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REVIEW

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Acinetobacter baumannii: biology and drug resistance — role of carbapenemases

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Abstract
Acinetobacter baumannii is a Gram-negative, glucose-non-fermenting, oxidase-negative coccobacillus, most commonly associated with the hospital settings. The ability to survive in adverse environmental conditions as well as high level of natural and acquired antimicrobial resistance make *A. baumannii* one of the most important nosocomial pathogens. While carbapenems have long been considered as antimicrobials of last-resort, the rates of clinical *A. baumannii* strains resistant to these antibiotics are increasing worldwide. Carbapenem resistance among *A. baumannii* is conferred by coexisting mechanisms including: decrease in permeability of the outer membrane, efflux pumps, production of beta-lactamases, and modification of penicillin-binding proteins. The most prevalent mechanism of carbapenem resistance among *A. baumannii* is associated with carbapenem-hydrolysing enzymes that belong to Ambler class D and B beta-lactamases. In addition, there have also been reports of resistance mediated by selected Ambler class A carbapenemases among *A. baumannii* strains. Resistance determinants in *A. baumannii* are located on chromosome and plasmids, while acquisition of new mechanisms can be mediated by insertion sequences, integrons, transposons, and plasmids. Clinical relevance of carbapenem resistance among strains isolated from infected patients, carriers and hospital environment underlines the need for carbapenemase screening. Currently available methods vary in principle, accuracy and efficiency. The techniques that deserve particular attention belong to both easily accessible unsophisticated methods as well as advanced techniques based on mass spectrometry or molecular biology. While carbapenemases limit the therapeutic options in *A. baumannii* infections, studies concerning novel beta-lactamase inhibitors offer a new insight into effective therapy. *(Folia Histochemica et Cytobiologica 2016, Vol. 54, No. 2, 61–74)*

Key words: *Acinetobacter baumannii*; virulence factors; drug resistance; carbapenems; carbapenemases; metallo-beta-lactamases; oxacillinases; CHDL; resistance detection; carbapenemase inhibitors

Introduction

Acinetobacter baumannii is a Gram-negative, nonmotile, glucose-non-fermenting oxidase-negative and increasingly important opportunistic pathogen. The bacterium can survive on solid and dry surfaces up to 5 months, which is attributed to: simplicity of its nutritional requirements, ability to grow in the wide range of temperatures and pH values, high degree of resistance to disinfectants and antiseptics, ability to form biofilm on abiotic substrata (environmental surfaces or medical devices, such as catheters or respiratory equipment) as well as biotic surfaces [1–5]. These features are likely to be a major contributing factor to nosocomial spread of the *A. baumannii* [5]. It is thought that *A. baumannii* infections are mostly acquired after exposure to contaminated hospital equipment or by direct contact with healthcare personnel that have been previously exposed to the microorganism [6]. The other important features responsible for *A. baumannii* clinical relevance are: high natural resistance as well as an outstanding ability to up-regulate innate and acquire foreign mechanisms of antimicrobial resistance [5].

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Clinical relevance

Since the 1980s the clinical importance of *A. baumannii* has radically emerged [7]. The increase of incidence of *A. baumannii* infections is believed to be attributed to advancements in medical procedures, which may result in rise of susceptible population of patients [8]. It was also noted that its antimicrobial resistance has progressively increased since the 1970s [7]. Consequently, *A. baumannii* has been described by Infectious Diseases Society of America as one of the most significant hospital pathogens [9]. The genus *Acinetobacter* belongs to *Moraxellaceae* family and consists of at least 21 described species, with *A. baumannii* as the most clinically relevant [10]. While *Acinetobacter* spp. phenotypical identification system was described, it does not distinguish all known so far *Acinetobacter* species [11]. Current *Acinetobacter* spp. taxonomy comprise *A. calcoaceticus–A. baumannii* complex (Abc) which includes: *A. calcoaceticus* (genomic species 1), *A. baumannii* (genomic species 2), *A. pittii* (genomic species 3) and *A. nosocomialis* (genomic species 13TU), that are all highly genetically related and difficult to distinguish phenotypically [10]. It is considered that more reliable methods of identification are represented by amplified 16S rRNA gene restriction analysis (ARDRA) and amplified fragment length polymorphism (AFLP) [12]. *Acinetobacter baumannii* can cause numerous acute hospital infections represented by: respiratory infections (in particular ventilator-associated pneumonia; VAP), bloodstream infections, urinary tract infections, skin and soft tissue infections, burn and surgical wound infections, endocarditis, meningitis, and osteomyelitis [7]. The risk factors that make patients prone to the colonisation and infection with *A. baumannii* comprise: considerable surgical procedures, major trauma, premature or old age, hospitalisation, antimicrobial therapy as well as medical treatment including mechanical ventilation, intravascular catheters, urinary catheters and drainage tubes [11]. This bacterium is reported as responsible for 2–10% of all Gram-negative hospital infections, mainly affecting critically ill patients, particularly hospitalised in intensive care units [7]. Furthermore, *A. baumannii* healthcare-associated infections may increase the risk of mortality from 8% to 40% [13]. The multicentre studies conducted by Hidron and co-workers revealed the growing occurrence of *A. baumannii* nosocomial infections, with 8.4% of VAP, 2.2% of central line-associated bloodstream infections, 1.2% of catheter-associated urinary tract infections and 0.6% of surgical site infections [14]. Despite the nosocomial infections, *A. baumannii* can also induce community-acquired infections (CA-Ab — community-acquired *A. baumannii*), which are reported predominantly in tropical regions of the world [15]. Pneumonia and bacteraemia are the most prevalent clinical syndromes related to *A. baumannii* infection in these settings. Community acquired pneumonia affects particularly individuals with underlying chronic obstructive pulmonary disease, renal failure, or diabetes mellitus, as well as heavy smokers or alcoholics [8]. As described by Dexter and co-authors CA-Ab pneumonia is characterised with fulminant course associated with a rapid onset of fever, acute respiratory symptoms and multi-organ dysfunction, with high mortality rate representing 64% [16]. Despite the hospital and community acquired *A. baumannii* infections, its occurrence appeared to be also related to injuries in war conflicts in *e.g.* Afghanistan, Iraq and Syria [17, 18] as well as natural disasters, such as earthquakes and tsunami [19, 20]. Epidemic spread of *A. baumannii* in above-mentioned extreme situations is attributed by some authors to substantial pressure on hospital emergency wards, which may result in failure in infection control precautions [7].

