REVIEW

Evolution of *Acinetobacter baumannii* infections and antimicrobial resistance. A review

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Abstract

The emergence of multi-drug resistant *Acinetobacter spp* involved in hospital-acquired infections, once considered an easily treatable pathogen, is troublesome and an immense burden for the modern medical systems worldwide. In the last 20 years the medical community recorded an increase in the incidence and severity of these infections as therapeutic means tend to be less and less effective on these strains. The ability of these bacteria to rapidly develop resistance to antimicrobial agents by continuously changing and adapting their mechanisms, their ability to survive for long periods of time

in the hospital environment and the multitude of transmission possibilities raises serious issues regarding the management of these complex infections. The future lies in developing new and targeted methods for the early diagnosis of *A. baumannii*, as well as in the judicious use of antimicrobial drugs. This review details the evolution of the pathogenicity of this microorganism, together with the changes that appeared in resistance mechanisms and the advancements in molecular testing for the early detection of infection.

Introduction

The critically ill patient as one of the biggest challenges in the medical world has a con-

stant need of complex management which also involves the prevention and treatment of associated infections. The length of stay in the ICU, the mechanical ventilation time, the invasive techniques used, as well as the nutrition strategy all make the critically ill patient susceptible to infection and influence the clinical outcome and mortality rates. It was proven that the mortality risk is 2.48 times higher in critically ill patients with hospital-acquired infections especially in the case of patients with respiratory disease [1].

Bacterial infections are a heavy burden on the medical systems and on patients worldwide, with the most threatening being pathogens of the respiratory tract, enteric pathogens and HIV-associated infections. Finding new treatment options represents a constant challenge for the medical community, as the fight is against the continuously evolving mechanisms of microbial resistance. New hope comes from the recent advances in the field of genetics, mainly DNA sequencing that offer the possibility of comparing the genomes of the same strains over a period of time in order to identify genetic changes signifying the development of adaptation techniques of the microorganism to certain drugs or to the environment [2]. Along the years research groups have struggled to elucidate the resistance mechanisms of certain pathogens involved in acute infections, such as *Neisseria meningitidis*, *Pseudomonas aeruginosa*, and *Acinetobacter baumannii*, with a soft focus on their genomic evolution.

Background

The alarming increase in the incidence of infections determined by opportunistic microorganisms, especially as epidemiologic events such as nosocomial infections, have determined researchers in the fields of microbiology and molecular biology to carry out studies on these certain types of pathogens. *Acinetobacter baumannii* is an ubiquitary bacterium that colonizes over 40% of healthy adults that come into contact, with a higher incidence seen in the medical personnel.

Belonging to a wider class called *Gammaproteobacteria*, germs in the *Acinetobacter* genus have long been considered as opportunists, usually involved in less severe infections. Genus *Acinetobacter* as we nowadays call it was first discovered in 1911 by Martinus Beijerinck, as a gram-negative bacterium [3]. Only in 1970 was this pathogen recognized as being significantly involved in hospital infections, but with a high sensitivity to common antimicrobials used at the time. However, in the last 30 years an increased incidence and severity of the in-

fections have been reported, especially in the case of hospital-acquired infections, raising a red flag related to the lack of therapeutic means for the ever-evolving multidrug-resistant strains of this bacterium. Together with technological advancements, in 1986 four new genomic species were proposed after using DNA-DNA hybridization: 3TU, 13 TU, *Acinetobacter baumannii*, and *Acinetobacter calcoaceticus* which together form the “*A. baumannii* complex” [4], as they share common properties that make them hard to distinguish from each other. *A. baumannii* infections associated with medical interventions were proven to have high-virulence which altered the belief that it was a low-virulence pathogen acting as an opportunist [5]. Their potential of rapidly developing resistance to antimicrobial agents, their capacity of surviving in the hospital environment for long periods of time, and the numerous transmission mechanisms have raised serious issues worldwide regarding the treatment and management of hospital-acquired *Acinetobacter* spp. infections [6]. Wright et al. carried out a genomic analysis of *A. baumannii* isolated from the same patient during their hospital stay and have proven that antibiotic treatment and host response led to enrichment in mutation [7].

