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MULTIRESISTANCE AND ENDEMIC STATUS OF *ACINETOBACTER BAUMANNII* ASSOCIATED WITH NOSOCOMIAL INFECTIONS IN A TUNISIAN HOSPITAL: A CRITICAL SITUATION IN THE INTENSIVE CARE UNITS

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ABSTRACT

Acinetobacter baumannii is often implicated in hospital outbreaks in Tunisia. It's a significant opportunistic pathogen associated with serious underlying diseases such as pneumoniae, meningitis and urinary tract infections. The aim of our study was to evaluate its degree of endemicity and its antibiotic resistance evolution essentially in the unit care where its isolation was predominant (57%).

This study used 3 methods: antibiotyping, RAPD using 2 primers VIL 1, VIL5 and PFGE with *ApaI* restriction enzyme. The presence of integron1 and 2 was also studied.

Antibiotyping showed that 92% of patients were resistant of all ß- lactams (except Imipenem) and that the resistance to Imipenem occurred in 47% of cases. RAPD profiles obtained with the 2 arbitrarily primers VIL1 and VIL5 gave respectively 5 and 4groups and PFGE fingerprinting patterns revealed 22 different pulsotypes. Integron 1 was present in 25% of unrelated strains and type 2 integron was not detected in any of the studied strains.

Among 204 strains, multiple and heterogeneous groups were detected with the genomic studies. In addition, any correlation was obtained with the antibiotyping results. These findings demonstrate the endemic status of *A. baumannii* in our hospital and the persistence of a large number of multiresistant strains in the unit's care. When outbreaks of *A. baumannii* occur, it's essential to develop restricted hygiene procedures and a serious surveillance of critical units such as ICU for very ill patients.

Key words: *A. baumannii*, epidemiology, antibiotic resistance, RAPD, PFGE

INTRODUCTION

The control of hospitals' acquired infections caused by multiply resistant Gram- negative bacilli is considered as a major problem in Tunisia (1, 21). It is now recognised that *A. baumannii* plays a significant role in the colonization and infection of hospitalized patients (18, 27, 25). They have been implicated in a variety of nosocomial infections including bacteria, urinary tract infections and secondary meningitis, but their predominant role is recognised in nosocomial pneumonia for patients confined to hospital intensive care units (ICUs) (8, 16, 28, 20) Such infections are often extremely difficult to treat

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for the clinician because of its widespread resistance to the major groups of antibiotics (5, 6, 16, 24).

MATERIAL AND METHODS

Strains of *A. baumannii*

A total of 204 strains were analyzed in this study. All were clinical isolates collected between 2004 and 2005 from puncture, pus and blood samples. Only one strain was selected from each patient and duplicates were excluded. ATCC 19606 reference strain was included in all methods. Identification of *Acinetobacter* was based on standard biochemical and morphologic characteristics. The *baumannii* species was performed by API 32GN automatic system (Biomerieux) and the Growth at 44°C. Strains were stored at -80°C in buffered peptone water with 10% glycerol.

Clinical information's

Clinical patients information were collected upon their admission in the intensive care. They were limited to 127 patients who were infected by *A. baumannii* after their admission in the intensive care unit (Table 1). In addition clinical features were noted on the day of clinically suspected infection.

Table 1. Characteristics of patients upon admission to the intensive care unit (ICU)

Parameters	Value (%)
Mean of age	50± 4
Number of males	58 (46)
Number of patients admitted for:	
Community	38 (30)
Wards	57 (45)
Another ICU	31 (24)
Number of underling infectious diseases:	
Pneumonia	45 (35)
Meningits	41 (32)
Urinary tract infection	12 (10)
Others	29 (23)
Number of indication for invasive mechanism:	
Ventilator mechanism	127 (100)
Catheterization	127 (100)
Sunder	127 (100)

Epidemiological data collection

In order to provide a complete description of our hospital, a few epidemiological data must be conferred. La Rabta university hospital is an institution accepting on average 23000 patients each year and has 960 beds and two units care (chirurgical and intensive care) that supports only 34 beds. In addition, our intensive care unit is available as well for all patients sent by other university hospitals or regional ones which don't have this unit. On average, 102 patients were infected or colonized by *A. baumannii* each year.

