Acinetobacter baumannii: global evolution of carbapenem-resistant and genotyping methods

Prata-Rocha, Mariana Lima[1]; Moreira, Michel Rodrigues[1]; Gontijo Filho, Paulo Pinto[1] and Melo, Geraldo Batista de[1]

[1]Uberlândia Federal University, Laboratory of Microbiology.

Acinetobacter spp., principally *Acinetobacter baumannii,* are problematic nosocomial pathogens often with multiresistance to different antimicrobial classes. Until recently, carbapenems remained active against nearly all *Acinetobacter* spp. isolates, but resistance is increasingly reported from many countries, caused by acquired metalo-β-lactamases (MBLs) or OXA-type (class D) carbapenemases. The OXA enzymes may be divided into four main subgroups, the acquired OXA-23_{like}, OXA-24_{like} and OXA-58_{like} and the chromosomally located intrinsic OXA-51_{like} associated with IS_{ABA-1} upstream. Other OXA enzymes recently reported included OXA-143 in *A. baumannii.* The presence of intrinsic bla OXA-51_{like} alleles does not correlate with the level of carbapenem resistance of the host isolate, apparently because this gene is regulated by insertion sequences such as ISAba1. Thus, resistance to carbapenems cannot be inferred from detection of bla OXA-51_{like} alleles. In contrast, alleles encoding OXA-23_{like}, OXA-24_{like} and OXA-58_{like} enzymes were consistently associated with resistance or, at least, with reduced susceptibility. Typing of these organisms can be important to assess cases of cross-infections or to identify the sources and modes of spread of the organisms or to assess the diversity and spread of these organisms over wide geographic areas. Early methods included phenotypic method, but the most frequent typing methods are genotypic methods like macrorestrition analysis by pulsed-field gel electrophoresis (PFGE), ribotyping, PCR based fingerprinting, AFLP analysis and multilocus sequence typing system.

Keywords carbapenemases, oxacilinase, *Acinetobacter baumannii*, typing methods

Acinetobacter spp. is a glucose non-fermentative Gram-negative coccobacillus commonly found in water and soil [1], was described in 1911 by Beijerinck [2], other studies have followed resulting in a total of 32 named and unnamed species today [3].

A phenotypic system for species identification has been proposed by Bouvet and Grimont [2, 4]. In particular, the genetically closely related species *A. baumannii* and the unnamed genomic species 3 and 13TU, together with *Acinetobacter calcoaceticus* combined in the *A. baumannii – A. calcoaceticus* (Acb) complex, are difficult to distinguish by phenotypic tests [5].

Acinetobacter spp. has emerged as one of the most resistant pathogens causing a multiple of community or nosocomial infections, associated with elevated morbidity and mortality, especially in critically ill, imunosuppressed patients and those treated with broad-spectrum antibiotics [6-11, 12, 13]. Carbapenems are the agents of choice to treat serious *A. baumannii* infections in many centres [14-18].

Exposure of *A. baumannii* to the selective pressure of potent antimicrobials in the Intensive Care Unit (ICU) has gradually led to a global prevalence of *A. baumannii* strains that are resistant to all β-lactams, including carbapenems [19].

Carbapenem resistance amongst *Acinetobacter* spp. has been increasing in the last decade, and outbreaks due to carbapenem-resistant *Acinetobacter* have been identified worldwide [6-11].

During the past decade, the terms "pandrug resistance", "extensively drug resistance" and "multidrug resistance (MDR)" have been commonly applied to *A. baumannii* strains to designate, respectively, resistance to all, to all but one or two, and to three or more classes of potentially effective antimicrobial agents [20, 21].

The evolution of multidrug resistance is relatively fast, as the main driving force is lateral gene transfer [22], a process influenced by a wide range of mobile genetic elements. Integrons have often been found in plasmids and/or transposons that enhance the spread of resistance genes [23]; the class 1 integrons are most frequently isolated from MDR pathogens, and the ongoing use of antibiotics has swelled their numbers in recent years [24].

Different mechanisms of resistance to carbapenems have been reported, including degradation of the drug by β-lactamase, diminished permeability of the outer membrane and, to a lesser extent, overexpression of efflux pumps and changes in the target site owing to modification of penicillin-binding proteins [9, 25]. Among these, β-lactamases are the major significant factors which contribute for resistance towards β-lactams. Usually, when the minimum inhibitory concentration (MIC) of antimicrobials is very high several resistance mechanisms are associated.

The β-lactamases are divided into four classes (A to D) [26]. Carbapenemase-producing *A. baumannii* has been reported in many countries worldwide in Europe, South America, North America, Australia and Asia [27-30].

Three ($bla_{IMP-like}$, $bla_{VIM-like}$ and bla_{SIM-1}) of the 6 known metallo-β-lactamases have been identified in IRAB (*A. baumannii* imipenem-resistant), but these are less prevalent than CHDLs (carbapenem-hydrolyzing class D beta-lactamases), which include $bla_{OXA23-like}$, $bla_{OXA-24like}$, $bla_{OXA-51like}$, $bla_{OXA-58like}$ and $bla_{OXA-143like}$. $Bla_{OXA51-like}$ is intrinsic to *A. baumannii*, while the others are acquired. An insertion sequence (IS_{Aba-1}) enhanced expression of the intrinsic

chromosomal $bla_{OXA\text{-}51like}$ gene of *A. baumannii* [31] and enhanced expression of the acquired $bla_{OXA\text{-}23}$ [32] and $bla_{OXA\text{-}58}$ [31]. The expression of OXA-51 was reported to be up to 8 times greater in the presence of $IS_{Aba\text{-}1}$ when compared to the absence of the promoter sequence [31, 33].