Microbiology – current classification and relevance in the clinical setting

The genus *Acinetobacter* encompasses opportunistic pathogens, gram-negative coco-bacilli in pairs, with dimensions between 1 and 1.5 µm that do not present mobility, and produce a catalase enzyme (catalase-positive) but does not produce cytochrome oxidase (oxidase-negative) or urease. Regarding nutritional mechanisms these pathogens are aerobic, non-fermenting, and non-fastidious [8]. More recent classification strategies have proposed to classify the genus *Acinetobacter* as part of the *Moraxellaceae* family and of the more extensive *Gammaproteobacteria* class.

The *A. baumannii* complex includes the three most clinically important species that have been involved in community-acquired and hospital-acquired infections in humans, as well as a fourth specie, *A. calcoaceticus*, frequently found in water and soil that does not inflict diseases in the human population. Apart from the “*Acinetobacter* complex” Bouvet and Grimont, and later Bouvet and Jeanjean [4], described further genospecies through DNA-hybridization including: *A. haemolyticus*, *A. johnsonii*, *A. junii*, *A. Iwoffii*, and *A. radioresistens*. In the last two decades additional species have been identified, but out of the total 10 only 3 are of human origin:

A. parvus, *A. schindleri*, and *A. ursingii* [9,10,11]. Up until now the following species of the *Acinetobacter* genus have been identified, with their type and reference strains mentioned in the brackets: *A. baumannii* (ATCC19606T) – with two genomic species, *A. baylyi* (DSM14961T), *A. bouvetii* (DSM 14961T), *A. calcoaceticus* (ATCC 23055T) - one genomic specie, *A. gerneri* (DSM 14967T), *A. grimontii* (DSM 14968T), *A. haemolyticus* (ATCC 17906T) with 4 genomic species, *A. johnsonii* (ATCC 17909T) with 7 genomic species, *A. junii* (ATCC 17908T) with 5 genomic species, *A. lowffii* (ATCC 15309, ATCC 9957) with 8, respectively 9 genomic species, *A. parvus* (NPH384T), *A. radioresistens* (IAM 13186T) with 12 genomic species, *A. schindleri* (NIPH1034T), *A. tandoii* (DSM 14970T), *A. tjernbergiae* (DSM 14971T), *A. twoneri* (DSM 14962T), *A. ursingii* (NIPH137T), *A. venetianus* (ATCC 31012, ATCC 19004, 10090 – close to 13TU) [4,9,12,13,14]

Identification of *Acinetobacter* strains

For the identification of *Acinetobacter* strains one must use a combination of phenotypic and genotypic tests that must be characterized by high reproducibility, good discrimination strength, and that are easily carried out and easy to interpret [15]. Samples in the case of ICU patients can be collected from different sites such as: lower respiratory tract secretions, urine, wound secretion, central lines, or even skin. Along the years this species has become more and more aggressive, as well as more resistant to even the most modern therapies. There are different methods for testing but unfortunately standard techniques used in the laboratory will only identify the genus and not the species [14]. Phenotypic identification methods such as the biochemical profile, phagocytic typology, surface antigens profile, antibiotic susceptibility etc. represent the expression of certain genetic structures, and therefore are susceptible to variability due to the influence of the culture environment, bacterial growth phase, spontaneous mutations [14]. Among the most used biotyping methods one of the most widely used in the clinical setting is the API 20NE and API 20E (Bio-Merieux, France) test. Complementary growth at 44 °C is sometimes required although the test has high reliability [15].

Molecular techniques

Genetic methods analyze the microorganism's genetic structure and include the polymorphism of the enzymatic restriction patterns of the total DNA, as well as the presence or ab-

sence of specific structures in the chromosomal or extra-chromosomal DNA sequence. Natural variability does not influence these methods as much but they can be affected by insertion, deletions, and point mutations. Fortunately the incidence of these defects is lower, and in many cases they can represent diagnostic or epidemiologic biomarkers.