Virulence factors

Till date, there have been only a few virulence factors (VF) described in *A. baumannii*. Recent analysis concerning genome sequencing, genetic manipulations and applications of animal models allow believing that current knowledge regarding additional factors will be broadened. Among virulence determinants responsible for the *A. baumannii* pathogenicity, there may be mentioned: lipopolysaccharide (LPS), capsular polysaccharides (CPS), *A. baumannii* outer membrane protein A (AbOmpA), outer membrane vesicles (OMV), phospholipase D (PLD), and biofilm [6, 21].

The studies concerning the role of *A. baumannii* LPS indicated the contribution of the surface carbohydrates residues in the virulence [22]. The endotoxins are also considered as a potent stimulators of inflammatory signalling in human monocytic cells, dependent on both TLR2 (toll-like receptor) and TLR4 receptors. Therefore, the pathology of *Acinetobacter* infections may be associated with exaggerated innate immune response to the LPS [23–25]. Further determinants implicated in the virulence of *A. baumannii* are capsular polysaccharides. Studies conducted by Russo and co-workers revealed that K1 capsule acquired from clinical isolate of *A. baumannii* AB307-0294 appeared to play an important role in *in vivo* as well as *in vitro* bacterial protection. The active protection of the CPS enables *A. baumannii* to avoid bactericidal activity of the complement. The authors

also suggested that the CPS protection against the effects of phagocytes or antimicrobial peptides requires further studies [26]. AbOmpA is considered to be one of the best-characterised *A. baumannii* virulence factors. It is responsible for the damage to the human airway cells *via* induction of the apoptosis. This process is mediated by the release of proapoptotic molecule cytochrome c and the apoptosis-inducing factor. Moreover, AbOmpA is believed to play a role in adherence, invasion of epithelial cells and may contribute to the dissemination of *A. baumannii* during infection [27, 28]. Furthermore, above-mentioned protein is also implicated in the surface motility, resistance to complement and biofilm formation [6, 21]. *Acinetobacter baumannii* as well as some other Gram-negative bacteria secrete outer membrane vesicles. OMVs have been reported to take part in delivering virulence factors to the interior of the host cells, facilitating horizontal gene transfer, and protecting bacterial cells from the host immune response [29]. Proteomic analysis of *A. baumannii* OMVs revealed the presence of more than 130 proteins including: AbOmpA, serine- as well as Zn-dependent proteases, phospholipases, bacterioferritin, Cu/Zn superoxide dismutase, catalase, and ferrichrome-iron receptor [30]. Further data published by Jun and co-workers demonstrated considerable role of *A. baumannii* OMVs in stimulation of innate immune response that may contribute to immunopathology of the infected host [31]. Another group of *A. baumannii* VF include phospholipases. These enzymes are associated with cleavage of the host cells phospholipids, therefore promoting the bacterial invasion [6]. Studies concerning the role of *A. baumannii* phospholipase D in murine model of pneumonia revealed that disruption of PLD results with reduction of the bacterial survival in serum, deficiency in epithelial cell invasion and diminished pathogenesis [32].

Biofilm is a multicellular complex with three-dimensional structure, relevant in the infection. While its formation comprises the surface of the host cells and indwelling medical devices, it plays an essential role in the pathogenesis. Moreover, the presence of biofilm reduces the antibiotic penetration, leading to the development of drug resistance [33–37]. The CsuA/BABCDE usher-chaperone pili assembly system has been involved in *A. baumannii* biofilm organization on abiotic surfaces [38]. The expression level of *csu* operon is regulated by the two-component system — BfmS/BfmR, comprising a sensor kinase and a response regulator [39]. Biofilm-associated protein (Bap), expressed on the surface of the bacterial cells, is implicated in cell-to-cell adhesion providing biofilm development and maturation on different substrata [40]. Furthermore, one of the most important components of exopolysaccharides constituting biofilm matrix was polysaccharide polymer poly-beta-1,6-*N*-acetylglucosamine (PNAG) that is crucial for maintaining the integrity of *A. baumannii* biofilm under nutrient limitation and other environmental stresses [41]. As mentioned above, the outer membrane protein OmpA (38 kDa) plays an important role in the pathogen attachment to the human cells and *Candida* filaments, as well as in biofilm development on plastics [42]. Another factor implicated in *A. baumannii* biofilm formation on abiotic surfaces is represented by 3-hydroxy-C12-HSL molecule, encoded by the *abaI* autoinducer synthase gene [43].

