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Antimicrobial Susceptibility of *Pseudomonas aeruginosa* Isolated from Cystic Fibrosis Patients in Northern Europe

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***Pseudomonas aeruginosa* is a major cause of morbidity and mortality in cystic fibrosis patients. This study compared the antimicrobial susceptibilities of 153 *P. aeruginosa* isolates from the United Kingdom (UK) ($n = 58$), Belgium ($n = 44$), and Germany ($n = 51$) collected from 118 patients during routine visits over the period from 2006 to 2012. MICs were measured by broth microdilution. Genes encoding extended-spectrum β-lactamases (ESBL), metallo-β-lactamases, and carbapenemases were detected by PCR. Pulsed-field gel electrophoresis and multilocus sequence typing were performed on isolates resistant to ≥3 antibiotic classes among the penicillins/cephalosporins, carbapenems, fluoroquinolones, aminoglycosides, and polymyxins. Based on EUCAST/CLSI breakpoints, susceptibility rates were ≤30%/≤40% (penicillins, ceftazidime, amikacin, and ciprofloxacin), 44 to 48%/48 to 63% (carbapenems), 72%/72% (tobramycin), and 92%/78% (colistin) independent of patient age. Sixty percent of strains were multidrug resistant (MDR; European Centre for Disease Prevention and Control criteria). Genes encoding the most prevalent ESBL (BEL, PER, GES, VEB, CTX-M, TEM, SHV, and OXA), metallo-β-lactamases (VIM, IMP, and NDM), or carbapenemases (OXA-48 and KPC) were not detected. The Liverpool epidemic strain (LES) was prevalent in UK isolates only (75% of MDR isolates). Four MDR sequence type 958 (ST958) isolates were found to be spread over the three countries. The other MDR clones were evidenced in ≤3 isolates and localized in a single country. A new sequence type (ST2254) was discovered in one MDR isolate in Germany. Clonal and nonclonal isolates with different susceptibility profiles were found in 20 patients. Thus, resistance and MDR are highly prevalent in routine isolates from 3 countries, with meropenem, tobramycin, and colistin remaining the most active drugs.**

Pulmonary infection represents a major cause of morbidity and mortality among cystic fibrosis (CF) patients (1). These patients are therefore regularly exposed to antibiotics for the treatment of infectious exacerbations as well as for the prevention of chronic colonization. *Pseudomonas aeruginosa* is one of the most prevalent bacterial species, especially in the adult population (2). It is well known for its genetic plasticity and capacity to accumulate resistance mechanisms, including acquisition of foreign genetic material (3). The percentage of patients colonized by *P. aeruginosa* has decreased in recent years (2), but with improved life expectancy, the absolute number of colonized patients has increased. It has also been proposed that multidrug-resistant (MDR) strains are more frequent in older patients, primarily due to cumulative exposure to antibiotics (2). A further reason for the spread of antibiotic resistance in CF patients is the dissemination of MDR clones. The Liverpool epidemic strain (LES), first described in 1996 (4), has proven particularly successful at acquiring resistance mechanisms over the years (5, 6) and at spreading from the United Kingdom (UK) to other countries, such as Canada, Spain, and Australia (7).

In this study, we compared the antimicrobial susceptibility of *P. aeruginosa* isolated from CF patients in the UK, where the MDR LES clone is known to be highly prevalent (5), with those of an equivalent number of strains collected in Germany and Belgium, where no specific survey has been published in recent years. We determined the presence of coresistance to unrelated antibiotic classes and its possible association with MDR clones. We found that resistance was high in the three countries but was not related to the dissemination of a specific MDR clone in Germany or Belgium. Carbapenems, tobramycin, and colistin remain the drugs most active against *P. aeruginosa* respiratory isolates. Importantly, no carbapenemases were detected in these strains.

MATERIALS AND METHODS

Bacterial isolates. A total of 153 clinical *P. aeruginosa* isolates were selected at random among those collected between 2006 and 2012 in 3 CF centers in Belgium (Hôpital des enfants malades Reine Fabiola/Erasme Hospital; $n = 44$), Germany (University Hospital of Münster; $n = 51$), and the UK (Queen's University of Belfast; $n = 58$) during routine visits.

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TABLE 1 *P. aeruginosa* collection (2006 to 2012)

Country	No. of isolates	No. of patients	Period of sampling
Belgium	44	38	2010
Germany	51	34	2012
United Kingdom	58	46	2006–2009
Total	153	118	

The details on the collection are 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 analyzed for some patients based on differences in their phenotypic appearance (see Fig. S1 in supplemental material).

Antibiotics. The following antibiotics were obtained as microbiological standards (with abbreviations and potencies shown in parentheses): amikacin disulfate (AMK; 74.80%), colistin sulfate (CST; 79.64%), piperacillin sodium (PIP; 94.20%), and ticarcillin disodium salt (TIC; 85.25%) from Sigma-Aldrich, St. Louis, MO; ciprofloxacin (CIP; 85.00%) from Bayer, Leverkusen, Germany; and tobramycin (TOB; 100%) from Teva, Wilrijk, Belgium. The remaining antibiotics were obtained as the corresponding branded product in Belgium for intravenous use and complied with the provisions of the European Pharmacopoeia with respect to content in active agent: ceftazidime as Glazidim (CAZ; 88.20%) from GlaxoSmithKline, Genval, Belgium; imipenem as Tienam (also containing cilastatin, which does not have any antibacterial activity) (IPM; 45.60%) from MSD, Brussels, Belgium; meropenem as Meronem (MEM; 74.00%) from AstraZeneca, Brussels, Belgium; and piperacillin-tazobactam as Tazocin (TZP; 97.00%) from Wyeth, Louvain-La-Neuve, Belgium (now part of Pfizer).

Susceptibility testing. MICs were determined by microdilution in cation-adjusted Mueller-Hinton broth by following Clinical and Laboratory Standards Institute (CLSI) recommendations, using *P. aeruginosa* ATCC 27853 as a quality control strain (8). Susceptibility was assessed according to the interpretive criteria of both the European Committee on Antimicrobial Susceptibility Testing (EUCAST) (9) and the CLSI (8). Isolates were considered multidrug resistant (MDR) if they were resistant to at least three antibiotic classes among those tested (penicillins/cephalosporins, carbapenems, fluoroquinolones, aminoglycosides, and polymyxins), according to European Centre for Disease Prevention and Control (ECDC) criteria (10).

Screening for extended-spectrum β-lactamases (ESBL) and carbapenemases. For all isolates ($n = 51$) showing MICs of >8 mg/liter for ceftazidime and meropenem, the bla_{TEM}, bla_{SHV}, and bla_{CTX-M} (groups 1, 2, and 9), bla_{VIM}, bla_{IMP}, bla_{KPC}, and bla_{NDM} gene families were detected by real-time multiplex PCR, using group-specific primers (references 11 to 13 and references therein). Genes encoding OXA (OXA-1, -2, -9, -10, -18, -20, -23, -24, -30, -48, -58, and -198), BEL (BEL-1 to -3), PER (PER-1 to -5 and -7), GES (GES-1 to -18), and VEB (VEB-1 to -7) enzymes were also detected by multiplex PCR.

Molecular typing. All MDR isolates in the collection showing coresistance to penicillins and/or cephalosporins and two other classes ($n = 56$) were characterized by pulsed-field gel electrophoresis (PFGE) analysis (14). In addition, 40 pairs of isolates collected simultaneously and in the same sample from 20 patients (see Fig. S1 in the supplemental material) but differing in their profiles of susceptibility to at least one class of antibiotics were also genotyped by PFGE to determine their genetic relatedness. The pulsotype classification criteria designated a pulsotype by one or two letters, including patterns showing zero to six DNA fragment differences (14). An epidemic pulsotype was defined as a pulsotype recovered from ≥2 patients, while a sporadic pulsotype was recovered only once.

Multilocus sequence typing (MLST) was performed on a representative strain of epidemic pulsotypes detected in ≥3 strains, as previously described (15). The reference LES B58 strain (4) was used as a control. MLST data were uploaded to the *P. aeruginosa* MLST Database (http://pubmlst.org/paeruginosa) for allele type and sequence type (ST) assignments (16).