A. baumannii species present indigenous plasmids and therefore plasmid analysis is one method that has been used for the epidemiological typing but only after taking into account that plasmids can be gained or lost in the transformation process [16].

Ribotyping, although labor-intensive and with limited discriminatory power, has been used to identify species of *Acinetobacter*. The method applied in the past in epidemiologic studies uses EcoRI, ClaI, and Sall in order to obtain the restriction of DNA; after this step electrophoresis must be carried out, followed by blotting and hybridization with a probe derived from *E. Coli* ribosomal RNA [9]. Because the technique is both expensive and time-consuming, and requires specific equipment it was replaced by pulse-field gel electrophoresis (PFGE).

Pulse-field gel electrophoresis (PFGE) remains the gold standard for identifying *Acinetobacter baumannii* [9]. It is a so-called comparative typing system as it uses Apal to impose restriction on chromosomal DNA [17], followed by electrophoresis for the separation of these fragments and by the comparison of the fingerprint profiles. Reproducibility between different laboratories is also possible which would help in the detection of epidemic strains in a certain hospital or even nation-wide [18].

Applied fragment length polymorphism analysis (AFLP analysis) is a semiautomated procedure and a powerful tool in determining taxonomy in bacterial strains, but being a method that requires high level equipment and standardization it is found in reference laboratories and it is not used in the daily practice for routine analysis of *A. baumannii* [19].

The sequence analysis for 16S rDNA genes and *rpoB* genes are molecular identification methods that are widely accepted, as well as the PCR-RFLP molecular technique. PCR Amplification of bla_{OXA-51} and CHROMagar *Acinetobacter*/MDR Technique are other tests that together with the antimicrobial susceptibility testing can aid in a correct and goal directed therapy for *A. baumannii* infections [20]. DNA extraction is another option, as well as PCR reactions.

As all gram-negative bacteria, the *Acinetobacter* species expresses lipopolysac-

charides (LPSs), present on the outer surface of the microorganism [21], that were shown to be of smooth phenotype after serological and chemical analysis [22-24]. Monoclonal antibodies (MAbs) against the LPSs of the *A. baumannii* strains were generated; MAb S48-3-13 and MAb S48-3-17 were found to react with certain strains [12]. Another pair of mouse monoclonal antibodies used against *A. baumannii* ATCC 19606 was reported by Baig et al [25], mainly F241G3sc2 and F241G6sc2 that have proven to be highly sensitive and demonstrated applicability in the clinical practice by use of ELISA testing and Western Blot analysis, and therefore can be used as rapid detection tools [25]. Moreover, in the last years, humanized MAbs have emerged as possible effective treatment options or prevention options for *A. baumannii* infections, especially with multi-drug resistant (MDR) or extensive-drug resistant (XDR) strains.

Another very important part is determining the sensitivity/resistance profile to antimicrobial drugs. This test can be applied to all strains and is easy to perform and interpret. The laboratory clinician will determine either the inhibition area, or the minimum inhibitory concentration (MIC). The resistance profile is very variable and can rapidly change in the same patient or in the same epidemiologic outburst. Another example of molecular and biochemical methods that are useful in the detection of antimicrobial resistance profiles is the detection of carbapenemase-producing *Acinetobacter baumannii*, as these strains have developed and evolved on a fast-raging pace over the last years. Two of these methods were described by Thoma et. al, CarbaAcinetoNP and BlueCarba Test, together with PCR testing for carbapenemases [26]. Testing the ability of producing large-spectrum betalactamases (BLSE) can also be achieved, through the double-disc method. Genotyping methods for MDR *A. baumannii* include: amplified fragment length polymorphism analysis (AFLP), PFGE, multiple locus variable number tandem repeat analysis (MLVA), single locus genotyping, resistance island typing, and next-generation whole-genome sequencing (NG WGS) with each having different genetic targets and specific features.

