Joanna Nowak, Agnieszka Pacholczyk, Violetta Petroniec, Beata Dziedzicka, Zofia Zwolska

Department of Microbiology, Institute of Tuberculosis and Lung Diseases in Warsaw, Poland
Head: Prof. Z. Zwolska M.D., P.hD.

The results of molecular epidemiologic investigations in patients infected with strains of the genus *Acinetobacter*

Abstract

Introduction: *Acinetobacter* spp. is an important opportunistic pathogen responsible for increasing number of nosocomial infections. The majority of infections are of epidemic origin, and treatment has become difficult because many strains are resistant to a wide range of antibiotics. The aim of this study was to investigate the local infections caused by various species of the genus *Acinetobacter*, occurring in the hospital wards IGiChP in periods of increased prevalence: August 2007 and February and March 2008.

Material and methods: Twenty three strains of *Acinetobacter* spp. were isolated from 19 patients residing in the same period and the same hospital ward (2007 — 13 strains from 11 patients, 2008 — 10 strains from 8 patients). *Acinetobacter* isolates obtained from these patients were characterized by phenotypic methods and genotypically by arbitrarily primed PCR (AP-PCR).

Results: All strains of *Acinetobacter* (n = 23) were multi-drug resistant. Used AP-PCR method showed 10 genotypes among the all strains. *Acinetobacter* spp. strains cultivated in 2007 and 2008 belonged to one genotype, came from patients hospitalized on the same wards, which confirms the transmission of infection in the patient's residence.

Conclusions: The same genotype *Acinetobacter baumannii* strains isolated from two patients in 2007, and two patients in 2008, showed the presence of bacteria in the hospital environment. In the present study, we also established the usefulness of AP-PCR in molecular epidemiological investigations.

Key words: *Acinetobacter* spp., nosocomial infections, epidemiologic investigations, AP-PCR

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Introduction

Nosocomial infections are a serious worldwide problem, both in terms of treatment and economics. The risk is closely associated with diagnostic and therapeutic procedures. According to statistics, the incidence of nosocomial infections in Poland ranges from 3–7% [1]. All medical facilities are required to have control programmes aimed at rapidly detecting a nosocomial infection, establishing its aetiology, and, if possible, determining its transmission routes.

Acinetobacter spp. are characterised by their widespread distribution in nature. They can survive in various environments, often ones that are poor in nutrients, and are highly resistant to antibiotics, disinfectants, and ultraviolet radiation [2–7]. *Acinetobacter* spp. have long been thought of as saprophytic microorganisms of no clinical relevance. In recent years, however, they have become one of the major causative pathogens of nosocomial infections [8, 9]. Infection is usually preceded by colonisation of the skin, mucous membranes, respiratory tract, and vascular catheters. This mainly applies to high-risk patients hospitalised at intensive care units (ICUs), surgical wards, oncology wards, and transplantation wards [10–12].

Molecular methods are increasingly used in microbiologic diagnostics, particularly in epidemiologic investigations [1, 13]. The genetic relation-

ship of the cultured bacterial strains is investigated using various techniques depending on the equipment available at a given facility and the knowledge possessed by its staff. The arbitrarily-primed polymerase chain reaction (AP-PCR) technique is commonly used. APR-PRC is based on DNA amplification using primers of randomly selected sequences. The use of this technique in investigations of infections caused by *Acinetobacter* spp. is very useful for determining their genetic diversity [1, 3, 13, 14].

During routine diagnostic activities we observed an increased incidence of *Acinetobacter* spp., which prompted us to perform molecular epidemiologic investigations and to determine whether the same strains were responsible for infections in different patients.

The aim of our study was to investigate local infections caused by various *Acinetobacter* species at the hospital wards of the Institute of Tuberculosis and Lung Diseases in Warsaw, Poland during two periods of their increased prevalence, namely in August 2007 and in February and March 2008.

Material and methods

We analysed 23 strains of various *Acinetobacter* species cultured from 19 patients (10 men and 9 women) hospitalised over the same period on the hospital wards of our Institute:

— August 2007: 13 strains from 11 patients hospitalised at the ICU of the Surgery Ward and Ward 1;

— February and March 2008: 10 strains from 8 patients hospitalised at the ICU of the Surgery Ward and Ward 2.

