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Epidemiology of *Acinetobacter baumannii* Infection in a University Hospital

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Abstract

Acinetobacter baumannii (*A. baumannii*) is an opportunistic pathogen in immunocompromised patients. Its natural strength and ability to develop new antibiotic resistance make it the most important emerging bacterial pathogen. The aim of this study is to determine, through a 6 year descriptive study (from 2010 to 2015), the evolution of the epidemiological profile of *A. baumannii* isolated at the Mohammed VI University Hospital in Marrakech, Morocco and evaluate levels of resistance to different antibiotics.This study noted an increasing frequency of *A. baumannii*, it represented 7% of all bacteria isolated during the study period (n=546) and 21% of all multi-resistant bacteria isolated since 2013. All hospital departments were concerned, with a clear predominance in intensive care units (62% of isolates). The main infections were pneumonia and bacteremia. An increase in resistance of *A. baumannii* isolates was observed for all antibiotics tested, mainly in intensive care units: ceftazidime (95%), imipenem (92%), ciprofloxacin (92%) and gentamicin (82%). These results confirm the multi-resistant nature of *A. baumannii* and its nosocomial character.

A. baumannii infection is synonymous with poor quality of care. Monitoring its dissemination and its eradication must be a top priority at our hospital.

Keywords: Epidemiology; *Acinetobacter baumannii*; Multidrug; Resistant bacteria; Resistance to antibiotics

Abbreviations

A. baumannii: *Acinetobacter baumannii*; ABMR: Multi-Drug Resistant *Acinetobacter baumannii*; ICU: Intensive Care Unit; MIC: Minimum Inhibitory Concentration; EUCAST: European Committee on Antimicrobial Susceptibility Testing; ONERBA: National Observatory of the Epidemiology of Bacterial Resistance to Antibiotics; CA-SFM: Antibiogram Committee of the French Society of Microbiology

Introduction

Acinetobacter baumannii is a Gram-negative coccobacillus. It is an opportunistic pathogen in immunocompromised patients, that attracted considerable attention for the last 40 years as the most important emerging bacterial pathogen [1,2]. This is explained by its ability to colonize the biotic and abiotic surfaces with high resistance to disinfectants but also to desiccation by formation of biofilms [3,4].

A. baumannii currently occupies an important place in hospital pathology globally, with an important role in potentially epidemic nosocomial infection. Indeed, Infections due to *A. baumannii* are frequent and severe with high morbidity and mortality [5,6]. *A. baumannii* is also characterized by its ability to over-regulate its innate resistance mechanisms, but above all to acquire new resistance mechanisms whose diversity is impressive with various genetic supports. Therefore, the therapeutic options for this bacteria are very limited [1,7].

Knowledge of local epidemiology is essential to monitor trends in antibiotic resistance, in order to determine the magnitude of this phenomenon, to adapt the protocols of probabilistic antibiotic therapy and to evaluate actions to fight this bacterium.

The aim of this study is to determine, through a 6 year descriptive study (from 2010 to 2015), the evolution of the epidemiological profile of *A. baumannii* isolated at the University Hospital Mohammed VI in Marrakech (Morocco) and evaluate levels of resistance to different antibiotics.

Materials and Methods

This is a retrospective and descriptive study that was carried out in the microbiology laboratory of the Mohamed VI University Hospital in Marrakech from 1 January 2010 to 31 Dec 2015.

We defined as Inclusion Criteria: all strains of *A. baumannii* isolated from diagnostic specimens from patients hospitalized in different departments. While the *A. baumannii* strains isolated from samples that were collected for epidemiological purpose as well as the duplicates were excluded.

The specimens studied were Pus, Urines, Catheters, Blood cultures, Respiratory specimens, Puncture fluids, etc. Isolation of the bacteria was carried out by culturing on enriched and selective agar media at 37°C. For 24 to 48 hours. Bacterial identification was based on standard morphological, cultural and biochemical characteristics [8,9]. While Biochemical Identification was done by API 20E and NE galleries of Bio-Mérieux (bioMérieux INDUSTRY, France).