Some of these genes have been reported to be encoded on plasmids, which contribute to the spread of resistance [14]. The source of this gene and the route of dissemination remain to be elucidated but there is evidence that this gene can be detected in the environment (air, soil and water) and in body lice, as well as in hospitals due to antibiotic selection pressure [1]. The emergence and spread in low-income countries is worrying since infections due to *A. baumannii* are associated with increased mortality as well as increased length of intensive care unit stay [1].

The loss of CarO OMP (outer membrane protein), which forms non-specific monomeric porin channels [34] is reported to cause carbapenem resistance in *A. baumannii* [35], though the prevalence of this mechanism has not been reported; combinations of resistance mechanisms have been observed too [34-37] though, again, the role of CarO loss is unclear in this context.

The distribution of *A. baumannii* carrying these different acquired CHDLs genes varies among different regions and even different hospitals [38, 39].

In Taiwan, in 2000, the imipenem-resistant rate in the intensive care units of 5 major hospitals was 22% [40], whereas in 2010, it was 66,8% [41]. Another study in Taiwan, in 2012, showed 48,8% of isolates resistant to imipenem and the most of the isolates carrying $bla_{OXA\text{-}23}$ (93,3%) and they had $IS_{Aba\text{-}1}$ upstream the $bla_{OXA\text{-}23}$ gene. All isolates contained $bla_{OXA\text{-}51}$. The presence of Tn2006 and Tn2008 in these isolates together with the diversity of the pulsotypes indicated that the preferred mechanism of spread of $bla_{OXA\text{-}23}$ was via transposons, but the spread of $bla_{OXA\text{-}23}$ via plasmid dissemination cannot be excluded with the use of current method [42].

In Taiwan, OXA-72 enzyme was the major mechanism of carbapenem resistance in *A. baumannii*, in 2009. OXA-72 enzyme-producing isolates were more resistant to carbapenems than those with $IS_{Aba\text{-}1}$-enhanced expression of the intrinsic OXA-51like enzyme [39].

In India, in 2012, the MIC determination showed that all the clinical isolates of *A. baumannii* were resistant to carbapenem, but the levels of resistance were different. Out of 26 clinical isolates, nine were highly resistant to imipenem (>32µg/mL) [43]. There has been a continuous rise in the resistance of *A. baumannii* to 35% compared to the incidence of ≈ 25% in 2004 [44]. Although imipenem resistance due to the presence of number of combination of efflux pump, OXA-66 and OXA-23 β-lactamase have previously been reported in the other part of world [28, 45] but imipenem is still a drug of choice.

One study in Brazil, in 2012, showed that 98% of carbapenem-resistant *Acinetobacter* strains were positive for genes encoding the enzyme OXA-51like, an enzyme that naturally exists in *A. baumannii* and has very weak carbapenem hydrolysing activity [46]. The same study found carbapenemase genes in 44 strains (88%), including 9 $bla_{OXA\text{-}51/OXA\text{-}23}$, 5 bla_{IMP} and 38 $bla_{OXA\text{-}143}$ [46]. Imipenem hydrolysing activity was detected in only three strains containing carbapenemase genes. The OMPs of 29KDa, 33-36KDa and 43KDa previously associated with carbapenem resistance were decreased or absent, highlighting the importance of alteration of OMPs as a mechanism of carbapenem resistance amongst *Acinetobacter* spp [46].

In Brazil, the OXA-23 like oxacillinases and the MBL IMP-1 were so far the most frequent carbapenemase described [9, 10, 11, 47]. Oxacillinases have a lower carbapenem hydrolysing activity compared with MBLs, but they are still the most frequent carbapenemases described in this genus of bacteria [48, 49].

In Minas Gerais, a state of Brazil, a total of 73 patients with 84 *A. baumannii* isolates were obtained in 2009-2010; that 59% were multidrug resistant, 11% were pan-resistant and 30% were non-MDR. Using the non-MDR isolates as the control group, the risk factors for the acquisition of MDR Acb were previous surgeries, the presence of co-morbidity (renal disease), use more than two devices, parenteral nutrition and inappropriate antimicrobial therapy [51].

The prevalence of $bla_{OXA\text{-}143like}$ is therefore unknown. A previous study found 76% isolates harboured the $bla_{OXA\text{-}143like}$ gene in Brazil [46]; however two recent studies in Brazil showed different results [52,53]. The first evaluated 30 carbapenem-resistant *A. baumannii* and showed that 70% carried the $bla_{OXA\text{-}143}$ gene [52] and the second evaluated 83 isolates of carbapenem-resistant *A. baumannii* and found the $bla_{OXA\text{-}143like}$ in only 8,4% of strains [53]. The others found $bla_{OXA\text{-}23like}$ with $bla_{OXA\text{-}143like}$ in 16% of strains [46].

The alleles encoding $bla_{OXA\text{-}58like}$ enzymes were found in isolates from UK together with alleles for a $bla_{OXA\text{-}51\ like}$ enzyme [54]. In Turkey, the authors found 54% isolates were harbouring both $bla_{OXA\text{-}51like}$ and $bla_{OXA\text{-}58like}$ and were carbapenem resistant [55]. Several reports have documented the implication of $bla_{OXA\text{-}23like}$ and $bla_{OXA\text{-}58like}$ genes in southern European countries including Turkey [56]. In Brazil, they detected only 1 strain in 310 strains with $bla_{OXA\text{-}58like}$ [57].

In Chinese hospital found 74% isolates harboured $bla_{OXA\text{-}23like}$ and $bla_{OXA\text{-}51like}$, 23,4% harboured only $bla_{OXA\text{-}51like}$ and 2,6% harboured $bla_{OXA\text{-}51like}$ and $bla_{OXA\text{-}58}$ [58]. Moreover, 4MBL genes, bla_{IMP}, bla_{VIM}, bla_{SIM}, bla_{NDM} were also search in all strains and negative results were acquired for all these MBL genes. All the isolates harbouring $bla_{OXA\text{-}23like}$ genes showed positive results when amplified with specific primers for $IS_{Aba\text{-}1}/bla_{OXA\text{-}23like}$ genes; the PCR products of $IS_{Aba\text{-}1}/bla_{OXA\text{-}51like}$ genes were only found in the 2 *A. baumannii* isolates that contained the $bla_{OXA\text{-}58like}$ genes. All isolates were negative for $IS_{Aba\text{-}1}/bla_{OXA\text{-}58like}$ genes [58].

