17 *In Vitro* Sensitivity of *Acinetobacter baumannii* and *Pseudomonas aeruginosa* to Carbapenems Among Intensive Care Unit Patients

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Abstract

Acinetobacter baumannii and *Pseudomonas aeruginosa* pathogens are the most common causes of fatal pneumonia among patients treated in Intensive Care Units (ICU). Carbapenems remain a group of antibiotics characterized by the highest effectiveness in treatment of heavy infections of the lower respiratory tract. This study compared *in vitro* sensitivity of *A. baumannii* and *P. aeruginosa* to three carbapenems: imipenem, meropenem and doripenem. The material was collected from 71 patients treated in the ICU from April 2009 to January 2010. Bronchial tree was the predominant source of samples. Fifty-four strains of *A. baumannii* and 17 strains of *P. aeruginosa* were analyzed. Sensitivity to carbapenems was interpreted in line with Clinical and Laboratory Standard Institute (CLSI) and European Committee for Antimicrobial Susceptibility Testing (EUCAST) criteria (imipenem and meropenem) or in compliance with the Food and Drug Administration (FDA) and CLSI guidelines (doripenem). We found that *A. baumannii* was significantly more often sensitive to imipenem than to doripenem and meropenem, but only according to the CLSI and FDA and not EUCAST criteria. The sensitivity of *P. aeruginosa* was higher to imipenem than to doripenem and meropenem, according to both CLSI and EUCAST criteria (64.7 %).

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We conclude that the EUCAST criteria demonstrate a higher rigor than those of CLSI and FDA in the determination of carbapenems sensitivity. Imipenem appears more effective than doripenem and meropenem in treatment of *A. baumannii* and *P. aeruginosa* infections.

Keywords
Acinetobacter baumannii • Carbapenems • *Pseudomonas aeruginosa* • Resistance • Sensitivity

1 Introduction

Infections, primarily due to pneumonia, account for the major cause of fatalities occurring in Intensive Care Units (ICU). Ventilator-associated pneumonia (VAP) occurs in 10–20 % of patients mechanically ventilated for more than 48 h and the mortality rate resulting from pneumonia is at a high level and ranges from 30 to 70 % (Kollef et al. 2005). High percentages of therapy failures in patients with infections treated in ICU are associated with improper choice of antibiotics for empiric therapy, whose spectrum does not cover the wide range of microorganisms and their mechanism of resistance. Empiric antibiotic therapy should follow the generally accepted current recommendations and consider the information about local pathogens and their susceptibility. The effectiveness of individual therapy regimens differs according to the country, region, and health institution, and depends markedly on the spectrum of microorganisms, antibiotics used earlier and the resultant mechanism of resistance (Koziol-Montewska et al. 2011).

Antibiotic therapy in ICU, including antibiotic therapy for VAP, should be a compromise between appropriate and early pharmacotherapy based on broad-spectrum antibiotics, knowledge of local epidemiology and susceptible bacteria, and fear of increasing resistance to the antibiotics presently available (Koziol-Montewska et al. 2011; Magnotti et al. 2008). Both delayed treatment and improper therapy (an antibiotic ineffective against infections producing pathogens, improper dose or pharmacodynamic parameters, and therapy duration) are associated with worse outcomes. Thus, surveillance of microorganisms responsible for infections in ICU should be strongly recommended and the microbiological pattern, including the susceptibility and resistance patterns of cultured bacteria, should be conducted and systematically repeated and analyzed in each ICU.

The common causes of infection in ICU are *Pseudomonas aeruginosa* and *Acinetobacter baumannii* pathogens (Koziol-Montewska et al. 2011; Magnotti et al. 2008; Kollef et al. 2005). In a prevalence study of infections in intensive care units conducted among 75 countries of the 5 continents, *Acinetobacter baumannii* has been found to be the fifth most common pathogen, although with a high variability among different countries (Vincent et al. 2009). Different surveillance studies have found this pathogen to be the fifth cause of pneumonia, after *P. aeruginosa*, in hospitalized patients, mainly in ICU (Jones 2010). In addition, these microorganisms are also frequently reported to cause other nosocomial infections such as bacteremia and urinary tract and surgical infections. In fact, *A. baumannii* was found to be the third most frequent cause of nosocomial bloodstream infection in a large multicenter study with an estimation of 34 % of all patients and 43 % in patients in ICU (Wisplinghoff et al. 2004). With respect to the treatment of *A. baumannii* infections, it is important to take into account the resistance profile involved to consider the different treatment options available.