RESULTS

MIC distributions. Table 2 shows the MIC distribution for 9 antipseudomonal drugs against 153 isolates collected from 118 CF patients originating from three different countries over the period from 2006 to 2012, together with the percentages susceptible and resistant based on both EUCAST and CLSI interpretive criteria. The corresponding MIC cumulative distributions are illustrated in Fig. S2 in the supplemental material. Resistance was high in this collection. Using the EUCAST or the CLSI resistance breakpoints, respectively, the rates of full resistance for the isolates were ≥71% or ≥54% for penicillins (ticarcillin, piperacillin, and piperacillin-

TABLE 2 MIC distributions for antipseudomonal antibiotics and corresponding percent susceptibility according to EUCAST or CLSI breakpoints[a]

	MIC (mg/liter)				Susceptibility according to: EUCAST[b]			CLSI[c]		
Antibiotic	Min	Max	50%	90%	% S	% I	% R	% S	% I	% R
TIC	1	>512	128	>512	16	NA	84	16	23	61
PIP	0.5	>512	256	>512	24	NA	76	24	15	61
TZP	0.5	>512	128	512	29	NA	71	29	17	54
CAZ	1	>512	64	512	31	NA	69	31	10	59
IPM	0.25	128	4	32	48	19	33	48	19	33
MEM	0.032	256	2	16	44	36	20	63	17	20
AMK	1	>512	32	128	22	17	61	39	15	46
TOB	0.064	>512	2	16	72	NA	28	72	12	16
CIP	0.064	64	1	8	24	20	56	44	29	27
CST	0.25	>512	1	4	92	NA	8	78	14	8

[a] Min, minimum; max, maximum; S, susceptible; I, intermediate; R, resistant; NA, not applicable (no I category); TIC, ticarcillin; PIP, piperacillin; TZP, piperacillin-tazobactam; CAZ, ceftazidime; IPM, imipenem; MEM, meropenem; AMK, amikacin; TOB, tobramycin; CIP, ciprofloxacin; CST, colistin.

[b] EUCAST breakpoints (in milligrams per liter): for TIC, S ≤ 16 and R > 16; for PIP, S ≤ 16 and R > 16; for TZP, S ≤ 16 and R > 16; for CAZ, S ≤ 8 and R > 8; for IPM, S ≤ 4 and R > 8; for MEM, S ≤ 2 and R > 8; for AMK, S ≤ 8 and R > 16; for TOB, S ≤ 4 and R > 4; for CIP, S ≤ 0.5 and R > 1; and for CST, S ≤ 4 and R > 4.

[c] CLSI breakpoints (in milligrams per liter): for TIC, S ≤ 16, I = 32 to 64, and R ≥ 128; for PIP, S ≤ 16, I = 32 to 64, and R ≥ 128; for TZP, S ≤ 16, I = 32 to 64, and R ≥ 128; for CAZ, S ≤ 8, I = 16, and R ≥ 32; for IPM, S ≤ 4, I = 8, and R ≥ 16; for MEM, S ≤ 4, I = 8, and R ≥ 16; for CIP, S ≤ 1, I = 2, and R ≥ 4; for AMK, S ≤ 16, I = 32, and R ≥ 64; for TOB, S ≤ 4, I = 8, and R ≥ 16; and for CST, S ≤ 2, I = 4, and R ≥ 8.

TABLE 3 Percent cross-resistance or coresistance among pairs of antibiotics and multivariate correlation between MICs of each pair of antibiotics for individual strains[a]

r value (multivariate correlation) \ Percentage of cross- or coresistance										
	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**	TOB	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

[a] Above the diagonal, figures correspond to the percentage of isolates categorized as resistant to the two antibiotics (row/column) using EUCAST breakpoints. Values in bold indicate combinations for which resistance is higher than 30%. The numbers below the diagonal correspond to the correlation coefficient between individual MICs for each pairs of antibiotics. Values higher than 0.75 are in bold. See Table 2, footnote *a*, for abbreviations of antibiotics and Fig. S4 in the supplemental material for the details of this analysis.

tazobactam), 69% or 59% for ceftazidime, 61% or 46% for amikacin, 56% or 27% for ciprofloxacin, ≥20% for carbapenems, and 28 or 16% for tobramycin. Full resistance to colistin was noted for only 8% of the isolates. Strains resistant to ceftazidime and meropenem were screened for the expression of frequent ESBLs, metallo-β-lactamases, and carbapenemases, which returned negative results.

Cross-resistance or coresistance. Cross-resistance or coresistance was examined among pairs of antibiotics. Cross-resistance is defined as the presence of 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 antibiotics, 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. Coresistance refers to the presence of different mechanisms of resistance in the same bacterial isolate and thus necessarily confers resistance to unrelated antibiotic classes (17). Ninety-four strains were considered MDR according the ECDC (10). The upper right part of Table 3 shows the percentage of strains showing cross-resistance or coresistance to pairs of antibiotics according to EUCAST criteria. About two-thirds of the strains were resistant to both penicillins and ceftazidime and more than 40% were resistant to penicillins and ceftazidime together with amikacin or ciprofloxacin. The rates of coresistance between any studied drug and tobramycin, meropenem, and colistin were lower than 28%, 20%, and 8%, respectively. Of note, only 4 strains in the whole collection were coresistant to meropenem, tobramycin, and colistin (see Fig. S3 in the supplemental material).

The lower left part of Table 3 shows the correlation coefficient between the individual MIC for each pair of antibiotics, with the corresponding multivariate analysis presented in detail in Fig. S4 in the supplemental material. The highest degrees of correlation (>0.75) between individual MICs were observed for ticarcillin versus ceftazidime, piperacillin versus piperacillin-tazobactam, ceftazidime versus piperacillin-(tazobactam), imipenem versus meropenem, and amikacin versus tobramycin, suggesting common mechanisms of resistance between these pairs of antibiotics. Yet differences in the intrinsic potency were nevertheless observed between these pairs of drugs throughout the collection (illustrated in Fig. S4 and associated Table B in the supplemental material): tazobactam reduced the MIC of piperacillin by a factor of 1.5 dilution, while ceftazidime MICs were 0.5 and 1 dilution lower than those of ticarcillin and piperacillin, respectively, and similar to those of piperacillin-tazobactam. Meropenem MICs were 1 dilution lower than those of imipenem, and tobramycin MICs were 3 dilutions lower than those of amikacin.

Typing of MDR isolates. Among the 94 MDR isolates, most were resistant to penicillins and/or cephalosporins. Only 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).

PFGE analysis was also performed on 40 isolates collected as pairs from 20 patients and displaying different susceptibility profiles (see Table S1 in the supplemental material). In 12 patients, the pair of *P. aeruginosa* isolates had the same pulsotype, while the 8 other patients had isolates with different pulsotypes.

Analysis per country or age group. Because of the genetic diversity observed between countries, we then examined the distribution of susceptible, intermediate (when applicable), and resistant isolates classified based on the country where they were collected (Fig. 1). Susceptibility rates differed among countries, with lower resistance in Belgium (significant for all antibiotics except ticarcillin and ciprofloxacin) and higher resistance in Germany and the UK (significant for piperacillin-tazobactam in Germany and for imipenem, ciprofloxacin, and colistin in the UK) than the mean value for the whole collection. There was no significant correlation between the patient's age when the isolate was

TABLE 4 Distribution of pulsotypes among the MDR *P. aeruginosa* clinical isolates

Country	No. of MDR strains	No. of pulsotypes		No. of strains in epidemic pulsotype								
		Sporadic	Epidemic	CA[a]	CK	CM	CD	H	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

[a] CA pulsotype corresponds to the LES epidemic clone pulsotype.

