Bachelor thesis:

Mechanisms of antibiotic resistance in *Acinetobacter baumannii*

Mart Bartelds
S2468263
Supervisor: Prof. dr. A. Driessen
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Abstract:

***A. baumannii* is an organism of great concern to modern health care. The main reason for it is its availability to become resistant against all known antibiotics. In this review the mechanisms behind drug resistance are described. The molecular mechanisms can be divided into four groups: reduced permeability, antibiotic modification, alteration of the targets or protecting the target by expressing a secondary protein. *A. baumannii* possesses mechanisms belonging to all four groups, but the most important is the first. *A. baumannii* is intrinsically very impermeable for many antibiotics and this leads to significant levels of multidrug resistance. To drugs that are capable of surpassing this first line of defence multiple mostly drug specific defence mechanisms are reported in clinical isolates. Although the genetic diversity among *A. baumannii* isolates is very high the molecular mechanisms behind the impermeablity are conserved. Therefore future drugs should be designed in such a way that they can permeate into the cell and evade the efflux systems.**

1) Introduction

On 27 February 2017 the WHO sounded the alarm to get attention for microbial multidrug resistance. They reported a list of twelve families of bacteria that should get the highest priority and Acitenobacter was on top of this list [WHO (2017)]. Antibiotic resistance development among clinical isolate of *A. baumannii* in China has been monitored in China over a period of 10 years [figure 1]. The study showed a constant high prevalence in resistance to most tested antibiotics, but also a sharp increase in resistance against tigyceline and iminiprem [Lei Gao (2017)]. In the WHO report the increase in iminiprem (which is a type of carbapenem) resistance was pointed out as very worrying.

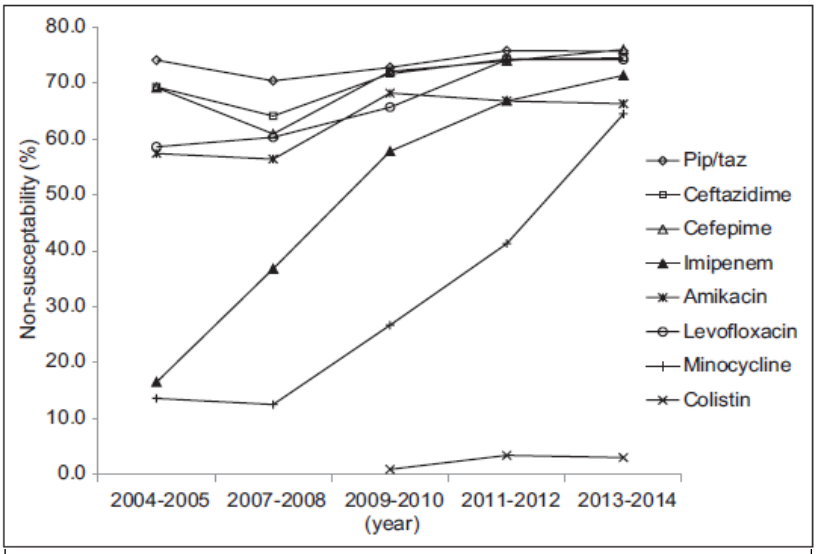

Figure 1: Development of occurence antiobiotic non-susceptibility The figure depicts the percentage of Chinese clinical isolates that were non-susceptible against the drugs listed in the legend. [Lei Gao (2017)]

This literature review aims at giving an overview of all mechanisms used by *A. baumannii* to become resistant against antibiotics. Using this overview an answer is given to what are the causes for multidrug resistance in *A. baumannii.* Also an explanation is given for the steep increase in carbapenem resistance and the relatively low occurrence of resistance against collistin. Finally directions are given for possible targets or processes at which future research should be focussed on.

1.1: General information

Although *A. baumannii* is not the only acinetobacter species that is known to infect humans it is the most common species and therefore the most relevant [Wong (2017)]. *Acinetobacter spp.* are gram-negative bacteria that are usually found in wet environments or colonializing human skin, however the natural habitat of *A. baumannii* is not known [Rahal (2000)].