Drug resistance

Acinetobacter baumannii is characterised by high level of intrinsic resistance to many groups of antimicrobials (*e.g.* glycopeptides, macrolides, lincosamides, and streptogramins) [44]. Moreover, this bacterium is able to develop resistance to all classes of antimicrobial agents used in the therapy. The process can be associated with genetic changes leading to membrane alterations, overexpression of efflux pumps (EP), overexpression of intrinsic antibiotic modifying enzymes, modifications of target sites for antimicrobial agents, and acquisition of novel resistance determinants. *Acinetobacter baumannii* strains enhanced by selective pressure of hospital environment may gain resistance via mutational changes as well as by horizontal gene transfer from other members of the species, genus, non-fermenters and/or Enterobacteriaceae family. Observed for a period of time, rapid accumulation of resistance determinants to multiple classes of antimicrobials among *A. baumannii* strains resulted in the elimination of penicillins, cephalosporins, aminoglycosides, quinolones, and tetracyclines as effective treatment options for many clinical isolates. Consequently, due to their good activity and low toxicity, carbapenems have left as one of the major therapeutic options in *A. baumannii* infections [9].

Carbapenems

Carbapenems are considered to play an essential role in antimicrobial therapy worldwide. Regarding antibacterial activity, they demonstrate the broadest spectrum among beta-lactam antibiotics. Therefore, carbapenems are often administered as "antibiotics of last-resort" or "last-line agents" in critically ill patients in the treatment a variety of infection.

Chemistry

The carbapenems differ from penicillins by the presence of carbon instead of sulphur in the position C-1 and possession of double bond between C-2 and C-3 in five-membered ring. The broad spectrum of antimicrobial agents is attributed to the natural resistance to different groups of beta-lactamases. In comparison to penicillins and cephalosporins, carbapenems possess trans-alpha-1-hydroxyethyl substituent at the 6 position, which results in high stability to beta-lactamases. The first described carbapenem was thienamycin, however the unstable nature of this compound resulted with the development of an *N*-formimidoyl derivative named imipenem. Nevertheless, imipenem as well as panipenem are degraded in brush border of renal tubules by dehydropeptidase-1 (DHP-1), forcing the co-administration of cilastatin or betamiprion, respectively [45]. Following carbapenems, such as meropenem, ertapenem, biapenem and doripenem, due to the presence of a methyl group in the 1-beta position, are not affected by DHP-1 inhibition. Meropenem as a derivative of thienamycin has a pyrrolidinyl substituent at 2 position, which is thought to provide the enhanced Gram-negative activity. Doripenem is a synthetic 1-beta-methylcarbapenem, differing from meropenem with the presence of sufamoylaminomethyl group. This modification increases doripenem activity towards non-fermentative Gram-negative bacilli [45, 46]. Another carbapenem, introduced to the therapy in 2001, is ertapenem. This broad-spectrum beta-lactam differs structurally from meropenem in a meta-substituted benzoic acid group at the 2 binding position. In consequence, increasing of the molecular weight and lipophilicity of the molecule was observed. Structural changes of the molecule also resulted in the increased half-life and decreased permeation through the Gram-negative bacteria cell wall [47].

Mechanism of action

The mode of action of carbapenems as well as other beta-lactam antibiotics is associated with the structure and biosynthesis of the bacterial cell wall [48]. Carbapenems enter the Gram-negative bacteria via outer membrane proteins (also described as porins), cross the periplasmic space and acetylate the penicillin-binding proteins (PBPs). PBPs are described as enzymes (*i.e.* peptidases, transglycosylases) responsible for the formation of peptidoglycan in the bacterial cell wall. Therefore, the bactericidal activity of carbapenems is associated with binding to PBP and, in consequence, the inhibition of peptide cross-linking and other peptidase reactions. Carbapenem binding of PBP leads to weakening of the peptidoglycan and may result with the cell burst due to the osmotic pressure. Furthermore, one of the essential features of the carbapenems is the ability to bind to multiple different PBPs [46].

Imipenem binds favourably to PBP2 and PBP1, while possess weak affinity towards PBP3. Meropenem affinity for the various PBPs differs between Gram-positive and Gram-negative bacteria. Among Gram-negative bacteria, this carbapenem binds preferentially to PBPs 2, 3 and 4, as well as exhibits strong affinity to PBPs 1a and 1b. Ertapenem binds primarily to PBP2 of *Escherichia coli* than PBP3, and has good affinity for PBP1a, 1b, 4 and 5. Doripenem has high affinity towards various PBPs exhibited by many bacterial species. This carbapenem presents high affinity for PBP2 and PBP3 in *Pseudomonas aeruginosa* and for PBP2 in *E. coli.* Doripenem is also considered to have increased antipseudomonal activity compared to imipenem. Taking into consideration the role of PBP in the mechanism of action of carbapenems, little is known about this processes in *A. baumannii* [21, 46].

Microbial activity

Carbapenems are beta-lactam antibiotics active against a wide range of Gram-positive and Gram-negative bacteria, including aerobes as well as anaerobes. However, in contrast, none of the carbapenems is clinically useful against *Enterococcus faecium* and *Stenotrophomonas maltophilia*. Generally, imipenem, panipenem and doripenem are slightly more active versus Gram-positive bacteria, while meropenem, biapenem, ertapenem, and doripenem are more potent against Gram-negative organisms. Taking into consideration carbapenem activity against *A. baumannii*, it has been shown that imipenem and doripenem are more potent than meropenem. Moreover, the MIC values of doripenem are lower than presented by imipenem and meropenem for *A. baumannii*. The studies concerning analysis of carbapenems MIC_{90} values among *Acinetobacter* spp., revealed more than eightfold higher MIC_{90} for ertapenem, comparing to other carbapenems [45–47].