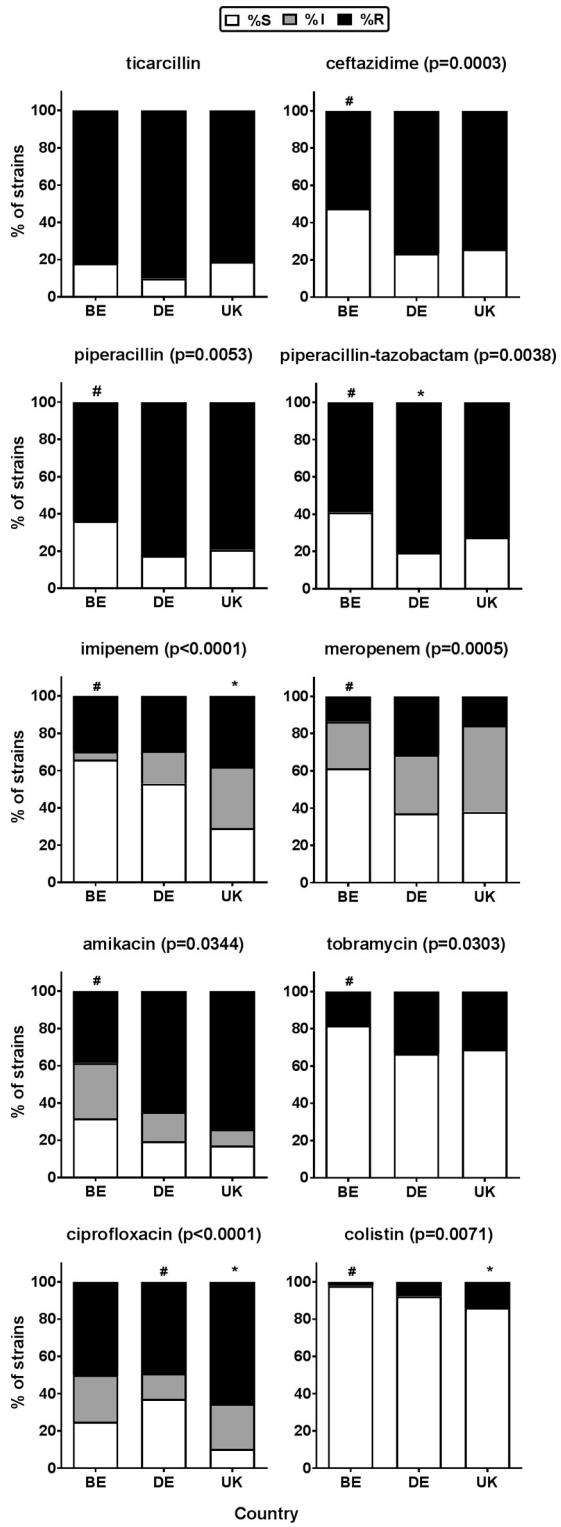

FIG 1 Comparison of the percentage of antibiotic resistance in the collection based on the country of origin of the strain (Belgium [BE]: $n = 44$; Germany [DE]: $n = 51$; United Kingdom [UK]: $n = 58$). Statistical analysis was done by chi square test (*P* values indicated are after the name of the antibiotic); Analysis of means of proportions was done with an α level of 0.05. *, the value is below the mean; #, the value is above the mean.

collected and the number of antibiotic classes to which the isolate was resistant (see Fig. S5 in the supplemental material).

DISCUSSION

In this study, we examined antibiotic susceptibility within a collection of *P. aeruginosa* isolates from CF patients in three northern European countries collected during routine examination, which provides a broader view than the majority of previous surveys, which have focused on a single country (18–20) or a single center (21–23). A key observation is that resistance rates were high in this population, confirming previous studies with CF patients (2), and notably much higher than that which has been reported for isolates collected in northern Europe from intensive care units (24–26). Resistance rates were also higher than those previously reported for strains from CF patients in a German survey from the University of Würzburg, except in the case of tobramycin (isolates collected in 2006 [27]), or in a multicentric study in the UK, except for meropenem and ciprofloxacin (isolates collected in 2000[28]). Moreover, a high degree of cross-resistance or coresistance among antibiotics was observed, which is important from both a pharmacological and a clinical perspective.

From a pharmacological perspective, we noticed, as expected, significant correlations between MICs 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 Fig. S4 and associated Table B in the supplemental material). Focusing on β-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 by tazobactam of the low basal levels of AmpC produced by the wild-type strains (30, 31). Likewise, a higher potency of ceftazidime than for penicillins and of meropenem than for imipenem is reported in wild-type EUCAST distributions (29). Thus, differences in potency among these pairs of drugs in our collection are likely to reflect differences in intrinsic activity rather than in vulnerability to resistance mechanisms. Remarkably, no carbapenemase production was apparent in this collection. The same finding was reported in two recent investigations of *P. aeruginosa* isolates collected over the same period as those examined here. The first of these studies was performed in Australia and examined successively a collection of 662 carbapenem-resistant isolates assembled in 2007 to 2009 from diverse CF centers and of 517 isolates collected in a single CF center in 2011 (32). The second study was performed in Brazil and analyzed isolates from 75 patients collected from 2010 to 2011 (19). In contrast, carbapenemases have been detected in 63 out of 217 *P. aeruginosa* isolates collected from CF patients in China (22). The prevalence of carbapenemase genes could, however, be different in other bacteria infecting CF patients, but there is no large survey published so far for other Gram-negative species (33, 34).

Thus, carbapenem resistance in CF European isolates is probably primarily mediated by the combined effect of AmpC and of a reduced accumulation (porin mutations and/or increased efflux) (35, 53). Of note, however, carbapenem resistance has previously been described for the LES clone (5), but the underlying mechanism(s) has not been investigated to date. For aminoglycosides, the higher potency of tobramycin over amikacin in our collection also reflects what is observed in MIC distributions of wild-type

strains assembled by EUCAST (29). Tobramycin has been described as a poorer substrate than amikacin for the efflux pump MexXY-OprM, considered responsible for natural and adaptive resistance to aminoglycosides in *P. aeruginosa* (36, 37).

Considering our findings from a clinical perspective, a high degree of cross-resistance was observed between penicillins and ceftazidime, which was expected. However, a high degree of coresistance was also apparent between these antibiotics and both ciprofloxacin and amikacin, resulting in 60% of the isolates being categorized as multidrug resistant. In contrast, meropenem, colistin, and, to a lesser extent, tobramycin were active against a large fraction of the isolates, with few strains coresistant to these three antibiotics. Tobramycin and colistin by inhalation are often considered first line for the eradication of early *P. aeruginosa* infection, and tobramycin is also considered first line for chronic therapies (38–40). High concentrations delivered by this route of administration may help to overcome resistance (41, 42).

We also noticed an important genetic diversity among multiresistant isolates collected in Belgium and Germany, while those collected in the UK belong in majority to the Liverpool epidemic strain (LES) clone. Global studies of *P. aeruginosa* population structure concluded 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 of the patients from the environment. However, a series of epidemic clones have been described (7), among which are the LES clone (4), representing 18 of the 24 MDR isolates collected in the UK in our study, and ST17 (7), which differs by only 1 nucleotide from ST958, found in the three countries we investigated. ST2254, the new ST we describe, was distinct from ST146 (LES clone; 5 alleles different) and ST958 or ST17 (6 alleles different).

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).

Although limited, differences in resistance rates between Belgium and the 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 consistent with the previously described phenotypic variability among LES isolates (6). ST958, represented in the three countries, is also found among the MDR clonal complexes (7). In the German collection, higher resistance is essentially related to the presence of more sporadic MDR clones than in the two other countries. We cannot exclude differences in therapeutic management of patients among these three centers that may influence resistance selection (48), but this specific aspect was not within the scope of our study.

Resistance rates were not higher in the older population than in children and young adults. The interpretation of these data needs to be done with caution because (i) we did not follow the evolution of susceptibility over time in single patients and (ii) we do not know the age of first colonization for each patient. With this limitation in mind, the fact that MDR isolates could be found in young people and susceptible isolates in adults may suggest that resistance depends on the initial susceptibility of the infecting strain. A link between emergence of resistance and early antibiotic use in CF patients is still controversial, even though it was underlined in the last report of the Cystic Fibrosis Foundation (2). A recent study in Australia showed that multiresistance in children is correlated with duration of intravenous antibiotic treatment, which was not the case for adults (18). A correlation with antibiotic usage irrespective of patient age (49) or with time after colonization (6) has also been proposed. In contrast, other studies following the evolution of antibiotic susceptibility in successive isogenic isolates from a single patient suggest that resistance can occur either sporadically (50) or without correlation with the time of isolation (51). In these cases, the presence of mutator variants seems to predetermine the risk of developing resistance over time (6).