A. baumannii has become resistant to almost all commonly used antimicrobial agents, including aminoglicosides, quinolones, and broad-

spectrum betalactamases, and multidrug or pandrug resistance strains, including strains resistant to carbapenems and occasionally colistin, have appeared (Rossolin and Mantegnoli 2008; Livermore et al. 2008). Data from many European centers show an increasing resistance of *P. aeruginosa* strains to carbapanems, conditioned mainly by beta-lactamase synthesis. In a tertiary hospital in Lithuania, the prevalence of carbapenem-resistant *P. aeruginosa* strains increased from 10 to 40 % (Vitkauskiene et al. 2011). Increasing resistance to carbapenems is a cause of concern because of nosocomial infections and it adversely affects clinical outcomes and adds to treatment costs.

The aim of the present study was to evaluate the effectiveness of three antibiotics of the carbapenems class, i.e., doripenem, imipenem and meropenem on the basis of *in vitro* sensitivity in compliance with the criteria of the Clinical and Laboratory Standards Institute (2008) and European Committee for Antimicrobial Susceptibility Testing (EUCAST) (2011) classification in case of imipenem and meropenem and of the Food and Drug Administration (FDA) and EUCAST in case of doripenem.

2 Methods

2.1 Collection and Processing of Biological Samples

The study was approved by the Ethics Committee of the Institute of Military Medicine in Warsaw, Poland. Fifty four strains of *A. baumannii*: 47 (87.0 %) from tracheal swabs, 3 (5.6 %) from wound swabs, 2 (3.7 %) from blood culture, 1 (1.9 %) from bronchoalveolar lavage, and 1 (1.9 %) from a drain site, and 17 strains of *P. aeruginosa*: 16 (94.1 %) from tracheal swabs and 1 (5.9 %) from pleural fluid culture, were subjected to analysis. The material was collected from 71 patients treated in the Clinic of Anesthesiology and Intensive Care of Institute of Military Medicine in Warsaw, Poland. All of the strains were isolated in the period from April 2009 to January 2010.

The isolated material was identified in an automated microbiology system VITEK 2 (bioMérieux) by means of GN cards, following the guidelines issued by manufacturer. The strains which had been identified were subjected to manual determination of Minimal Inhibitory Concentration (MIC) value by means of Etest® bioMérieux gradient strips which measure the concentration of a given antibiotic (the analyzed concentration range for imipenem 0.002–32 mg/L, meropenem 0.002–32 mg/L, doripenem 0.002–32 mg/L) on the Müeller-Hinton plates (bioMérieux, France). The MIC limit values, meant for classifying the strain as sensitive or resistant according to the American CLSI and FDA criteria (Food and Drug Administration 2011; Clinical and Laboratory Standards Institute 2008) and European EUCAST guidelines (The European Committee on Antimicrobial Testing. EUCAST criteria 2011) were presented in Table 17.1.

The next stage of the study was a genetic analysis conducted in the Institute of Molecular Microbiology of the National Medicines Institute in Warsaw, Poland. Owing to predominance of *A. baumannii* isolates, 10 randomly selected strains of this particular isolate became subject to molecular genotyping (*P. aeruginosa* isolates were not subject to typing) by means of a referential method based on *Restriction Fragment Length Polymorphism* (restriction enzyme Apal) of the genome using the procedure of *Pulsed-Field Gel Electrophoresis* (RFLP-PFGE) (Fig. 17.1).

2.2 Statistical Analysis

Chi^2 test was applied to calculate statistical drug sensitivity of bacterial strains studied with respect to particular carbapenems and classification standards. A $p < 0.05$ was determined as

Table 17.1 MIC values of *A. baumannii* and *P. aeruginosa* strains to carbapenems according to CLSI (imipenem, meropenem), FDA (doripenem) and EUCAST (imipenem, meropenem, doripenem) classification

	Imipenem mg/L		Meropenem mg/L		Doripenem mg/L	
Bacterial strain	Sensitivity	Resistance	Sensitivity	Resistance	Sensitivity	Resistance
MIC values according to CLSI and FDA classifications						
Acinetobacter baumannii	≤4	≥16	≤4	≥16	≤1	>1
Pseudomonas aeruginosa	≤4	≥16	≤4	≥16	≤2	>2
MIC values according to EUCAST classification						
Acinetobacter baumannii	≤2	>8	≤2	>8	≤1	>4
Pseudomonas aeruginosa	≤4	>8	≤2	>8	≤1	>4