Susceptibility to antibiotics

Isolates were tested by disk diffusion method for susceptibility to ticarcillin (75µg), ticarcillin+ clavulanic acid (75/l0µg), ceftazidim (30 µg), cefepim (30µg), aztreonam (30µg), imipenem (10µg), tobramycin (10µg), kanamycin

(30µg), gentamycin (10UI), amikacin (30µg), pefloxacin (5µg), levofloxacin (5µg), ofloxacin (5µg), colistin (50µg), tetracyclin (30µg) and fosfomycin (50µg). The Antibiotics sensitivities were subjected to analysis with normalized values of the diameters of inhibition zones within the CA-SFM (comité de l'antibiogramme, Société Française de microbiologie) norms. Antibiograms were also determined by the disk inhibition method for 8 selected antibiotics shown to be useful for distinguishing *Acinetobacter* cluster: gentamycin, imipenem, tobramycin, amikacin, tetracyclin, ciprofloxacin, ceftazidim and rifampicin (23). Plates were incubated 24h at 37°C. The diameters of inhibition zones were normalized and subjected to cluster analysis. Isolates were classified on the basis of similarities according to the squared euclidean distance. The classification was obtained by the unweighted - pair group method using the arithmetic-average clustering criteria and Taxotron software (Grimont, Institut Pasteur, Paris, France).

PCR detection of Integron 1 and 2

Presence of integron 1 and 2 was detected by PCR using the 5' and 3' conserved segments a described previously (19). Primers used were 5'CS (GGCATCCAAGCAGCAAG) and 3'CS (AAGCAGACTTGACCTGA). Integron 2 was also detected using 2 primers; imA2s (ACCTTTTTGTCGCATA TCCGTG) and imAcs2 (TACCTGTTCTGCCCGTATCT). Amplified DNA products were resolved by electrophoresis in agarose 1.5% gels.

Random Amplified polymorphic DNA Analysis (RAPD)

RAPD was performed for all collected strains as described previously (12). Isolates were cultured overnight on nutritive agar, genomic DNA was extracted by phenol- chlorophorm method. 3 arbitrarily primers namely; VIL 1 (5' CCGCAGC CAA 3'), VIL5 (5' AACGCGCAAC 3') were used. Cluster analysis was performed by the unweighted pair group method with mathematic averaging UPGMA (1 % tolerance, 1 % Dice coefficient) and the cut off was fixed to 90% of similarity, using Molecular Analysing Fingerprinting software (Bio-Rad Laboratories).

Pulsed Field Gel Electrophoresis (PFGE)

PFGE was performed for all studied strains using a consensus protocol for *A. baumannii* typing (22). DNA fragments run for 19 h at 14°C in a CHEF DR-III apparatus (Bio-Rad). Clusters were analyzed with Molecular Analyst Fingerprinting software (Bio-Rad Laboratories). The classification was performed by the unweighted pair group method using mathematic averaging UPGMA (1% tolerance, 1% Dice coefficient). The cut off was fixed to 90% of similarity.

RESULTS

Epidemiology of outbreaks

The outbreaks outlined in Figure 1 occurred essentially in intensive care units (57% in intensive care and 32% in surgery). The clinical information's concerned 127 strains.

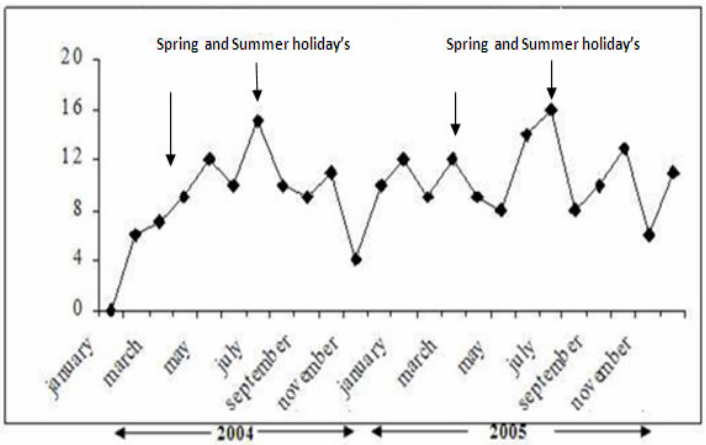

Figure 1: Occurrence of *A. baumannii* strains during the study period (2004-2005)

They included brief explosive periods and prolonged endemic episodes. The same endemic situation was observe

during the 2 years study. These outbreaks were amplified by many conditions: a) the humidity rises during the summer season and sweating by patients and staff is expected to be increased, b) the increasing of *A. bauamnni*i infections was increasing during the holiday's period (March to September) when the personnel was limited under the unit's hospital. This factor may favour the colonization of this pathogen in the skin and hence the rate of its infection.

