Review Article

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Pharmacology Section

Acinetobacter baumannii: A Brief Account of Mechanisms of Multidrug Resistance and Current and Future Therapeutic Management

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ABSTRACT

Acinetobacter baumannii, a non-motile, glucose non fermentative, oxidase negative, encapsulated, gram-negative coccobacillus, has recently gained importance because of its increasing resistance to the available antibiotics. Three main mechanisms of resistance in *A. baumannii* are: enzymes inactivating antibiotics, reduced entry into the target site of bacteria and alteration of the target or cellular functions due to mutations. Multi-drug resistant *A. Baumannii*, including carbapenam resistant *A. Baumannii*, are posing a potential threat to mankind by causing lethal infections, especially in ICU set up and in patients who are on ventilators, for which our conventional antibiotics were not shown to be effective. Many reports have indicated carbapenam resistance among *A. Baumannii* and only colistin and tigecyclyne have shown some promise in combating this lethal microorganism.

Keywords: A. *baumannii,* MDR-Ab, CRAB, Colistin

INTRODUCTION

The genus, *Acinetobacter*, as has been currently defined, comprises gram-negative, strictly aerobic, non-fermenting, non-fastidious, non-motile, catalase-positive, oxidase-negative bacteria. This genus has undergone significant taxonomic modifications over the last 30 years. Its most important representative, *Acinetobacter baumannii*, has recently emerged as one of the most troublesome pathogens in health care setups. Its clinical significance, especially over the past 15 years, has been propelled by its remarkable ability in upregulating or acquiring resistance determinants, thus making it one of the organisms which threaten the current antibiotic era [1].

Acinetobacter baumannii (A. baumannii) is an encapsulated gram-negative coccobacilli containing proteins, namely porins and efflux channels, on the outer cell membrane, which mainly contribute to their resistance mechanisms [2]. However, as compared to other gram negative bacteria, it has fewer and smaller porin channels, which thereby decrease its cell permeability and increase its antibiotic resistance [3]. It was also discovered that the cell wall of the bacteria changes according to the environmental conditions, thus causing an increase in its thickness when it is placed in a very dry conditions, thereby again providing extra resistance at high temperatures also [4].

A. baumannii is generally considered to cause opportunistic infections and it is found to be non-pathogenic in healthy individuals [5]. Most of the cases are usually seen in the intensive care units (ICUs) of hospitals, in patients with deprived immunity and in those who are on various invasive equipments, like ventilator machines and catheters (causing various infections such as pneumonia, meningitis, septicaemia, and urinary tract infections). The irrational use of antibiotics in the ICU set up and the various bacterial mechanisms of resistance contribute to summation of resistance function for this untreatable, risky microorganism [6]. Risk factors [4-6] for colonization or infection with multidrug-resistant *A. baumannii* are:

- Prolonged length of hospital stay, exposure to an intensive care unit (ICU).
- Receipt of mechanical ventilation.
- Prolonged exposure to antimicrobial agents.
- Recent surgical and invasive procedures, and
- Underlying severe illnesses.

There is rising concern about antimicrobial resistance among Acinetobacter species since the past decade [7]. Presence of the porin channels, efflux mechanisms and the non static behaviour of the bacteria in hot and humid conditions equip the species with extensive antimicrobial resistance [8].

Mechanisms of Resistance

The three main mechanisms of resistance are [9].

1. Enzymes inactivating antibiotics.
2. Reduced entry into the target site of bacteria.
3. Alteration of the target or cellular functions due to mutations.

1. **Enzymes inactivating antibiotics:** The enzymes inactivating the drugs are the beta-lactamases that hydrolyze and confer resistance against various groups of drugs, namely the penicillins, synthetic cephalosporins, and carbapenems. Carbapenem resistance was noticed due to a large number of class D, OXA-type inactivating enzymes [10] and some class B metallo–beta-lactamases (MBLs), which provided a significant threat of easily transferable locations of the enzymes in the bacterial gene [11].
2. **Reduced entry into the target site of bacteria:** The presence of porin channels and other outer membrane proteins helps in the delivery of the drugs into the target proteins, for their antibiotic action. Unluckily, the porin channels are smaller and lesser in the *A. baumannii* strains, which prevent the entry of the drug molecules, which confer the resistance which is seen in case of carbapenems [12]. So, the porins and beta- lactamases work together to confer resistance. Along with these factors, efflux pumps also contribute to the resistance pattern, by throwing the drugs out of the targets. Point mutations occurring in the genes coding for the target proteins, namely the enzymes or the porin channels, decrease

the affinity or up-regulating cellular functions involved in the production of efflux pumps. Change in affinity for binding was seen in case of colistin resistance [13].

3. *Alteration of the target or cellular functions due to mutations*: The changes in the membrane binding and changes in bacterial targets due to point mutations in gyrA and parC topoisomerase enzymes confer resistance against quinolones. So, finally, selective pressure exerted by the use of broad-spectrum antimicrobials and transmission of strains among patients may be the causes of the emergence of resistance [12,13].