Clinical use

As MDR (multidrug-resistant) pathogens emerge worldwide, in many cases carbapenems remain the "last-resort" antimicrobials used in empirical as well targeted therapy of severe infections. Furthermore, increasing phenomenon of carbapenem resistance limits available clinical options and stresses the need of development of combined therapy. Carbapenems can be combined with other antimicrobials to achieve an effective and safe therapy for serious *A. baumannii* infections [21, 46]. The possible advantages of combined therapy in comparison to monotherapy

comprise a broader antibacterial spectrum, synergistic effects, and decreased risk for emerging resistance during therapy.

Many combinations have recently been reported in the scientific literature. *In vitro* study conducted by Fernández-Cuenca and co-workers revealed the additive effect of imipenem and azithromycin in the group of clinical strains of *A. baumannii* [49]. Moreover, Principe and co-authors demonstrated *in vitro* synergistic activity of tigecycline in combination with imipenem against tigecycline non-susceptible *A. baumannii* strains [50]. Another study concerning combination therapy showed synergistic antibacterial effect of imipenem with colistin among 100% of analysed imipenem- and meropenem-resistant *A. baumannii* isolates. Furthermore, the triple combinations of meropenem, sulbactam and colistin exhibited synergy against 96.7% of MDR *A. baumannii* [51]. Further studies concerning the combination of meropenem and ciprofloxacin revealed a small synergistic effect against the *A. baumannii* intensive care unit strains [52]. The research of Kiffer and co-workers showed that the combinations of meropenem and sulbactam may show synergism or partial synergism for analysed MDR *A. baumannii* isolates [53]. In the recent study, a combination of meropenem with minocycline was synergistic to tested XDR (extensively drug-resistant) *A. baumannii* isolates, but neither showed bactericidal activity alone. Furthermore, the authors observed that also colistin and meropenem presented synergistic effect and showed bactericidal activity against all tested strains [54, 55].

Carbapenem resistance

While carbapenems representing group 2 (meropenem, imipenem–cilastatin and doripenem) have been regarded as antimicrobials of "last-resort" in MDR *A. baumannii* infection therapy, recently a rapid increase in the rates of carbapenem resistant *A. baumannii* was observed [5]. Studies performed by National Healthcare Safety Network showed carbapenem resistance among 33% of *A. baumannii* strains derived from medical centres in the United States of America [14]. Moreover, Reddy and co-authors revealed the disturbing tendency of sharp increase in the rates of CRAb (carbapenem-resistant *A. baumannii*), from 1% in 2003, to 58% in 2008, associated with a more than twofold rise in occurrence of *A. baumannii* isolates [56]. A multinational study showed that *A. baumannii* imipenem susceptibility rates were higher for strains isolated from Europe and North America than those from Latin America and the Asia–Pacific Rim [57]. Another microbiological surveillance reports have revealed substantial rates of multidrug resistance in *A. baumannii*, which suggest that this bacterium became a growing public-health problem nowadays [8, 58]. Growing prevalence of carbapenem-resistant *A. baumannii* isolates is considered as a threat to healthcare and patient safety worldwide, significantly reducing the ability to cure the infections. The progressive spread of CRAb strains is resulting in an urgent need for efficient detection, surveillance and guidance for infection prevention and control [58].

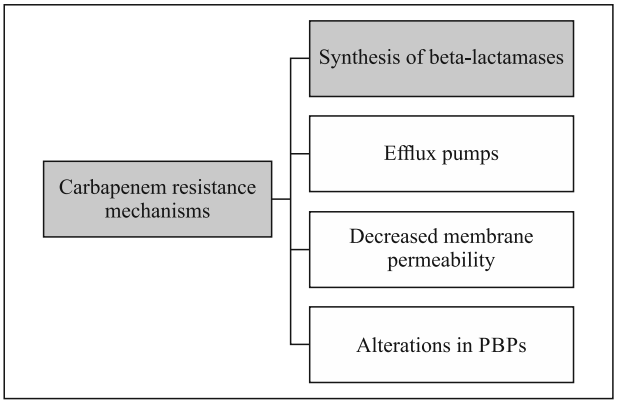

Figure 1. Mechanisms responsible for *Acinetobacter baumannii* carbapenem resistance. PBPs — penicillin-binding proteins.

Mechanisms mediating *A. baumannii* resistance to carbapenems are presented in the Figure 1. The fusion of these mechanisms may result in high levels of carbapenem resistance in *A. baumannii* strains [46].

Non-enzymatic mechanisms

While carbapenems enter the bacterial cell *via* certain types of porins, their reduced expression play a role in the resistance to these antibiotics. The most characterised porin among *A. baumannii* is the carbapenem-associated OMP (CarO) [10]. According to Catel-Ferreira and co-workers, decrease in the expression of CarO results in the reduction of susceptibility to imipenem [59]. Another carbapenem-associated OMP is 33- to 36-kDa protein. Carbapenem resistance in the epidemic strain analysed by del Mar Tomás and co-workers was considered to be caused by the loss of 33- to 36-kDa OMP [60]. Efflux pumps extrude antimicrobials from the bacterial cells, which may result in resistance to a wide spectrum of antimicrobial agents as well as disinfectants. While five families of EP are associated with increased resistance of bacteria, only three of them are represented in *A. baumannii*: multidrug and toxic compound extrusion (MATE) family, the major facilitator superfamily (MFS), and

the resistance-nodulation-cell division (RND) family. In *A. baumannii* the major efflux pump associated with carbapenem resistance is AdeABC. This EP belongs to RND family and is comprised of three-components: AdeA — membrane fusion protein, AdeB — inner membrane protein channel, and AdeC — outer membrane protein channel [61]. Overexpression of this EP is regulated by *adeS* and *adeR* genes contributing to increased resistance to antimicrobials, inter alia: meropenem, fluoroquinolones, tetracyclines, chloramphenicol as well as aminoglycosides [10]. Another mechanism responsible for carbapenem resistance in *A. baumannii* is related to alterations in penicillin-binding proteins. This mechanism confers resistance to beta-lactam antimicrobials in many bacterial species, however the data concerning this phenomenon in *A. baumannii* are limited. Although several PBP proteins have been described in *A. baumannii* so far, their role is attributed only to low-level carbapenem resistance [62, 63]. Furthermore, studies concerning CRAb strains with multiple mechanisms responsible for this process, revealed reduced expression of selected PBP proteins [63].