Our study has a number of limitations, primarily linked to the fact that samples collected during periodic routine examinations may not correspond to the first *P. aeruginosa* infections in these patients. Moreover, as we did not have the history of antibiotic use in these patients, we could not determine if there was a potential link between antibiotic usage and subsequent development of resistance. Nevertheless, this collection reflects the situation CF clinicians face daily, where they have to select antibiotics based on susceptibility testing performed on current isolates. In this context, our data may lead to three clinically meaningful conclusions. First, susceptibility testing is important to perform even for newly infected patients, because they can be colonized very early by MDR clones. Second, these tests should be performed on more than one colony (especially if different phenotypes are evidenced on culture plates), because of potential population heterogeneity with respect to susceptibility profiles (52). Third, prudent use of highly active drugs should be promoted in order to preserve their efficacy. This implies the use of optimized doses if administered by conventional routes or administration by inhalation to ensure high local concentrations that could minimize the risk of selection of resistance.

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REFERENCES

1. **Elborn JS.** 29 April 2016. Cystic fibrosis. Lancet http://dx.doi.org/10.1016/S0140-6736(16)00576-6.
2. **Cystic Fibrosis Foundation.** 2014. Patient registry 2014 annual data report. Cystic Fibrosis Foundatiohn, Bethesda, MD. https://www.cff.org/2014_CFF_Annual_Data_Report_to_the_Center_Directors.pdf/.
3. **Mesaros N, Nordmann P, Plesiat P, Roussel-Delvallez M, Van Eldere J, Glupczynski Y, Van Laethem Y, Jacobs F, Lebecque P, Malfroot A,**

Tulkens PM, Van Bambeke F. 2007. Pseudomonas aeruginosa: resistance and therapeutic options at the turn of the new millennium. Clin Microbiol Infect **13:**560–578. http://dx.doi.org/10.1111/j.1469-0691.2007.01681.x.

4. **Cheng K, Smyth RL, Govan JR, Doherty C, Winstanley C, Denning N, Heaf DP, van Saene H, Hart CA.** 1996. Spread of beta-lactam-resistant Pseudomonas aeruginosa in a cystic fibrosis clinic. Lancet **348:**639–642. http://dx.doi.org/10.1016/S0140-6736(96)05169-0.
5. **Ashish A, Shaw M, Winstanley C, Ledson MJ, Walshaw MJ.** 2012. Increasing resistance of the Liverpool epidemic strain (LES) of Pseudomonas aeruginosa (Psa) to antibiotics in cystic fibrosis (CF)—a cause for concern? J Cyst Fibros **11:**173–179. http://dx.doi.org/10.1016/j.jcf.2011.11.004.
6. **López-Causapé C, Rojo-Molinero E, Mulet X, Cabot G, Moya B, Figuerola J, Togores B, Perez JL, Oliver A.** 2013. Clonal dissemination, emergence of mutator lineages and antibiotic resistance evolution in Pseudomonas aeruginosa cystic fibrosis chronic lung infection. PLoS One **8:**e71001. http://dx.doi.org/10.1371/journal.pone.0071001.
7. **Oliver A, Mulet X, Lopez-Causape C, Juan C.** 2015. The increasing threat of Pseudomonas aeruginosa high-risk clones. Drug Resist Updat **21-22:**41–59.
8. **Clinical and Laboratory Standards Institute.** 2015. Performance standards for antimicrobial susceptibility testing; 25th informational supplement. CLSI document M100-S25. Clinical and Laboratory Standards Institute, Wayne, PA.
9. **European Committee on Antimicrobial Susceptibility Testing.** 2015. Breakpoint tables for interpretation of MICs and zone diameters, version 5.0. http://www.eucast.org/clinical_breakpoints/.
10. **Magiorakos AP, Srinivasan A, Carey RB, Carmeli Y, Falagas ME, Giske CG, Harbarth S, Hindler JF, Kahlmeter G, Olsson-Liljequist B, Paterson DL, Rice LB, Stelling J, Struelens MJ, Vatopoulos A, Weber JT, Monnet DL.** 2012. Multidrug-resistant, extensively drug-resistant and pandrug-resistant bacteria: an international expert proposal for interim standard definitions for acquired resistance. Clin Microbiol Infect **18:**268–281. http://dx.doi.org/10.1111/j.1469-0691.2011.03570.x.
11. **Naas T, Poirel L, Karim A, Nordmann P.** 1999. Molecular characterization of In50, a class 1 integron encoding the gene for the extended-spectrum beta-lactamase VEB-1 in Pseudomonas aeruginosa. FEMS Microbiol Lett **176:**411–419.
12. **Glupczynski Y, Bogaerts P, Deplano A, Berhin C, Huang TD, Van Eldere J, Rodriguez-Villalobos H.** 2010. Detection and characterization of class A extended-spectrum-beta-lactamase-producing Pseudomonas aeruginosa isolates in Belgian hospitals. J Antimicrob Chemother **65:**866–871. http://dx.doi.org/10.1093/jac/dkq048.
13. **Bogaerts P, Naas T, El Garch F, Cuzon G, Deplano A, Delaire T, Huang TD, Lissoir B, Nordmann P, Glupczynski Y.** 2010. GES extended-spectrum beta-lactamases in Acinetobacter baumannii isolates in Belgium. Antimicrob Agents Chemother **54:**4872–4878. http://dx.doi.org/10.1128/AAC.00871-10.
14. **Deplano A, Denis O, Poirel L, Hocquet D, Nonhoff C, Byl B, Nordmann P, Vincent JL, Struelens MJ.** 2005. Molecular characterization of an epidemic clone of panantibiotic-resistant Pseudomonas aeruginosa. J Clin Microbiol **43:**1198–1204. http://dx.doi.org/10.1128/JCM.43.3.1198-1204.2005.
15. **Curran B, Jonas D, Grundmann H, Pitt T, Dowson CG.** 2004. Development of a multilocus sequence typing scheme for the opportunistic pathogen Pseudomonas aeruginosa. J Clin Microbiol **42:**5644–5649. http://dx.doi.org/10.1128/JCM.42.12.5644-5649.2004.
16. **Jolley KA, Maiden MCJ.** 2010. BIGSdb: scalable analysis of bacterial genome variation at the population level. BMC Bioinformatics **11:**595. http://dx.doi.org/10.1186/1471-2105-11-595.
17. **Périchon B, Courvalin P.** 2009. Antibiotic resistance, p 193–204. *In* Schaechter M (ed), Encyclopedia of microbiology. Elsevier, San Diego, CA.
18. **Smith DJ, Ramsay KA, Yerkovich ST, Reid DW, Wainwright CE, Grimwood K, Bell SC, Kidd TJ.** 2016. Pseudomonas aeruginosa antibiotic resistance in Australian cystic fibrosis centres. Respirology **21:**329–337. http://dx.doi.org/10.1111/resp.12714.
19. **Ferreira AG, Leao RS, Carvalho-Assef AP, da Silva EA, Firmida Mde C, Folescu TW, Paixao VA, Santana MA, de Abreu e Silva FA, Barth AL, Marques EA.** 2015. Low-level resistance and clonal diversity of Pseudomonas aeruginosa among chronically colonized cystic fibrosis patients. APMIS **123:**1061–1068. http://dx.doi.org/10.1111/apm.12463.
20. **Llanes C, Pourcel C, Richardot C, Plesiat P, Fichant G, Cavallo JD, Merens A.** 2013. Diversity of beta-lactam resistance mechanisms in cystic fibrosis isolates of Pseudomonas aeruginosa: a French multicentre study. J Antimicrob Chemother **68:**1763–1771. http://dx.doi.org/10.1093/jac/dkt115.
21. **Luna RA, Millecker LA, Webb CR, Mason SK, Whaley EM, Starke JR, Hiatt PW, Versalovic J.** 2013. Molecular epidemiological surveillance of multidrug-resistant Pseudomonas aeruginosa isolates in a pediatric population of patients with cystic fibrosis and determination of risk factors for infection with the Houston-1 strain. J Clin Microbiol **51:**1237–1240. http://dx.doi.org/10.1128/JCM.02157-12.
22. **Li Y, Zhang X, Wang C, Hu Y, Niu X, Pei D, He Z, Bi Y.** 2015. Characterization by phenotypic and genotypic methods of metallo-beta-lactamase-producing Pseudomonas aeruginosa isolated from patients with cystic fibrosis. Mol Med Rep **11:**494–498.
23. **Parkins MD, Glezerson BA, Sibley CD, Sibley KA, Duong J, Purighalla S, Mody CH, Workentine ML, Storey DG, Surette MG, Rabin HR.** 2014. Twenty-five-year outbreak of Pseudomonas aeruginosa infecting individuals with cystic fibrosis: identification of the prairie epidemic strain. J Clin Microbiol **52:**1127–1135. http://dx.doi.org/10.1128/JCM.03218-13.
24. **Riou M, Carbonnelle S, Avrain L, Mesaros N, Pirnay JP, Bilocq F, De Vos D, Simon A, Pierard D, Jacobs F, Dediste A, Tulkens PM, Van Bambeke F, Glupczynski Y.** 2010. In vivo development of antimicrobial resistance in Pseudomonas aeruginosa strains isolated from the lower respiratory tract of intensive care unit patients with nosocomial pneumonia and receiving antipseudomonal therapy. Int J Antimicrob Agents **36:**513–522. http://dx.doi.org/10.1016/j.ijantimicag.2010.08.005.
25. **Fihman V, Messika J, Hajage D, Tournier V, Gaudry S, Magdoud F, Barnaud G, Billard-Pomares T, Branger C, Dreyfuss D, Ricard JD.** 2015. Five-year trends for ventilator-associated pneumonia: correlation between microbiological findings and antimicrobial drug consumption. Int J Antimicrob Agents **46:**518–525. http://dx.doi.org/10.1016/j.ijantimicag.2015.07.010.
26. **Micek ST, Wunderink RG, Kollef MH, Chen C, Rello J, Chastre J, Antonelli M, Welte T, Clair B, Ostermann H, Calbo E, Torres A, Menichetti F, Schramm GE, Menon V.** 2015. An international multicenter retrospective study of Pseudomonas aeruginosa nosocomial pneumonia: impact of multidrug resistance. Crit Care **19:**219. http://dx.doi.org/10.1186/s13054-015-0926-5.
27. **Valenza G, Tappe D, Turnwald D, Frosch M, Konig C, Hebestreit H, Abele-Horn M.** 2008. Prevalence and antimicrobial susceptibility of microorganisms isolated from sputa of patients with cystic fibrosis. J Cyst Fibros **7:**123–127. http://dx.doi.org/10.1016/j.jcf.2007.06.006.
28. **Pitt TL, Sparrow M, Warner M, Stefanidou M.** 2003. Survey of resistance of Pseudomonas aeruginosa from UK patients with cystic fibrosis to six commonly prescribed antimicrobial agents. Thorax **58:**794–796. http://dx.doi.org/10.1136/thorax.58.9.794.
29. **European Committee on Antimicrobial Susceptibility Testing.** 2015. Antimicrobial wild type distributions of microorganisms. http://www.eucast.org/mic_distributions_and_ecoffs/.
30. **Giwercman B, Lambert PA, Rosdahl VT, Shand GH, Hoiby N.** 1990. Rapid emergence of resistance in Pseudomonas aeruginosa in cystic fibrosis patients due to in-vivo selection of stable partially derepressed beta-lactamase producing strains. J Antimicrob Chemother **26:**247–259. http://dx.doi.org/10.1093/jac/26.2.247.
31. **Lister PD, Gardner VM, Sanders CC.** 1999. Clavulanate induces expression of the Pseudomonas aeruginosa AmpC cephalosporinase at physiologically relevant concentrations and antagonizes the antibacterial activity of ticarcillin. Antimicrob Agents Chemother **43:**882–889.
32. **Tai AS, Kidd TJ, Whiley DM, Ramsay KA, Buckley C, Bell SC.** 2015. Molecular surveillance for carbapenemase genes in carbapenem-resistant Pseudomonas aeruginosa in Australian patients with cystic fibrosis. Pathology **47:**156–160. http://dx.doi.org/10.1097/PAT.0000000000000216.
33. **Trancassini M, Iebba V, Citera N, Tuccio V, Magni A, Varesi P, De Biase RV, Totino V, Santangelo F, Gagliardi A, Schippa S.** 2014. Outbreak of Achromobacter xylosoxidans in an Italian cystic fibrosis center: genome variability, biofilm production, antibiotic resistance, and motility in isolated strains. Front Microbiol **5:**138.
34. **Leão RS, Pereira RHV, Folescu TW, Albano RM, Santos EA, Junior LGC, Marques EA.** 2011. KPC-2 carbapenemase-producing Klebsiella pneumoniae isolates from patients with cystic fibrosis. J Cyst Fibros **10:**140–142. http://dx.doi.org/10.1016/j.jcf.2010.12.003.
35. **Tomás M, Doumith M, Warner M, Turton JF, Beceiro A, Bou G, Livermore DM, Woodford N.** 2010. Efflux pumps, OprD porin, AmpC beta-lactamase, and multiresistance in Pseudomonas aeruginosa isolates