A. baumannii is an opportunistic pathogen that causes a large range of mostly nosocomial infections of immune-compromised or traumatized patients. But an increasing number of community acquired infections is reported. An important factor that makes *A. baumannii* such resilient pathogen is its resistance against all kinds of environmental resistance [Dijkshoorn (2007)].
In this review the focus is put on the mechanisms responsible for clinical levels of antibiotic resistance in *A. baumannii.* Clinical resistance differs from resistance in the lab in the concentrations that can be used and in the environment that the bacterium is in. The first factor is caused by the possible toxicity of antibiotics, allowing a limited dosage, and the break-down of the medicine by the human body, resulting in a lower effective concentration. The second factor is caused by the human immune system, which tries to fight of the infection on its own. Therefore the conditions under which the bacterium is exposed to antibiotic stress are less optimal than those in a lab environment. The ability of bacteria to get enough nutrients and evade the immune systems depends on virulence factors. Although these virulence factors are quite closely involved in antibiotic resistance these are not discussed in this review, but more information about them can be found in the review of Nowak *et al.*.

Another important property of *A. baumannii* is that it is excellent at acquiring new genes from its environment. As a consequence there is huge genetic variation in clinical isolates. In this report it was tried to give an overview of all mechanisms found in *A. baumannii* so also those that are not widely distributed. It was attempted to indicate the importance and prevalence of the mechanisms in all strains, however knowledge about this is limited.

1.2: Outline
In the following report, first all antibiotic resistance mechanisms *A. baumannii* possesses will be reported. The mechanisms are divided into four groups similar to what was done in a review by Blair *et al.* about mechanisms of antibiotic resistance in general. After categorizing all mechanisms a chapter will be devoted to the regulation of antibiotic gene expression as this is inseparable from resistance mechanisms. In the discussions chapter the relative relevance of the different mechanisms will be discussed and based on this the questions mentioned earlier will be answered. Finally some potential directions for research and clinical options will be mentioned.
This report differentiates itself from many other reviews that can be found in *A. baumannii* , because it gives a detailed up-to-date description of all mechanisms *A. baumannii* processes, without focussing on a specific group of antibiotics.

2) Reduced permeability

The first mechanism described by Blair *et al*. is via reduced access to the bacteria. Most antibiotics sabotage processes which either take place in the cytosol or in the periplasmic place (beta-lactams). Therefore almost all antibiotics need to cross the outer membrane before they can function. Lipophilic and amphiphilic antibiotics can cross the membrane via diffusion, but this process is hindered by negatively charged lipopolysaccharide (LPS).
LPS is the main component of the outer leaflet of most Gram-negative bacteria consisting of three components: lipid-A, a core oligo-saccharide and a repeating carbohydrate called an O-antigen. It must be mentioned that a debate is going on whether A. baumannii possesses the O-antigen and it seems like the component is more likely to be a lipo-oligosaccharide (LOS) than LPS [Weber (2015)]. However since most literature still uses LPS to describe the component and the difference does not matter for the discussion, I will use this annotation later on to discuss its role in antibiotic resistance.