Enzymatic mechanisms

Bacterial enzymes responsible for hydrolytic inactivation of different groups of beta-lactam antibiotics, such as penicillins, cephalosporins, monobactams and carbapenems are represented by beta-lactamases. These enzymes are categorised according to the sequence homology into four molecular classes: A, B, C and D.

Carbapenem resistance can be mediated by hyperproduction or derepression of Ambler class C beta-lactamases (AmpC beta-lactamases), selected ESBL (extended-spectrum beta-lactamases) and carbapenemases [64]. In accordance with the current knowledge the most significant mechanism of carbapenem resistance in *A. baumannii* is associated with carbapenemases, the most versatile family of beta-lactamases. The Figure 2 shows the clinically relevant carbapenemases occurring among *A. baumannii*.

Based on the participation of divalent cations in enzyme activation, carbapenemases can be segregated into metallo-carbapenemases (class B) and non-metallo-carbapenemases (class A, C and D) [65]. Both groups of enzymes comprise the resistance to carbapenems by breaking the amide bond of the beta-lactam ring, however the mechanism of this process differs substantially. The hydrolysis of carbapenems mediated by metalloenzymes involves a water molecule, which is coordinated to a divalent cation (zinc) in order to activate and disrupt the beta-lactam ring. It is worth emphasizing that metallo-beta-lactamases do not form the covalent acyl-enzyme intermediate [65, 66].

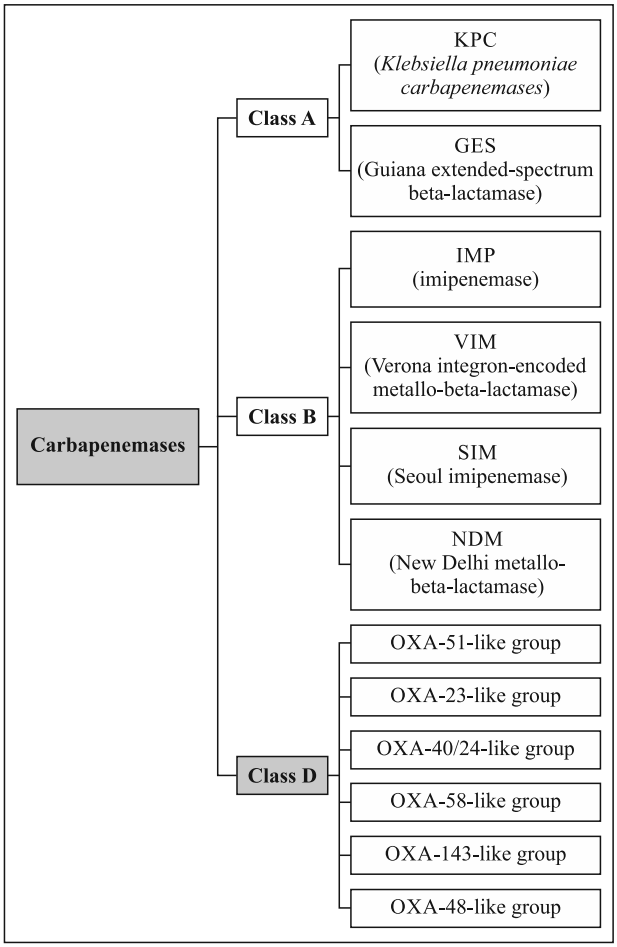

Figure 2. Clinically relevant carbapenemases occurring among *Acinetobacter baumannii.*

The hydrolytic inactivation of carbapenem antibiotics performed by non-metallo-carbapenemases includes acylation and deacylation reactions. In the first step, a conserved serine located in the active site of enzymes, attacks the beta-lactam amide bond, assembling an acyl-enzyme complex. This intermediate is hydrolysed afterwards by a deacylating water molecule, forming the hydrolysed product that is released from the active site of the enzyme [65]. It is considered that carbapenem hydrolysing class D beta-lactamases (CHDL) are the most common factor of carbapenem resistance in *A. baumannii* strains. These enzymes are referred as OXAs (oxacillinases) due to their ability to hydrolyse isoxazolylpenicillin — oxacillin much faster than benzylpenicillin [67, 68]. Among *A. baumannii* there have been identified so far six groups of OXA carbapenemases represented by: OXA-51-like, OXA-23-like, OXA-40/24-like, OXA-58-like, OXA--143-like, and OXA-48-like [9, 69].

The groups, occurrence, structure, catalytic mechanism and inhibitors of *A. baumannii* carbapenemases have been thoroughly described in the Supplementary File 1. The acquired carbapenem-hydrolysing OXA-type beta-lactamases carried by *Acinetobacter baumannii* strains have been listed in Supplementary Table 1 and the metallo-beta-lactamases reported among *A. baumannii* strains — in Supplementary Table 2.

