among LES isolates (6). The ST958 represented in the three countries is also found among the MDR clonal complexes (7). In the German

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288	collection, higher resistance is essentially related to the presence of more sporadic MDR
289	clones than in the two other countries. We cannot exclude differences in therapeutic
290	management of patients among these three centers that may influence resistance selection
291	(48) but this specific aspect was not within the scope of our study.
292	
293	Resistance rates were not higher in the older population than in children/young adults. The
294	interpretation of these data need to be cautious because (a) we did not follow the evolution
295	of susceptibility over time in single patients and (b) we do not know the age of first
296	colonization for each patient. With this limitation in mind, the fact that MDR isolates could be
297	found in young people and susceptible isolates in adults may suggest that resistance
298	depends on the initial susceptibility of the infecting strain. A link between emergence of
299	resistance and early antibiotic use in CF patients is still controversial, even though
300	underlined in the last report of the Cystic Fibrosis Foundation (2). A recent study in Australia
301	showed that multiresistance in children is correlated with duration of intravenous antibiotic
302	treatment, which was not the case for adults (18). A correlation with antibiotic usage
303	irrespective of patient's age (49) or with time after colonization (6) has also been proposed.
304	In contrast, other studies following the evolution of antibiotic susceptibility in successive
305	isogenic isolates from a single patient suggest either that resistance can occur sporadically
306	(50) or without correlation with the time of isolation (51). In these cases, the presence of
307	mutator variants seems to predetermine the risk of developing resistance over time (6).
308	
309	Our study has a number of limitations, primarily linked to the fact that samples collected
310	during periodic routine examinations may not correspond to the first isolates of
311	P. aeruginosa infections in these patients. Moreover, as we did not have the history of
312	antibiotic use in these patients, we could not determine if there was a potential link between
313	antibiotic usage and subsequent development of resistance. Nevertheless, this collection
314	reflects the situation CF clinicians face daily, where they have to select antibiotics based on
315	susceptibility testing performed on current isolates. In this context, our data may lead to

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316	three clinically-meaningful conclusions. First, susceptibility testing is important to perform
317	even in newly infected patients, because they can be colonized very early by MDR clones.
318	Second, these tests should be performed on more than one colony (especially if different
319	phenotypes are evidenced on culture plates), because of potential population heterogeneity
320	with respect to susceptibility profiles (52). Third, prudent use of highly active drugs should
321	be promoted in order to preserve their efficacy. This implies the use of optimized doses if
322	administered by conventional routes or administration by inhalation to insure high local
323	concentrations that could minimize the risk of selection of resistance.

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#### 532 533 Table 1: P. aeruginosa collection (2006-2012)

Country	Number of isolates	Number of patients	Period of sampling			
Belgium	44	38	2010			
Germany	51	36	2012			
United Kingdom	58	46	2006-2009			
Total	153	120				

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#### Table 2: MIC distributions for antipseudomonal antibiotics and corresponding percentage of susceptibility according to EUCAST or 537 538

## **CLSI** breakpoints

		Susceptibility according to								
Antibiotic			EUCAST "	CLSI <sup>b</sup>						
	min	max	MIC <sub>50</sub>	MIC <sub>90</sub>	% S	% I	%R	% S	%I	%R
Ticarcillin (TIC)	1	>512	128	>512	16	NA	84	16	23	61
Piperacillin (PIP)	0.5	>512	256	>512	24	NA	76	24	15	61
Piperacillin- tazobactam (TZP)	0.5	>512	128	512	29	NA	71	29	17	54
Ceftazidime (CAZ)	1	>512	64	512	31	NA	69	31	10	59
Imipenem (IPM)	0.25	128	4	32	48	19	33	48	19	33
Meropenem (MEM)	0.032	256	2	16	44	36	20	63	17	20
Amikacin (AMK)	1	>512	32	128	22	17	61	39	15	46
Tobramycin (TOB)	0.064	>512	2	16	72	NA	28	72	12	16
Ciprofloxacin (CIP)	0.064	64	1	8	24	20	56	44	29	27
Colistin (CST)	0.25	>512	1	4	92	NA	8	78	14	8