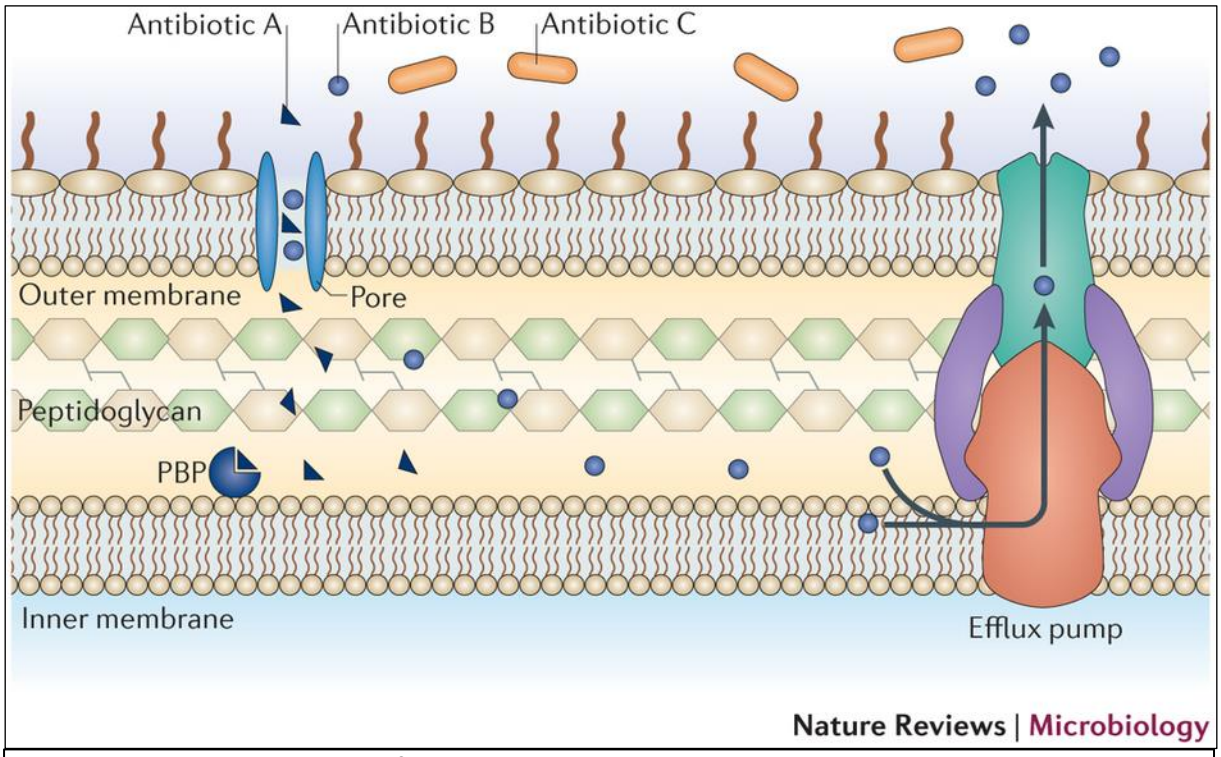

Figure 2: Intrinsic mechanisms of resistance.
"The figure shows an overview of intrinsic resistance mechanisms. The example shown is of β-lactam antibiotics targeting a penicillin-binding protein (PBP). Antibiotic A can enter the cell via a membrane-spanning porin protein, reach its target and inhibit peptidoglycan synthesis. Antibiotic B can also enter the cell via a porin, but unlike Antibiotic A, it is efficiently removed by efflux. Antibiotic C cannot cross the outer membrane and so is unable to access the target PBP." [Blair (2015)]

More hydrophilic antibiotics cannot cross the membrane and are dependent on membrane channels, called porins. If antibiotics manage to cross these barriers a second line of defence are efflux pumps. These pumps actively export out of the cell and therefore they effectively contribute to reduced permeability of the bacteria (**Figure 2**) [Blair (2015)].

2.1: Porin expression

2.1.1: OmpA

The major porin forming protein in *A. baumannii* is OmpA, which is also called HMP-AB in some literature. OmpA was found to be a slow non selective porin, which results in a low general permeability of the outer membrane. The general permeability is similar to that of the related species *P. aeruginosa*, but much lower than that of *E. coli* [Sugawara (2012)]. The low permeability of the outer membrane is the first intrinsic line of defence against many hydrophilic antibiotics.

A secondary mechanism of action for OmpA was hypothesized by Smani *et al.*. They found a decrease in MIC value for several antibiotics in the OmpA knock-out strain and they found that an efflux pump inhibitor resulted in a similar decrease. Therefore they concluded that OmpA was involved in certain resistance phenotypes and that the mechanism was likely coupled to efflux pumps. In contrast Sugawara and Nikaido found no decrease in MIC values for two of the tested antibiotics, despite using the same *A. baumannii* strain. Moreover knock-out of the major porin may cause membrane disruption and altered expression of other proteins. Therefore I do not find it conclusive that OmpA is involved in antibiotic resistance via this secondary mechanism.

