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Spread of Multidrug Resistant *Acinetobacter baumannii* in a Teaching Hospital

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Abstract: We describe an outbreak of *Acinetobacter baumannii* (16 stains) in intensive care units at Charles Nicolle hospital of Tunis over a 5 month period (March to July 2005). The antimicrobial susceptibility was determined by disc diffusion test and the genetic relatedness of isolates was done by RAPD analysis. Two strains not related to the outbreak were used for the discrimination of the technique. Samples were collected from blood (44%), materials (31%), pus (6.5%), urines (6.5%) and respiratory tract (12.5%). Antibiotic resistance pattern showed 2 different profiles. However, genotyping revealed 3 different profiles suggesting 3 different clones. Molecular typing of isolates revealed 3 distinct profiles represented, respectively by 8, 7 and one isolates. The major profile was the profile A found in 5 patients and in materials. It was appeared firstly in intensive care unit I, then in the 2 other units (II and III). The profile B was observed also in the 3 units. However, the profile C was found in one patient in unit I. These data emphasize the need for active surveillance for multidrug-resistant *Acinetobacter baumannii* and the value of molecular typing of strains in hospital settings to investigate spread of infection.

Key words: *Acinetobacter baumannii*, multidrug-resistant, outbreak, intensive care units

INTRODUCTION

A. baumannii is an important opportunistic pathogen widely distributed in the hospital environment and responsible for a variety of nosocomial infections especially in patients from intensive care units (Seifert *et al*., 2005).

Extensive use of antimicrobials has contributed to the emergence and increase in the number of Multidrug Resistant *A. Baumannii* isolates (MRAB).

During the last decade, hospital-acquired infections involving MRAB isolates have been reported, often in association with contamination of the hospital equipment or cross-contamination by the colonized hands of patient-attending personnel (Bou *et al*., 2000; Bergogne and Towner, 1996).

A wide spread of MRAB occurred in different intensive care units of Charles Nicolle hospital of Tunis from March to July 2005 was investigated for the relatedness between isolates.

MATERIALS AND METHODS

Bacterial strains: During the study period, 16 clinical isolates of *A.baumannii* were recovered from patients hospitalized in 3 intensive care units (I, II, III). An epidemiologic study of the isolates has been undertaken based on their antibiotic resistance profiles and the genotyping.

Susceptibility testing: Antimicrobial susceptibility testing was done by disc diffusion method. Seventeen antibiotics were tested: Ticarcillin, ticarcillin/clavulanic acid, piperacillin, piperacillin/tazobactam, ceftazidime, aztreonam, imipenem, meropenem, cefepime, cefsulodin, amikacin, gentamicin, tobramycin, nalidixic acid, ofloxacin, ciprofloxacin and trimethoprim-sulfamethoxazole. Quality control was performed by testing *Escherchia coli* ATCC 25922, *Staphylococcus aureus* ATCC 25923 and *Pseudomonas aeruginosa* ATCC 27853.

Genotyping: Genomic DNA was extracted by Instagene and was studied by RAPD using EricII primer.

Reaction mixtures (50 μL) contained 10 μL of genomic DNA, 0.2 mM of desoxynucleotide triphosphate, 1 μM of oligonucleotide, 2.5 mM $MgCl_2$, 1 X buffer and 2.5 U of Taq polymerase (Promega).

Amplification was performed in a thermocycler programmed for 40 cycles of 1mn at 95°C, 1mn at 25°C and 1 mn at 72°C.

RAPD products were separated by electrophoresis in 2% agarose gel with 0.5 TBE running buffer at 100V for 1 h. Molecular size standard PGEM DNA marker was included on gel. Isolates which differed by 2 or more bands were considered sufficiently divergent (Cambell *et al*., 2000). Duplicated RAPD analysis was performed for each strain to assess reproductibility.

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Table 1: Origin and characteristics of *A. baumannii* strains isolated from materials and patients in intensive care units at Charles Nicolle Hospital