Carbapenemase encoding genes: genetic location transfer and mechanisms of control

Carbapenemase encoding genes can be carried within *A. baumannii* genome on chromosome and/or plasmids. Furthermore, genetic determinants of carbapenemases are often associated with mobile genetic elements, such as insertion sequences (IS), integrons, transposons, resistance islands (RI), and plasmids, often contributing to their acquisition, dissemination and regulation among bacterial isolates [21].

Insertion sequences

Insertion sequences, also described as insertion sequence elements are DNA segments not exceeding in size 2500 bp, and therefore considered to be the smallest mobile DNA elements. IS can contribute to resistance by: providing additional promoters in order to amplify the expression of certain genes, disarranging the coding sequences of particular genes, and allowing the dissemination of gene cassettes among bacterial strains [21]. According to the Reference Centre for Bacterial Insertion Sequences (ISfinder), 30 different types of IS were described in *A. baumannii* so far [70]. The insertion sequence of particular importance and prevalence is represented by IS*Aba1*. It is considered that the first report of *A baumannii* IS*Aba1* concerned the isolate from 2001, however it turned out that the sequences corresponding to this element had been reported previously. While the presence of IS*Aba1* has been found in association with numerous antimicrobial resistance determinants, its role in mobilization of OXA-type carbapenemases has been reported in particular [69]. The association of IS*Aba1* and OXA-type carbapenemases encoding genes includes intrinsic as well as acquired enzymes. Turton and co-authors suggested that the location of IS*Aba1* sequence upstream of *bla*OXA-51-like is providing the gene promoter that may result in resistance to carbapenems [71]. The IS*Aba1* has been also found in association with $bla_{\text{OXA-23-like}}$ and $bla_{\text{OXA-58-like}}$ genes [71–73]. Nevertheless, several authors have suggested that overexpression of these CHDLs may be also associated with the presence of IS*Aba1* flanking these genes, and therefore may result with decreased susceptibility to carbapenems [72, 74, 75].

Integrons

Integrons are genetic elements that are capable of capturing antibiotic resistance determinants and able to promote their transcription and expression [76]. In recent decades it has turned out that integrons play a vital role in the acquisition and dissemination of antibiotic resistance genes, particularly among Gram-negative bacteria [77]. Integrons can be classified into several classes according to the relative homology of integrase encoding gene (*intI*) [77]. It is considered that the occurrence of integrons among *A. baumannii* strains may suggest their high epidemic potential. More than five classes of integrons have been described so far, with the class 1 integrons to be the most prevalent among multidrug-resistant *A. baumannii* clinical strains worldwide [78–80]. Moreover, it has been noted so far that among *A. baumannii* isolates class 1 integrons can carry only metallo-beta-lactamases, neither OXA nor KPC carbapenemase encoding genes. An example of imipenem-resistant *A. baumannii* strain carrying $bla_{\text{IMP-5}}$ gene within class 1 integron was reported by Da Silva and co-workers [81]. Also the studies of Huang and co-authors revealed the presence of multidrug-resistant *A. baumannii* isolates carrying within the class 1 integron the $bla_{\text{VIM-11}}$ gene [82]. Additionally, the occurrence of $bla_{\text{SIM-1}}$ was detected in class 1 integrons among seven clinical isolates of *A. baumannii* from Korea [83]. While the genetic determinants of IMP, VIM and SIM-type enzymes were found on class 1 integrons of *A. baumannii*, bla_{NDM} have not been found till date [84].

Resistance islands

Bacterial resistance islands are defined as a particular region within the genome harbouring a high concentration of horizontally transferred antimicrobial resistance genes [9, 21]. There have been numerous RI described among *A. baumannii* strains so far, represented by AbaR1, AbaR3, AbaR4, AbaR5-Aba19, AbaR25, and others [85–87]. The first *A. baumannii* resistance island (AbaR1) was reported in multidrug-resistant AYE strain, in 2006 by Fournier and co-workers [88]. The whole-genome sequencing of the strain revealed the presence of 86 kb resistance island, which harboured a cluster of 45 resistance genes, including $bla_{\text{OXA-69}}$ (member of $bla_{\text{OXA-51-like}}$ group) [88]. Studies concerning the group of carbapenem-resistant *A. baumannii* isolates in Latvia disclosed the prevalence of AbaR25 resistance island. The *Acinetobacter*

baumannii strain carrying the AbaR25 (variant of AbaR4) was linked to international clone II/ST2 and carried within the resistant island the $bla_{\text{OXA-23-like}}$ carbapenemase gene [87]. Furthermore, studies concerning CRAb isolates from Asia revealed the presence of two AbaR4-type resistance islands (D36 and AB210) that carried $bla_{\text{OXA-23-like}}$ genes [89].

Plasmids

Plasmids are circular or linear double-stranded DNA molecules distinct from chromosomal DNA, often transferred by means of conjugation [90, 91]. Studies concerning the sequence analysis of *A. baumannii* plasmid replicons revealed the differences comparing to other prokaryotic species, suggesting that *A. baumannii* strains may carry distinct set of plasmid types [92, 93]. It is believed that the most clinically relevant carbapenemases are often associated with plasmids [68]. While *A. baumannii* plasmids may vary in size as well as genetic content, Bertini and co-authors proposed their classification based on the replicase gene sequences [92]. Towner and co-authors analysing the group of 96 MDR *A. baumannii* clinical isolates from 17 European countries, confirmed the presence at least 1 (with a maximum of 4) out of 19 replicases (*rep*) gene groups (GR) among all clinical strains tested. The largest group of strains belonged to GR6 (repAci6; 93 isolates), and the variations in *rep* gene content were even presented among epidemiologically related isolates. Further analysis of co-occurrence of *rep* genes and CHDL encoding genes revealed the association of $bla_{\text{OXA-58-like}}$ genes (22 strains) with repAci1, repAci3, repAci4, and repAciX genes, while $bla_{\text{OXA-40-like}}$ (6 strains) were related to repAci2 and p2ABSDF0001, and $bla_{\text{OXA-23-like}}$ (8 isolates) were correlated with repAci1 [93].

