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Review

Molecular biology for the identification and diagnostic of *Acinetobacter baumannii* infections

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Abstract

The presence of *Acinetobacter baumannii* as an opportunistic pathogen has become a major concern in healthcare settings. This bacterium is frequently responsible for a range of infections, including pneumonia, meningitis, bloodstream infections, urinary tract infections, and wound infections….etc, in individuals with weakened immune systems. Its multidrug-resistant nature, particularly to carbapenem antibiotics, makes it a difficult pathogen to treat. Molecular biology provides a solution to these challenges, offering a vital tool in the identification and diagnosis of *A. baumannii* infections. The rapid and specific detection methods offered by molecular biology, such as polymerase chain reaction (PCR), allow for a quicker and more accurate diagnosis. Additionally, the characterization of antibiotic-resistant genes in *A. baumannii* isolates using molecular biology methods is critical in guiding effective treatment and preventing the spread of antibiotic-resistant strains. In conclusion, the integration of molecular biology into the diagnostic and treatment process for *A. baumannii* infections has been transformative, providing a crucial tool for prompt and effective treatment and controlling the spread of antibiotic-resistant strains of this opportunistic pathogen.

Keywords:
Molecular biology, *Acinetobacter baumannii*, infection, genotyping

1. Introduction

The advent of molecular typing in the identification and characterization of microorganisms constitutes a major advance in the management of several infections [1]. We then speak of molecular

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diagnosis carried out by techniques of research and analysis of the genome of an organism. This is no longer the privilege of certain specialized laboratories or specific centers of excellence, but it is becoming the daily practice of several laboratories allowing molecular diagnosis even in outpatient practice [2]. Such methods allow early diagnosis of the disease in its acute phase before the appearance of antibodies. In some cases, the pathogen is cultured for a long time, is not or is difficult to cultivate: *Legionella pneumophila*, *Treponema pallidum*, *Mycoplasma genitalium* and *Haemophilus ducreyi*. On the other hand, the differentiation between bacterial species is not always easy due to the similarity of phenotypic characters. Only gene differentiation allows reliable identification and appropriate management [2].

The typical example of this situation is when the incriminated agents are part of the *Acinetobacter calcoaceticus-A.baumannii* complex. The latter are the cause of serious infections mainly in fragile subjects, with a pronounced and ascending resistance to antibiotics at the origin of an epidemic of resistance [3]. Knowing that in Algeria, *A. baumannii* occupies, according to the Algerian Antimicrobial Resistance Network (AARN), the fourth place among the multiresistant bacteria, affecting the imipenem (65% resistance) which is prescribed in first intention in this case of infection [4]. Therefore, genotyping must be generalized for the management of the diagnosis of emerging and re-emerging bacterial infections. It allows orientation and a much more appropriate and rapid therapeutic choice and lays the foundations for better knowledge [5]. This work reviews the importance of molecular techniques in the diagnostic approach of *A. baumannii* infections.

2. Bacteriology

The *Acinetobacter* genus was first described in 1911 by the Dutch microbiologist Beijerinck under the name *Micrococcus calcoaceticus* [6]. In 1954, and following the work of Brisou and Prévot, the genus designation *Acinetobacter* (from the Greek akinetos: "immobile") was proposed for the first time to separate immobile bacteria from mobile bacteria of the *Achromobacter* genus [7]. The classification of these bacteria then underwent many changes until the 1980s with the appearance of modern genotypic methods [8].

Currently according to molecular biology techniques this genus includes more than 50 species [9]. The majority are non-pathogenic environmental microorganisms. The species most incriminated in nosocomial infections is *A. baumannii*, especially in intensive care units [10].

A.baumannii is a non-fermenting Gram-negative coccobacillus. Aerobic strict, immobile, asporulated and sometimes encapsulated, oxidase negative and catalase positive [11, 12] of easy culture this genus is nutritionally undemanding and grows on most ordinary mediums [11]. It multiplies at incubation temperatures of 20°C to 37°C, with optimum growth temperatures ranging between 33 and 35°C [13].

Its preferred habitat is then the infected and/or colonized patient. It is also found in the close environment of the patient: on catheters, mechanical ventilation and aerosol therapy devices, taps, basins and infusion bottles, etc. respiratory, but also at the level of the intestinal mucosa [14].

The ability of *A. baumannii* to survive in a hospital setting and its multiresistance to antibiotics makes it a nosocomial dangerous pathogen, causing serious infections by the immunocompromised patients [15]. These infections are often difficult to treat and are responsible for additional morbidity and mortality with an increase in the duration and cost of hospitalization [15].