from cystic fibrosis patients. Antimicrob Agents Chemother **54:**2219–2224. http://dx.doi.org/10.1128/AAC.00816-09.
36. **Morita Y, Tomida J, Kawamura Y.** 2012. MexXY multidrug efflux system of Pseudomonas aeruginosa. Front Microbiol **3:**408.
37. **Islam S, Oh H, Jalal S, Karpati F, Ciofu O, Hoiby N, Wretlind B.** 2009. Chromosomal mechanisms of aminoglycoside resistance in Pseudomonas aeruginosa isolates from cystic fibrosis patients. Clin Microbiol Infect **15:**60–66. http://dx.doi.org/10.1111/j.1469-0691.2008.02097.x.
38. **Langton Hewer SC, Smyth AR.** 2014. Antibiotic strategies for eradicating Pseudomonas aeruginosa in people with cystic fibrosis. Cochrane Database Syst Rev **11:**CD004197.
39. **Elborn JS, Hodson M, Bertram C.** 2009. Implementation of European standards of care for cystic fibrosis—control and treatment of infection. J Cyst Fibros **8:**211–217. http://dx.doi.org/10.1016/j.jcf.2009.03.001.
40. **Mogayzel PJJ, Naureckas ET, Robinson KA, Mueller G, Hadjiliadis D, Hoag JB, Lubsch L, Hazle L, Sabadosa K, Marshall B.** 2013. Cystic fibrosis pulmonary guidelines. Chronic medications for maintenance of lung health. Am J Respir Crit Care Med **187:**680–689.
41. **Döring G, Flume P, Heijerman H, Elborn JS.** 2012. Treatment of lung infection in patients with cystic fibrosis: current and future strategies. J Cyst Fibros **11:**461–479. http://dx.doi.org/10.1016/j.jcf.2012.10.004.
42. **Flume PA, VanDevanter DR.** 2015. Clinical applications of pulmonary delivery of antibiotics. Adv Drug Deliv Rev **85:**1–6. http://dx.doi.org/10.1016/j.addr.2014.10.009.
43. **Pirnay JP, Bilocq F, Pot B, Cornelis P, Zizi M, Van Eldere J, Deschaght P, Vaneechoutte M, Jennes S, Pitt T, De Vos D.** 2009. Pseudomonas aeruginosa population structure revisited. PLoS One **4:**e7740. http://dx.doi.org/10.1371/journal.pone.0007740.
44. **Ashish A, Paterson S, Mowat E, Fothergill JL, Walshaw MJ, Winstanley C.** 2013. Extensive diversification is a common feature of Pseudomonas aeruginosa populations during respiratory infections in cystic fibrosis. J Cyst Fibros **12:**790–793. http://dx.doi.org/10.1016/j.jcf.2013.04.003.
45. **Workentine ML, Sibley CD, Glezerson B, Purighalla S, Norgaard-Gron JC, Parkins MD, Rabin HR, Surette MG.** 2013. Phenotypic heterogeneity of Pseudomonas aeruginosa populations in a cystic fibrosis patient. PLoS One **8:**e60225. http://dx.doi.org/10.1371/journal.pone.0060225.
46. **Darch SE, McNally A, Harrison F, Corander J, Barr HL, Paszkiewicz K, Holden S, Fogarty A, Crusz SA, Diggle SP.** 2015. Recombination is a key driver of genomic and phenotypic diversity in a Pseudomonas aeruginosa population during cystic fibrosis infection. Sci Rep **5:**7649. http://dx.doi.org/10.1038/srep07649.
47. **Jorth P, Staudinger BJ, Wu X, Hisert KB, Hayden H, Garudathri J, Harding CL, Radey MC, Rezayat A, Bautista G, Berrington WR, Goddard AF, Zheng C, Angermeyer A, Brittnacher MJ, Kitzman J, Shendure J, Fligner CL, Mittler J, Aitken ML, Manoil C, Bruce JE, Yahr TL, Singh PK.** 2015. Regional isolation drives bacterial diversification within cystic fibrosis lungs. Cell Host Microbe **18:**307–319. http://dx.doi.org/10.1016/j.chom.2015.07.006.
48. **Cramer N, Wiehlmann L, Ciofu O, Tamm S, Hoiby N, Tummler B.** 2012. Molecular epidemiology of chronic Pseudomonas aeruginosa airway infections in cystic fibrosis. PLoS One **7:**e50731. http://dx.doi.org/10.1371/journal.pone.0050731.
49. **Mouton JW, den Hollander JG, Horrevorts AM.** 1993. Emergence of antibiotic resistance amongst Pseudomonas aeruginosa isolates from patients with cystic fibrosis. J Antimicrob Chemother **31:**919–926. http://dx.doi.org/10.1093/jac/31.6.919.
50. **Valenza G, Radike K, Schoen C, Horn S, Oesterlein A, Frosch M, Abele-Horn M, Hebestreit H.** 2010. Resistance to tobramycin and colistin in isolates of Pseudomonas aeruginosa from chronically colonized patients with cystic fibrosis under antimicrobial treatment. Scand J Infect Dis **42:**885–889. http://dx.doi.org/10.3109/00365548.2010.509333.
51. **Ho SA, Lee TWR, Denton M, Conway SP, Brownlee KG.** 2009. Regimens for eradicating early Pseudomonas aeruginosa infection in children do not promote antibiotic resistance in this organism. J Cyst Fibros **8:**43–46. http://dx.doi.org/10.1016/j.jcf.2008.08.001.
52. **Foweraker JE, Laughton CR, Brown DFJ, Bilton D.** 2005. Phenotypic variability of Pseudomonas aeruginosa in sputa from patients with acute infective exacerbation of cystic fibrosis and its impact on the validity of antimicrobial susceptibility testing. J Antimicrob Chemother **55:**921–927. http://dx.doi.org/10.1093/jac/dki146.
53. **Chalhoub H, Sáenz Y, Rodriguez-Villalobos H, Denis O, Kahl BC, Tulkens PM, Van Bambeke F.** High-level resistance to meropenem in clinical isolates of *Pseudomonas aeruginosa* in the absence of carbapenemases: role of active efflux and porin alterations. Int J Antimicrob Agent, in press.