The strains came from different clinical samples: bronchoscopy samples (10), sputum (4), blood (4), endotracheal tube swabs (2), drains (2), and a wound swab (1). We investigated phenotypic traits (drug susceptibility, biochemical traits) and genetic relationship. Identification of the cultured strains and antibiotic susceptibility testing were performed on an automated system: Phoenix (BD). DNA was isolated using a GenElute Bacterial Genomic Kit, Mini (Sigma).

AP-PCR was performed using ReadyMix™ Taq PCR Reaction Mix With $MgCl_2$ (Sigma) and AP2-5'GTTTCGCTCC3' and AP4-5'AAGAGCCCGT3' primers. The amplification conditions were as follows: initial denaturation at 94°C for 5 minutes, followed by 35 cycles of 15 seconds at 94°C, 30 seconds at 35°C, and 2 minutes at 72°C. After the final elongation, 10 minutes at 72°C, the samples were cooled down to 4°C.

Results

The predominating species among the investigated *Acinetobacter* spp. originating from the respiratory tract was *Acinetobacter baumannii* (16 strains, 70%) (Table 1).

All the strains (n = 23) were characterised by a very low susceptibility to the tested drugs and were only susceptible to carbapenems (74%, 17 strains), the following aminoglycosides: tobramycin (61%, 14 strains) and gentamycin (57%, 13 strains), and to Unasyn (ampicillin/sulbactam; 30%, 7 strains).

The molecular analysis of the genetic relationship of the 23 strains of *Acinetobacter* spp. isolated in 2007 (13 strains) and 2008 (10 strains) revealed the presence of 10 genotypes (Table 2): genotype I was present in 4 patients, genotype II in 4 patients, genotype III in 2 patients, genotype IV in 3 patients, and genotypes V to IX and genotype X exhibited unique patterns (Figures 1 and 2). *Acinetobacter* strains cultured from two different materials from the same patient had an identical molecular pattern (Figure 1, lanes 1 and 2 and lanes 11 and 12; Figure 2: lanes 2 and 3).

A comparison of all the analysed strains revealed the presence of a common genotype for two *Acinetobacter baumannii* strains isolated from two patients in 2007 (lanes 5 and 7 in Figure 1) and three strains isolated from two patients in 2008 (lanes 2, 3 and 5 in Figure 2) at the ICU. The analysis shows that this is the same nosocomial strain which has been present in the hospital environment since at least 2007.

We found no relationship between the genotype and the pattern of antibiotic resistance. Most of the analysed strains of the same amplification pattern, both in the first and in the second period, were characterised by different drug resistance. The differences were related to one or two drugs. This phenomenon may be associated with long-term antibiotic treatment and/or exchange of the genetic material between the strains. Genotype III from 2007 is an exception (lanes 9 and 10 in Figure 1) where the antibiotic resistance patterns were identical. The *Acinetobacter baumannii* strains cultured in 2007 and 2008 of the same genotype originated from patients hospitalised on the same wards (Department of Surgery and ICU). It may therefore be assumed that the infection was transmitted within the patients' places of residence.

Discussion

Nosocomial infections are difficult to avoid and require constant monitoring on hospital wards.

Table 1. Species of *Acinetobacter* spp. isolated from 19 patients hospitalized in IGIChP during the study period

Year	Hospital ward	Clinical material	Species
2007	Surgery	Bronchoscopy (2) Drain (2) Wound swab (1)	*Acinetobacter baumannii* (5)
	ICU	Blood (3) Tracheostomy tube (2)	*Acinetobacter baumannii* (1) *Acinetobacter baumannii/calcoaceticus complex* (1) *Acinetobacter lwoffii/haemolyticus* (1) *Acinetobacter baumannii* (2)
	Ward I	Sputum (2) Bronchoscopy (1)	*Acinetobacter baumannii/calcoaceticus complex* (2) *Acinetobacter lwoffii/haemolyticus* (1)
2008	Surgery	Bronchoscopy (3)	*Acinetobacter baumannii* (2) *Acinetobacter baumannii/calcoaceticus complex* (1)
	ICU	Bronchoscopy (3) Blood (1)	*Acinetobacter baumannii* (4)
	Ward II	Sputum (2) Bronchoscopy (1)	*Acinetobacter baumannii* (1) *Acinetobacter baumannii/calcoaceticus complex* *Acinetobacter baumannii* (1)