The drug susceptibly testing was determined by diffusion of antibiotic discs in agar medium, according to the recommendations

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Table 1: Distribution of *A. baumannii* in isolated bacterial species.

Bacterial species	Number	Percentage (%)
Acinetobacter baumannii	**546**	**7**
Enterobacteria	2964	38
Staphylococcus aureus	1248	16
Streptococci	1170	15
Pseudomonas aeruginosa	624	8
Other species	1248	16
TOTAL	7800	100

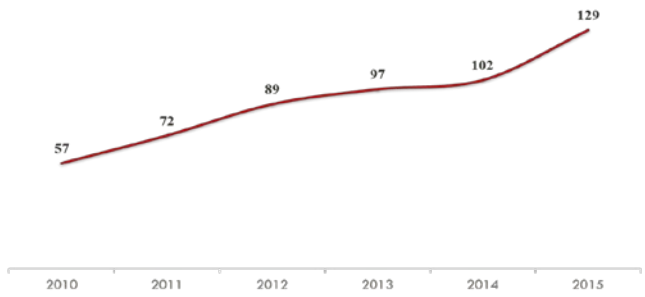

Figure 1: Evolution of the number of *A. baumannii* isolates between 2010 and 2015.

of the Antibiogram Committee of the French Society of Microbiology [10].

The antibiotic discs tested were: Ticarcillin (TIC), Ticarcillin/Clavulanic Acid (TCC), Piperacillin (PIP), Piperacillin/Tazobactam (TZP), Aztreonam (ATM), Cefotaxime (CRT), Ceftriaxone (CRO), Ceftazidime (CAZ), Cefepime (FEP), Imipenem (IPM), Meropenem (MEM), Gentamicin (GM), Tobramycin (TM), Amikacin (AN), Netilmicin (NET), Ciprofloxacin (CIP), Colistin (CS), Tetracycline (TET), Cotrimoxazole (SXT) (Oxoid Inc, Canada).

Multi-drug resistance in *A. baumannii* is often defined by a resistance affecting at least one agent in three or more antimicrobial categories [11]. This resistance is observed in the presence of any reduction in critical diameters or MICs of antibiotics according to the recommendations established by CASFM / EUCAST [10].

Results

Epidemiology of *A. baumannii* at the Marrakech University Hospital between 2010 and 2015

Among all the samples collected from the different study sites of the hospital, *A. baumannii* accounted for 7% of all germs isolated during this period (n=546). Enterobacteria occupied the first place followed by *Staphylococcus aureus*, Streptococci, Pseudomonas aeruginosa and finally *A. baumannii* (Table 1). Within the genus, *Acinetobacter, baumannii* dominated the profile and accounted for 98% of all species isolated in the genus Acinetobacter. The other species were mainly represented by the two species: *A. calcoaceticus and A. lwoffii.*

The frequency of isolation of *A. baumannii* has increased markedly over the past 6 years, from 57 strains in 2010, 72 strains in 2011, 89 strains in 2012 and 97 strains in 2013, 102 strains in 2014 to Reach 129 strains in 2015, almost double the number of strains

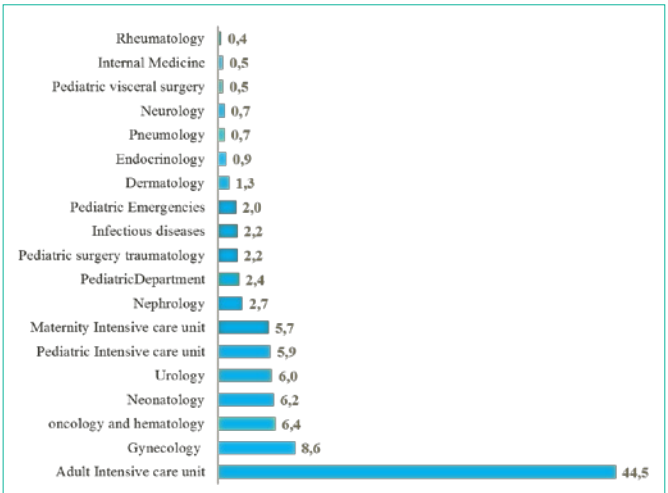

Figure 2: Distribution of *A. baumannii* strains according to departments by percentage.