In Nigeria found 60% isolates harboured the bla_{OXA-23} gene, while none of the strains harboured bla_{OXA-24}, bla_{OXA-58} [59].

In Algeria found all strains positive for bla_{OXA-51} and IS_{Aba-1} genes. 92% isolates harboured a bla_{OXA-23} gene, 4% (1 strain) harboured a bla_{OXA-58} gene and 1 strain harboured both bla_{OXA-23} and bla_{OXA-58} genes. None of strains harboured bla_{IMP}, bla_{VIM}, bla_{OXA-24}, bla_{KPC} or bla_{NDM-1} genes [60].

Molecular characterization and strain typing of such epidemic bacteria is important for the detection of the sources and mode of spread, which is a main step in order to design targeted infection control strategies.

Among the most common methods currently used for genotyping of *A. baumannii* are pulsed-field gel electrophoresis (PFGE) [61-63], amplified fragment length polymorphism (ALFP) analysis [64, 65], multilocus variable number tandem repeat analysis (MLVA) [66-68], multilocus sequence typing (MLST) [69-72], other PCR based and sequence based methods [73-76] and whole genome sequencing (WGS) analysis [77-79].

Molecular typing by PFGE analysis has been standardised for *A. baumannii* and used to study clonal outbreaks and local epidemiology [61-63].

Macrorestriction analysis of bacterial genomes resolved by pulsed-field gel electrophoresis is frequently referred to as the gold standard method for typing bacteria. By this method, genomic DNA is digested with low-frequency cutting enzymes and the resulting, relatively large fragments are separated by pulsed-field gel electrophoresis (PFGE) in which the electric field periodically changes between spatially distinct electrodes. Different electrophoresis instruments have been developed of which the contourclamped homogenous electric field (CHEF) systems (Bio-Rad) with a hexagonal array of electrodes are the most widely used. PFGE is a robust method which allows to compare samples on different gels, both within and between laboratories which is exemplified by a PFGE profile based network for recognition of food-borne disease-causing bacteria (http://www.cdc.gov/pulsenet/) [61, 62].

Since the development of the concept in the early 1990s, RAPD analysis and other variants of PCR based fingerprinting have been used methods for epidemiologic typing bacteria [80]. Primers and conditions for typing an organism vary between laboratories. Randon amplified polymorphic DNA (RAPD) analysis is based on amplification of random fragments using short arbitrary primers and low stringency conditions. Repetiteve extragenic palindromic (REP) and enterobacterial repetitive intergenic consensus (ERIC) fingerprinting are based on amplification of spacer regions between repeat motifs using outwardly directed primers at high stringency. Usually, DNA preparations prepared by boiling cells in a simple lysis solution [81] are sufficient to start with amplified fragments can be separated by agarose gel electrophoresis or by the use of an automated sequencing system if fragments are labelled with a fluorescent dye [82].

PCR and sequence based methods are frequently used for genotyping of *A. baumannii* and might be more appropriate for strain phylogeny and large scale epidemiology. The DiversiLab ^{TM} typing method is a repetitive extragenic plalindromic PCR (rep-PCR) analysis that is able to identify eight distinct clonal lineages distributed worldwide that include international clones I,III [80, 83].

Traditional ribotyping (as opposed to PCR ribotyping based on comparison of patterns of PCR products of the 16S-23S rRNA intergenic spacer region), has been used in numerous studies to type *Acinetobacter* strains. Genomic DNA is digested by a restriction enzyme and the obtained fragments are separated by agarose electrophoresis, and then transferred to a membrane, followed by hybridization with a labelled probe specific for the ribosomal operon. This method allows to compare profiles between laboratories, was pioneered for the Acb complex by Gerner-Smidt [84] who used EcoRI, ClaI and SalI as restriction enzymes and a digoxigenin-11-UTP labelled cDNA probe derived from 16S and 23S rRNA of *Escherichia coli*.

AFLP^{TM} is the patent name for a highly sensitive DNA fingerprinting method by which DNA is digested with restriction enzymes followed by selective amplification, electrophoretic separation of fragments and visualization. Epidemiologic and typing system concordance of the method was demonstrated for a set of well described *Acinetobacter* strains from different outbreaks [85]. A limitation of AFLP analysis is that the fingerprints are not exchangeable between laboratories, probably mainly due to differences in fragment separation systems.

The general view is that PCR based fingerprinting is a highly useful method for rapid local outbreak analysis, but not suited for setting up data bases for longitudinal or interlaboratory comparison [86]. Comparison of the PCR ribotyping with PFGE to type 73Acb complex strains revealed that ribotyping was less discriminatory than PFGE, but that the ribotypes were useful for species identification [87].

A long-range PCR followed by DNA sequence analysis has been used to determine the organisation and gene content of AbaR-type resistance islands, large genomic regions transposed into the comM gene locus, that were found in MDR *A. baumannii* strains assigned to international clonal lineages I and II and contained genes involved in resistance to drugs and metals [76].

Trilocus sequence based typing (3LST) involves amplification and sequencing of three genes (*ompA*, *csuE* and $bla_{OXA-51like}$) that are under selective pressure and has frequently been used to identify clonal lineages of outbreak strains of *A. baumannii* that were assigned to six different sequence groups (SGs) 1-6 corresponding to the international clonal lineages identified by other methods [62, 75].