3. *A. baumannii* major infections

In recent decades worldwide *A.baumannii* has emerged as one of the main responsible of severe nosocomial infections, particularly in intensive care units. These infections include pneu monia, bacteremia, infections of the urinary tract, skin, soft tissues and external meningitis … etc [16].

3.1. Pneumonia

Nosocomial pneumonia is the most common clinical manifestation of *A. baumannii* infection. These infections occur oftenly mechanical ventilated patients in intensive care units. This infection is thought to be the result of air respiratory tract colonization by this bacterium. The mortality rate of ventilator-associated pneumonia caused by *A. baumannii* has been reported to be between 40% and 70% [17].

Several risk factors of pneumonia due to *A. baumannii* under mechanical ventilator have been highlighted: advanced age, immunosuppression, chronic lung diseases, surgical context, recent use of antibiotics and the presence of endobronchial materials (intubation tube or tracheostomy tube) or a gastric tube [18, 19].

A. baumannii can also cause community-acquired pneumonia, occurring mainly in tropical areas (Asia and Australia). These infections are frequently observed in subjects with chronic respiratory disease or other comorbidities such as renal failure, diabetes, hepatic cirrhosis, alcohol intoxication or neoplastic pathologies [8]. These pneumonias are more serious than those acquired in the hospital environment and are more often associated with multiorgan failure or disseminated intravascular coagulation [20]. The risk factors associated with community-acquired pneumonia are age, smoking, alcoholism, chronic bronchitis, diabetes, cancer and a history of pneumonia. Community-acquired pneumonia is generally fulminant, with a mortality rate that can exceed 60% due to a delay in appropriate therapeutic management [21, 23].

3.2. Sepsis

A large national surveillance survey carried out in the United States from 1995 to 2002 placed *A. baumannii* in the tenth place of the microorganisms most frequently responsible for sepsis [24].

A. baumannii can cause bacteremia which is most often of traumatic, surgical origin, related to catheters, or other implantable devices. They occur late on average 26 days after hospitalization. It is therefore difficult to know whether the high mortality rate is linked to the type of bacteria in question or to the underlying pathologies [24].

The bacteremic risk depends on several factors, in particular the type of catheter and its physico-chemical composition, its insertion site, the frequency of its use and the duration of the catheterization. This risk increases with the number of lumens and the frequency of its manipulation and decreases with the use of polyurethane or silicone elastomer catheters which reduce the risk of bacterial adhesion [25]. Nosocomial septicemia represents the most serious infections among *A.baumannii* infections. The mortality rate linked to this type of infection is around 16% outside intensive care, while it can vary between 34 and 43% in intensive care units [26].

3.3. Skin and soft tissue infections

They frequently occur in critically ill patients with underlying comorbidities and are often accompanied by bacteremia and sepsis syndrome, usually with a predominance of polymicrobial infections [27]. *A.baumannii* is responsible for nearly 2% of skin and soft tissue infections acquired in intensive care units [3]. This bacterium was well known in the burn services. A Tunisian study carried out in a burns resuscitation unit shows that *A. baumannii* represent on average 12.2% of the bacterial ecology of the service [28].

3.4. Urinary tract infections

These generally occur in the presence of a urinary catheter and vary in incidence from one service to another. In intensive care units *A. baumannii* is responsible for less than 2% of urinary tract infections [29]. These infections are classified among the most frequent infections due to *A. baumannii* during the first years of observation. Their decreasing trend is probably linked to the improvement of probe systems. Probe withdrawal is usually the effective measure applied [30].

3.5. Meningitis

A. baumannii is an increasingly important cause of meningitis, with the majority of cases occurring in patients recovering from neurosurgical procedures [17] with a mortality rate greater than 15% [31].

A retrospective study identified 51 cases of postsurgical *A. baumannii* meningitis in two tertiary care hospitals between 1990 and 2004. These cases represented 10.9% of all meningitis cases in these facilities and had a crude mortality of 33% [32]. A similar study evaluating post-surgical *A. baumannii* meningitis in 28 patients reported a crude mortality of 71% [33].

The clinical features of *A. baumannii* meningitis match those of bacterial meningitis caused by other organisms and include fever, altered consciousness, headache, and seizures [32].

The management of meningitis and/or ventriculitis due to *A. baumannii* MDR remains a difficult therapeutic problem due to the limited penetration of antibiotics into the cerebrospinal fluid [34].

3.6. Other infections

In addition to the infections mentioned above, *A. baumannii* is an uncommon cause of endocarditis, the majority of which is related to the use of prosthetic valves and intravascular catheters. It can also be the cause of peritonitis, osteomyelitis, ophthalmia or post-surgery keratitis [17].