Antimicrobial susceptibility of *Pseudomonas aeruginosa* isolated from Cystic Fibrosis patients through Northern Europe

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SUPPLEMENTARY MATERIAL

Table S1: MIC of isolates collected simultaneously in individual patients and classified according to their PFGE pattern

Patient ID	Country	PFGE	Age	Collection date	TIC	PIP	TZP	CAZ	IMI	MER	CIP	AMK	TOB	CST
pairs with identical PFGE patterns														
AD	UK	CA	18	13-09-06	**128**	**256**	2	**16**	4-8	2	**2**	8-16	1-2	2
AD	UK	CA	18	13-09-06	**32**	8	0.5	8	4-8	2	**2**	**64**	**8**	4
JP	UK	YY	19	10-05-06	16	8-16	4	1-2	1	0.125	0.5	**32-64**	4	1
JP	UK	YY	19	10-05-06	8	2	2	8	1-2	2-4	**8**	**256**	**128**	**16**
BAM75	BE	CV	35	26-09-10	**128**	**>512**	**512**	**>512**	2-4	1-2	**32**	16	2	2
BAM75	BE	CV	35	26-09-10	**32**	4-8	8	2	0.5	0.5	1	16	2	0.5
BCM75	BE	CP	35	02-09-10	**512**	**>512**	**128**	**>512**	**32**	**16**	**32**	**>512**	**>512**	4
BCM75	BE	CP	35	02-09-10	**512**	**>512**	**128**	**512**	**32**	**16**	**8**	4	0.5	1
JSF89	BE	CM	21	12-10-10	**>512**	**>512**	**512**	**256**	**32**	**16**	**2-4**	**64**	2	1
JSF89	BE	CM	21	12-10-10	**512**	**>512**	**256**	**512**	**32**	**16**	**8**	**128**	**16**	0.25
RCF62	BE	CB	48	09-09-10	**128**	**256**	**128**	**32**	**32**	8	**4**	16	1	1
RCF62	BE	CB	48	09-09-10	**64**	**128**	**32**	**8-16**	2	1	**2**	**32-64**	2	1
127	DE	YI	31	10-07-12	**>512**	16	16	8	**32-64**	8	1	**64**	**8-16**	1
127	DE	YI	31	10-07-12	**512**	**>512**	**512**	**256**	8	8	0.5	**64**	**8**	0.5
178	DE	CD	49	27-07-12	1	2	2	1	1	0.25	1	4	0.5	1
178	DE	CD	49	27-07-12	**>512**	**>512**	**>512**	**>512**	**128**	**256**	**8**	16	2	0.5
205	DE	CR	36	09-08-12	**>512**	**>512**	**>512**	**64**	2	**32**	**4**	16	1	1
205	DE	CR	36	09-08-12	**256**	**512**	**256**	**64**	2	**32**	**4**	**32**	2	**256**
158	DE	WY	23	18-07-12	2	4	4	4-8	1-2	0.5	**4**	**64**	**16**	4
158	DE	WY	23	18-07-12	**64**	**32-64**	**32**	4-8	1	0.25	**2**	**64**	**8**	2-4
191	DE	CK	36	03-08-12	**128**	**>512**	**512**	**>512**	1	0.25	**2**	**512**	**32**	2
191	DE	CK	36	03-08-12	**256**	**>512**	**256**	**>512**	0.5	0.5	0.25	**64**	4	2-4
208	DE	H	32	09-08-12	1	**256**	**256**	**256**	**16**	8	**32**	**256**	**64**	2-4
208	DE	H	32	09-08-12	**256**	**>512**	**512**	**>512**	1-2	0.25	**2**	**512**	**128**	2

pairs with different PFGE patterns														
AON	UK	WT	41	2007	**64**	**64**	**32**	4-8	**16**	4-8	**2**	4-8	0.25	1
AON	UK	CQ and CA	41	2007	**>512**	**512**	**256**	**128**	**16**	8	**2**	**64**	2	**128**
AW	UK	CA	48	2007	**>512**	**512**	**256**	**512**	**16-32**	**32**	**2**	**64**	4-8	**64**
AW	UK	WG	48	2007	**>512**	**>512**	**512**	**512**	**64**	**32**	**8**	**64**	4	1-2
CC	UK	CA	22	11-10-06	**>512**	**256-512**	**128**	**512**	8	2	**2**	**64**	4	2
CC	UK	CS	22	11-10-06	**>512**	**>512**	**>512**	**>512**	**32**	**16-32**	**8-16**	**>512**	**128**	1-2
CI	UK	WL	24	2007	**512**	**>512**	**256**	**256**	1	0.5	1	**64**	2	1-2
CI	UK	CA	24	2007	**>512**	**>512**	**512**	**512**	**16**	8	**2**	**256**	**32**	4
LS	UK	WN	20	2007	**64**	8	8	4	1	1	**2**	8	0.5	1
LS	UK	CA	20	2007	**>512**	**>512**	**512**	**512**	**16**	8	0.5-1	**128**	4-8	4
143	DE	WI	29	12-07-12	**64**	**512**	**256**	**128**	2	0.5	0.5	**128**	**16**	2
143	DE	CV	29	12-07-12	16	4	4	2-4	1	0.25	0.25-0.5	**32-64**	4	1
192	DE	WS	48	04-08-12	**32**	**32-64**	16	**16**	8	4	0.5	16	1-2	2
192	DE	CO	48	04-08-12	**>512**	**256**	**256**	**>512**	2	**32**	1	8	1	0.5
195	DE	WP and CJ	31	06-08-12	**128**	**256**	**256**	**16**	8	**16-32**	**64**	**64**	4	0.25
195	DE	CJ	31	06-08-12	**256**	4	4	8	2	**16**	**4**	**32**	1-2	0.5

values in bold: above the EUCAST R breakpoint

Difference in MIC conferring resistance to one of the two isolates (MIC > EUCAST breakpoint for one of the isolates in the pair)