The clinical information's shown in Table 2 concerned only patients infected in the unit care.

Table 2. Clinical features in relation of colonization with *A. baumannii* strains in ICU

Parameters	Value (%)
Duration of mechanical ventilation (average)	11± 2 days
Number of patients with prior antimicrobial therapy	96 (75)
Mean temperature (°C)	38.5± 2
Number of patients with organ failure:	
Respiratory	127 (100)
Cardiovascular	45 (35)
Renal	57 (45)
Hepatic	12 (10)
Mean total number of organ affected	27 (22)
Number of death	66 (52)

In the 127 studied cases, 54% were female. The mean age was 57 years. The majority of patients presented with acute onset of fever, shortness of breath, and cough (90%). 57 patients (45%) had acute deterioration of renal function, 45 (35%) developed cardiovascular problems and 127 (100%) developed respiratory failure requiring mechanical ventilation after the onset of *A. baumannii* bacteraemia. The development of these 3 complications was a significant predictor of mortality. We can submit the hypothesis that an association between the number of invasive procedures used and mortality might be found. In fact, mechanical ventilation, central venous and urinary catheterization had been given to all patients confined in the ICU before the first bacteraemia episode. The antibiotherapy prescribed generally in the all cases was the same: association of an aminoglyoside (Amikacine) and Imipenem.

Susceptibility to antibiotics

Antibiotyping tested by disk diffusion method showed a multiresistance to a large range of antibiotics. In fact, 92% of patients were resistant of all ß- lactams (except Imipenem); the resistance to Imipenem occurred in 47% of cases. 38% were multiresistant to all antibiotics tested except colistin and 51% of resistance to aminosids. A greater variety of strains seem to have achieved high levels of resistance to a wider range of antibiotics. Infact, the consumption average of Imipenem had doubled during the two years study (2500g/year in 2003 to 5000g/ year in 2005). The degree of relatedness between *A. baumannii* isolates in term of the 8 antibiotics sensitivities was determined by cluster analysis of the diameters of inhibition zones. 10 clusters were distinguished with the taxotron software which represents a primary classification of the isolates according to their resistance to antibiotics.

Integron PCR results

Integron 1 was present in 25% of unrelated strains within 3 different types; 800bp (13%), 2.1kb (5%) and (800bp+2.1kb) (7%). All the strains who gave two bands (800bp+2.1kb) were

multiresistant to antibiotics. Further PCR could help us to determinate their gene cassette assortments. Type 2 integron was not detected in any studied strain.

RAPD

RAPD profiles obtained with the 2 arbitrarily primers VIL1 and VIL5 gave respectively 5 and 4 groups. These groups recovered strains of the two years study confirming the endemic status of these bacteria in our hospital. In addition, we showed that some profiles disappear the second year study and in contrast others epidemic profile became endemic the second year (Figure 2). This method is useful as a first, effective and fast approach to detect epidemic strains in our hospital.