MLST is the gold standard method to investigate the population structure and global epidemiology of bacteria. A systematic sequence based approach, multilocus sequence typing (MLST), characterizes isolates of bacterial species

using fragmens of sequences of seven house-keeping genes. For each house-keeping gene, the different sequences within a bacterial species are assigned as distinct alleles and, for each isolate, the alleles at each of seven loci make up its allelic profile or sequence type (ST). Two MLST schemes exist for *Acinetobacter* that able to type both *A. baumannii* and non-*baumannii Acinetobacter*, namely PubMLST and the Pasteur's MLST database [69-71], with only three loci in common. The Pasteur's MLST scheme [71] although more recently described, already includes 587 *Acinetobacter* isolates.

Current typing technologies have been useful in revealing relationships between isolates of *A. baumannii* but they are unable to resolve differences between closely related isolates from smallscale outbreaks, where chains of transmission are often unclear [19]. The recent availability of whole-genome sequencing (WGS) permits detailed investigation of genetic differences between bacterial isolates belonging to a single species and gave insight into the nature of genetic changes between isolates under antibiotic selection pressure [19]. These studies aimed to elucidate the role of mobile genetic elements in the transfer of antibiotic resistance genes and substantiate the rate of genetic alterations associated with resistance acquisition. The currently available results point to a high degree of genome plasticity, rapid emergence of antibiotic resistance, and significant genetic differences between closely related isolates [19].

Comparative genomic analysis of whole-genome sequences of *A baumannii* strains highlighted differences in genome organisation of *A. baumannii* epidemic clones assigned to differente PFGE genotypes or sequence types (STs) and defined the pools of genes assigned to the core and accessory genome in *A. baumannii* [88, 89].

The recent decades have faced the validation and application of a wide array of methods for typing acinetobacters. Most studies combined several methods to identify strains, which is a recommended strategy since the outcome of a single method may not allow for unambiguous strain identification. Phenotypic methods are no longer widely used, although in clinical practice epidemic strains will be initially identified on the basis of their antibiogram profile and biochemical profile. For unambiguous typing, genotypic methods are indispensible.

For evaluation the global epidemiology of *A. baumannii* it is important to determine the dynamics of disease transmission at local/hospital or country/national level using several typing methods. All these studies reinforce the need to monitor the epidemiology and its associated antimicrobial resistances. A network of reference laboratories in different regions could be established for harmonise the protocols using panel of reference strains, that would generate an understanding of the importance of high-risk clones in the international dissemination.

References

[1] Fournier PE, Vallenet D, Barbe V, Audic S, Ogata H, Poirel L, Richet H, Robert C, Mangenot S, Abergel C, Nordmann P, Weissenbach J, Raoult D, Claverie JM. Comparative genomics of multidrug resistance in *Acinetobacter baumannii. PLoS Genet* 2006; 2:e7.

[2] Bouvet PJM, Grimont PAD. Taxonomy of the genus *Acinetobacter* with the recognition of *Acinetobacter baumannii* sp. nov., *Acinetobacter haemolyticus* sp. nov., *Acinetobacter johnsonii* sp. nov., and *Acinetobacter junii* sp. nov. and emended descriptions of *Acinetobacter calcoaceticus* and *Acinetobacter lwoffii.* Int J Syst Bacteriol 1986; 36: 228-40.

[3] Nemec A, Dijkshoorn L, Cleenwerck I, De Baere T, Janssens D, Van Der Reijden TJ, Jezek P, Vaneechoutte M. *Acinetobacter parvus* sp. nov. a small-colony-forming species isolated from human clinical specimens. *Int J Syst Evol Microbiol* 2003; 53: 1563;7.

[4] Bouvet PJ, Grimont PA. Identification and biotyping of clinical isolates of *Acinetobacter. Ann Inst Pasteur Microbiol* 1987; 138: 569-78.

[5] Gerner-Smidt P, Tjernberg I, Ursing J. Reliability of phenotypic tests for identification of *Acinetobacter* species. *J Clin Microbiol* 1991; 29: 277-82.

[6] Higgins PG, Dammhayn C, Hackel M, Seifert H. Global spread of carbapenem-resistant *Acinetobacter baumannii*. *J Antimicrob Chemother* 2010; 65: 233-8.

[7] Bratu S, Landman D, Martin DA, Georgescu C, Quale J. Correlation of antimicrobial resistance with β-lactamases, the OmpA-like porin, and efflux pumps in clinical isolates of *Acinetobacter baumannii* endemic to New York City. *Antimicrob Agents Chemother* 2008; 52: 2999-3005.

[8] Zarrili R, Casillo R, Di Papolo A, Tripodi MF, Bagattini M, Cuccurulio S, Crivaro V, Ragone E, Mattei A, Galdieri N, Triassi M, Utili R. Molecular epidemiology of a clonal outbreak of multidrug-resistant *Acinetobacter baumannii* in a university hospital in Italy. *Clin Microbiol Infect* 2007; 13: 481-9.

[9] Carvalho KR, Carvalho-Assef AP, Peirano G, Santos LC, Pereira MJ, 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: 25-8.

[10] Gales AC, Pfaller MA, Sader HS, Hollis RJ, Jones RN. Genotypic characterization of carbapenm-nonsusceptible *Acinetobacter* spp. isolated in Latin America. *Microb Drug Resist 2004;* 10:286-91.

[11] Sader HS, Castanheira M, Mendes RE, Toleman M, Walsh TR, Jones RN. Dissemination and diversity of metallo-β-lactamases in Latin America: report from the SENTRY Antimicrobial Surveillance Program. *Int J Antimicrob Agents* 2005; 25: 57-61.

[12] Chen SJ, Chao TF, Chiang MC, Kuo SC, Chen LY, Chiang DH, Yin T, Chen TL, Fung CP. Predictors of mortality in surgical patients with *Acinetobacter baumannii* bacteremia. *J Microbiol immunol infect* 2011; 44: 209-14.

