ORIGINAL ARTICLE

MIC score, a new tool to compare bacterial susceptibility to antibiotics application to the comparison of susceptibility to different penems of clinical strains of *Pseudomonas aeruginosa*

Cédric Bretonnière^{1,2}, Adeline Maitte¹, Jocelyne Caillon¹, Gilles Potel¹, David Boutoille¹, Cédric Jacqueline¹ and Christophe Guitton²

This study aimed to compare the susceptibility to carbapenems (imipenem, meropenem and doripenem) of clinical strains of *Pseudomonas aeruginosa*. It also studied whether susceptibility to imipenem or meropenem could predict, reliably, susceptibility to doripenem. Pseudomonal strains were collected from respiratory specimens, half of them from cystic fibrosis patients. MICs were determined according to European Committee on Antimicrobial Susceptibility Testing recommendations. Carbapenems were compared according to the susceptible, intermediate or resistant categories. A new approach also allowed comparing these carbapenems in a 'MIC score' taking into account the differences in breakpoints between drugs. One hundred thirty-nine strains were studied. They were found to be statistically more susceptible to meropenem than to the two other drugs. However, this difference was small: less than one dilution between the agents. This study also highlighted a significant correlation between susceptibility to penems taken in pairs. However, susceptibility to imipenem or meropenem did not reliably predict susceptibility to doripenem. Despite potential differences in resistance mechanisms, the *Pseudomonas aeruginosa* strains showed close susceptibility to three carbapenems. This was true for both cystic fibrosis patients and others. However, there were variations between strains. That justifies MICs to be determined for each of the three penems. This might be useful in case of elevated MICs and/or for potentially difficult-to-treat infections such as pneumonia in patients with cystic fibrosis patients.

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INTRODUCTION

Pseudomonal infections are common, especially in specific populations such as in intensive care patients or cystic fibrosis (CF) patients.^{1–4} As with other species, physicians treating *Pseudomonas aeruginosa* are facing an increase in resistant strains.^{5–8} This is concerning as there are relatively few treatment options.^{1,9,10} The carbapenems are a class of antibiotics that are currently very active against these strains.^{11–13}

Doripenem is the newest penem.^{14,15} Some studies have compared the different penems; however, they are relatively few.^{16,17} In addition, none of them really takes into account differences in breakpoints between agents.¹⁸ The primary endpoint was therefore to compare imipenem, meropenem and doripenem while taking account of these specific breakpoints, thanks to a simple and original method. MIC-based comparisons will be replaced by this new breakpoint-based scoring system. In addition, doripenem is not routinely tested in laboratories for strains of *Pseudomonas*.¹⁹ A secondary endpoint of the present study was therefore to test the hypothesis that the results of the susceptibility of a given strain to imipenem or meropenem may predict susceptibility to doripenem.

MATERIALS AND METHODS

Bacterial strains

One hundred and thirty-nine strains of *Pseudomonas aeruginosa* were studied. These bacteria were isolated from various respiratory specimens from different patients admitted to the University Hospital of Nantes. This is a 3000 bedded University Hospital providing care for a population of one million inhabitants. After exclusion of duplicate and redundant samples, over a 6-month period, 67 consecutive strains from patients with CF were analyzed. Seventy-two strains from non-CF patients were also studied at the same time.

Antimicrobial drugs

The tested antibiotics were imipenem/cilastatin (Merck Sharp & Dohme-Chibret, Paris, France), meropenem (AstraZeneca, Rueil-Malmaison, France) and doripenem (Janssen-Cilag, Issy-les-Moulineaux, France). Stock solutions (2000 mcg ml⁻¹) of each antibiotic were prepared from commercial powders of the antibiotics according to manufacturer's recommendations. Thus, cilastatin was also in the used solution but a correction was applied in order to have a final concentration of antibacterially active imipenem of 2000 mcg ml⁻¹.