Pathogenicity and pathogenesis of *Acinetobacter* spp

The species of the *Acinetobacter* genre can be involved in hospital-acquired infections, the most commonly identified in the clinical setting being *A. baumannii*, more specifically the genospecies included in the "*Acinetobacter baumannii* complex", that cannot be distin-

guished biochemically [27]. This pathogen is able to infect and survive on hospital surfaces for long periods of time and therefore lead to hospital-acquired infections in susceptible patients and nosocomial outbreaks [8,28]. The vast majority of clinically relevant infections appear in hosts with a compromised immune system, where the virulence factors allow colonization and infection. Among these virulence factors we mention: production and release of exopolysaccharides, the LPS that can contribute to its pathogenicity through their mutagenic properties and their ability to induce TNF-alpha synthesis [29]. Mortality rates in hospitalized patients infected with this pathogen reach 26%, while mortality in the ICU patient group reaches 43% [30].

Acinetobacter baumannii has been isolated from all culture sites, but the most common infection and colonization site remains the inferior respiratory tract. It is the main pathogen incriminated in ventilator-associated pneumonia (VAP) but lately it has been proven that it can also lead to community-acquired pneumonia in patients that present with further risk factors such as COPD, alcoholism, heavy smoking, and diabetes, with a high prevalence in Asia as well as Australia [31]. Among the risk factors for developing an infection with *A. baumannii* Arodino et al. [32] mentioned bronchoscopy and oxygen therapy as invasive procedure performed outside the ICU or before the admission in the ICU, together with obesity or malnutrition, severe liver disease, and the type and duration of antibiotic therapy. Apart from respiratory infections cause by *A. baumannii*, other types and localizations of infections are also possible such as meningitis, bloodstream and central-line associated, skin or wound.

A. baumannii also has a horizontal transmission pattern, from one patient to the other when they are hospitalized in close vicinity and it is also capable of colonizing surfaces which apart from hospital furniture can also refer to feeding tubes. Wrong manipulation and lack of hygiene will therefore lead to the spread of the infection. In the ICU wards the main source for the infection are catheters of all types, equipment used for mechanical ventilation and other type of ventilatory support, and suction devices [9].

The pulmonary colonization and infection begins through the adherence to host cells that can be followed by the formation of a biofilm, dependent on the existence of pilli on the bacterial outer membrane. *A. baumannii* forms biofilms on both biotic and abiotic surfaces which makes the microorganism metabolically inert. It is because of this mechanism that vir-

ulence is increased as antibiotics cannot penetrate or act on metabolically inert organisms [8]. Biofilm production is deeply influenced by other virulence factors such as the family of BAP proteins (biofilm associated protein), OmpA, and BAP-like proteins [33].

AbOmpA (outer membrane protein) is a major membrane protein that is highly immunogenic and leads to an increase expression of cytochrome c which is a proapoptotic molecule. High concentrations of AbOmpA induce apoptosis of dendritic and epithelial cells that leads to the destruction of the mucosa and allows the bacteria to penetrate deeper tissues [34]. In low concentrations AbOmpA stimulates the expression of CD80, CD86, CD40, Major Histocompatibility Complex (MHC) class I, and MHC class II, as well as the production of interleukin 1 (IL1); it also determines epithelial cells to become more responsive to ligands by increasing the surface expression of Toll-Like Receptor 2 (TLR 2) which plays an important role in the pulmonary host defense mechanism. The membrane LPS of *A. baumannii* has been proven to be involved in the pathogenesis of sepsis by inducing pro-inflammatory cytokine synthesis and by binding to CD14 and the TLR4/MD-2 complex [35].

Neutrophil depression significantly reduces the cytokine levels showing that these cells play a crucial role in the pro-inflammatory response of the lung. Recruiting a high number of neutrophils in the upper and lower airway and in the lung parenchyma was correlated with the eradication of *A. baumannii* infection in the lungs [36]. Macrophages on the other hand are the first defense line and involved in the phagocytosis in the lower airways. They express a variety of cellular recognition molecules and studies have proven that they have a direct role in the local defense against *A. baumannii* through a NO-dependent mechanism that need an intact and functional microfilament and microtubule system in order to function adequately [37]. Understanding the virulence mechanisms of *A. baumannii* can facilitate the development of new immunotherapies, as well as new antibiotics.