isolated in the first year of the study (Figure 1). The distribution of *A. baumannii* isolates by sex, showed a male predominance with 63% isolates. The sex ratio Male/Female was: 1,7. The majority of *A. baumannii* strains were isolated in adult subjects in 70% of cases. While 30% of *A. baumannii* isolates were collected from children (<15 years of age).

The *A. baumannii* found with a clear predominance in intensive care units with 62% of *A. baumannii* isolates, followed by departments of medicine 20%, then Surgical departments with 18% of isolates. The adult intensive care unit represented the first origin of the isolated *A. baumannii* 44.5% (Figure 2). The distribution of the *A. baumannii* isolates according to the nature of the samples revealed the predominance of the strains in the protected distal specimens, followed by the cytobacteriological examinations of the urine. The main infectious sites at which *A. baumannii* was most frequently isolated were pneumonia, bacteremia, supportive infections and urinary tract infections.

Epidemiology of antibiotic resistance of *A. baumannii* isolates at the Marrakech University Hospital between 2010 and 2015

During the study period, *A. baumannii* accounted for 18% of isolated multi-resistant bacteria. The extended beta lactamase secreting enterobacteria remained the most frequently isolated multi-resistant bacteria. The presence of ESBL was confirmed by a qualitative method according to the CASFM standards [10]. The qualitative method can consist in the use of the synergy between two discs on the standard antibiogram: a disc of cefotaxime, ceftazidime or cefepime and a disc containing clavulanic acid (amoxillin+clavulanic acid: AMC), 30 mm apart from the cephalosporin discs. The presence of an ESBL is expressed by the appearance of a synergy in "bouchon de champagne" shaped image. However, when clinical isolates producing ESBL have mechanisms of β-lactam resistance such as hyper production of cephalosporinase, the detection of the synergy image is facilitated by performing a standard antibiogram on Mueller-Hinton agar supplemented with 250 mg/L cloxacillin (cephalosporinase inhibitor) [10].

The frequency of isolation of ABMR experienced a clear increase

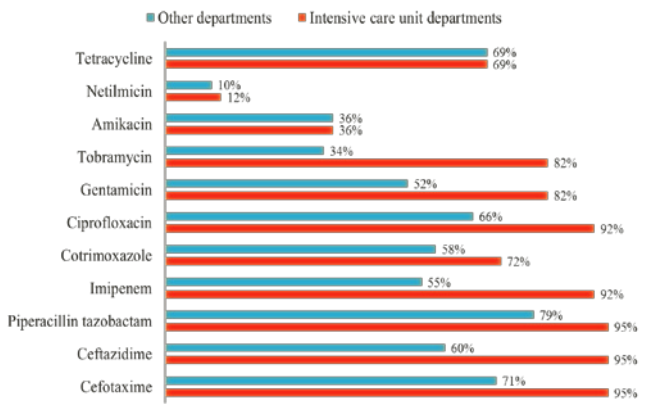

Figure 3: Resistance profile of *A. baumannii* isolates in intensive care units comparing to other hospital departments.