<sup>a</sup> EUCAST breakpoints (NA: not applicable [no I category]): **TIC** S≤16 R>16; **PIP** S≤16 R>16; **TZP** S≤16 R>16; **CAZ** S≤8 R>8; **IPM** S≤4 R>8; **MEM** S≤ 2 R>8; **AMK** S≤ 8 R>16; **TOB** S≤ 4 R>4; **CIP** S≤ 0.5 R>1; **CST** S≤ 4 R>4.

<sup>b</sup> CLSI breakpoints: TIC S≤16, I=32-64, R≥128; PIP S≤16, I=32-64, R≥128; TZP S≤16, I=32-64, R≥128; CAZ S≤8, I=16, R≥32; IPM S≤4, I=8, R≥16; MEM S≤4, I=8, R≥16; CIP S≤1, I=2, R≥4; AMK S≤16, I=32, R≥64; TOB S≤4, I=8, R≥16; CST S≤2, I=4, R≥8.

S: susceptible; I: intermediate; R: resistant

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#### Table 3: Percentage of co-resistance among pairs of antibiotics and multivariate correlation between MIC values of each pair of

544 antibiotics for individual strains.

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545 Above the diagonal, figures correspond to the percentage of isolates categorized as resistant to the two antibiotics (row/column) using

546 EUCAST breakpoints. Values highlighted in bold indicate combinations for which resistance is higher than 30 %.

The numbers below the diagonal correspond to the correlation coefficient between individual MIC values for each pairs of antibiotics. Values higher than 0.75 are highlighted in bold characters. See Table 2 for abbreviations of antibiotics and Figure S4 for the details of this analysis.

TIC	68	71	69	31	20	54	25	48	8			
0.78	CAZ	68	65	29	20	48	24	42	7			
0.72	0.88	PIP	71	31	20	52	24	45	7			
0.73	0.86	0.94	TZP	30	20	50	24	42	7			
0.53	0.47	0.47	0.45	IPM	16	24	12	24	4			
0.66	0.55	0.48	0.54	0.80	MEM	14	7	18	3			
0.37	0.46	0.40	0.36	0.34	0.26	AMK	28	38	8			
0.26	0.40	0.31	0.28	0.29	0.17	0.90	тов	22	5			
0.26	0.30	0.27	0.28	0.39	0.43	0.31	0.31	CIP	6			
0.18	0.16	0.14	0.11	0.13	0.04	0.32	0.34	0.01	CST			
	0.78           0.72           0.73           0.53           0.66           0.37           0.26	0.78         CA2           0.72         0.88           0.73         0.86           0.53         0.47           0.66         0.55           0.37         0.46           0.26         0.40	0.78         CAZ         68           0.72         0.88         PIP           0.73         0.86         0.94           0.53         0.47         0.47           0.66         0.55         0.48           0.37         0.46         0.40           0.26         0.30         0.27	0.78         CAZ         68         65           0.72         0.88         PIP         71           0.73         0.86         0.94         TZP           0.53         0.47         0.47         0.45           0.66         0.55         0.48         0.54           0.37         0.46         0.40         0.36           0.26         0.40         0.31         0.28	0.78         CAZ         68         65         29           0.72         0.88         PIP         71         31           0.73         0.86         0.94         TZP         30           0.53         0.47         0.47         0.45         IPM           0.66         0.55         0.48         0.54         0.80           0.37         0.46         0.40         0.36         0.34           0.26         0.40         0.21         0.28         0.29	0.78         CAZ         68         65         29         20           0.72         0.88         PIP         71         31         20           0.73         0.86         0.94         TZP         30         20           0.53         0.47         0.47         0.45         IPM         16           0.66         0.55         0.48         0.54         0.80         MEM           0.37         0.46         0.40         0.36         0.34         0.26           0.26         0.40         0.31         0.28         0.29         0.17           0.26         0.30         0.27         0.28         0.39         0.43	0.78         CAZ         68         65         29         20         48           0.72         0.88         PIP         71         31         20         52           0.73         0.86         0.94         TZP         30         20         50           0.53         0.47         0.47         0.45         IPM         16         24           0.66         0.55         0.48         0.54         0.80         MEM         14           0.37         0.46         0.40         0.36         0.34         0.26         AMK           0.26         0.40         0.31         0.28         0.29         0.17         0.90           0.26         0.30         0.27         0.28         0.39         0.43         0.31	0.78         CAZ         68         65         29         20         48         24           0.72         0.88         PIP         71         31         20         52         24           0.73         0.86         0.94         TZP         30         20         50         24           0.53         0.47         0.47         0.45         IPM         16         24         12           0.66         0.55         0.48         0.54         0.80         MEM         14         7           0.37         0.46         0.40         0.36         0.34         0.26         AMK         28           0.26         0.40         0.31         0.28         0.29         0.17         0.90         TOB           0.26         0.30         0.27         0.28         0.39         0.43         0.31         0.31	0.78         CAZ         68         65         29         20         48         24         42           0.72         0.88         PIP         71         31         20         52         24         45           0.73         0.86         0.94         TZP         30         20         50         24         42           0.53         0.47         0.47         0.45         IPM         16         24         12         24           0.66         0.55         0.48         0.54         0.80         MEM         14         7         18           0.37         0.46         0.40         0.36         0.34         0.26         AMK         28         38           0.26         0.40         0.31         0.28         0.39         0.43         0.31         0.31         CIP			