[13] Chiang MC, Kuo SC, Chen YC, Lee YT, Chen TL, Fung CP. Polymerase chain reaction assay for the detection of *Acinetobacter baumannii* in endotracheal aspirates from patients in the intensive care unit. *J Microbiol Immunol Infect* 2011; 44: 106-10.

[14] Sevillano E, Gallego L, García-Lobo JM. First detection of the OXA-40 carbapenemase in P. aeruginosa isolates, located on a plasmid also found in *A. baumannii. Pathol Biol* (Paris) 2009; 57: 493–5.
[15] D'Andrea MM, Giani T, D'Arezzo S, Capone A, Petrosillo N, Visca P, Luzzaro F, Rossolini GM. Characterization of pABVA01, a plasmid encoding the OXA-24 carbapenemase from Italian isolates of *Acinetobacter baumannii*. *Antimicrob Agents Chemother* 2009; 53: 3528–33.
[16] Lolans K, Rice TW, Munoz-Price LS, Quinn JP. Multicity outbreak of carbapenem resistant*Acinetobacter baumannii* isolates producing the carbapenemase OXA-40. *Antimicrob Agents Chemother* 2006; 50: 2941–5.
[17] Merino M, Acosta J, Poza M, Sanz F, Beceiro A, Chaves F, Bou G. OXA-24 carbapenemase gene flanked by XerC/XerD-like recombination sites in different plasmids from different *Acinetobacter* species isolated during a nosocomial outbreak. *Antimicrob Agents Chemother* 2010; 54: 2724–7.
[18] Quinteira S, Grosso F, Ramos H, Peixe L. Molecular epidemiology of imipenem resistant *Acinetobacter haemolyticus* and *Acinetobacter baumannii* isolates carrying plasmid-mediated OXA-40 from a Portuguese hospital. *Antimicrob Agents Chemother* 2007; 51: 3465–6.
[19] Zarrilli R, Pournaras S, Giannouli M, Tsakris A. Global evolution of multidrug-resistant *Acinetobacter baumannii* clonal lineages. *Int J Antimicrobial Agents* 2013; 41: 11-19.
[20] Durante-Mangoni E, Zarrilli R. Global spread of drug-resistant *Acinetobacter baumannii*: molecular epidemiology and management of antimicrobial resistance. *Future Microbiol* 2011; 6: 407-22.
[21] Magiorakos AP, Srinivasan A, Carey RB, Carmeli Y, Falagas ME, Giske CG, Harbarth S, Hindler JF, Kahlmeter G, Olsson-Liljequist B, Paterson DL, Rice LB, Stelling J, Struelens M, Vatopoulos A, Weber JT, Monnet DL. Multidrug-resistant, extensively drug-resistant and pandrug'resistant bacteria: an international expert proposal for interim standard definitions for acquired resistance. *Clin Microbiol Infect* 2012; 18: 268-81.
[22] Roy CP, Ingold A, VAnegas N, Martinez E, Merlino J, Merkier AK. Dissemination of multiple drug resistance genes by class 1 integrons in *Klebsiella pneumonia* isolates from four countries: a comparative study. *Antimicrob Agents Chemother* 2011; 55: 3140-9.
[23] Poirel L, Carrer A, Pitout JD, Nordman P. Integron mobilization unit as a source of mobility of antibiotic resistance genes. *Antimicrob Agents Chemother* 2009; 53: 2492-8.
[24] Labbate M, Case RJ, Stokes HW. The integron/gene cassette system: an active player in bacterial adaptation. *Methods Mol Biol* 2009; 532: 103-25.
[25] Pournaras S, Markogiannakis A, Ikonomidis A, Kondyli L, Bethimouti K, Maniatis AN, Legakis NJ, Tsakris A. Outbreak of multiple clones of imipenem-resistant *Acinetobacter baumannii* isolates expressing OXA-58 carbapenemase in na intensive care unit. *J Antimicrob Chemother* 2006; 57: 557–61.
[26] Bush K, Jacoby GA, Medeiros AA. A functional classification scheme for blactamases and its correlation with molecular structure. *Antimicrob Agents Chemother* 1995; 39: 1211-33.