Table 2. Genotypes of Acinetobacter spp. isolated from patients in 2007 and 2008

2007 year	Genotypes					
	I	II*	III	V	VI	VII
Number of patients	4	2	2	1	1	1

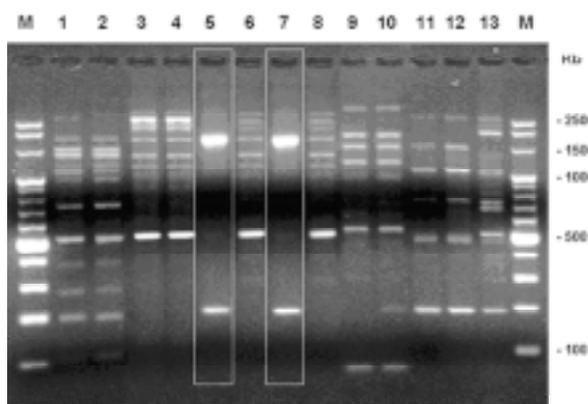| 2008 year	Genotypes				
	IV	II*	VIII	IX	X
Number of patients	3	2	1	1	1

*the same genotype in 2007 and 2008

Figure 1. AP-PCR products of *Acinetobacter* spp. isolates in 2007. Lane M: molecular marker (100 bp); lanes 3, 4, 6 and 8 — genotype I; lane 5 and 7 — genotype II (common to strains from 2007 and 2008); lane 9 and 10 — genotype III; lane 1 and 2 (the same patient), lane 11 and 12 (the same patient) and lane 13 — unique genotypes
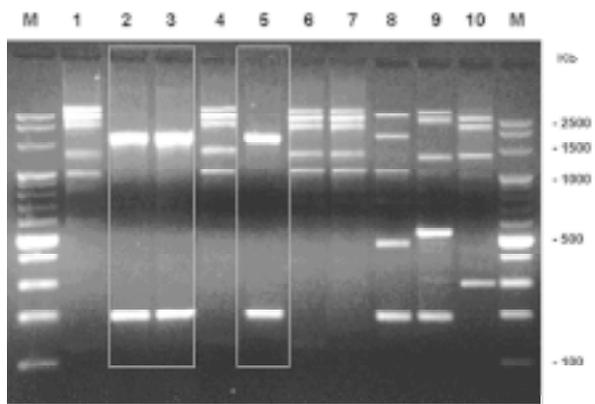

Figure 2. AP-PCR products of *Acinetobacter* spp. isolates in 2008. Lane M: molecular marker (100 bp); lanes 1, 4 (the same patient) and lane 6, 7 — genotype IV; lane 2, 3 (the same patient) and lane 5 — genotype II (common to strains from 2007 and 2008); lane 8, 9, 10 — unique genotypes

Patients hospitalised on surgical wards and in intensive care units are at higher risk of nosocomial infections due to the presence of indwelling vascular and urinary catheters and surgical wounds, which are favourable environments for pathogenic bacterial strains, and due to the use of broad-spectrum antibiotics, which promotes the emergence of resistant strains [1, 10].

Acinetobacter spp. are of great significance in nosocomial infection statistics [2, 7, 15, 16]. Infections with these opportunistic pathogens may take various clinical forms, such as pneumonia, urinary tract infections, wound infections, and sepsis [7, 11, 12, 16]. Falagas et al. [17] demonstrated the effect of inappropriate treatment of infections caused by *Acinetobacter* spp. on the mortality rate of patients infected by multidrug-resistant strains (25.8%).