2.1.2: Specific porins

To get enough nutrients organisms such as *A. baumannii* and *P. aeruginosa* express other porins which are more selective but have a higher permeability. These selective porins are shown to be used by antibiotics to enter the cell and down-regulation of some specific antibiotic permeable porins can increase resistance against these agents. In several reviews the following porins are coupled to antibiotic resistance [Lee (2017)] or more specific carbapenem resistance [Poirel 2011, Vila (2007)]: CarO, a 32-36 kDa protein and an OrpD like 43 kDa protein.

CarO was long believed to be the most important carbapenem transporting porin, because in multiple carbapenem resistance strains CarO was no longer expressed. Moreover in the same research was shown that when susceptible strains were grown under carbapenem pressure, resistance was developed accompanied by loss of CarO [Mussi (2005)]. Also [Catel-fereirra (2011)] showed it functioned as an ion-channel and that iminiprem inhibited the ion flux, suggesting a carbapenem binding site. However in recent research the crystal structure was resolved and it showed that CarO did not form a channel through the entire membrane, but would be able to function as a channel for some small molecules. The researchers concluded that carbapenem transport would be unlikely and they confirmed this with liposome swelling experiments using purified CarO [Zahn (2015)]. CarO was also shown to be a hub of protein interaction by Wu *et al.* therefore it seems more likely that CarO contributes to carbapenem resistance in a more elusive fashion than being a channel used by it.

One study linked the 32-36 kDa outer membrane protein to carbapenem resistance. Deletion of this protein lead to an increase in carbapenem resistance and susceptibility was regained after re-expression of the protein via a plasmid [Tomas (2005)]. However based on the CarO discussion care should be taken with respect to the mechanism, unfortunately no more literature concerning this protein was found.

A change in expression of a 43 kDa outer membrane protein, which had a significant level of homology with OrpD of *P. aerigunosa*, was found in drug resistance strains [Dupont (2005)]. In 2016 the structure was resolved by Zahn *et al.* (2016) together with three other outer membrane proteins of *A. baumannii*. In this study it was also concluded that the 43 kDa OrpD like protein was likely the main drug transporting porin of *A. baumannii*.

Several other porins are mentioned in the review of Lee *et al.* but these were identified in studies where the expression levels of resistant strains was compared to the expression of susceptible strains. However, since antibiotic resistance is multifactorial, the expression of many proteins is altered in resistant strains [Hua (2014)]. Therefore the evidence of involvement in antibiotics transport of these proteins was found too meagre to conclude that these outer membrane proteins are porins involved in (multi)drug transport. Nevertheless it is highly unlikely that the porins discussed above are the only drug transporting porins.

2.2: Efflux pumps

Efflux pumps are single membrane proteins or complexes of multiple proteins that can either pump a very broad range of substrates (multi-drug) or a very narrow range of substrates (drug-specific) out of the cell. The transport effectively results in a reduced permeability, because the drugs are transported out of the cell before they can perform their inhibitory work [Blair (2015)]. All drug efflux systems are found in five (super) families: The resistance-nodulation-division (RND) family, the major facilitator superfamily (MFS), the multidrug and toxic compound extrusion (MATE) family, the small multidrug resistance (SMR) family and the ATP binding cassette (ABC) family [Vila (2007)].

All systems actively transport drugs and use either an ion-gradient over the membrane or ATP-hydrolysis as an energy source. All transporters are located in the cytosolic membrane, since the energy is only available there, but members of the RND family can actually transport drugs over the outer membrane as well (**Figure 3**) [Kumar (2005)].