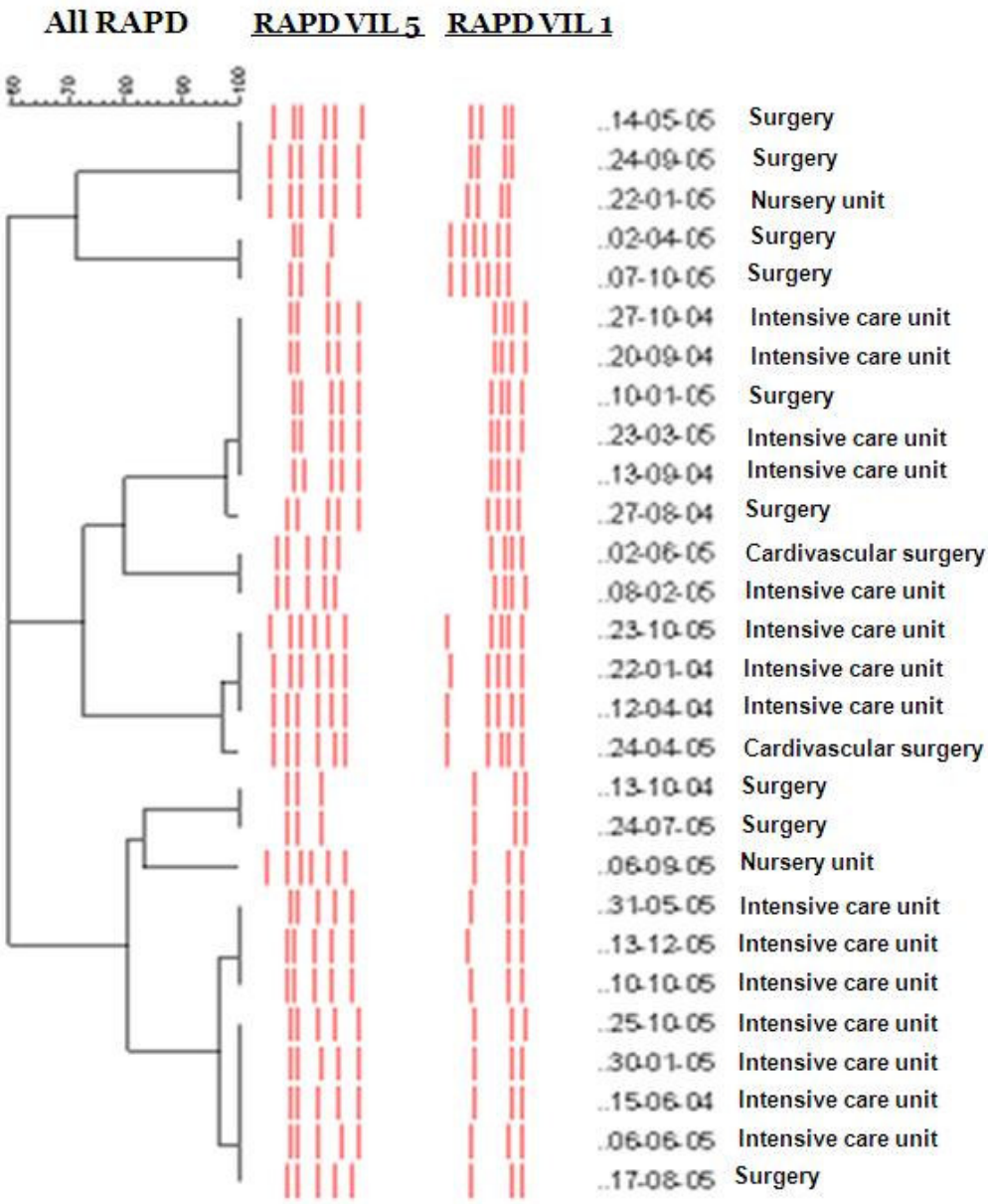

Figure 2. RAPD profiles of some epidemic strains that were occurred during (2004- 2005) vil1 and vil 5: primers used

PFGE

The most frequent PFGE patterns of *A. baumannii* isolates investigated and generated in our laboratory is illustrated in Figure 3 (pulsotypes A, B, C). Pulsotypes of the 204 *A. baumannii* strains revealed 22 different pulsotypes. The presence of such temporally and epidemiologically related and unrelated strains in two years study demonstrated the endemic situation in our hospital. Each PFGE pattern yielded 15 to 20 bands. Three groups, A ,B and C were markedly predominant, as these were represented by 22%, 27% and 10% respectively and were dispatched essentially in intensive care but in separate period. Some sporadic strains with unique pulsotype profile were showed.

In addition, comparison of some profiles revealed minor differences (% of similarity ≥90) suggesting that strains may be have derived from a common ancestral strain.

The genomic typing using PFGE revealed groups that RAPD couldn't distinguish. This demonstrates the sensitivity of this typing method.

