Prevalence of Acinetobacter baumannii bacteremia in intensive care units of Ibn Rochd University Hospital, Casablanca

Assiya El Kettani1,2*, Fakhreddine Maaloum1,2, Idrissa Diawara1,2, Khalid Katfy1,2, Nadia Harrar2, Khalid Zerouali1,2, Houria Belabbes1,2, Naima Elmdaghri1,2

1Department of Microbiology, Faculty of Medicine and Pharmacy, Hassan II University of Casablanca, Morocco
2Bacteriology-Virology and Hospital Hygiene Laboratory, University Hospital Centre Ibn Rochd of Casablanca, Morocco

Received: July 2017, Accepted: November 2017

ABSTRACT

Background and Objectives: Acinetobacter baumannii bacteremia are grave because of the multi-resistance of the organism to antibiotics. This study aimed to determine the prevalence of A. baumannii isolated from blood cultures and to describe their antibiotic resistance patterns.

Materials and Methods: A retrospective longitudinal study was conducted on blood cultures between 2010 and 2014 from all Ibn Rochd University Hospital intensive care units; it was based on the exploitation of microbiology laboratory database (duplicates were excluded). Isolation and identification of A. baumannii were performed according to standard techniques of bacteriology and susceptibility testing as recommended by the CLSI. PCR was used to detect β-Lactamase genes, blaOXA-51, blaOXA-23.

Results: Among the 4232 samples received at the laboratory, 2402 (56.8%) were positive. Negative coagulase Staphylococcus was isolated in 21.6% of cases followed by A. baumannii (9.2%), and K. pneumoniae (9.1%). A. baumannii strains were resistant to most antibiotics tested: imipenem (75.7%), ceftazidim (85.4%), cefotaxim (98.6%), gentamicin (78.1%), amikacin (63.5%) and ciprofloxacin (88.2%). All A. baumannii strains, resistant to carbapenem, tested were positive for blaOXA-51 genes and 87.5% expressed the blaOXA-23 genes.

Conclusion: A. baumannii was the second germ frequently isolated from blood cultures in intensive care units. It was multi-resistant to antibiotics. The strengthening of hospital hygiene measures and surveillance of antibiotic resistance is needed to limit the spread of germs and to optimize the management of antibiotics.

Keywords: Acinetobacter baumannii, Bacteremi, Antibiotic resistance, blaOXA-51, blaOXA-23

INTRODUCTION

Acinetobacter baumannii is a Gram-negative pathogen. It is frequently associated with nosocomial infections (bacteremia, pneumonia, meningitis and urinary tract infections). It was also recognized worldwide as an emerging cause of nosocomial outbreaks and listed by the American Society of

*Corresponding author: Dr. Assiya El Kettani, Department of Microbiology, Faculty of Medicine and Pharmacy, Hassan II University of Casablanca, Morocco; Bacteriology-Virology and Hospital Hygiene Laboratory, University Hospital Centre Ibn Rochd of Casablanca, Morocco.
Tel: +212619094322
Email: assiyaellkettani@gmail.com
Infectious Diseases (IDSA) as one of the six most hazardous microorganisms (1). \textit{A. baumannii} bacteremia in intensive care units (ICUs) are responsible of a significant morbidity and mortality and create a therapeutic problem due to the multidrug resistance of the organism to antibiotics (2, 3). \textit{A. baumannii} have a natural resistance to antibiotics, but also an acquired resistance by production of enzymes: the \( \beta \)-lactamases and especially metallo-\( \beta \)-lactamase and oxacillinas and by efflux pumps and changing porins (4). Carbapenem hydrolyzing \( \beta \)-lactamases belonging to oxacillinases enzymes are the main enzymes contributing to the inactivation of imipenem in \textit{A. baumannii} (5, 6).

The aim of this study was to determine the prevalence of \textit{A. baumannii} isolated from blood cultures performed in intensive care units of Ibn Rochd University hospital at Casablanca-Morocco during the last five years (2010-2014) and to describe the evolution of the organism’s resistance to antibiotics. Then, to detect the presence of Oxacillinas: OXA-23 and OXA-51 among the isolates showing resistance to imipenem.

MATERIALS AND METHODS

Samples collection (Patients samples). A retrospective longitudinal study was carried out on blood cultures from all ICUs in Ibn Rochd University Hospital between 2010 and 2014. It was based on the exploitation of the Microbiology laboratory database (duplicates of the same patient were excluded). All bacteria including \textit{A. baumannii} identified by the standard bacteriological methods in the routine diagnosis of bacteremia from all ICUs were examined. PCR was used to target the \textit{bla_{OXA-23}} and \textit{bla_{OXA-51}} among the imipenem resistant isolates \textit{A. baumannii}.