Strains	Date	Wards[a]	Samples	Resistance to antibiotics[b]	Antibiotic resistance profile[c]	RAPD profiles[d]
1	20/03/05	IC I	Material Catheter	Tic, Tcc, Pip, Tzp, Caz, Imp, Mem, Fep, Azt, Cfs, Gm,Tm, AN, NA, Ofx, Cip, Sxt	Carb R	A
2	11/04/05	IC III	Blood	Tic, Tcc, Pip, Tzp, Caz, Imp, Mem, Fep, Azt, Cfs, Gm, AN, NA, Ofx, Cip, Sxt	Carb R	A
3	12/04/05	IC I	Blood	Tic, Tcc, Pip, Tzp, Caz, Imp, Mem, Fep, Azt, Cfs, Gm, AN, NA, Ofx, Cip, Sxt	Carb R	A
4	18/04/05	IC I	Material Catheter	Tic, Tcc, Pip, Tzp, Caz, Fep, Azt, Cfs, Gm, AN, NA, Ofx, Cip, Sxt	C-P	A
5	18/05/05	IC I	Respiratory tract	Tic, Tcc, Pip, Tzp ,Caz, Imp, Mem, Fep, Azt, Cfs, Gm, AN, NA, Ofx, Cip, Sxt	Carb R	C
6	26/05/05	IC I	Blood	Tic, Tcc, Pip, Tzp, Caz, Imp, Mem, Fep, Azt, Cfs, Gm, Tm, AN, NA, Ofx, Cip, Sxt	Carb R	B
7	23/05/05	IC I	Blood	Tic, Tcc, Pip, Tzp, Caz, Imp, Mem, Fep, Azt, Cfs, Gm, Tm, AN, NA, Ofx, Cip, Sxt	Carb R	B
8	23/05/05	IC I	urine	Tic, Tcc, Pip, Tzp, Caz, Fep, Azt, Cfs, Gm, Tm, NA, Ofx, Cip, Sxt	C-P	A
9	17/06/05	IC I	Material Catheter	Tic, Tcc, Pip, Tzp, Caz, Fep, Azt, Cfs, Gm, Tm, NA, Ofx, Cip , Sxt	C-P	B
10	22/6/05	IC III	Blood	Tic, Tcc, Pip, Tzp, Caz, Fep, Azt, Cfs, Gm, Tm, NA, Ofx, Cip, Sxt	C-P	A
11	8/07/05	IC II	Blood	Tic, Tcc, Pip, Tzp, Caz, Imp, Mem Fep, Azt, Cfs, Gm, Tm, AN NA, Ofx, Cip	Carb R	B
12	12/07/05	IC I	Material Urinary catheter	Tic, Tcc, Pip, Tzp, Caz, Fep, Azt, Cfs, Gm, Tm, NA, Ofx, Cip, Sxt	C-P	A
13	8/07/05	IC II	Respiratory tract	Tic, Tcc, Pip, Tzp, Caz, Imp, Mem, Fep, Azt, Cfs, Gm ,Tm, AN, NA, Ofx, Cip	Carb R	B
14	21/07/05	IC III	Pus	Tic, Tcc, Pip, Tzp, Caz, Imp, Mem, Fep, Azt, Cfs, Gm, Tm, AN, NA, Ofx, Cip, Sxt	Carb R	B
15	15/07/05	IC I	Blood	Tic, Tcc, Pip, Tzp, Caz, Imp, Mem ,Fep, Azt, Cfs, Gm, AN, NA, Ofx, Cip, Sxt	Carb R	A
16	27/05/05	IC I	Material Catheter	Tic, Tcc, Pip, Tzp, Caz, Imp, Mem, Fep, Azt, Cfs, Gm, Tm, AN, NA, Ofx, Cip, Sxt	Carb R	B
17	23/12/05	Maternity ward	urine	----	W	D
18	12/08/05	cardiology	urine	Tic, Tcc, Pip, Tzp, Caz, Imp, Mem, Fep, Azt, Cfs, Gm, Tm, AN, NA, Ofx, Cip, Sxt	Carb R	E

[a] Intensive care units I, II and III (IC I, IC II, IC III). [b]Abreviations:Tic, ticarcillin; Tcc, ticarcillin-clavulanic acid; Pip, piperacillin ; Tzp , Pip-tazobactam ; Caz, Ceftazidime; Imp , Imipenem ; Mem, Meropenem; Fep,cefepime; Atm, Aztreonam; Cfs, Cefsulodine; Gm,Gentamicin; Tm, Tobramicin; AN, Amikacin; NA, Nalidixic Acid; Ofx : ofloxacin; Cip, Ciprofloxacin ; Sxt, trimethoprim + sulfamethoxazole, [c] Carb R: Carbapenem Resistant ; C-P: Cephalosporinase-Penicillinase ; W : wild type, [d] Letters (A,B,C,D,E) indicate strains of unrelated lineage

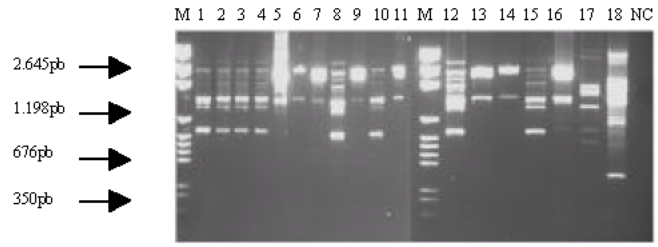

Fig. 1: RAPD pattern of *A. baumanni*i isolates with primer EricII.
Lane M: Benchtop PGEM DNA marker,
Lane 1-16: RAPD patterns of the 16 *A. baumannii* tested,
Lane 17-18: RAPD patterns of 2 unrelated strains, Lane NC : Negatif Control

RESULTS

The results of this study were summarized in Table 1. *A. baumanni*i was isolated from blood (44%), materials (31%), pus (6.5%), urines (6.5%) and respiratory tract (12.5%). MRAB isolates were recovered from 3 intensive care units of Charles Nicolle hospital: one general intensive care unit (IC I: 11 isolates) and 2 surgical units (IC II: 2 isolates and IC III: 3 isolates). The first isolate was recovered from the IC I in March 2005 and spread mainly in this unit.