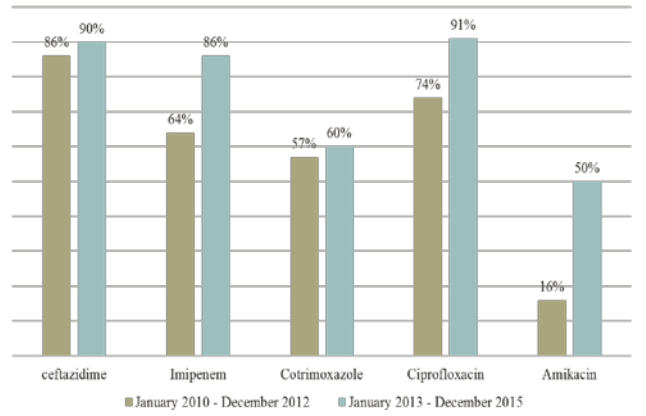

Figure 4: Evolution of *A. baumannii* isolates resistance for the antibiotics that showed significative change between 2010 and 2015.

between the period of 2010-2012 where *A. baumannii* represented 15% of multi-resistant bacteria and the period of 2013 to 2014 and 2015 when the rate reached 21% of all isolated multi-resistant bacteria.

The problem of bacterial resistance to antibiotics arises mainly in Enterobacteria resistant to third generation cephalosporin followed by ABMR that ranks second.

Of the 340 strains of *A. baumannii* isolated from intensive care units between 2010 and 2015, 95% of the strains isolated were resistant to cefotaxime, ceftazidime and piperacillin tazobactam. 92% of *A. baumannii* isolated were resistant to imipenem and 92% were also resistant to ciprofloxacin. The rate of resistance to gentamicin was 82% and 64% of the isolates retained sensitivity to amikacin. 82% of the strains remained sensitive to netilmicin. 72% of the isolated strains were resistant to cotrimoxazole (Figure 3).

The resistance rate observed in *A. baumannii* isolates from other hospital departments was lower than in the intensive care unit such for gentamicin, fluoroquinolones, imipenem and Third generation cephalosporins (Figure 3).

An increase in the resistance of *A. baumannii* isolates was observed for all antibiotics tested between 2010 and 2015.

The resistance of *A. baumannii* isolates to imipenem increased from 64% to 86% between the period 2010-2012 and the period 2013-2015. Resistance to amikacin also increased between the two periods from 16% to 50%.

The same observation was noted for ciprofloxacin for which the resistance of the isolated strains changed from 74% to 91% (Figure 4).

Tigecycline was tested in 50 strains of *A. baumannii* during 2015. Intermediate susceptibility to Tigecycline was noted in 11 strains, two strains showed resistance and 74% retained sensitivity to tigecycline.

Discussion

A. baumannii strains accounted for 7% of all bacteria isolated within the laboratory from 2010 to 2015. This result is close to the rate reported by a study carried out at the FES University Hospital in 2015 (Morocco) [12]. On the other hand, The prevalence survey carried out on many French hospitals in 2011 and the 2014 annual report of ONERBA, showed very low rates which are less than 1% for all departments combined [8,13]. This geographic variability in the distribution of *A. baumannii* within isolated bacterial species between cities and countries is related to differences in the use of antibiotics, infection control policies, but especially hygiene and disinfection [14].

A. baumannii accounted for 98% of the isolated Acinetobacters, which is consistent with data from the literature that finds the predominance of this strain with similar rates. Indeed, *A. baumannii* is the most incriminated Acinetobacter in the nosocomial infections by its capacity to colonize and to survive in the hospital environment [5,15,16].

A. baumannii infections were more frequent in the male population than female with a sex ratio of 1.7. This masculine predominance is reported by several national, Maghreb and international studies [17-21], but some studies have reported a female predominance [22,23]. The male predominance can be explained by the fact that *A. baumannii* is often associated with underlying conditions such as smoking, alcoholism, diabetes as well as other pneumopathies [17,21].