Antimicrobial susceptibility testing. Antimicrobial susceptibility testing was done for all \textit{A. baumannii}. Disk diffusion test was used to determine the susceptibility of isolates to ceftazidim (30 \( \mu \)g), 30 \( \mu \)g cefotaxim (30 \( \mu \)g), 30 \( \mu \)g amikacin (30 \( \mu \)g), 10 \( \mu \)g gentamicin (10 \( \mu \)g), ciprofloxacin (5 \( \mu \)g), ampicillin/sulbactam (10 \( \mu \)g), piperacillin/tazobactam (85 \( \mu \)g), netilmicin (10 \( \mu \)g), Tobramycin (10 \( \mu \)g), trimethoprim/sulfamethoxazol (25 \( \mu \)g), tetracycline (30 \( \mu \)g), imipenem (10 \( \mu \)g). E-test was used to determine the MICs for colistin (Biomérieux Marcy l’Etoile France). \textit{E. coli} ATCC 25922 was used as a quality control strain. Results were interpreted according to the breakpoints recommended by the Clinical and Laboratory Standards Institute (CLSI, 2014).

Preparation of DNA and PCR. All \textit{A. baumannii} resistant to imipenem were grown on Mueller–Hinton (MH) agar plates (Bio-Rad, Marnes-la-Coquette, France) for 18-24 hours at 37°C, bacterial cells were suspended in 500 \( \mu \)l of ultrapure water. Suspension was heated at 100°C for 10 min and immediately frozen at 0°C for 5 min. 300 \( \mu \)l of supernatant were then recovered after centrifugation of 14000 g for 10 min. Supernatant containing DNA was stored at -20°C until further use (5).

PCR protocol described by Sandle et al. (6) was used for detection of \textit{bla_{OXA-23}} and \textit{bla_{OXA-51}} among the imipenem resistant isolates.

Statistical analysis. Data were analyzed with Epi-Info 7 (Centers for Disease Control, Atlanta, Georgia, USA) and Microsoft Excel. The chi square test or Fisher’s exact test was performed to compare proportions. Differences were considered significant if the \( p \)-value is <0.05.

RESULTS

During the study period, a total of 4232 non-duplicate blood cultures from 4232 patients were received at the laboratory. Of these, 2402 blood cultures (56.8%) were positive. Negative coagulase Staphylococcus was isolated in 21.6% of cases followed by \textit{A. baumannii} (9.2%) and \textit{K. pneumoniae} (9.1%) (Fig. 1).

The prevalence of \textit{A. baumannii} strains increased from 7% in 2010 to 10% in 2011, 2012 and 2013, and then decreased slightly in 2014 (9%). \textit{A. baumannii} strains were resistant to: imipenem (75.7%), ceftazidime (85.4%), cefotaxime (98.6%), gentamicin (78.1%), ciprofloxacin (88.2 %), the netilmicin (14%) and colistin (1%) (Fig. 2).

The evolution of antibiotic resistance was determined for ceftazidim, cefotaxim, imipenem and ciprofloxacin. It was relatively stable for the third generation cephalosporins and fluoroquinolones. However, it increased for imipenem, from 50% in 2010 to 86% in 2013 (\( p=0.0003 \)) and then decreased slightly in 2014 (75%) (\( p=0.35 \)) (Fig. 3).
As for the genotypic characterization of *A. baumannii* strains resistant to imipenem, determined by PCR, all *A. baumannii* strains were positive for *bla*\textsubscript{OXA-51} genes and 87.5% (n=147) expressed the *bla*\textsubscript{OXA-23} genes (Fig. 4).

**DISCUSSION**

In our study, *A. baumannii* was the second germ frequently isolated from blood cultures in ICUs between 2010 and 2014 (9.2%). It was characterized by a multi-resistance to the antibiotics tested (C3G, aminoglycosides, fluoroquinolones and imipenem). It was however, sensitive to colistin in 99% of cases.

The evolution of antibiotic resistance during the study period was increasing especially for imipenem (from 50% in 2010 to 75% in 2014). All isolates of *A. baumannii*, resistant to carbapenem, were positive for *bla*\textsubscript{OXA-51} genes and 87.5% were positive for *bla*\textsubscript{OXA-23}.