[27] Acosta J, Merino M, Viedma E, Poza M, Sanz F, Otero JR, Chaves F, Bou G. Multidrugresistant *Acinetobacter baumannii* harboring OXA-24 carbapenemase, Spain. *Emerg Infect Dis* 2011; 17: 6.
[28] Hu WS, Yao S, Hsieh CF, Liu C, Lin J. An OXA-66/OXA-51-like carbapenemase and possibly an efflux pump are associated with resistance to imipenem in *Acinetobacter baumannii*. *Antimicrob Agents Chemother* 2007; 51(11): 3844-52.
[29] Costa L, Coelho MJM, Souza H, Castro M, Stier CJN, Bragagnolo KL, Rea-Neto A, Penteado-Filho SR, Livermore DM, Woodford N. Outbreak of carbapenem-resistant *Acinetobacter baumannii* producing the OXA-23 enzyme in Curitiba, Brazil. *J Clin Microbiol* 2003; 41: 3403-6.
[30] Papa A, Koulourida V, Souliou E. Molecular epidemiology of carbapenemresistant *Acinetobacter baumannii* in a newly established greek hospital. *Microb Drug Res* 2009; 15: 4.
[31] Turton JF, Ward ME, Woodford N, Kaufmann ME, Pike R, Livermore DM, Pitt TL. The role of ISAba1 in expression of OXA carbapenemase genes in Acinetobacter baumannii. FEMS Microbiol Lett 2006; 258: 72–7.
[32] Poirel, L., Nordmann, P., 2006. Genetic structures at the origin of acquisition and expression of the carbapenem-hydrolyzing oxacillinase gene bla OXA-58 in *Acinetobacter baumannii*. *Antimicrob. Agents Chemother*. 50, 1442–1448.
[33] Ahmed, H., Benjamin, A.E., Kevin, J.T., Sebastian, G.B.A., 2010. Characterization of epidemiologically unrelated *Acinetobacter baumannii* isolates from fourcontinents by use of multilocus sequence typing, pulsed-field gel electrophoresis, and sequence-based typing of blaOXA-51-like genes. *J. Clin. Microbiol*. 48, 2476–2483.
[34] Siroy A, Molle V, Lemaitre-Guillier C et al. Channel formation by CarO, the carbapenem resistance-associated outer membrane protein of *Acinetobacter baumannii*. *Antimicrob Agents Chemother* 2005; 49: 4876–83.
[35] Mussi MA, Limansky AS, Viale AM. Acquisition of resistance to carbapenems in multidrug-resistant clinical strains of *Acinetobacter baumannii*: natural insertional inactivation of a gene encoding a member of a novel family of b-barrel outer membrane proteins. *Antimicrob Agents Chemother* 2005; 49: 1432–40.
[36] Hirtier C, Poirel L, Lambert T. Contribution of acquired carbapenem-hydrolyzing oxacillinases to carbapenem resistance in *Acinetobacter baumannii*. *Antimicrob Agents Chemother* 2005; 49: 3198-202.
[37] Hu WS, Yao SM, Fung CP. An OXA-66/OXA-51 like carbapenemase and possibly an efflux pump are associated with resistance to imipenem in *Acinetobacter baumannii. Antimicrob Agents Chemother* 2007; 51: 3844-52.
[38] Huang LY, Chen TL, Lu PL, Tsai CA, Cho WL, Chang FY, Fung CP, Siu LK. Dissemination of multidrug-resistant, class 1 integron-carrying *Acinetobacter baumannii* isolates in Taiwan. *Clin Microbiol Infect* 2008; 14: 1010-9.
[39] Lu PL, Doumith M, Livermore DM, Chen T-P,Woodford N. Diversity of carbapenem resistance mechanisms in *Acinetobacter baumannii* from a Taiwan hospital: spread of plasmid-borne OXA-72 carbapenemase. *J Antimicrob Chemother* 2009; 63: 641-7.
[40] Hsueh PR, Liu YC, Yang D, Yan JJ, Wu TL, Huang WK, Wu JJ, Ko WC, Leu HS, Yu CR, Luh KT. Multicenter surveillance of antimicrobial resistance of major bacterial pathogens in intensive care units in 2000 in Taiwan. *Microb Drug Resist* 2001; 7: 373-82.