Among non-fermenting bacteria, *Acinetobacter* spp. are the second-most-common microorganisms isolated from clinical samples [2, 7, 10, 11, 18]. These reports are consistent with our previous findings. The bulletins published since 1998 covering the bacterial spectrum of respiratory diseases managed at our Institute indicate an increasing proportion of *Acinetobacter* spp. in nosocomial infections (from 3.9% in 43 patients in 2001 to 5.5% in 75 patients in 2006). In 90% of the cases they were predominantly isolated in samples from the respiratory tract. According to numerous reports, *Acinetobacter* spp. are often isolated from patients hospitalised in intensive care units [2, 7, 15, 16, 19]. This is consistent with our study presented here and is most likely associated with the lack of antibacterial immunity in patients (immunosuppression, metabolic defects), long hospitalisation, and invasive diagnostic and therapeutic procedures.

Infections caused by multidrug-resistant *Acinetobacter* spp. are difficult to treat due to the limited therapeutic options [5, 11, 12, 14, 20]. This problem affects the whole world, including Poland. Ranjbar et al. [14] reported high resistance to antibiotics among 21 strains of *Acinetobacter baumannii*. The strains were only susceptible to tobramycin and amikacin (50%), piperacillin plus tazobactam (66%), and colistin (100%). Lahiri et al. [11] also showed the highest resistance of these strains to amikacin and netilmicin (73%), and piperacillin and cefotaxime (55%).

Studies conducted in Polish hospitals have shown that the most active antibiotics against *Acinetobacter* spp. species are imipenem, netilmicin, ampicillin plus sulbactam, and cefoperazone plus sulbactam [2, 16]. These results are comparable to ours. Among all the investigated strains, 74% were susceptible to carbapenems and more than 50% were susceptible to aminoglycosides. Despite the occasional resistance to carbapenems, these drugs are considered the most active against *Acinetobacter* spp. [10, 12, 15]. Colistin also proves effective, although its toxicity largely limits its use in clinical practice [21].

Recent reports suggest the high efficacy of combination treatment, mainly combinations of a β-lactam and an aminoglycoside or a fluoroquinolone. This treatment reduces the emergence of resistant strains and increases the efficacy of antibiotic therapy [9].

An important role in combatting nosocomial infections is played by the microbiology laboratory which monitors the drug resistance of pathogens by detecting resistance mechanisms. Molecular methods, which use unique genetic material and constant for each of the microorganisms, are becoming more and more frequently utilised by the laboratories.

The importance of using assays based on nucleic acid amplification by PCR in epidemiologic investigations has been increasing for more than a decade. According to Kura et al. [13], the use of AP-PCR for differentiating *Acinetobacter* spp., among other microorganisms, enables rapid identification of epidemiological risks. The high discriminatory value makes AP-PCR very useful in the analysis of local epidemics and infections caused by pathogenic microorganisms. The method does not require knowledge of the bacteria's genome, as primers of any nucleotide sequence specifically bind to complementary regions of matrix DNA. Gel electrophoresis of the amplification product visualises differences between the investigated strains [1, 3, 13, 14].

The AP-PCR method we used, demonstrated the presence of an identical molecular pattern for two *Acinetobacter baumannii* strains isolated in 2007 and three isolated in 2008. The strains came from patients hospitalised at the ICU. This proves that infection occurred at the patient's place of residence and that *Acinetobacter baumannii* has been present in the hospital environment since at least 2007. It is most likely associated with the living requirements of *Acinetobacter* spp. and their ability to colonise patients and medical equipment [1, 4–6, 8, 17]. Most commonly the infection is transmitted by direct contact during nursing and therapeutic procedures. If the sanitary regime is not complied with, asymptomatic carriers or infected individuals are a potential risk [1, 8, 10, 15, 16].

Horizontal transmission of *Acinetobacter* spp. strains requires greater surveillance of nosocomial infections and stricter sanitary regimes.

Conclusions

1. The same molecular pattern of *Acinetobacter baumannii* strains isolated from two patients in 2007 and from two in 2008 confirms the presence of this microorganism in the nosocomial environment.
2. Molecular testing coupled with determination of drug resistance should be the responsibility of laboratories as part of nosocomial infection surveillance activities.

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