What is Multi-Drug Resistant *A. Baumannii* (MDR-Ab)?

A. baumannii Is labelled as MDR-Ab when it is resistant to more than two of the following five classes of antibiotics [1,14].

1. Antipseudomonal cephalosporins (ceftazidime or cefepime).
2. Antipseudomonal carbapenems (imipenem or meropenem)
3. Ampicillin/sulbactam.
4. Fluoroquinolones (ciprofloxacin or levofloxacin) and
5. Aminoglycosides (gentamicin, tobramycin, or amikacin).

In the past years, carbapenems were considered as the most important agents for the treatment of infections caused by MDR-*A. baumannii*. Carbapenem resistant A. baumannii (CRAB) is now emerging as a potential threat [15,16] and it is usually resistant to almost all antimicrobial classes except colistin and tigecycline, which have shown some promise against this organism [15,16].

The most important mechanism of carbapenem resistance in *A. baumannii* is enzyme inactivation by the production of beta-lactamases, which hydrolyze the carbapenams. These hydrolyzing enzymes include metallo-β-lactamases (which have been sporadically reported in some parts) and class D β-lactamases (widespread). The main gene clusters responsible for this resistance are *bla*OXA-23-, *bla*OXA-24/40-, and *bla*OXA-58-like gene clusters. They are identified either in the chromosome or in plasmids of *A. Baumannii* strains [15]. Another mechanism of reduced susceptibility to carbapenems are:

- Altered penicillin-binding proteins and porins and
- Upregulation of the efflux system.

These factors may together lead to a high-level carbapenam resistance in these bacteria.

Treatments Options for Drug Susceptible and MDR-Ab, Including CRAB:

Sulbactam: Beta-lactamase inhibitors possess the greatest intrinsic bactericidal activity against *A. baumannii* isolates. This was evident from the study of Urban et al., [17], in which a clinical efficacy wise improvement was observed in 9 out of 10 seriously ill patients who were on mechanical ventilation, with the combinational ampicillin-sulbactam being given at a dosage of 3 g of amicillin and 1.5 g of sulbactam IV, three times a day. The clinical improvement was also comparable with other system involvements like pneumonia and blood stream infections [18]. In blood stream infections caused by this strain, treatment with a combination of sulbactam and ampicillin was found to be statistically significant in reducing the mortality, which was seen in a study conducted in Israel in situations of multi-drug resistance [19]. In meningitis, the combination was not found to be so beneficial. The dosage recommended is around 6g/day in divided doses with normal renal parameters.

Carbapenems: Carbapenems were found to have an excellent intrinsic bactericidal activity and they remain the most effective treatment against beta-lactamases and infections caused by multidrug-resistant *A. baumannii*. In some studies [20, 21], variations in the resistance patterns against imepenem and meropenem were seen . Discordant results have been obtained between the various carbapenems against a few isolates of *A. baumannii* [22] However, increasing numbers of CRAB isolates have been reported, which dramatically reduce the existing therapeutic options and pose a potential threat to public health. This resistant type *A. baumannii* is known to cause serious central nervous system (CNS) infections i.e. meningitis and ventriculitis, especially in patients undergoing neurosurgical procedures or head trauma. Significant mortality rates (20%-27% have been reported in different case series [23].

Aminoglycosides: Among aminoglycosides, amikacin and tobramycin are agents that retain activity against many *A. baumannii* isolates. There is a concern regarding its toxicity profile and as with other drugs, their resistance were growing with increased toxicity. A study [24] which compared the activity and toxicity of tobramycin against colistin demonstrated no statistical significance between these two in mortality also. In a study done by Hallal A et al., [5], the efficacy and safety of inhaled and intravenous tobramycin were compared, in which inhaled tobramycin proved to be little better than the intravenous one.

Polymyxins: in the past, the use of colistin was limited because of toxicity concerns and increasing availability of newer and safer antibiotics. However, in recent times, with increasing incidence of MDR-*Ab,* lack of effective antibiotics and reasonable activity of colistin against MDR-*Ab* have made it a useful drug against this organism [26].

Colistin and polymyxin B are the polymyxins used for the multidrug resistant *A.baumannii*. Colistin is most commonly used drug among the two. The toxicity (mainly, the nephrotoxicity) is the main concern in this group. It has been used for carbapenem-resistant cases, either IV polymyxin plus an intrathecal or intraventricularpolymyxin or aminoglycoside, with or without IV rifampin [27]. The dosing recommended by the Infectious Diseases Society of America for adults is 10 mg daily of colistin or 5 mg daily of polymyxin B [28].

Tigecycline: Tigecycline is a new glycylcycline agent which has shown bacteriostatic activity against MDR- *Acinetobacter* species [29]. A high level of resistance has now been documented by this drug, which has shown overexpression of efflux pumps in the strain. [30] In recent studies, *Acinetobacter* isolates with a decreased susceptibility to tigecycline, due to overexpression of a multidrug efflux pumps, was documented [31,32], but still, tigecycline is an effective alternative for salvage therapy when it is properly administered by experts.