[41] Taiwan Nosocomial Infections Surveillance System (TNIS) Available at: http://www2.cdc.gov.tw/ct.asp?xItemZ7773&ctNodeZ948&mpZ5. Accessed June, 2012.
[42] Lee MH, Chen TL, Lee YT, Huang L, Kuo SC, Yu KW, Hsueh PR, Dou HY, Su IJ, Fung CP. Dissemination of multidrug-resistant *Acinetobacter baumannii* carrying BlaOXA-23 from hospitals in central Taiwan. *J Microbiology, Immunology and Infection.* 2012. Available at: http://dx.doi.org/10.1016/j.jmii.2012.08.006. Accessed June, 2012.
[43] Tiwari V, Kapil A, Moganty RR. Carbapenem-hydrolyzing oxacillinase in high resistant strains of *Acinetobacter baumannii* isolated from India. *Microbial Pathogenesis*. 2012; 53: 81-86.
[44] Gupta E, Mohanty S, Sood S, Dhawan B, Das BK, Kapil A. Emerging resistance to carbapenems in a tertiary care hospital in north India. *Ind J Med Res* 2006; 124(1): 95-8.
[45] Yang HY, Lee HJ, Suh JT, Lee KM. Outbreaks of Imipenem resistant Acinetobacter baumannii producing OXA-23 b-Lactamase in a tertiary care hospital in Korea. *Yonsei Med J* 2009; 50: 764-70.
[46] Mostachio AK, Levin AS, Rizek C, Rossi F, Zerbini J. High prevalence of OXA-143 and alteration of outer membrane proteins in carbapenem-resistant *Acinetobacter* spp. isolates in Brazil. 2012; 39: 396-401.
[47] Clinical and Laboratory Standards Institute. Performance standards for antimicrobial susceptibility testing: twenty-first informational supplement. Document M100-S21. Wayne, PA: CLSI; 2011.
[48] Jeong HW, Cheong HJ, Kim WJ, Kim MJ, Song KJ, Song JW, Kim HS, Roh KH. Loss of the 29- kilodalton outer membrane protein in the presence of OXA-51-like enzymes in *Acinetobacter baumannii* is associated with decreased imipenem susceptibility. *Microb Drug Resist* 2009; 15: 151–8.
[49] Yang SC, Chang WJ, Chang YH, Tsai YS, Yang TP, Juan CW, Shiau MY. Prevalence of antibiotics resistance and OXA carbapenemases genes in multidrug-resistant *Acinetobacter baumannii* isolates in central Taiwan. *Eur J Clin Microbiol Infect Dis* 2010; 29: 601–4.
[50] Prata-Rocha ML, Gontijo-Filho PP, Melo GB. Factors influencing survival in patients with multidrug-resistant *Acinetobacter baumannii* infection 2012; 16(2): 245-9.
[51] Antônio CS, Neves PR, Medeiros M, Mamizuka EM, Elmor De Araújo, MR, Lincopan N. High prevalence of carbapenem-resistant *Acinetobacter baumannii* carrying the blaOXA-143 gene in Brazilian hospitals. *Antimicrob Agents Chemother* 2011; 55:1322-3.
[52] Werneck JS, Picão RC, Girardello R, Cayo R, Margutti V, Dalla-Costa L. Low prevalence of blaOXA0143 in private hospitals in Brazil. *Antimicrob Agents Chemoter* 2011; 55: 4494-5.
[53] Woodford N, Ellington MJ, Coelho JM, Turton JF, Ward ME, Brown S, Amyes SGB, Livermore DM. Multiplex PCR for genes encoding prevalent OXA carbapenemases in *Acinetobacter* spp. *Int J of Antimicrob Agents* 2006; 27:351-353.
[54] Metan G, Sariguzel F, Sumerkan B, Dijkshoom, T. Clonal diversity and high prevalence of OXA-58 among *Acinetobacter baumannii* isolates from blood cultures in a tertiary care centre in Turkey. *Infection, Genetics and Evolution* 2013; 14: 92-97.
[55] Marque S, Poirel L, Héritier C, Brisse S, Blasco MD, Filip R, Coman G, Naas T, Nordmann P. Regional occurrence of plasmid-mediated carbapenem-hydrolyzing oxacilinase OXA-58 in *Acinetobacter* spp. in Europe. *J Clin* Microbiol 2005; 43: 4885-4888.
[56] Gusatti CS. Caracterização de isolados clínicos e ambientais de *Acinetobacter* sp.: presença de IS_{Aba1} e diversidade de $bla_{OXA-51like.}$ 2011. 94f. Dissertação (Mestrado em microbiologia agrícola e do ambiente) – Instituto de Ciências Básicas da Saúde, Universidade Federal do Rio Grande do Sul, Porto Alegre, 2011.
[57] Dai W, Huang S, Sun S, Cao J, Zhang L. Nosocomial spread of carbapenem-resistant *Acinetobacter baumannii* (types ST75 and ST137) carrying blaOXA-23like gene with an upstream ISAba-1 in a Chinese Hospital. *Inf, Genetics and Evolution* 2013; 14:98-101.
[58] Olaitan AO, Berrazeg M, Fagade OE, Adelowo OO, Alli JA, Rolain JM. Emergence of multidrug-resistant *Acinetobacter baumannii* producing OXA-23 carbapenemase, Nigeria. *Int J Infet Dis* 2013, Available at:: http://dx.doi.org/10.1016/j.ijid.2012.12.008. Accessed March, 2013.
[59] Touati M, Diene SM, Racherache A, Dekhil M, Djahoudi A, Rolain J. Emergence of blaOXA-23 and blaOXA-58 carbapenemase encoding genes in multidrug-resistant *Acinetobacter baumannii* isolates from university Hospital of Annaba, Algeria. *Int J Antimicrob Agents.* 2012; 40:84-93.
[60] Seifert H, Dolzani L, Bressan R, van der Reijden T, van Strijen B, Stefanik D, Heersma H, Dijkshoorn L. Standardization and interlaboratory reproducibility assessment of pulsed-field gel electrophoresis-generated fingerprints of *Acinetobacter baumannii*. *J Clin Microbiol* 2005; 43: 4328–35.
[61] Giannouli M, Cuccurullo S, Crivaro V, Di Popolo A, Bernardo M, Tomasone F, et al. Molecular epidemiology of multidrug-resistant *Acinetobacter baumannii* in a tertiary care hospital in Naples, Italy, shows the emergence of a novel epidemic clone. *J Clin Microbiol* 2010; 48: 1223–30.
[62] Zarrilli R, Vitale D, Di Popolo A, Bagattini M, Daoud Z, Khan AU, Afif C, Triassi M. A plasmid-borne blaOXA-58 gene confers imipenem resistance to *Acinetobacter baumannii* isolates from a Lebanese hospital. *Antimicrob Agents Chemother* 2008; 52: 4115–20.
[63] Dijkshoorn L, Nemec A, Seifert H. An increasing threat in hospitals: multi drug resistant *Acinetobacter baumannii*. *Nat Rev Microbiol* 2007; 5: 939–51.
[64] Dijkshoorn L, Aucken H, Gerner-Smidt P, Janssen P, Kaufmann ME, Garaizar J, Ursing J, Pitt TL. Comparison of outbreak and nonoutbreak *Acinetobacter baumannii* strains by genotypic and phenotypic methods. *J Clin Microbiol* 1996; 34: 1519–25.
[65] Turton JF, Matos J, Kaufmann ME, Pitt TL. Variable number tandem repeat loci providing discrimination within widespread genotypes of *Acinetobacter baumannii*. *Eur J Clin Microbiol Infect Dis* 2009; 28: 499–507.
[66] Pourcel C, Minandri F, Hauck Y, D'Arezzo S, Imperi F, Vergnaud G, Visca P. Identification of variable-number tandem-repeat (VNTR) sequences in *Acinetobacter baumannii* and interlaboratory validation of an optimized multiple-locus VNTR analysis typing scheme. *J Clin Microbiol* 2011; 49: 539–48.