In this study, more than 70% of *A. baumannii* strains were isolated in adult subjects, which join literature, in particular in a study carried out at the Sahloul University Hospital in Tunisia, in addition to a prospective cohort carried out on several hospitals in Spain which report the predominance in elderly subjects with averages of more than 60 years [21]. Multiple underlying morbidities and multi-organ failure, which are most often associated with advanced age, favor immunosuppression and consequent prolonged hospitalization, especially in intensive care units. This explains why these subjects are more likely subject to nosocomial infections [19].

A large proportion of strains of *A. baumannii* (62%) were hospitalized in Intensive care units, with adult ICU as leader (44%). These results are in line with the literature since ICU is still the most proving service for *A. baumannii* infection but with variable rates. The rate found in this study is close to that reported at the Casablanca University Hospital in 2015 (59%), lower than that reported in Fez in 2015 (82%), but still higher than that reported in Spain in 2014 (46%) [12,21,24]. Patients in ICU departments have a higher risk of

Table 2: Comparison of carbapenem resistance rates.

	Our study	YAHYAOUI Fez 2015	ZEROUALI Casablanca 2015	Algeria 2014	Tunisia 2011	France 2014	Greece 2014	Norway 2014	Brazil 2011
Carbapenems resistance rate (%)	78	97	74	79	34.5	15.2	90.6	0	69

developing *A. baumannii* infection, which is explained by the severity of underlying conditions, prolonged hospitalization, broad spectrum antibiotherapy and the use of multiple invasive procedures [21,22].

The protected distal specimens represented the main isolation site of *A. baumannii* with a rate of 33%. This result joins several studies that report rates similar to or higher than ours, 33% in Casablanca in 2015, 37% in Fez in 2015, 48.3% in Spain in 2014 and 46% in Brazil in 2011 [5,12,21,24].

Analyzing the evolution of *A. baumannii* according to the years of study, an increase in upward curve of the number of strains isolated was observed. A thesis carried out in 2014 in Algeria on the 3 university hospitals of Tlemcen, Oran and Sidi Bel Abbes as well as the report of ONERBA 2014 report different findings with curves of evolution without particular trend [17,19]. The growing trend in this study may reflect the importance of the hospital pool, hygiene practices and the misuse of antibiotics, highlighting the need to develop comprehensive strategies to control these infections [14].

A. baumannii is the second multi-resistant bacterium isolated in our study after the extended beta lactamase secreting Enterobacteria, this positioning joins that of the literature but with different rates. The frequencies reported by studies carried out in Rabat in 2012 (26.5%), Fez in 2015 (27%) and Algeria in Tlemcen in 2014 (28.6%) remain higher than the rate reported in this study. However, this frequency of 18% in this work remains higher than that reported in ONERBA's 2014 annual report (6.7%). The evolution of the place of *A. baumannii* within the multi-resistant bacterium has been marked by the increase of its frequency of isolation from 15% during the 2010-2012 period to 21% in 2015, whereas the annual report of The ONERBA reports a variable evolution with a frequency close to 6% between 2010 and 2012 [12,13,15,25].

The results in terms of resistance to antibiotics found in this study were alarming. Resistance rates were very high for beta-lactams, up to 95% for ICU services and around 70% for other services. Recent national and international studies have shown similar results with high levels of resistance to beta-lactams, in particular in a study conducted in Casablanca in 2015 (91%), in Fez in 2015 (97%), in the 2014 Algerian network report surveillance of antibiotic resistance (90%), as well as in a Spanish study of 2010 (93.9%) and an Iranian study carried out in 2012 [12,21,24,26,27]. The report published by ONERBA in France in 2014 revealed lower resistances ranging from 17% to 35% [13]. Indeed *A. baumannii* naturally has mechanisms of resistance to beta-lactams to which will be added its ability to easily acquire resistance [28,29].

For carbapenems, the isolation of resistant strains was very high, reaching 92% in ICU with an average of 78% for all departments combined. These percentages are similar to those reported in Casablanca, Fez, Algeria, Greece and Brazil; On the other hand, low rates have been reported in Tunisia, France and particularly in Scandinavian countries such as Norway (Table 2) [5,12-14,24,26,30].