*A. baumannii* is considered as an opportunistic pathogen that can survive in austere conditions. It is responsible of an increasing rate of severe nosocomial infections. They affect especially immunocompromised patients, exposed to prolonged stays in ICUs and having a previous exposure to antibiotics; carbapenems and 3rd generation cephalosporins are the most involved, followed by fluoroquinolones, aminoglyco-
sides and metronidazole (2, 7). Other factors that are associated with the occurrence of *A. baumannii* bacteremia are: assisted ventilation, central catheterization, urinary catheters, and nasogastric probes (8).

The distribution of bacteria in our study is consistent with a previous study in the same hospital in which *A. baumannii* represented 13% of the bacteria isolated from blood cultures (9). It should be noted that the negative coagulase staphylococci are contaminants in the majority of cases and only 10% to 30% were clinically significant (10). Nevertheless, this distribution contrasts with data from a French study where *S. aureus* (18.4%), *E. coli* (15.4%) and *S. epidermidis* (14.4%) accounted for almost a half (48.3%) of blood cultures isolated microorganisms; *Candida albicans* represented 2.5%. *A. baumannii* was rarely isolated (0.6% of microorganisms responsible of nosocomial infections in all sites) (11).

Resistance to the 3rd generation cephalosporins and ciprofloxacin, in Algeria, Tunisia and France, is over than 50% (11-14) as it was in our study.

Resistance to imipenem (treatment of choice for *A. baumannii* infections) is variable: 3.3% in France, 60% to 89.9% in Tunisia and 48% in Algeria (11-14). Our study objectified an increased resistance from 50% in 2010 to 86% in 2013 with a slight decrease in
The acquired carbapenem resistance in *A. baumannii* is often associated with carbapenemase production; IMP, VIM and SIM-type metallo-β-lactamase production or the OXA-24, OXA-23 and OXA-58 type class D carbapenemases. But also with the over production of natural oxacillinase (OXA-51) (15). OXA-23 and OXA-51 are considered as the most prevalent among *A. baumannii* carbapenem resistance (16). In our study, all *A. baumannii* strains were positive for *bla*_{OXA-51} and 87.5% (n=147) for *bla*_{OXA-23}.

Strains of *A. baumannii* harbouring OXA-23 enzymes have been identified in Brazil, Argentina and Colombia (17-21). Moreover, carbapenemases belonging to OXA-23 subgroup have been detected in Europe, Australia, Tahiti, China, Korea, Singapore, Vietnam, USA, Libya and Pakistan (22) Mobilization of the *bla*_{OXA} genes is determined by the presence of insertion sequences and transposons, and therefore has a high potential to spread (23). That is why it is essential to conduct molecular genotyping studies as well as characterizing the carbapenemases found in specific geographic areas, to highlight molecular evolution of both genes and resistant clones.

The multi-drug resistance of this germ has led to a renewed interest in colistin, to the association of antibiotics (rifampicin-colistin, colistin-imipenem) and the use of new antibiotics such as tigecycline to overcome therapeutic impasses (24).

**CONCLUSION**

*A. baumannii* was the second germ that was frequently isolated in blood cultures of intensive care units in the last five years in Ibn Rochd University Hospital. It was multi-resistant to antibiotics with a particular increasing resistance to imipenem. The strengthening of hospital hygiene measures and antibiotic resistance surveillance is needed to limit the spread of multi resistant bacteria and to optimize the management of antibiotic therapy.