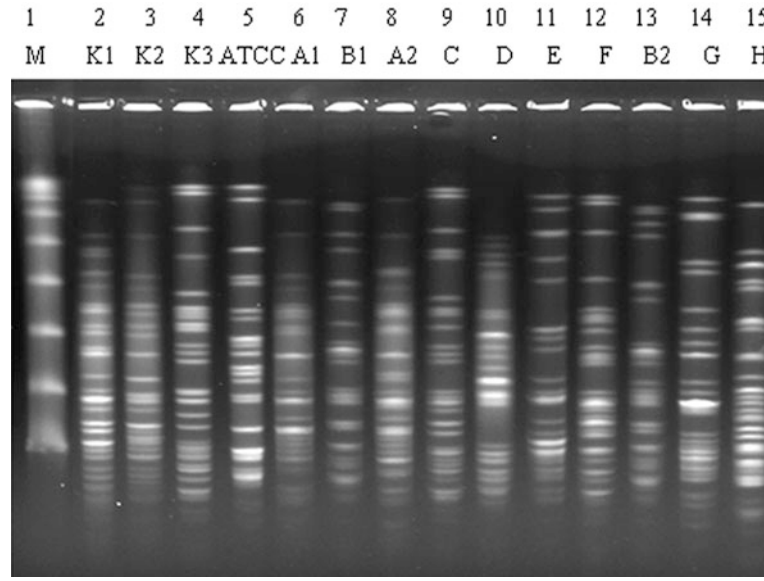

Fig. 17.1 PFGE of ApaI restriction of representative *A. baumannii* isolates, demonstrating PFGE type A (A1, A2), type B (B1, B2), patterns C, D, E, F, G, H. Also in this figure, M – DNA lambda ladder PFGE marker (New England BioLabs, USA); K1,K2,K3 – controls; ATCC – isolate marker *A. baumannii* ATCC 19606

significant. Calculations were performed using program Statistica PL (license number SN: SP 7105488009 G51).

3 Results

As a result of the genetic study conducted, 10 patterns of electrophoretic separations were identified for *A. baumannii*. Proximity analysis of the electrophoretic patterns revealed 2 pairs of mutually related isolates: type A (subtype A1, A2) and type B (subtype B1 and B2). The remaining isolates demonstrated unique separation patterns C, D, E, F, G, and H.

Table 17.2 demonstrates sensitivity of *A. baumannii* and *P. aeruginosa* strains to carbapenems according to the American and European criteria and Table 17.3 illustrates the comparison of *A. baumannii* and *P. aeruginosa* sensitivity to carbapenems. The percentage of *A. baumannii* strains sensitive to doripenem following the guidelines of both classifications was identical – 37.0 %, whereas the proportion of sensitive *P. aeruginosa* strains differed between both classifications – 64.7 % according to the FDA and 47.1 % according to the EUCAST criteria (Table 17.2).

The percentage of *A. baumannii* strains sensitive to imipenem according to the CLSI criteria was higher than that in case of the EUCAST classification – 68.5 % vs. 50.0 %, whereas the percentage of sensitive *P. aeruginosa* strains studied in compliance with both classifications was identical (Table 17.2). The proportion of

Table 17.2 Sensitivity of *A. baumannii* and *P. aeruginosa* strains to doripenem (according to FDA and EUCAST criteria), imipenem and meropenem (according to CLSI and EUCAST criteria)

		FDA	EUCAST
Bacterial strain (*n*)	Sensitivity to doripenem	n (%)	n (%)
Acinetobacter baumannii (*n* = 54)	Sensitive	20 (37.0)	20 (37.0)
	Intermediate	–	20 (37.0)
	Resistant	32 (59.3)	12 (22.2)
	No data	2 (3.7)	2 (3.7)
Pseudomonas aeruginosa (*n* = 17)	Sensitive	11 (64.7)	8 (47.1)
	Intermediate	–	4 (23.5)
	Resistant	5 (29.4)	4 (23.5)
	No data	1 (5.9)	1 (5.9)
		CLSI	EUCAST
Bacterial strain (*n*)	Sensitivity to imipenem	n (%)	n (%)
Acinetobacter baumannii (*n* = 54)	Sensitive	37 (68.5)	27 (50.0)
	Intermediate	6 (11.1)	15 (27.8)
	Resistant	11 (20.4)	12 (22.2)
Pseudomonas aeruginosa (*n* = 17)	Sensitive	11 (64.7)	11 (64.7)
	Resistant	6 (35.3)	6 (35.3)
		CLSI	EUCAST
Bacterial strain (*n*)	Sensitivity to meropenem	n (%)	N (%)
Acinetobacter baumannii (*n* = 54)	Sensitive	29 (53.7)	15 (27.8)
	Intermediate	11 (20.4)	16 (29.6)
	Resistant	14 (25.9)	23 (42.6)
Pseudomonas aeruginosa (*n* = 17)	Sensitive	11 (64.7)	7 (41.2)
	Intermediate	–	4 (23.5)
	Resistant	6 (35.3)	6 (35.3)

n – number of strains

bacterial strains sensitive to meropenem was higher according to the CLSI criteria than that in case of the EUCAST classification – 53.7 % vs. 27.8 % for *A. baumannii* and 64.7 % vs. 41.2 % for *P. aeruginosa* (Table 17.2).