Mechanisms of antimicrobial resistance and their evolution pattern

From once being only an opportunistic bacterium, easy to treat even with narrow-spectrum antibiotics *A. baumannii* has evolved to being capable of mutations associated both with antimicrobial therapies and with the host response. Since 1928, with the great discovery of Alexander Fleming – Penicillin – people have thought that they discovered a “magic weapon”

that would eradicate infections from the face of the Earth, not knowing the capability of bacteria of counteracting this fight. The first penicillin-resistant microorganisms have been reported only one year after the first administration of penicillin, and methicillin soon followed the same pattern. With regard to *A. baumannii* up until 1970 infections were easily treatable but high rates of resistance emerged as early as 1975. In the late years many valuable drugs are no longer efficient in the treatment of these infections, including cephalosporins, tetracycline, most aminoglycosides and ceftazidime [9,38]. The current therapeutic regimens include carbapenems, although recent studies have shown increased resistance, fluoroquinolones, and sometimes broad-spectrum cephalosporins if the microorganism is susceptible. Even in susceptible strains in the late years MICs have increased [39]. Based on their research Marvig et al., concluded that the population of *A. baumannii* includes at least nine distinct clonal lineages worldwide [40].

In their study, Hua et al. [41] carried out the analysis of 172 genomes of isolated *A. baumannii*, from 86 different patients to research the evolution of the microorganism within its host and they have shown that worldwide *A. baumannii* comprises of three major international lineages: EC I, EC II, and EC III. Out of the three the dominant one is EC II with its subgroup ICL-II that represents the carbapenem-resistant *A. baumannii*. After analyzing the single nucleotide polymorphism (SNPs) for within-patient diversity they have shown that over 15 genes has been mutated more than one time. A gene with a significant role in capsule synthesis, *ptk* has been shown to be mutated the most, with the mutant microorganism presenting higher motility levels. Another mutation was that of *adeRS* that increased the resistance to tigecycline. Hua et al have also shown that *A. baumannii* is resistant to major antimicrobial drugs and that the resistance increases together with its evolution within the host [41].

Since the 1980s the spread of *A. baumannii* infections has been a problem across Europe, with outbreaks in hospitals in western European countries such as Germany, the Netherlands, France and Italy [42]. After identifying the epidemic strains infecting these hospitals it was proven that transmission is also possible between hospitals, most probably via transfers of infected or colonized patients [43]. The possibility of spread of multi-drug resistance *A. baumannii* is possible not only from a patient to the other or from one hospital to the other, but also at a national and even international level, a fact that was proven by many different stud-

ies. Turton et al have described the multi-hospital epidemic given by the "Southeast clone" and by the Oxa-23, Oxa-1, and Oxa-2 clones (all MDR-A. *baumannii*) which infected patients in English hospitals and disseminated through Southeast England [44]. Another example is the epidemic that affected northern and southeastern France, where a VEB-1 ESBL A. *baumannii* clone spread in a total of 55 medical centers [45]. Moreover, hospital outbreaks in Northern Europe, Eastern Europe and other EU countries such as Greece and Spain were reported with three international clones of A. *baumannii*, mainly the European clone I, II, and III. Epidemiologists and researchers could not find the link between the outbreaks given by these European clones [46].

The resistance mechanisms to Cephalosporins in the case of A. *baumannii* is given by the natural production of Acinetobacter-derived cephalosporinase (also called AmpC β -lactamase or, shorter, ADC). In this case the production of ADC cannot be repressed, and what makes it even more dangerous is the fact that it can even be enhanced by the insertion of certain sequences in the gene which in the end leads to a high level of resistance [47, 48]. Certain strains of A. *baumannii* also have the capacity of producing, apart from ADC, the extended-spectrum β -lactamase (ESBL) which also renders them resistant to cephalosporins [49].