**REFERENCES**

1. Boucher HW, Talbot GH, Bradley JS, Edwards JE, Gilbert D, Rice LB, et al. Bad bugs, no drugs: no escape! An update from the Infectious Diseases Society of America. *Clin Infect Dis* 2009; 48: 1-12.
2. Lee HY, Chen CL, Wu SR, Huang CW, Chiu CH. Risk factors and outcome analysis of *Acinetobacter baumannii* complex bacteremia in critical patients. *Crit Care Med* 2014;42:1081-1088.
3. Esterly JS, Griffith M, Qi C, Malczynski M, Postelnick MJ, Scheetz M H. Impact of carbapenem resistance and receipt of active antimicrobial therapy on clinical outcomes of *Acinetobacter baumannii* bloodstream infections. *Antimicrob Agents Chemother* 2011; 55: 4844-4849.
4. Rumbo C, Gato E, Lopez M, Ruiz de Alegria C, Fernandez-Cuenca F, Martinez-Martinez L, et al. Contribution of efflux pumps, porins, and β-lactamases to multidrug resistance in clinical isolates of *Acinetobacter baumannii*. *Antimicrob Agents Chemother* 2013;57:5247-5254.
5. Higgins PG, Lehmann M, Wisplinghoff H, Seifert H. gyrB Multiplex PCR To Diferentiate between *Acinetobacter calcoaceticus* and *Acinetobacter* genomic species. *J Clin Microbiol* 2010;48:4592-4594.
6. Sandle T, Babenko D, LavrinenkoA, Azizov I, Cheyča A. The current state of PCR approach in detection and identification of carbapenem hydrolysis β-lactamases genes. *EJPPS* 2014; 19:153-164.
7. Falagas ME, Kopterides P. Risk factors for the isolation of multi-drug-resistant *Acinetobacter baumannii* and *Pseudomonas aeruginosa*: a systematic review of the literature. *J Hosp Infect* 2006; 64:7-15.
8. Zhou HY, Yuan Z, Du YP. Prior use of four invasive procedures increases the risk of *Acinetobacter baumannii* nosocomial bacteremia among patients in intensive care units: a systematic review and meta-analysis. *Int J Infect Dis* 2014;22:25-30.
9. Hassoune S, Nani S, Ouhadous M, Aalloula O, Benbachir M, Maaroufi A. Incidence des bactériémies nosocomiales dans les services à haut risque du centre hospitalier universitaire de Casablanca (Maroc). *Prat Organ Soins* 2012;43(1):19-24.
10. Le groupe Rémic de la Société Française de Microbiologie. Rémic Référentiel en microbiologie médicale 4ème édition 2010.
11. Le groupe Rémic de la Société Française de Microbiologie. Rémic Référentiel en microbiologie médicale 4ème édition 2010.
12. Thabet L, Zoghlami A, Boukadida J, Ghanem A, Messadi AA. Comparative study of antibiotic resistance in bacteria isolated from burned patients during two periods (2005-2008, 2008-2011) and in two hospitals (Hospital Aziza Othmana, Trauma and Burn Center). *Tunis Med* 2013; 91:134-138.
13. Elhani D, Elhani I, Aouni M. Résistance chez les bacilles gram négatif. Où en sommes nous? Tunis Med 2012; 90: 680-685.

14. Bakour S, Touati A, Sahli F, Ameur AA, Haouchine D, Rolain JM. Antibiotic resistance determinants of multidrug-resistant Acinetobacter baumannii clinical isolates in Algeria. Diagn Microbiol Infect Dis 2013;76:529-531.

15. Ehlers MM, Hughes JM, Kock MM. Prevalence of Carbapenemases in Acinetobacter baumannii. IN-TECH Open Access Publisher 2012.

16. Ciftci IH, Asik G, Karakece E, Oksuz L, Yagci S, Sesli Cetin E, et al. Distribution of blaOXA genes in Acinetobacter baumannii strains: a multicenter study. Mikrobiyol Bul 2013;47:592-602.

17. Higgins P, Dammhayn C, Hackel M, Seifert H. Global spread of carbapenem-resistant Acinetobacter baumannii. J Antimicrob Chemother 2010; 65: 233-238.

18. Villegas MV, Kattan JN, CorreaA, Lolans K, Guzman A. M, Woodford N, et al. Dissemination of Acinetobacter baumannii Clones with OXA-23 Carbapenemase in Colombian Hospitals. Antimicrob Agents Chemother 2007;51:2001-2004.

19. Merkier A, Catalano M, Ramirez MS, Quiroga C,orman B, Ratier L, et al. Polyclonal spread of blaOXA-23 and blaOXA-48 in Acinetobacter baumannii isolates from Argentina. J Infect Dev Ctries 2008; 2: 235-240.

20. Carvalho KR, Carvalho-Assef APDA, Peirano G, Dos Santos LCG, Pereira MJF, Asensi MD. Dissemination of multidrug-resistant Acinetobacter baumannii genotypes carrying blaOXA-23 collected from hospitals in Rio de Janeiro, Brazil. Int J Antimicrob Agents 2009; 34: 25-28.

21. SchimithBier KE, Luiz SO, Scheffer MC, Gales AC, Paganini MC, Nascimento AJ, Carignano E, et al. Temporal evolution of carbapenem-resistant Acinetobacter baumannii in Curitiba, southern Brazil. Am J Infect Control 2010; 38: 308-314.

22. Peleg A, Seifert H, Paterson D. Acinetobacter baumannii: emergence of a successful pathogen. Clin Microbiol Rev 2008; 21: 538-582.

23. Opazo A, Dominguez M, Bello H, Amyes SGB, González-Rocha G. OXA-type carbapenemases in Acinetobacter baumannii in South America. J Infect Dev Ctries 2012; 6:311-316.

24. Fishbain J, Peleg AV. Treatment of Acinetobacter Infections. Clin Infect Dis 2010;51:79-84.