A. baumannii strains demonstrated the highest sensitivity to imipenem according to both classifications (Table 17.3). However, this observation was statistically significant only when the CLSI guidelines were considered. Sensitivity of *P. aeruginosa* to all studied carbapenems assessed both with CLSI and FDA classifications was not appreciably different (Table 17.3).

4 Discussion

Gram-negative bacterial infections, especially those described as multiple drug resistance (MDR) strains pose major epidemiological risk as far as nosocomial infections are concerned. The most common pathogens causing such infections are non-fermenting Gram-negative coccobacilli of the *Pseudomonas* spp. and *Acinetobacter* spp. genus and of the *Enterobacteriaceae* spp. family. The literature shows that a substantial fraction of infections acquired in ICU have been due to Gram-negative bacteria, including *P. aeruginosa* (14.2 %) and *A. baumannii* (15.3 %) (Kübler et al. 2004).

Carbapenems, which were first introduced in the 1980s, proved to be the most effective group of antibiotics, and in many cases they were used as a last resort in treating infections induced by Gram-negative bacteria above outlined. Unfortunately, in recent years carbapenems have appeared less effective in a considerable number of infections. Carbapenem resistance in *Acinetobacter* and

Table 17.3 Comparison of *A. baumannii* and *P. aeruginosa* sensitivity to carbapenems according to CLSI, FDA, and EUCAST criteria

Sensitivity of *A. baumannii* according to CLSI and FDA	Doripenem ($n = 52$)[a] n (%)	Meropenem ($n = 54$) n (%)	Imipenem ($n = 54$) n (%)	p
Sensitive	20 (38.5)	29 (53.7)	37 (68.5)	0.008
Intermediate + resistant	32 (61.5)	25 (46.3)	17 (32.5)	
Sensitivity of *A. baumannii* according to EUCAST	Doripenem ($n = 52$)[a] n (%)	Meropenem ($n = 54$) n (%)	Imipenem ($n = 54$) n (%)	P
Sensitive	20 (38.5)	15 (27..8)	27 (50.0)	0.06
Intermediate + resistant	32 (61.5)	39 (72.2)	27 (50.0)	
Sensitivity of *P. aeruginosa* according to CLSI and FDA	Doripenem ($n = 16$)[b] n (%)	Meropenem ($n = 17$) n (%)	Imipenem ($n = 17$) n (%)	P
Sensitive	11 (68.8)	11 (64.7)	11 (64.7)	0.96
Intermediate + resistant	5 (31.2)	6 (35.3)	6 (35.3)	
Sensitivity of *P. aeruginosa* according to EUCAST	Doripenem ($n = 16$)[b] n (%)	Meropenem ($n = 17$) n (%)	Imipenem ($n = 17$) n (%)	P
Sensitive	8 (50.0)	7 (41.2)	11 (64.7)	0.38
Intermediate + resistant	8 (50.0)	10 (58.8)	6 (35.3)	

[a]In two cases sensitivity to doripenem was not determined
[b]In one case sensitivity to doripenem was not determined

Pseudomonas infections is increasingly observed worldwide and constitutes a sentinel event for emerging antimicrobial resistance (Roca et al. 2012; Poirel and Nordman 2006).

The results of a recent study on bacterial resistance to antibiotics, based on the data collected in 35 ICU in 13 European countries indicate that *A. baumannii* resistance to imipenem ranged from 0 % in Estonia and Sweden, 10–20 % in the majority of other countries, to 38 % in Turkey, and as much as 90 % in Malta. *P. aeruginosa* resistance to the drug was estimated at the level of 13 % in Estonia to 48 % in Turkey. The growth in prevalence of antibiotic resistant bacteria is commonly associated with increased consumption of antibiotics. The lowest consumption, i.e., 426–638 DDD/1,000 beds, was registered in Switzerland; while in other European countries it was approximately 1,254 DDD/1,000 beds. An interesting fact remains that no correlation between the use of antibiotics and a number infections induced by the so-called alert pathogens occurring in ICUs has been observed, since that greatly depends on a constant inflow of patients infected with such pathogens (Hanberger et al. 2009).