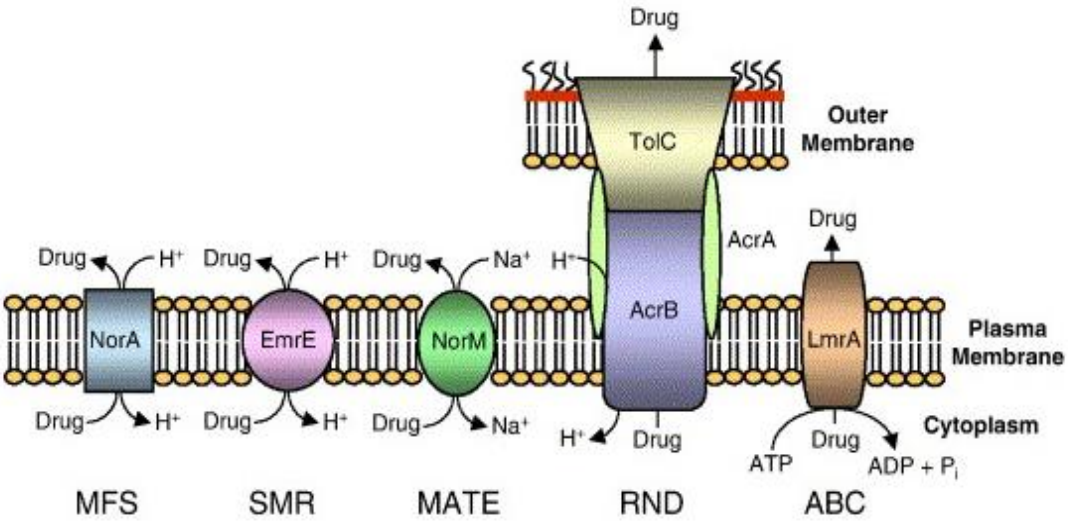

Figure 3: "Schematic representation of the different drug efflux mechanisms
Schematic illustration of the main types of bacterial drug efflux pumps. Illustrated are Staphylococcus aureus NorA, a member of the major facilitator superfamily (MFS); Escherichia coli EmrE, a member of the small multidrug resistance (SMR) superfamily; Vibrio parahaemolyticus NorM, a member of the multidrug and toxic compound extrusion (MATE) superfamily; E. coli AcrAB–TolC, a member of the resistance-nodulation-cell division (RND) superfamily; and Lactococcus lactis LmrA, a member of the ATP-binding cassette (ABC) superfamily." **[Kumar (2005)]**

Multi-drug efflux systems are often chromosomally located and constitutively expressed at low levels. These systems are thought to function as housekeeping enzymes and contribute to the intrinsic membrane impermeability, but when overexpressed their contributions can lead to significant levels of resistance. Drug specific efflux systems are mostly located on plasmids or mobile genetic elements and acquiring such gene is often enough to achieve clinical relevant levels of drug-resistance [Poole (2007)]. The systems that can lead to overexpression and the regulatory mechanisms are discussed further in the regulatory chapter. In this chapter each family of efflux systems will be discussed shortly based on an example found in *A. baumannii.* For a more complete list of different efflux systems I would refer you to the review written by Lee *et al.* (2017).

2.2.1: RND-family

The major multidrug efflux systems in *A. baumannii* belong to the RND-family. RND-family efflux systems are composed of three proteins (an inner membrane transporter, an outer membrane protein and a periplasmic adaptor protein) and can transport drugs over the outer membrane as well (**Figure 3**)[Nikaido (2009)]. Therefore these efflux pumps work synergistically with the mechanism for membrane permeability reduction discussed in the previous chapter [Vila (2007)]. A complementary working can also be described since the substrates of these efflux systems are often lipophilic or amphiphilic [Marquez (2005)] and porins regulate the flux of the more hydrophilic antibiotics, so together they can limit the entrance of practically every antibiotic.

RND family transporters can also transport drugs from the periplasmic space (not shown in **Figure 3**) and can therefore contribute to reduced susceptibility towards beta-lactam antibiotics.
The most important multi drug transporter is AdeABC, which reduces the permeability of many antibiotics.

2.2.2: MFS
Multiple efflux systems belonging to the major facilitator superfamily are found in *A. baumannii.* Among these are specific pumps for chloramphenicol and tetracyclines.[Lee (2017)] Although the latter can also be transported by multidrug transporters belonging to the RND-family the main efflux mechanism is via a pump belonging to the *tet* family. [Poole (2007)] Two members of this family are found in *A. baumannii*, Tet(A) and Tet(B). In a slightly out-dated study it was found that most of the clinical isolates that were tetracycline resistant either Tet(A) or Tet(B) was present, indicating that it is an important gene for tetracycline resistance. However nowadays another group of antibiotics, called glycyl cyclines, is known, which cannot be transported by either of the systems [Vila (2007)] , but can be transported by RND-type multidrug efflux systems [Poirel (2011)].