4. Basic molecular methods for identification of *Acinetobacter*

Genotyping remains the best way to support the insufficient phenotypic identification of species of the genus *Acinetobacter*. The DNA-DNA hybridization technique represents the current reference method for the identification of this genus. However, it is a restricted method and available only in specialized reference laboratories [35]. This lack is filled by newly developed and applied molecular tools. Among them, those based on the amplification of a specific gene, based on the comparison of DNA fragments obtained after separation by electrophoresis, or those based on the analysis of sequenced DNA fragments. We present here the main techniques used.

4.1. PCR methods targeting specific A. baumannii gene

4.1.1. PCR targeting the $bla_{OXA-51-like}$ gene

This is a gene, naturally present on the chromosome of *A.baumannii*, coding for the production of oxacillinase. The ability of this gene to resist carbapenems can only be induced when it is associated upstream with the insertion sequence (IS) of IS*Aba1*. The latter are short repetitive and transposable sequences that are difficult to detect [36]. Turton et al., in 2006 reported for the first time that the demonstration of the blaOXA-51-like gene is a reliable and rapid means for the identification of *A. baumannii* [37]. Then the search for this gene for the diagnosis of this species has been widely applied [38]. However, this method is no longer sufficient due to the detection of this type of gene in other species of the same genus and the presence of mutants of this same gene [36, 39].

4.1.2. Multiplex PCR targeting the gyrB gene

It is a gene encoding the β subunit of DNA gyrase. Its analysis is a promising tool for differentiating certain species of *Acinetobacter* due to its high specificity and the ease of implementation of the technique. The PCR products of the gyrB gene are of different sizes for each species,

allowing them to be distinguished from each other [40]. In this field, the first multiplex PCR was reported by Higgins et al., in 2007. They use 3 types of primers: Sp2F, Sp4R and Sp4F in order to differentiate between *A. baumannii* and *A. nosocomialis*. For which both species produce a 294 bp amplicon and only *A. baumannii* produces a 490 bp amplicon [41]. In 2010, the same authors described a second multiplex PCR to distinguish two species (*A. pittii* and *A. calcoaceticus*) from the *A. calcoaceticus A. baumannii* complex. This new PCR uses two pairs of primers (D16- D8) and (D14 - D19) which identify *A. pittii* and *A. calcoaceticus* respectively [42].

Given the reproducibility of this approach, it becomes easy to effectively discriminate between the different species belonging to the *A. calcoaceticus-A.baumannii* complex confirmed in 2014 by the work of lee and collaborators [43].

4.1.3. PCR targeting the recA gene

It is also a more specific rapid and less expensive multiplex PCR technique. It is done by using a pair of primers amplifying a 425 bp region of recombinase A (recA) and serving as an internal PCR control and a another pair amplifying a 208 bp fragment of the 16S-23S rDNA intergenic spacer region (ITS or 16S-23S rRNA intergenic spacer) specific to *A.baumannii*. This strategy allows the diagnosis of *A.baumannii* (two bands) and other species of the genus *Acinetobacter* (one band) [44, 45].

4.2. Methods based on the analysis of DNA fragment

4.2.1. Amplified Ribosomal DNA Restriction Analysis (ARDRA)

It is a technique that consists of an amplification of a specific locus followed by an enzymatic restriction by five types of enzymes: CfoI, AluI, MboI, RsaI, MspI and separation of the fragments obtained by migration on electrophoresis gel. The most commonly studied gene is the one that encodes 16S ribosomal DNA. The identification of the species is made by compar-ing the profiles to databases. ARDRA is an inexpensive technique, easily usable and with goodinterlaboratory reproducibility [46].

4.2.2. Amplified Fragment Length Polymorphism (AFLP)

It is a technique of genetic fingerprinting of any DNA molecule regardless of its origin. It is very useful for the identification of *Acinetobacter*, in particular species of the *A. calcoaceticus-A.baumannii* complex [4]. The AFLP takes place according to the following steps, the first is based on the amplification by PCR of a DNA fragment previously digested using two restriction enzymes, one with a high cut-off frequency (EcoRI) and the other at low cut-off frequency (MseI). In the second step, a ligation of adapters to the restriction fragments, then a selective amplification of these fragments was carried out with specific primers, and finally, a separation of the amplicons by migration on electrophoresis gel and profile analysis [47].

4.2.3. Ribotyping

This is a method that detects genetic polymorphisms located within genes coding for ribosomal RNAs (16S and 23S) and their adjacent DNA sequence [48]. Its effectiveness in the identification and genotyping of bacterial strains has already been demonstrated. In this technique, the DNA is digested using restriction enzymes then the fragments are separated by electrophoresis and transferred to a membrane. This is followed by hybridization with a specific labeled probe. Non-radioactive labels are used making this technique easy to perform in a well-equipped microbiology laboratory [49].