The EMA withdrew the marketing authorization of doripenem mid-2014. Nevertheless, the drug remains available in Japan or in the United States.

¹UPRES EA 3826, Faculté de Médecine, Université de Nantes, Nantes, France and ²CHU Nantes, PHU3, Service de Réanimation Médicale Polyvalente, Nantes, France
Correspondence: Dr C Bretonnière, UPRES EA 3826, Faculté de Médecine, Université de Nantes, 1 rue Gaston Veil, 44035 Nantes, France.
E-mail: cedric.bretonniere@chu-nantes.fr

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Minimum inhibitory concentrations

MICs were determined by the agar dilution method on Mueller–Hinton as recommended by the European Committee on Antimicrobial Susceptibility Testing (EUCAST).²⁰ In brief, a series of increasing antibiotic concentrations, from 0.03 to 64 mg l⁻¹, was prepared for each agent. The inoculum for each strain was standardized at 0.5 Mac Farland then diluted to 1:10, so that each of the Steers multipoint inoculator laid 10⁴ CFU on the agar. After 18 h of incubation at 35 °C, the MIC for each strain was defined as the lowest concentration of antibiotic leaving no growth or less than three colonies at each inoculation of corresponding points.

The current EUCAST recommendations for imipenem, meropenem and doripenem were used to classify *P. aeruginosa* strains as susceptible (S), intermediate (I) or resistant (R; Table 1).

Ethical committee

For this study without clinical data, the local ethical committee ruled out the need for informed consent.

Statistical analysis—MIC score

The first part of the analysis consisted of the comparison of the susceptibility of the *P. aeruginosa* strains to each of the three carbapenems. The categories (susceptible, intermediate or resistant) were first compared by Fischer's exact test.

To take into account the differences in breakpoints, a MIC score defined as follows was used. A score of zero is assigned to an intermediate strain. A negative score of -1 is assigned to a strain with a MIC to the upper terminal of the susceptible class. A score of -2 is assigned if the strain has an MIC one dilution lower than the upper terminal of the susceptible class, a score of -3 if the strain has a MIC two dilutions lower than the upper terminal of the susceptible class and so on. On the contrary, a positive score indicates a resistant strain. The correspondence between these scores and actual MICs are summarized in Table 2. Presenting MICs that way allows the comparison between the three antibiotics, despite their different breakpoints. The observed difference between the average scores for two out of the three studied antibiotics (Student's *t*-test) reflects the gap in dilutions between these two agents. If this difference is statistically different from zero, it is concluded that there is a difference between the two drugs.

In a second step, we looked at whether the results of the susceptibility to one of the three antibiotics could serve as surrogates for the two other agents: if so, for instance, it could be proposed to test the susceptibility to imipenem and extrapolate the results to meropenem and doripenem. The correlation between the MIC scores was tested using the Spearman's correlation test. For each pair of penems, the scattergrams were built and presented. To predict doripenem

susceptibility, the surrogate value of imipenem or of meropenem was investigated in terms of error rates as follows: a very major error was defined as the percent of isolates susceptible to one or two agents and resistant to doripenem, a major error was defined as the percent of isolates resistant to one or two agents and susceptible to doripenem, and minor errors were any categorical disagreements that resulted in an intermediate for one carbapenem and either susceptibility or resistance for doripenem. A testing methodology is usually defined as acceptable when the sum of very major errors and major errors is 3% or less. In addition, minor errors must be 10% or less. Surrogate predictive value was also assessed as described previously. Surrogate predictive value was defined as the percentage of meropenem (or imipenem)-susceptible isolates that were susceptible to doripenem.

Given the particular population of our study, we will focus on some analyses in subgroups (patients CF vs non-CF patients).

All statistical analyses were performed using Microsoft Office Excel 2010 and IBM SPSS Statistics 19.0. *P*-value <0.05 were considered significant.