[67] Minandri F, D'Arezzo S, Antunes LC, Pourcel C, Principe L, Petrosillo N, Visca P. Evidence of diversity among epidemiologically related carbapenemaseproducing *Acinetobacter baumannii* strains belonging to international clonal lineage II. *J Clin Microbiol* 2012; 50: 590–7.

[68] Bartual SG, Seifert H, Hippler C, Luzon MA, Wisplinghoff H, Rodrìguez-Valera F. Development of a multilocus sequence typing scheme for characterization of clinical isolates of *Acinetobacter baumannii*. *J Clin Microbiol* 2005; 43: 4382–90.

[69] Wisplinghoff H, Hippler C, Bartual SG, Haefs C, Stefanik D, Higgins PG, Seifert H. Molecular epidemiology of clinical *Acinetobacter baumannii* and *Acinetobacter* genomic species 13TU isolates using a multilocus sequencing typing scheme. *Clin Microbiol Infect* 2008; 14: 708–15.

[70] Diancourt L, Passet V, Nemec A, Dijkshoorn L, Brisse S. The population structure of Acinetobacter baumannii: expanding multiresistant clones from an ancestral susceptible genetic pool. *PLoS ONE* 2010; 5: 1003-4.

[71] Gogou V, Pournaras S, Giannouli M, Voulgari E, Piperaki ET, Zarrilli R, Tsakris A. Evolution of multidrug-resistant *Acinetobacter baumannii* clonal lineages: a 10 year study in Greece (2000–2009). *J Antimicrob Chemother* 2011; 66: 2767–72.

[72] Ecker JA, Massire C, Hall TA, Ranken R, Pennella TT, Agasino Ivy C, Blyn LB, Hofstadler SA, Endy TP, Scott PT, Lindler L, Hamilton T, Gaddy C, Snow K, Pe M,Fishbain J, Craft D, Deye G, Riddell S, Milstrey E, Petruccelli B, Brisse S, Harpin V, Schink A, Ecker DJ, Sampath R, Eshoo MW. Identification of Acinetobacter species and genotyping of *Acinetobacter baumannii* by multilocus PCR and mass spectrometry. *J Clin Microbiol* 2006; 44: 2921–32.

[73] Decker BK, Perez F, Hujer AM, Hujer KM, Hall GS, Jacobs MR, Gebreyes WA, Zoll ST, Massire C, Eshoo MV, Ecker DJ, Rather PN, RA. Longitudinal analysis of the temporal evolution of *Acinetobacter baumannii* strains in Ohio, USA, by using rapid automated typing methods. PLoS ONE 2012; 7: 334-43.

[74] Turton JF, Gabriel SN, Valderrey C, Kaufmann ME, Pitt TL. Use of sequencebased typing and multiplex PCR to identify clonal lineages of outbreak strains of *Acinetobacter baumannii*. *Clin Microbiol Infect* 2007; 13: 807–15.

[75] Post V, White PA, Hall R. Evolution of AbaR-type genomic resistance islands in multiply antibiotic-resistant *Acinetobacter baumannii*. *J Antimicrob Chemother* 2010; 65: 1162–70.

[76] Adams MD, Goglin K, Molyneaux N, Hujer KM, Lavender H, Jamison JJ, MacDonald IJ, Martin KM, Russo T, Campagnari AA, Hujer AM, Bonomo RA, Gill SR. Comparative genome sequence analysis of multidrug-resistant *Acinetobacter baumannii*. *J Bacteriol* 2008; 190: 8053–64.

[77] Lewis T, Loman NJ, Bingle L, Jumaa P, Weinstock GM, Mortiboy D, Pallen MJ High-throughput whole-genome sequencing to dissect the epidemiology of *Acinetobacter baumannii* isolates from a hospital outbreak. *J Hosp Infect* 2010; 75: 37–41.

[78] Hornsey M, Loman N, Warheam DW, Ellington MJ, Pallen MJ, Turton JF, Underwood A, Gaulton T, Thomas CP, Doumith M, Livermore DM, Woodford N. Whole-genome comparison of two *Acinetobacter baumannii* isolates from a single patient, where resistance developed during tigecycline therapy. *J Antimicrob Chemother* 2011; 66: 1499–503.

[79] Carretto E, Barbarini D, Dijkshoorn L, van der Reijden TJ, Brisse S, Passet V, et al. Widespread carbapenem resistant *Acinetobacter baumannii* clones in Italian hospitals revealed by a multicenter study. *Infect Genet Evol* 2011; 11: 1319–26.

[80] Vaneechoute M, Elaichouni A, Maquelin K, Claeys G, Van Liedekerke A, Louagie H, Verschraegen G, Dijkshoorn L. Comparison of arbitrarily primed polymerase chain reaction and cell envelope protein electrophoresis for analysis of *Acinetobacter baumannii* and *A. junii* outbreaks. *Res Microbiol* 1995; 146: 457-65.

[81] Grundmann HJ, Towner KJ, Dijkshoorn L, Gerner-Smidt P, Maher M, Seifert H, Vaneechoutte M. Multicenter study using standardized protocols and reagents for evaluation of reproducibility of PCR-based fingerprinting of *Acinetobacter* spp. *J CLin Microbiol* 1997; 35: 3071-7.

[82] Zander E, Nemec A, Seifert H, Higgins PG. Association between β lactamase-encoding blaOXA-51 variants and DiversiLab rep-PCR-based typing of *Acinetobacter baumannii* Isolates. *J Clin Microbiol* 2012; 50: 1900–4.

[83] Gerner-Smidt P. Ribotyping of the *Acinetobacter calcoaceticus-Acinetobacter baumannii* complex. *J Clin Microbiol* 1992; 30: 2680-5.

[84] Janssen P, Dijkshoorn L. High resolution DNA fingerprinting of *Acinetobacter* outbreak strains. *FEMS Microbiol Lett* 1996; 142: 191-4.

[85] Dijkshoorn L. Two decades experience of typing *Acinetobacter* strains: evolving methods and clinical applications. *Antibiotiques* 2006; 8: 108-116.

[86] Seifert H, Gerner-Smidt P. Comparison of ribotyping and pulsed field gel electrophoresis for molecular typing of *Acinetobacter* isolates. *J CLin Microbiol* 1995; 33: 1402-7.

[87] Zarrilli R, Giannouli M, Rocco F, Loman NJ, Haynes AS, Constantinidou C, Pallen MJ, Triassi M, Nocera PP. Genome sequences of three *Acinetobacter baumannii* strains assigned to ST2, ST25 and ST78 multilocus sequence typing genotypes. *J Bacteriol* 2011; 193: 2359–60.

[88] Di Nocera PP, Rocco F, Giannouli M, Triassi M, Zarrilli R. Genome organization of epidemic *Acinetobacter baumannii* strains. *BMC Microbiol* 2011; 11: 224.