Difference in MIC of at least 2 dilutions between the two isolates in the pair, but no change in S/R categorization

Figure S1: Distribution of replicates in the collection (total number of patients = 118)

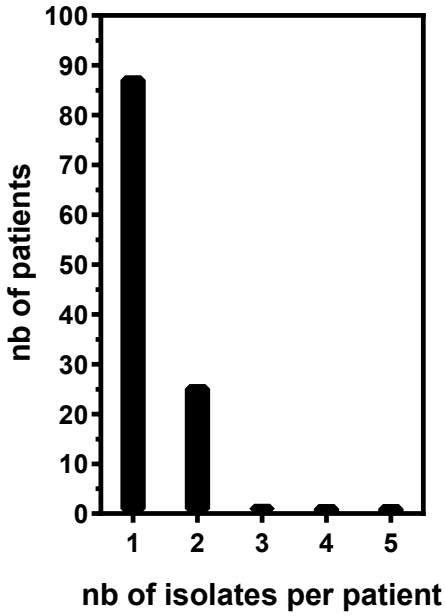


Figure S2: MIC distributions for the antibiotics under study (n=153)
CST: Colistin; CIP: Ciprofloxacin TOB: Tobramycin; AMK: Amikacin; MEM: Meropenem; IPM: Imipenem; CAZ: Ceftazidime; TZP: Piperacillin-tazobactam; PIP: Piperacillin; TIC: Ticarcillin

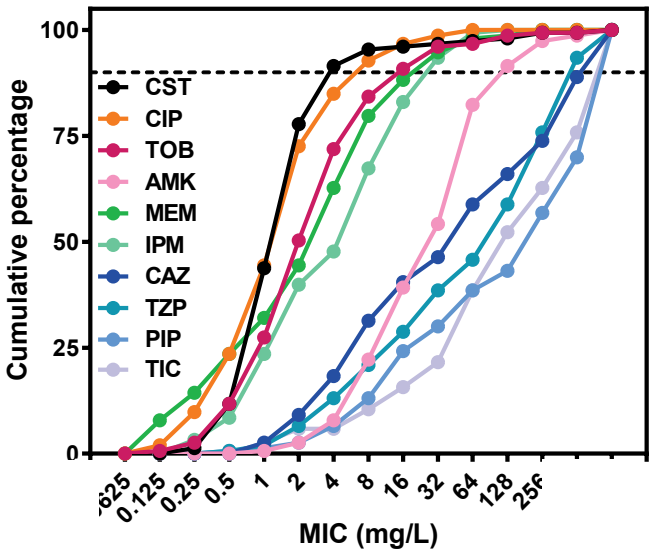


Figure S3

Tridimensional plot analysis of individual MICs of colistin (CST), meropenem (MEM) and tobramycin (TOB) among the whole collection.

Strains resistant (as per EUCAST criteria) to two or three of these antibiotics are highlighted in specific colors. MICs are expressed as log_2 of their value.

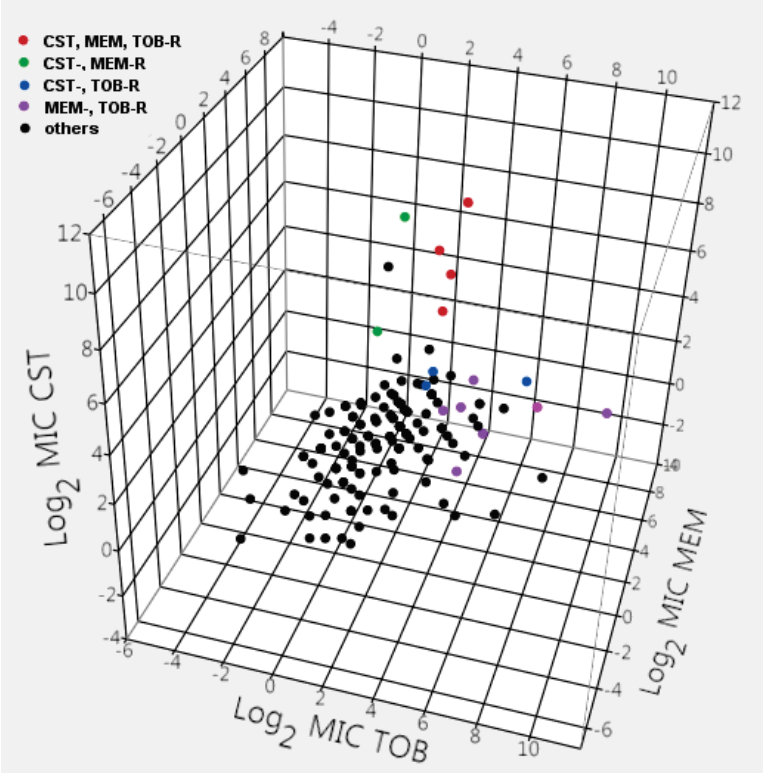
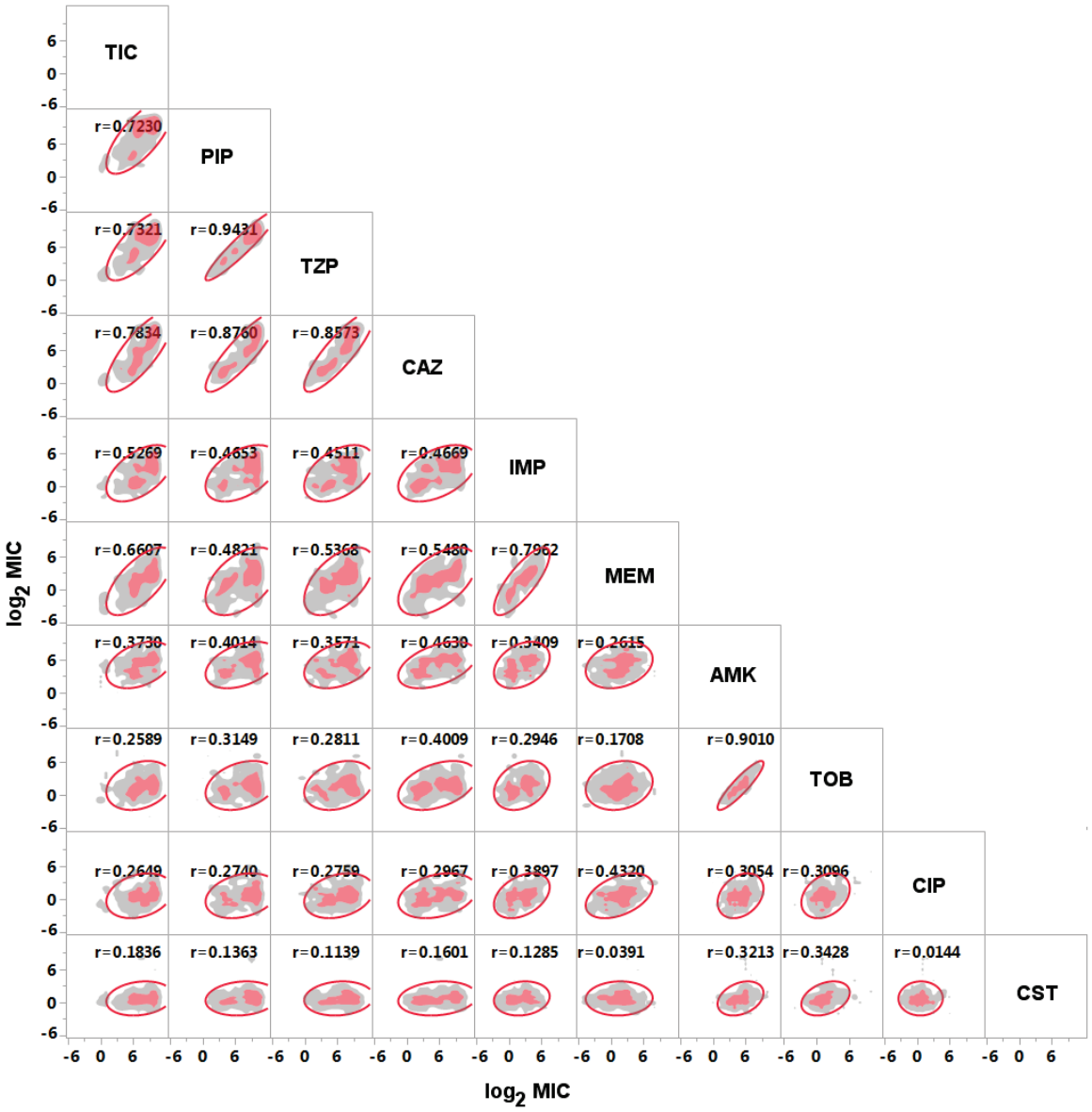