Methods of detection of carbapenemases

The clinical relevance of rapid detection of carbapenemases among *A. baumannii* strains is crucial due to selection of appropriate antibiotic therapy as well as prevention of the development of the outbreaks. Till date, there have been introduced a number of methods, differing in principles, accuracy and time of detection.

Phenotypic methods

Among phenotypic tests used in the detection of carbapenemases, methods based on the inhibition of the enzyme activity by specified inhibitors deserve particular attention. The culture-based techniques of metallo-beta-lactamases detection are, inter alia represented by double-disk synergy test and Etest MBL (bioMeriéux, France). These methods, enabling to detect MBL carbapenemases in routine microbiology practice, utilize EDTA as the enzymes inhibitor [94, 95]. Another culture-based method, used for detection of carbapenemases among *A. baumannii* is represented by KPC combined disk assay. This technique utilizes boronic acid compound (BAC) as KPC inhibitor [96, 97].

Biochemical methods

Very recently, a novel and promising technique for the rapid and accurate detection of carbapenemases has been developed. The Carba NP test uses isolated bacterial colonies and is based on *in vitro* hydrolysis of imipenem. The carbapenemase activity is detected by colour change of pH indicator resulting from the hydrolysis of imipenem into a carboxylic derivative, leading to a decrease of the pH value [98–100]. While the application of Carba NP test in carbapenemase detection among *Acinetobacter* spp. strains encountered certain difficulties, a modified version of the assay — the CarbAcineto NP test, has been developed. Dortet and co-authors performed the analysis of specificity and sensitivity of the CarbAcineto NP test and revealed 100% and 94.7%, respectively [101]. Another method based on hydrolysis of carbapenem beta-lactam ring is Rapid CARB Blue Kit (Rosco Diagnostica, Taastrup, Denmark). The assay uses Tienam (commercially available imipenem) (Merck Sharp & Dohme, France) as a substrate for carbapenemases and bromothymol blue as a pH indicator. The Rapid CARB Blue Kit has been developed for detection of carbapenemase among *Acinetobacter* spp., *Enterobacteriaceae* and *Pseudomonas* spp. strains [102, 103].

An additional approach to carbapenemase detection is associated with application of the ultraviolet (UV) spectrophotometry. The assay is performed for overnight cultures of *A. baumannii* which are centrifuged and sonicated, and then followed by UV spectrophotometric measurement (wavelength value of 297 nm for imipenem) of specific activity of carbapenemases. While this technique is considered to be efficient in detection of VIM, IMP and SIM carbapenemase producers, it is regarded to be unsuitable for testing for CHDL and NDM carbapenemases [104].

Another promising method of carbapenemase detection is based on the MALDI-TOF MS (matrix-assisted laser desorption/ionization time of flight mass spectrometry). According to the definition presented by Wieser and co-authors the method is based on the ionization of cocrystalized sample material by short laser pulses, and then so formed ions are accelerated and their time of flight is measured in a vacuum flight tube [105]. The application of MAL-

DI-TOF MS in the detection of carbapenemases is based on the identification of native imipenem and its natural metabolite, both molecules are identified according to their different masses. In the studies by Kempf and co-workers the carbapenemase detection by MALDI-TOF MS method was carried among 106 *A. baumannii* clinical isolates from France and Algeria. According to the authors, the method revealed a sensitivity and specificity of 100%. While this technique is considered to be rapid and effective, it requires particularly expensive equipment and specialized laboratory personnel [106].

Molecular methods

Among techniques used in the detection of carbapenemases, nucleic acid based methods deserve particular attention. These methods are considered to be the "gold standard" characterised with high specificity and sensitivity. Currently, the most of the molecular techniques used in the detection of carbapenemases are based on the polymerase chain reaction (PCR). A single end-point PCR is one of the first molecular methods used in epidemiological as well as resistance studies [107].

While single end-point PCR allows detection of only one gene (per reaction), the multiplex PCR technique enables identification of different target genes in one reaction. Several multiplex PCR assays detecting carbapenemases in *A. baumannii* strains were described so far. Woodford and co-authors developed the multiplex PCR technique which allows identification and distinguishing of alleles encoding four groups of OXA carbapenemases — OXA-23--like, OXA-40/24-like, OXA-58-like, and OXA-51-like among *Acinetobacter* spp. [108]. Another reliable and rapid method used in the detection of carbapenemases was developed by Poirel and co-workers. The authors defined and evaluated the technique that allows detecting 11 carbapenemase encoding genes (bla_{IMP}, bla_{VIM}, bla_{NDM}, bla_{SPM}, bla_{AIM}, bla_{DIM}, bla_{GIM}, bla_{SIM}, bla_{KPC}, bla_{BIC}, and bla_{OXA-48}) in three multiplex PCR reactions. Application of the optimized conditions in each reaction mixture allowed obtaining distinct PCR amplicons for respective carbapenemase genes [109].