Antibiotic susceptibility: All isolates were multidrug resistant with 2 different profiles of resistance to

β-lactams: 10 isolates were resistant to all β-lactams including carbapenems and 5 strains were resistant to penicillins and cephalosporins. The rates of associated resistance to other antibiotics were quinolones (100%), gentamicin(100%), amikacin(87.5%), tobramycin(68.75%) and trimethoprim-sulfamethoxazole (93.75%).

Genotyping: RAPD analysis performed with EricII primer showed 3 different profiles from the 16 studied isolates designed A (8 isolates), B (7 isolates) and C (one isolate) (Fig. 1). A, B represented the 2 major clones. The 3 clones were firstly observed in the unit I, then clones A and B in unit III and only clone B in unit II.

DISCUSSION

A. baumannii is an important nosocomial pathogen, particularly in intensive care units and multiresistance has long been a problem for this species (Afzal *et al*., 2001). During the last decade, nosocomial infections caused by MRAB have been reported (Hsueh *et al*., 2000, 2002). Carbapenem resistance has now becoming common and few therapeutic options remain against such resistant organisms (Turton *et al*., 2005).

Over a 5-month period, an increased number of infections and colonisation associated with strains of *A. baumannii* exhibiting the same multiresistant antibiotype has been observed at Charles Nicolle hospital suggesting the occurrence of an outbreak.

Antibiotic resistance is an important factor in nosocomial spread. In fact, the prevalence of imipenem resistance in *A. baumannii* has been increased in our hospital (11% in 2004 to 42% in 2005). The SENTRY surveillance from 1997 to 1999 reported 11% of resistance to carbapenems in Europe, Canada, the United States, Latin America and the Asia Pacific (Higgins *et al*., 2004; Gales *et al*., 2001).

Antibiotic resistance pattern showed 2 different profiles however genotyping revealed 3 different profiles suggesting 3 different clones. The major profile was the profile A found in 5 patients and 3 times in materials. Clone A of MRAB appeared firstly in intensive care unit I, then in the 2 other units. The spread of MRAB in the 3 units may be related to the circulation of the intensive care doctors between the 3 units that shared a common medical staff. The presence of A.*baumannii* in the environmental samples (catheter, urinary catheter) and detection of infected patients in the intensive care units during the outbreak period suggested that the spread of the epidemic strain was related to cross-transmission via the hand of health care workers and patients. The dissemination of this pathogen is facilated by its prolonged survival on inanimate surfaces. In fact, A. baumannii is a well-recognized opportunistic pathogen that gives rise to nosocomial infections and outbreaks, particularly in the intensive care units as have been described elsewhere (Seifert *et al*., 2005; Wilks *et al*., 2006).

Molecular typing plays an important role in epidemiological study of *A. baumannii* and the relatedness between isolated strains (Seifert *et al*., 2005).

Numerous outbreaks of nosocomial infections with carbapenem-resistant *A. baumannii* have been reported from many other countries (Bou *et al*., 2000; Afzal and Livermose, 1998; Cobella *et al*., 2000; Koelman *et al*., 1998). Recent data indicate that several successful epidemic A. baumannii strains circulate in Europe and a better understanding of the diversity within the species and the emergence of epidemic clones is urgently needed (Seifert *et al*., 2005).

Other researchers, have on the contrary reported great diversity among epidemic A. b aumannii strains without evidence of interregional spread (Sefert *et al*., 1994).

Risk factors for acquisition of this organism include prolonged hospital stay, intravascular catheterization and treatment with broad-spectrum antibiotics (Koelman *et al*., 1998).

Epidemiological investigations of A. baumannii have depended upon the insufficient discriminatory capacity of phenotypic markers, such as antimicrobial susceptibility testing. Molecular typing is a method of choice to identify an outbreak.

Various genotypic methods have been developed for the typing of Acinetobacter, including ribotyping, macrorestriction analysis by Pulsed-Filed Gel Electrophoresis (PFGE) and Randomly Amplified Polymorphic DNA (RAPD).

Among these, PFGE is regarded as the gold standard of epidemiological typing (Seifert *et al*., 2005). However, RAPD-fingerprinting may provide a useful and rapid identification technique for the epidemiological investigation of a hospital outbreak as demonstrated in our study.

CONCLUSION

MRAB is an important pathogen in ICU causing serious nosocomial infections and outbreaks. The increasing rates of resistance of *A. baumannii* to the major antimicrobial drugs make early identification and control of hospital outbreaks mandatory. Strict surveillance and adequate environment cleaning are essential to prevent recurrent outbreaks in intensive care

units and the strengthening of handwashing and restricted clinical use of broad spectrum ß-lactams including carbapenems are valuable measures to control an outbreak.

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