The identification of *Acinetobacter* species by this method was initiated in 1992 by Gerner-Smidt [50]. This technique has been automated and marketed under the name Riboprinter™ (Dupont Qualicon, USA) and has been widely used in several studies with the EcoRI enzyme. Although ribotyping is laborious, this method is robust for the identification of *Acinetobacter* with good correlation with ARDRA and AFLP. Results can be compared between laboratories and *Acinetobacter* species can be differentiated [40].

4.3. Methods based on the analysis of a DNA sequence

4.3.1. 16S ribosomal DNA gene sequencing

This gene encodes the 16S subunit of ribosomal RNA (rRNA) is mainly used in taxonomic and phylogenetic studies because of its structure, which is highly conserved in all bacteria. Indeed, it consists of a succession of conserved domains, sites of complementarity for the universal primers used for sequencing this gene, and other portions of sequences specific to a group of bacteria, called signature sequences (species, genus, family). Its sequencing allows the identification of species during routine microbiological evaluation. The results of the sequencing of the gene coding for this gene are compared with those of public databases (GenBank) and with known type strains. Identification of a species is made only when a group of strains share more than 98.7% homology at the level of DNA sequences encoding 16S rDNA. The usefulness of this gene in *Acinetobacter* is a subject of debate [51] . In a study by Dijkshoorn and Nemec in 2008 the similarity values of the type and reference strains vary between 94.1% and 99.6%, with only 3.7% of the values above the taxonomic threshold of 98.7% [52]. Chan et al., in 2012 showed that this technique was unable to correctly differentiate species of the same genus with a cut-off value above 99% [53]. In order for this identification technique to be more discriminating, in 2008, Vaneechoutte and De Baere proposed new thresholds for the identification of species of this genus with a similarity value of 99.7% or more can define the identity of the species. On the other hand, a value below 99.6% may indicate that these two strains belong to different species [54].

4.3.2. rpoB gene Sequencing

This gene encodes the β subunit of RNA polymerase. It seems to be the best way to correctly identify species of the genus *Acinetobacter*. Sequencing of this gene shows the presence of two polymorphic regions named 1 and 2 located within the gene, and two variable regions adjacent to the end, called rpIL-rpoB (301-310 bp) and rpoB-rpoC (86-177 bp). La Scola et al., in 2006 proposed to sequence zone 1 as a rapid scheme for the identification of *Acinetobacter* (partial sequencing of the rpoB gene) [55].

In 2009, Gundi et al., validated the usefulness of zone 1 for the identification of *Acinetobacter* including the *A. calcoaceticus-A.baumannii* complex. The inter-species similarity of rpoB zone 1 sequences within this complex is between 88.3% and 96.9% [50]. Then, several authors show the usefulness of this zone in the identification of *A. baumannii* [43] [56]. In addition, this molecular method allows rapid and exact identification, provided that a sequencer is available.

4.3.3. Sequencing of the 16S-23S ribosomal DNA intergenic spacer

The region separating the gene encoding the 16S subunit from that encoding the 23S subunit of ribosomal RNA is an unstable region, its size depends on the bacterial species considered. The size variability is very minimal within the same bacterial species, but increases when comparing different species belonging to the same genus. The analysis of these intergenic sequences makes it possible to discriminate between the different members belonging to the *A. calcoaceticus-A.baumannii* complex. The principle is based on PCR amplification of the intergenic region and its flanking regions using universal primers 1512F (5'-GTCGTAACAAGGTAGCCGTA-3')and 6R (5'- GGGTTYCCCCRTTCRGAAAT -3' where Y is C or T, and R is A or G), fol- lowed by sequencing to specify the nucleotide sequence and its length. Indeed, the intra-speciessimilarity of the sequences of this region within this complex varies between 0.99 and 1 [57]. Thanks to its reliability and speed, this method manages to discriminate between species of the *A. calcoaceticus-A.baumannii* complex, provided that a sequencer is available in the laboratory.

5. Conclusion

A. baumannii is a major nosocomial bacterium, responsible for a wide range of severe infections in weakened patients. This pathogen has become problematic in hospital departments due to its ability to persist, ease of spread and its capability to emerge new strains multi-resistance to antibiotics and in particular to carbapenems. Thus, diagnostic support must be fast and precise. Therefore, various molecular biology techniques in particular PCR methods targeting specific *A. baumannii* gene and methods based on the analysis of DNA fragments and DNA sequences, are the tool of choice to quickly detect and characterize this pathogen. The application of these techniques has a positive impact on patients care especially the limitation of the *A. baumannii*

infection diffusion, the durations of hospitalization and the cost. Hence, the avoidance of an epidemic resistance caused by *A.baumannii*. These methods must be the daily routine in laboratories having the mission of diagnosing bacteria resistant to antibiotics.

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