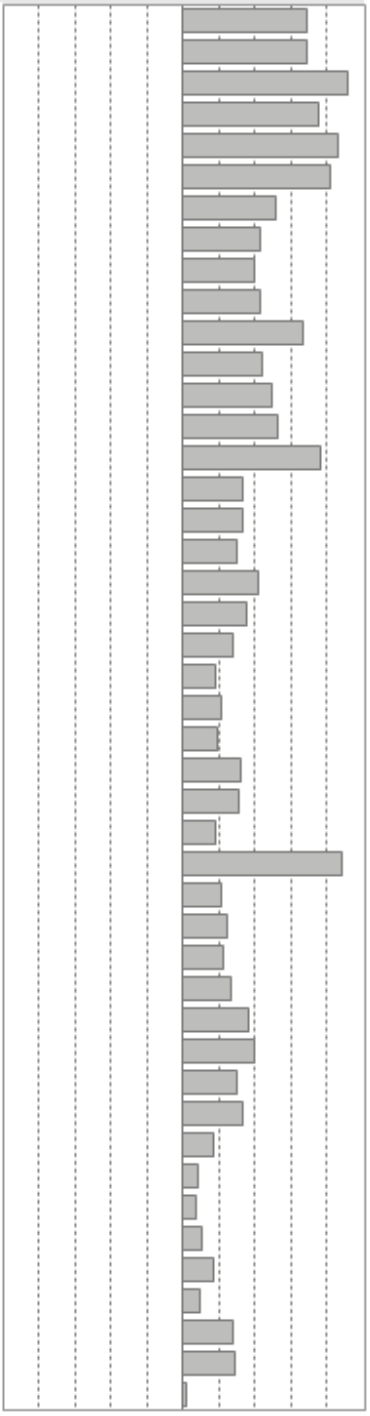
Figure S4: multivariate correlation analysis of MICs for pairs of antibiotics.
The graphs show the density plots and density ellipse (α: 95%) for individuals pairs of MICs (expressed in as log_2 of values) together with the correlation coefficient (r). The tables below the graph show respectively the details of statistical analyses for pairwise correlations and the equation of the linear correlation for antibiotic pairs with r values > 0.75.
TIC: Ticarcillin; PIP: Piperacillin; TZP: Piperacillin-tazobactam: CAZ: Ceftazidime; IPM: imipenem; MEM: Meropenem; AMK: Amikacin; TOB: Tobramycin; CIP: Ciprofloxacin; CST: Colistin



A. statistical analyses for pairwise correlations

Pairwise Correlations

Variable	by Variable	Correlation	Count	Lower 95%	Upper 95%	Signif Prob
PIP	TIC	0.6943	153	0.6019	0.7683	<.0001*
TZP	TIC	0.6888	153	0.5952	0.7640	<.0001*
TZP	PIP	0.9147	153	0.8844	0.9373	<.0001*
CAZ	TIC	0.7587	153	0.6822	0.8188	<.0001*
CAZ	PIP	0.8628	153	0.8159	0.8985	<.0001*
CAZ	TZP	0.8256	153	0.7675	0.8703	<.0001*
IPM	TIC	0.5199	153	0.3937	0.6268	<.0001*
IPM	PIP	0.4271	153	0.2879	0.5486	<.0001*
IPM	TZP	0.4016	153	0.2594	0.5267	<.0001*
IPM	CAZ	0.4370	153	0.2990	0.5570	<.0001*
MEM	TIC	0.6671	153	0.5686	0.7467	<.0001*
MEM	PIP	0.4476	153	0.3111	0.5661	<.0001*
MEM	TZP	0.4971	153	0.3674	0.6078	<.0001*
MEM	CAZ	0.5321	153	0.4078	0.6370	<.0001*
MEM	IPM	0.7680	153	0.6939	0.8260	<.0001*
AMK	TIC	0.3389	153	0.1904	0.4721	<.0001*
AMK	PIP	0.3306	153	0.1814	0.4648	<.0001*
AMK	TZP	0.3013	153	0.1498	0.4390	0.0002*
AMK	CAZ	0.4207	153	0.2807	0.5431	<.0001*
AMK	IPM	0.3591	153	0.2125	0.4898	<.0001*
AMK	MEM	0.2824	153	0.1296	0.4222	0.0004*
TOB	TIC	0.1878	153	0.0300	0.3365	0.0201*
TOB	PIP	0.2215	153	0.0651	0.3672	0.0059*
TOB	TZP	0.1903	153	0.0326	0.3388	0.0185*
TOB	CAZ	0.3287	153	0.1794	0.4632	<.0001*
TOB	IPM	0.3153	153	0.1649	0.4514	<.0001*
TOB	MEM	0.1869	153	0.0290	0.3356	0.0207*
TOB	AMK	0.8853	153	0.8454	0.9154	<.0001*
CIP	TIC	0.2171	153	0.0605	0.3633	0.0070*
CIP	PIP	0.2492	153	0.0943	0.3924	0.0019*
CIP	TZP	0.2247	153	0.0684	0.3701	0.0052*
CIP	CAZ	0.2650	153	0.1109	0.4065	0.0009*
CIP	IPM	0.3631	153	0.2169	0.4934	<.0001*
CIP	MEM	0.4021	153	0.2600	0.5271	<.0001*
CIP	AMK	0.3062	153	0.1551	0.4433	0.0001*
CIP	TOB	0.3330	153	0.1841	0.4670	<.0001*
CST	TIC	0.1782	153	0.0201	0.3276	0.0275*
CST	PIP	0.0873	153	-0.0723	0.2426	0.2831
CST	TZP	0.0760	153	-0.0837	0.2319	0.3506
CST	CAZ	0.1043	153	-0.0553	0.2587	0.1993
CST	IPM	0.1768	153	0.0186	0.3263	0.0288*
CST	MEM	0.1020	153	-0.0576	0.2565	0.2095
CST	AMK	0.2808	153	0.1279	0.4208	0.0004*
CST	TOB	0.2883	153	0.1359	0.4275	0.0003*
CST	CIP	0.0211	153	-0.1381	0.1791	0.7961



B. Equations of linear correlations for antibiotics pairs when r values are > 0.75

X variable	Y variable	slope	Y intercept (log_2)
TZP	PIP	0.94	1.4
TZP	CAZ	0.90	0.1
PIP	CAZ	0.92	-0.9
TIC	CAZ	0.87	-0.5
IPM	MEM	0.95	-0.7
AMK	TOB	0.99	-3.2

- All slopes are close to 1, denoting that MICs to both X and Y antibiotics increase in parallel.
- A Y intercept
    - close to 0 indicates that MICs of X and Y are globally similar;
    - close to +1, that MIC of Y are globally 1 doubling dilution higher than those of X;
    - close to -1, that MICs of Y are globally 1 doubling dilution lower than those of X.

Figure S5
Multiresistance as a function of patient's age.
The graph shows the number of antibiotic classes (among penicillins, cephalosporins, carbapenems, fluoroquinolones, aminoglycosides, polymyxins) to which each isolate is resistant (MICs > EUCAST "R" breakpoint) as a function of the patient's age pooled in categories (left) or individually but according to the country of origin (UK: United Kingdom; BE: Belgium; DE: Germany).
Statistical analysis: left panel: one-way ANOVA: p= 0.467; right panel: Pearson coefficient for the whole collection: 0.047