On the other hand, the production of carbapenemases is the most important mechanism for resistance of A. *baumannii* to carbapenems. Carbapenemases can be produced by the microorganism naturally, such as the group of OXA-51 chromosomally encoded carbapenemases, but they can also be acquired. In the same manner as for the ADS, resistance can be reinforced through an insertion sequence upstream of OXA-51 gene which will lead to increased MICs for carbapenems [9,50]. In the recent years A. *baumannii* has acquired carbapenemases in the non-OXA group, that up until recently were specific for the *Enterobacteriaceae*.

Sulbactam, which used to be a solution for the β -lactamase producing group, is given as a combination with either ampicillin or cefaperazone, but it also has its own activity against A. *baumannii*. However, in the last decades through the production of TEM-1 β -lactamase and through low expression of penicillin-binding protein (PBP2), A. *baumannii* has managed to develop resistance to this drug [51,9].

The activity of aminoglycosides is affected by the ability of the bacteria to produce a variety of aminoglycoside-modifying enzymes

[52]. This class of antimicrobials exerts their activity through the inhibition of protein synthesis. This is achieved by binding to the ribosomal RNA (16S) of the ribosomal subunit (30S) but A. *baumannii* is capable of producing ArmA which is a 16S ribosomal RNA methyltransferase [9], protecting the aminoglycoside-binding site from binding the drug [53].

Colistin is an antimicrobial with bactericidal activity and exerts its effect by binding to lipid A, an endotoxin of gram-negative bacteria, and the interior layer of the LPS. Modification of lipid A lead to resistance to colistin, by the addition of phosphoethanolamine [56]. Another mechanism that renders colistin ineffective is the total loss of the LPS [57].

A. *baumannii* also presents both moderate and high-level resistance to fluoroquinolones. As a mechanism of action they bind to the topoisomerase IV and DNA gyrase [9], therefore disrupting the synthesis of DNA and inducing cell death. The microorganism substitutes amino acids in the genes that encode target proteins for the quinolone resistance determining region [54], this leading to high levels of resistance to fluoroquinolones. Moderate levels of resistance are achieved by the overexpression of active efflux pumps [55,9].

Conclusions

The emergence of multi-drug resistant *Acinetobacter spp* involved in hospital-acquired infections is troublesome and an immense burden for the modern medical systems worldwide, especially because of the ever-developing strains capable of ESBL and carbapenemases production that led to pan-resistance (extensive resistance to carbapenems, aztreonam, all cephalosporins, aminoglycosides, and fluoroquinolones). These strains have a high potential of survival for long periods of time in the hospital environment, leading to contamination of medical equipment and prosthetic devices.

The risk factors for hospital-acquired infections with multi-drug resistant A. *baumannii* include admission in the intensive care unit, mechanical ventilation, invasive procedure and surgical interventions, using prosthetic devices, as well as previous antibiotic treatment with carbapenems, 3rd generation cephalosporins, and quinolones.

Modern testing for A. *baumannii* strains and for the resistance profiles should be available to all healthcare authorities. However, genetic testing is still not accessible everywhere, but with future reduction of costs for the DNA library preparation could make genomics a tool that could be used for routine surveillance [2].

As a future perspective population genomics will help researchers and clinicians altogether to better understand both the exact etiology but also the epidemiology and evolving possibilities of infections caused by *A. baumannii*.

Therapeutic options are limited as the microorganism continues to evolve and to develop new and more complex resistance mechanisms to modern antimicrobials. Hospital-acquired infections with *A. baumannii* lead to a worse clinical outcome of the patients, increasing the financial burden related to medical costs because of longer stays in the ICU and on other hospital wards and complex antimicrobial treatments. Passive and active surveillance as well as prevention and control methods could reduce the rate of *A. baumannii* infections. Therefore surveillance of the antimicrobial therapy in each hospital, with guidelines respecting the resistance profiles, and constant training of all medical personnel regarding prevention methods would have a favorable impact on the issue.

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