Percentage of cross- or co-resistance

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#### 550 Table 4: Distribution of pulsotypes among the MDR *P. aeruginosa* clinical isolates

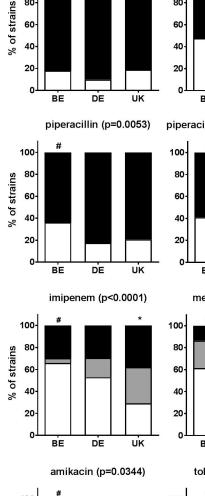
	Number of MDR strains	Number of pulsotypes		Number of strains in epidemic pulsotype									
Country		Sporadic	Epidemic	CA <sup>a</sup>	СК	СМ	CD	н	ww	YI	CJ	YY	
Belgium	10	3	4	0	0	2	2	0	2	0	0	1	
Germany	22	11	5	0	2	0	0	3	0	2	2	2	
United Kingdom	24	5	2	18	0	0	0	0	0	0	0	1	

551 <sup>a</sup> CA pulsotype corresponds to the LES epidemic clone pulsotype

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- 553 **Figure 1:** Comparison of the percentage of antibiotic resistance in the collection based on
- the country of origin of the strain (Belgium (BE): n=44; Germany (DE): n=51; United
- 555 Kingdom (UK): n=58). Statistical analysis: Chi Square test (p values indicated after the
- 556 name of the antibiotic); Analysis of means of proportions with  $\alpha$  level of 0.05: \* denotes a
- 557 value below the mean and  $^{\#}$ , above the mean.

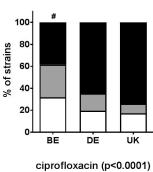


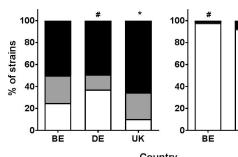
ticarcillin

100-

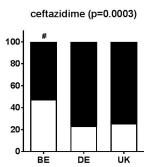
80

60



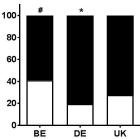




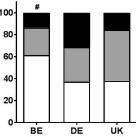


🗆 %S 🗖 %I 🔳 %R

piperacillin-tazobactam (p=0.0038)



meropenem (p=0.0005)



tobramycin (p=0.0303)