There are slight differences in the properties of imipenem, meropenem, and doripenem. The distinction between the first two mentioned is of no clinical significance. However, in case of doripenem, the most recently developed carbapenem effective mainly against Gram-negative bacteria, especially *Pseudomonas aeruginosa*, the difference is quite important. A study which analyzed *in vitro* sensitivity of 6,000 bacteria to imipenem and meropenem demonstrated a lower MIC value for meropenem toward *Enterobacteriaceae* and *Pseudomonas aeruginosa* than that for imipenem. On the other hand, imipenem was characterized by a lower MIC value for *A. baumannii*. A Meropenem Yearly Susceptibility Test Information Collection (MYSTIC) study indicated that meropenem is more effective toward *Acinetobacter* spp., *Pseudomonas* spp. and *Enterobacteriaceae* spp. than imipenem, whereas the latter was more effective toward *E. faecalis* (Hoban et al. 1993).

A major multicenter study on the usefulness of doripenem in treating hospital-acquired pneumonia (HAP) demonstrated that its effectiveness is similar to that of imipenem 68.3 vs. 64.2 %; whereas regarding *P. aeruginosa* it was higher – 80.0 vs. 42.9 %, respectively, although that result

failed to be of statistical significance (Chastre et al. 2008). The above mentioned results apply to bacteria cultivated from samples collected in ICUs, which means bacterial strains of the highest possible resistance. Genetic studies of *Acinetobacter* strains demonstrated a diversity of electrophoretic separations; confirming that the subject for analysis was not a single, separate type of bacteria.

In the present study, sensitivity of the bacterial strains was interpreted in compliance with two separate criteria, the American and European criteria. That, in our opinion, contributes to clinical importance of the study. Adopting the EUCAST criteria, more rigorous regarding the category of sensitivity, seems justifiable in the assessment of antibiotic treatment in ICUs. This particularly applies to sensitivity of *P. aeruginosa* to doripenem, *P. aeruginosa*, and *A. baumannii* to meropenem, and also sensitivity of *A. baumannii* to imipenem. Our data showed that in both classifications *A. baumannii* demonstrated a higher sensitivity to imipenem; however, statistical significance only applied to the CLSI guidelines. No differences regarding the sensitivity of *P. aeruginosa* to the three carbapenems studied emerged. Also, the finding of other authors (Pillar et al. 2008) of higher *in vitro* sensitivity of doripenem than that that of imipenem and meropenem toward *P. aeruginosa* was not confirmed.

The present study shows that, considering an *in vitro* assessment, carbapenems remain an effective group of antibiotics as far as treating *P. aeruginosa* and *A. baumannii* infection is concerned. This result is consistent with the observation of Koziol-Montewska et al. (2011) that in a pool of VAP pathogens, carbapenems (imipenem, meropenem, doripenem) seem to be the drugs for empiric antibiotic therapy. The question why imipenem, which is the oldest carbapenem, demonstrates a better effectiveness toward the bacteria analyzed than the two remaining carbapenems cannot be answered unequivocally. From the standpoint of pharmacotherapy, one of the better properties of imipenem is its ability to bind with penicillin binding proteins (PBP-2) of bacteria regardless of concentration, whereas in case of meropenem such combination is only possible in higher concentrations (Hanberger et al. 2009).

It should be underlined that multidrug resistant/carbapenem resistant strains of *A. baumannii* and *P. aeruginosa* are associated with treatment challenges, which emphasize the importance of preventing and controlling the dissemination of the strains (Poirel and Nordman 2006). Infection control measures, such as culture surveillance with recognition of susceptibility and resistance patterns, contact precautions, cohorts, source identification, and environmental control should all be introduced to prevent dissemination of multidrug resistant microorganisms (Romanelli et al. 2009; Urban et al. 2003). Hospitals, mainly ICUs, should introduce the antibiotic policy based on permission system to control the use of carbapenems which can efficiently suppress the incidence of drug resistance bacteria (Ikeda et al. 2012).

5 Conclusions

The EUCAST criteria, as opposed to the CLSI and FDA guidelines, demonstrate a higher rigor regarding the category of sensitivity for carbapenems that remain an effective group of antibiotics in treatment of *A. baumannii* and *P. aeruginosa* infections. Imipenem was confirmed *in vitro* to be more effective an antibiotic in comparison with doripenem and meropenem in such infections.

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Conflicts of Interest The authors declare no conflicts of interest in relation to this article.

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