Another step forward in the molecular diagnostics was the introduction of real-time PCR (also known as quantitative PCR). While the end-point PCR requires gel electrophoresis in amplicon detection, the real-time PCR technique utilizes amplification and detection in a single step. The product detection can be obtained according to two main approaches: applying a dye that binds to double-stranded DNA (*e.g.* SYBR Green, EvaGreen) or implementing sequence specific probes (*e.g.* TaqMan®, Molecular Beacons, Scorpion) [107, 110]. Furthermore, real-time PCR in comparison to end-point PCR method allows to obtain a quantitative result and also to perform the reaction in a shorter period of time. An example of the application of real-time PCR in carbapenemase detection among *A baumannii* isolates was reported by Pasanen and co-workers. The authors described the multiplex real-time PCR assay that detected carbapenemase genes for KPC, VIM, IMP, GES-1/-10, OXA-48, NDM, GIM-1, SPM-1, IMI/NMC-A, SME, CMY-10, SFC-1, SIM-1, OXA-23-like, OXA--40/24-like, OXA-58, and IS*Aba1*-OXA-51-like in just two separate reactions. This SYBR Green based assay demonstrated the good performance detecting relevant carbapenemases among clinical isolates of *A. baumannii* [111]. Despite the SYBR Green chemistry based real-time PCR methods, sequence specific TaqMan® probes are applied in carbapenemase detection among *A. baumannii* strains. Huang and co-workers described the multiplex TaqMan® real-time PCR assay for simultaneous detection of four carbapenem-resistance genes — $bla_{OXA-23-like}$, $bla_{OXA-51-like}$, $bla_{OXA-40/24-like}$, and $bla_{OXA-58-like}$. The assay demonstrated a high specificity, suggesting its application in early diagnosis of carbapenem resistant *A. baumannii* [112].

Another method of gene amplification is represented by loop-mediated isothermal amplification assay (LAMP). This technique is based on autocycling strand displacement DNA synthesis performed under isothermal conditions in the presence of *Bst* DNA polymerase. Solanki and co-workers evaluated the application of LAMP method in the detection of bla_{NDM-1} and bla_{KPC} genes among carbapenem resistant Gram-negative isolates, including *A. baumannii*. The authors compared the LAMP assay with phenotypic and PCR based methods. The studies revealed that LAMP technique appeared to be more sensitive than conventional PCR, indicating the presence of bla_{NDM-1} and bla_{KPC} genes among four strains that were not detected by PCR. The authors also concluded that LAMP method with its high sensitivity and short turnaround time could be considered as a rapid and accurate point-of-care assay in the detection of NDM and KPC carbapenemases [113, 114].

Yet another latest molecular technique is the DNA microarray, also known as DNA chip or bio-chip. This method is based on hybridization of nucleic acid sample to a large set of oligonucleotide probes, which are attached to a solid surface [115]. DNA microarray allows for the simultaneous analysis of multiple genes; therefore, it could be applied in the identification of various resistance as well as virulence genes among tested strains within one assay. Dally and co-work-

Table 3. Selected methods used in the detection of carbapenemases in *Acinetobacter baumannii*

Group	Method	References
Phenotypic	Double-disk synergy test Disk combination method Etest MBL	[94] [97] [95]
Biochemical	CarbAcineto NP test Rapid CARB Blue Kit UV spectrophotometry MALDI-TOF MS	[101] [102] [104] [106]
Molecular	PCR Real-time PCR LAMP DNA chip	[108, 109] [111, 112, 117] [113, 114] [116, 118, 119]

ers developed a microarray which allows detecting 91 target sequences of resistance determinants among multidrug-resistant *A. baumannii* strains within 4 hours from bacterial culture to the result. Among resistance determinants included in the study, carbapenemase genes were represented by: $bla_{\text{OXA-23}}$, $bla_{\text{OXA-37}}$, $bla_{\text{OXA-40}}$, $bla_{\text{OXA-48}}$, $bla_{\text{OXA-51}}$, $bla_{\text{OXA-58}}$, bla_{KPC}, bla_{VIM}, $bla_{\text{IMP-1}}$, $bla_{\text{IMP-2}}$, bla_{SIM}, bla_{GES}, and bla_{NDM}. In the group of 60 *A. baumannii* strains the carbapenemase genes were detected among: 14, 13, 11, and 8 strains, carrying $bla_{\text{OXA-23-like}}$, $bla_{\text{OXA-40/24-like}}$, $bla_{\text{OXA-58-like}}$, and MBL genes, respectively. Furthermore, 10 isolates harboured deregulated $bla_{\text{OXA-51-like}}$ genes due to IS*Aba1* integration. Application of the microarray assay in the detection of carbapenemases revealed complete concordance with singleplex PCR provided by German National Reference Centre for Gram-negative Pathogens. Taking into consideration microarray's reliability and short handling time, it can be applied as a fast and reliable tool in resistance studies concerning both adequate treatment selection of intensive therapy patients as well as epidemiological studies [116]. The overview of mentioned above methods is presented in Table 3.

Conclusions

Over the last decades *A. baumannii* has emerged as an important nosocomial pathogen. While carbapenems have long been considered as an effective antimicrobial against *A. baumannii* infections, emerging resistance has been causing substantial difficulties in the treatment of this nosocomial pathogen worldwide. Currently, the most relevant mechanism of *A. baumannii* carbapenem resistance is associated with the beta-lactams hydrolysing enzymes represented by OXA-type and MBL carbapenemases. While laboratory detection and identification of carbapenemases among *A. baumannii* is troublesome, numerous promising methods have been described. The techniques which deserve particular attention belong to unsophisticated screening methods that can be used in routine microbiological laboratory, modern methods based on mass spectroscopy, and molecular biology, which enable detection of different carbapenemases in one run. While carbapenemase-producing *A. baumannii* strains are often multidrug-resistant, studies concerning carbapenemases inhibitors may offer a new insight into effective treatment of MDR *A. baumannii*.

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