Combination therapy: In the setting of an increased number of *A. baumannii* infections, treatment of those caused by carbapenem-resistant strains, susceptible only to colistin, has become a major problem during the past years. So, an appropriate combination therapy is of great importance, for tackling these infections

In a study, combination therapy with a carbapenem and a sulbactam led to favourable clinical outcomes in four critically ill patients who presented with MDR- *Ab* bacteraemia. The authors also conducted an in vitro study which showed that this combination was synergistic and that it showed an enhanced antibacterial activity against MDR-*A. baumannii*. Thus, a carbapenem-sulbactam combination can serve as an alternative in setups where colistin and tigecycline are not available for clinical use [33].

The combination of polymixins and intra-ventricular aminoglycosides has been considered as a good option for treating meningitis caused by CRAB species. But due to lack of newer agents and increasing resistance to the available antibiotics, it will be very difficult to treat meningitis caused by Acinetobacter in the future [27].

In another study, the combination of a rifampicin/imipenem was tried for the treatment of CRAB infections, but disappointing results were obtained [34].

In a study, the authors compared monotherapy and combination therapy with ampicillin/sulbactam, doripenem and tigecycline against MDR-*Ab* using an in vitro pharmacodynamic model. Although specific combination regimens displayed an additive

activity at aggressive doses against these MDR-*Ab*, none of the regimens was able to maintain reductions in colony forming units against the more resistant isolates [35].

Recent data has suggested that glycopeptides, in particular, vancomycin, may have a unique activity against laboratory-adapted and clinical strains of *A. baumannii*, alone and in combination with colistin. In an in vivo study, the authors studied the effect of combinations of vancomycin, colistin, and doripenem on clinical strains of CRAB and found promising results. Their findings suggested that regimens containing vancomycin may confer a therapeutic benefit against infections caused by CRAB [36].

Future Drug Strategies

New β-lactamase inhibitors [1,37].

Compounds targeting the β-lactamases, especially the Ambler class B MBLs, can play an important role in halting the emergence of carbapenem resistance in *A. baumannii*. Their structures and catalytic mechanisms of these enzymes are zinc dependent and hence, they are more difficult to tackle with current β-lactamase inhibitors. Newer agents that are able to chelate the active Zn^{2+} site can turn out to be a promising therapy against this notorious organism. But several challenges are present, which make it difficult e.g presence of significant differences in the active site architecture between MBL types, the ability to develop a pan-MBL inhibitor. Also, MBLs have homologous mammalian enzymes and therefore, they can increase the risk for significant toxicity. Despite these problems, the development of compounds that target these enzymes continues.

Inhibitors of aminoglycoside-modifying enzymes and multidrug efflux pumps [1,38,39]

The inhibitors of both aminoglycoside-inactivating enzymes and multidrug efflux pumps have also been troubled by diverse targets, with bacteria often harbouring multiple enzyme or pump types. More recently, cationic antimicrobial peptides that are capable of inhibiting both aminoglycoside phosphotransferases and acetyl transferases have been described. The importance of multidrug efflux pumps in *A. baumannii* is increasingly being recognized, with tigecycline being recently identified as a substrate of the RND-type pump Ade ABC. Through a large-scale in vitro screening, a range of efflux pump inhibitors has been identified (plant alkaloids and some synthetic compounds). Unfortunately, progress has been slow, with agents such as phenyl-arginine-β-naphthylamide showing a very good in vitro response, but being disappointing due to their toxicity concerns. Another major concern is that a variety of efflux systems are available in gram-negative organisms, which lead to a compensatory upregulation of noninhibited pumps.

Eukaryotic antimicrobial peptides [40-42]

These cationic peptides are ubiquitous elements of the innate immune response in a variety of invertebrate, plant, and animal species. They primarily act by disturbing the cell membranes and they have a structure and charge profile similar to those of the polymyxins, but the final steps in pathogen lethality are different. This mechanistic difference is clinically attractive and it has been well illustrated by the susceptibility of polymyxin-resistant *A. baumannii* strains to such peptides. Bactericidal activity against *A. baumannii* has been reported, on using both in vitro and in vivo models. Combination studies, as determined by fractional inhibitory indexes, demonstrated that magainin II (an antimicrobial peptide) acts synergistically with β-lactams against MDR- *Ab*.

CONCLUSION

From above discussion, it is clear that multidrug-resistant *Acinetobacter* infections are associated with extremely high crude mortality rates and most commonly, in severely ill patients. These infections are associated with increased times on mechanical ventilation, in ICUs, and in hospitals. Treatment options are limited; carbapenems and colistin are the current agents of choice for the most drug-resistant infections. The roles of other agents and combination therapy remains unclear. The development of new medications, properly conducted clinical trials of existing regimens and their combinations, more research and preventive measures for reducing the transmission of multidrug-resistant *Acinetobacter* infection, are urgently needed.

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