RESULTS

MICs for were available for 139 strains (Figure 1). MIC₅₀ were 4, 2 and 1 µg ml⁻¹, respectively for imipenem, meropenem and doripenem and MIC₉₀ were 32 mcg ml⁻¹ for imipenem and 16 for both meropenem and doripenem. The distribution of the three categories (susceptible, intermediate and resistant) was statistically different between the three antibiotics, not only for the entire cohort but also for the two subgroups (CF and non-CF patients; see electronic Supplementary Material).

The comparison of the MIC scores revealed that the *P. aeruginosa* strains in our study were statistically more susceptible to meropenem than to the other two drugs. The average difference in MIC scores between meropenem and imipenem was 0.4 (95% confidence interval: 0.20–0.66, *P*<10⁻³) and 0.6 (95% confidence interval: 0.39–0.76, *P*<10⁻⁶) between meropenem and doripenem. There was no statistical difference in susceptibility between imipenem and doripenem (0.1 (95% confidence interval: -0.08 to 0.32), *P*>0.05).

Because of the differences in breakpoints such as defined by EUCAST, the MIC score zero is assigned to two classes of MIC for meropenem against only one for imipenem or doripenem (Table 2). For that reason, the score has been forced by assigning +1 to the upper class of the intermediate class for meropenem (and the following scores are shifted accordingly). The observed difference between the agents remained statistically significant; meropenem remaining superior to doripenem (data non shown). Similarly, the lowest MICs of doripenem could disadvantage doripenem. But, even if the scoring system is changed censoring extreme values (minimum score of -6 and maximum score of 3 for the three study drugs), we found the same differences between the agents (data not shown).

Significant correlations were found when two agents were compared with each other. These results are shown in Figure 2.

The prediction of susceptibility to doripenem from tests to meropenem or imipenem was not easy. Indeed, there was variability between the three penems as evidenced by the scattergrams presented in Figure 3. Thus, in this cohort of *P. aeruginosa* neither doripenem

Table 1 Breakpoints for the three carbapenems following EUCAST's recommendations

	MIC breakpoints (mg l ⁻¹)	
	S	R
Imipenem	≤4	>8
Meropenem	≤2	>8
Doripenem	≤1	>2

Abbreviation: EUCAST, European Committee on Antimicrobial Susceptibility Testing.

Table 2 Correspondence between MIC scores and actual MICs

MIC (µg ml ⁻¹)	0.03	0.06	0.125	0.25	0.5	1	2	4	8	16	32	64
Imipenem	-8	-7	-6	-5	-4	-3	-2	-1	0	1	2	3
Meropenem	-7	-6	-5	-4	-3	-2	-1	0	0	1	2	3
Doripenem	-6	-5	-4	-3	-2	-1	0	1	2	3	4	5

This transformation of MICs into MIC scores allows comparison between the three agents, taking into account the different MIC breakpoints.

nor meropenem can serve as perfect surrogate marker for doripenem. Indeed, although very major or major errors were rare, minor errors occurred in over 10% of cases (Table 3).

DISCUSSION

Doripenem, a relatively new drug, originally appeared as a promising antibiotic, probably more efficient than previously developed penems.^{17,19,21–23} This was noticed even in populations of patients for whom antibiotic use can be very complex such as CF patients.^{1,24} However, data specifically comparing the susceptibility of

Pseudomonas aeruginosa to the three available carbapenems are lacking. None takes into account differences in breakpoints.^{18,25,26} The new scoring system that we propose is original and very easy to implement. It seems interesting because it allows to make 'a real difference in dilutions' (incorporating the difference in breakpoints) between two agents and it seems that this concept may be useful to clinicians or to clinical microbiologists. This score can be criticized. Nevertheless, even when correcting points possibly disfavoring doripenem, the same differences were observed. The results seem then quite robust.