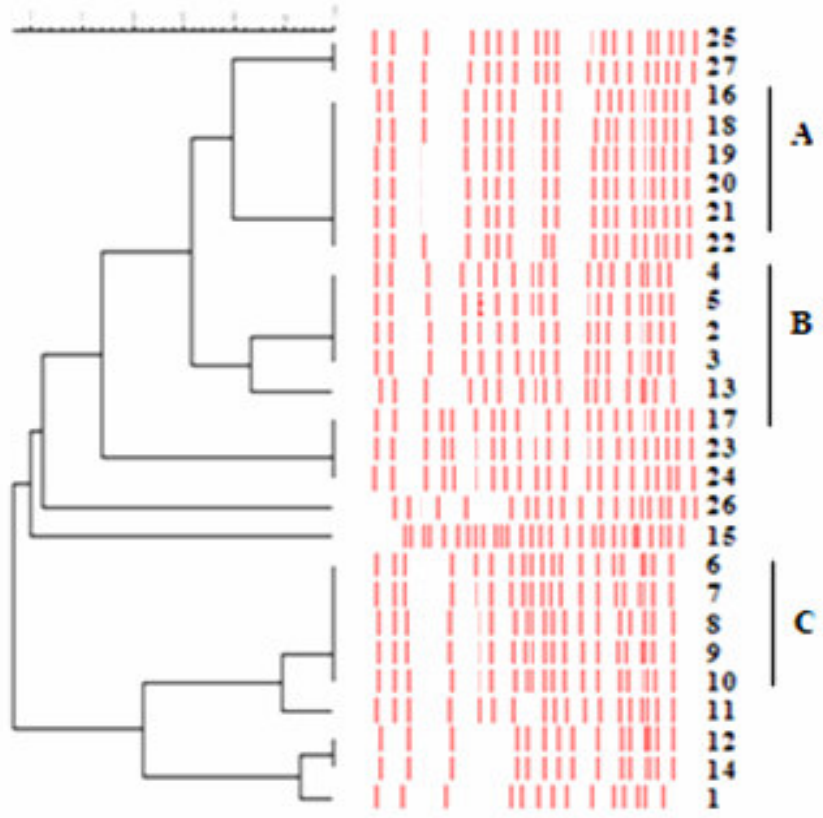

Figure 3. PFGE profiles and dendrogram of some epidemic *A.baumannii* strains isolated between the two years study digested with Apa I

DISCUSSION - CONCLUSION

The combined typing results showed a good correlation between the genomic method typing (RAPD and PFGE) with almost more discrimination with PFGE typing. Infact, each RAPD group was despatched under 3 or 4 PFGE profiles. We

also showed some sporadic strains that had a unique profile and that were essentially sensitive to all antibiotics.

Our results also indicate that the RAPD technique represents a rapid and simple means of typing of *A. baumanni.* However, PFGE, the gold standard of epidemiological methods, was indispensable for more discrimination and detection of epidemic strains.

These results don't correlate with the antibiotyping. Infact, many genomic clusters comprising more than one isolate contained strains with more than one antibiogram. This may be explained by horizontal transfer of gene resistance between strains due to the endemic situation. In addition, the genomic groups were heterogeneous comprising some unrelated strains. Some epidemics strains persisted during the two years period which was in accordance with our antibiotic resistance behaviour and his rapid dissemination between patients.

This study had demonstrated many factors that may contribute to enhance the colonization and infections due to *A. baumannii* in our hospital.

Whether conditions and higher relative humidity during summer season may be considered as important factor that contribute to the prolonged survival of this bacteria and correlate with the peaks periods of its isolation. This finding was supported by further studies (4,10). During 4 years retrospective survey in Hong Kong, seasonal variation in the isolation of *Acinetobacter ssp.* corresponded to a peak period from July through October (the hot, humid summer season) during which increased numbers of *Acinetobacter ssp*. were isolated. In addition, it was known that *A. baumannii* is a bacteria that have prolonged life time survival to dry surfaces (10). Jennane *et al* (11) had demonstrated that the best disinfection in our hospital was obtained after 5minutes of bacterial contact with aldehyde, phenol and amphotere. In addition, different nonbiotic dry environmental sources have been implicated as routes of transmission, including reusable medical equipment and various components of hospital beds. A great variety of strains seem to have achieved high levels of resistance to a wide range of antimicrobial combinations possibly as a result of less restricted use of antibiotic. This finding was demonstrated by Tunisian studies (21). Brahmi *et al* (3) had tested the efficacy of local antibiotic policy in a Tunisian IUC which result in the decrease of carbapenems resistant *Enterobacteriacea*, antibiotherapy cost and length of stay. The sensibility to antibiotics was studied in our hospital (11) and it was demonstrated that a combination of Amikacin-Imipenem was effective against all strains (13). However, recent papers demonstrated that *A. baumannii* had also developed a resistance to colistin who was considered as the last universally active drug (7,14). This finding conduce to the possibility of use Cecropin A- Melittin peptides as alternative chemotherapy. This resistance to colistin was not yet observed in Tunisia.

The occurrence of this pathogen in our hospital environment demonstrated that ICU units and surgery wards were the most affected. This result confirmed the opportunistic behaviour of this bacteria described in the literature (3, 26) and others Tunisian hospitals (15).

In conclusion, when outbreaks of *A. baumannii* occur, it's essential to develop a restricted hygiene procedures and a serious surveillance of critical units such as ICU on very ill patients.

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