The place of carbapenems as a reference antibiotic in *A. baumannii* infection has been questioned since the emergence from the 1990s of numerous epidemics due to imipenem resistant strains described in several countries with no particular geographical distribution. This resistance is mainly related to the production of oxacillinases having carbapenemase activity [19,31].

Resistance to aminoglycosides varied by 82% for gentamicin and tobramycin, 36% for amikacin and only 12% for netilmicin, which remains the most effective aminoside. These results are close to those found in the literature in the Maghreb and other underdeveloped and developing countries as well as certain European countries (Greece, Croatia, Italy and Romania). However, very low rates were found in Scandinavian countries reporting rates around 3% [13,14,26,27,30].

The rate of resistance to ciprofloxacin reported in this study was 82% for all departments and reached 92% for strains from ICU. This rate was close to that reported in Casablanca, Algeria and Spain with respective rates of 85%, 81% and 89% [21,24,26]. This rate even reaches 99% in a national study conducted at the university hospital of Fes in 2015 and reaches 95% in Greece in 2013. Elsewhere, data from the European network for surveillance of antibiotic resistance report very low percentages with a rate of 13.6% In France and 0% in Norway [8,17].

As for colistin, which is often the only therapeutic alternative for carbapenem-resistant *A. baumannii* strains. Our strains have retained a constant sensitivity on the standard antibiogram but the confirmation by the MIC determination has not been made [32]. Several studies confirm this result but with somewhat higher resistance rates, in particular the study carried out at the Casablanca, which reported a resistance rate of 2%, a thesis carried out in Algeria reported a resistance of 6% at the Tlemcen University Hospital and a study carried out in Spain in 2010 reported a rate of 0.3% [15,21,24]. EARS's Report 2014 has identified a rate of 5% [14].

Of the 50 strains tested in 2015, 4% of the strains showed resistance to tigecycline. These results are in line with several studies that report resistances ranging from 0 to 12%x [33-37]. However, a meta-analysis carried out in 2016 calls into question the intake of this antibiotic in the *A. baumannii infection*, noting the absence of any significant difference in therapeutic response, mortality and duration of hospitalization [38]. These data must be supplemented by a larger series of strains tested.

Analysis of the evolution of *A. baumannii* resistance to antibiotics showed an increase in resistance rates over the years of study. This increasing trend is reflected in several published studies and severely increases the risk of impasse in treatment. In particular, an Algerian thesis speaks of a significant increase in the resistance to the Imipenem from 50.6% in 2009 to 75% in 2012 and in Tunisia with the increase in resistance from 2% in 2000 to 33% in 2007. Than the ONERBA Network in France, which reports an evolution of resistance to the imipenem from 1% in the year's 2000 to 21.5% in 2012. The same

observation is noted in Spain with an evolution from 66.2% in 2000 to 82.7% in 2010 [13,15,21,30].

The same finding was noted for the other antibiotics especially amikacin and ciprofloxacine [13-15,21].

Conclusion

A. baumannii have an important role in hospital pathology due to its great capacity to colonize and persist in the hospital environment, its increasing frequency, its pathogenic potential and its ability to continuously acquire resistance. Therefore, these bacteria should imperatively be the subject of national surveillance programs in all countries.

The results of this study showed the importance of nosocomial infection due to *A. baumannii* within our hospital. This study made it possible to note the increasing frequency of *A. baumannii* isolates between 2010 and 2015. Their levels of resistance to antibiotics have been very high. Faced with this alarming situation, which severely limits the therapeutic arsenal and increases the risk of impasse in treatment, it is imperative to rationalize the use of antibiotics and improve hygiene measures. *A. baumannii* remains the most feared agent in nosocomial infection because it is a difficult opponent to control and eliminate and the optimal treatment of infections with multidrug resistant strains remains to be established.

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