It is interesting to note that in two previous experimental studies, we have demonstrated a really good efficacy of meropenem; this at doses that might seem a little low today.^{27,28}

Thus, we demonstrate a difference between the three penems, for the first time in the favor of meropenem. This difference is statistically significant. However, is it clinically relevant? We do not think so. An average difference of 0.4 or 0.6 in dilutions does not seem sufficient to be a decision criterion for the choice of one drug versus another. Indeed, classical PK/PD parameters remain key determinants in the choice of one antibiotic.^{29–31} But, to date, the scoring system is not validated for PK/PD calculations.

Finally, there is little difference between the three drugs. This was not necessarily expected. The oprD mutations are often involved in resistance to imipenem but not for meropenem or doripenem. However, the mechanisms of resistance in *Pseudomonas* are much more complex.³² Thus, several mechanisms may be involved simultaneously to explain resistance to carbapenems such as production of a metallo- β -lactamase, overexpression of efflux pumps. The three agents can thus be affected (class effect).

Our aim was also to try to determine whether the susceptibility test to one penem could replace tests with the other drugs in this class.

Figure 1 Distribution of the *Pseudomonas aeruginosa* strains ($n=139$) according to their susceptibility to penems. Numbers for each class are detailed. χ^2 -test revealed statistical differences in the distribution of the susceptibility among the three penems ($P < 10^{-6}$).

Figure 2 Correlation between MIC scores for the three penems taken in pairs. graph **a** shows the correlation between imipenem (x axis) and doripenem (y axis). For each pair (graph **a**, **b** or **c**) the association is highly statistically significant ($P < 10^{-6}$). A full color version of this figure is available at *The Journal of Antibiotics* journal online.

Figure 3 MIC scattergrams of the three penems taken in pairs against 139 *Pseudomonas aeruginosa*. European Committee on Antimicrobial Susceptibility Testing (EUCAST) breakpoints are indicated by dashed lines. One MIC for doripenem was not available so that the first scattergram presents.

Table 3 Categorical agreement, error rates and surrogate predictive value for imipenem and meropenem to predict doripenem interpretative criteria against 139 *Pseudomonas aeruginosa*

Surrogate antibiotic	Categorical agreement (%)	Error rates (%)			Surrogate predictive value
		Minor	Major	Very major	
Imipenem	87.8%	11.5%	0.7%	0.0%	100.0%
Meropenem	74.1%	25.2%	0.0%	0.7%	92.0%

For antimicrobial classes that contain more agents (penicillins, cephalosporins, fluoroquinolones), it is well recognized that in fact only one or two agents have to be tested and extrapolations are acceptable. Using such surrogates is, of course, cost effective. It is interesting here as doripenem is not routinely available in all laboratories. The major or very major errors are effectively low level (Table 3). Nevertheless, the rate of minor errors is much higher so that it seems difficult to recommend using imipenem or meropenem as surrogate markers instead of doripenem. The surrogate predictive value is excellent but it is not a recognized indicator yet.¹⁹

However, our study has limitations. The main limitations are first that it is a single-center study and second that it has a relatively small sample size. Nevertheless, we believe that this ‘snapshot’ is accurate since in the baseline study for doripenem, each country ($n=16$) was represented by an average of only five centers resulting in a total of only about 150 PA strains per country.¹⁷ In addition, it is interesting to note that the breakpoints vary depending on the organization that determines them (EUCAST, FDA or CLSI) and our results according to EUCAST would probably be different if other breakpoints were applied.

CONCLUSION

In conclusion, we showed that, in our cohort of *Pseudomonas aeruginosa* (with a significant number of CF patients), differences in susceptibility between the penems exist without being major. Because of these differences, the MIC should be determined for the three drugs when facing a potentially difficult-to-treat infection (resistance involving several classes of antibiotics, specific infectious sites...). Finally, the MIC score could be a very useful tool for clinicians, and epidemiologists, to compare clinical strains. It would be, for example, really helpful to recommend an antibiotic, rather than another, for an empirical treatment when susceptibility testing results are not yet available.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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