2.2.3: MATE-family
The only known efflux system in *A. baumannii* belonging to the MATE family is AbeM. [Lee (2017), Vila (2007)]
AbeM is considered a multidrug-transporter, although it is particularly good at transporting (hydrophilic) flouroquinolones [Su (2005)]. Although most drug transporters in this family use a Sodium gradient as the example in **Fout! Verwijzingsbron niet gevonden.**, AbeM actually uses the proton motive force as energy source. Also the importance of efflux systems belonging to this family is small in comparison with that of the RND family in gram-negative species [Kuroda (2009)].

2.2.4: SMR-family
In *A. baumannii* the only efflux system belonging to the SMR family is AbeS. AbeS is a multidrug efflux system that was shown to contribute to a decreased susceptibility by generating a knock-out strain [Srinivasan (2009)]. The MIC-values of several antibiotics decreased in this knock-out strain, but based on the level of decrease it can also be concluded that it functions as a complementary mechanism.

2.2.5: ABC-transporters
This is the only family of which no drug transporters are found in *A. baumannii*. So, although ABC-transporters function via quit different than the other drug transporters, this mechanism will not be discussed in this review. Lean *et al.* state in a table that they found an ABC multidrug transporter, however they do not expand on this finding in their article. Moreover in other literature only one article was found linking ABC-transporters to antibiotic resistance and this was not as drug transporter. [Hua (2014)] . Further according to Vila *et al.* these systems are rare in gram-negative organisms.

3) Enzymatic antibiotic modifications

Antibiotics that managed to surpass the membrane and efflux pumps face a second line of defence: enzymatic modification (**Figure 4**). The first inactivation mechanism is hydrolysis, which works by destroying the functional group of the antibiotic. The second mechanism is modification via a group transfer reaction. In this case a chemical group is placed on a free OH or NH_2 group of an antibiotic making it unable to bind to the target site [Blair (2015)].

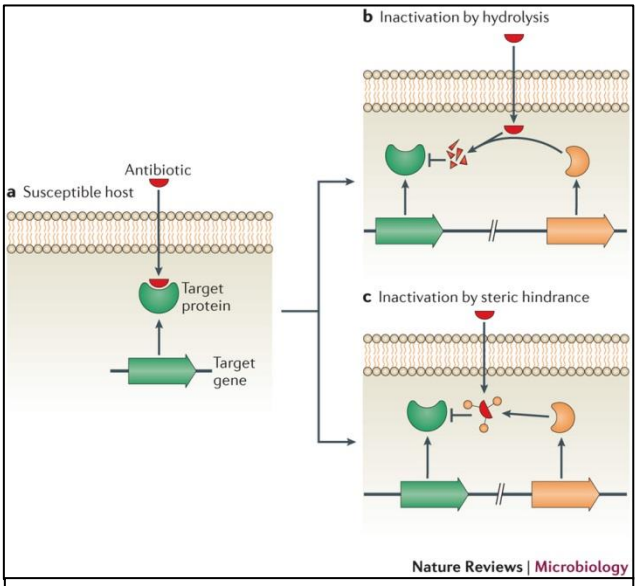

Figure 4 : **"Direct interactions with antibiotics** **a** | A susceptible host with a target that is efficiently inhibited by an antibiotic. **b** | Acquisition and production of an enzyme that destroys the antibiotic (for example, β-lactamases) prevents binding to the target and confers resistance. **c** | Acquisition and production of an enzyme that modifies the structure of the antibiotic (for example, aminoglycoside-modifying enzymes) can also prevent binding to the target and confer resistance." [Blair (2015)]

3.1: Hydrolysis

The most important group of antibiotic modifying enzymes are the beta-lactamases, who hydrolyse the beta-lactam ring of beta-lactam antibiotics. Since the first use of penicillin, beta-lactamases were found in pathogens leading to resistance. This development lead to new beta-lactam antibiotics capable of circumventing these beta-lactamases, which in turn resulted in new beta-lactam degrading enzymes. As a result, an enormous diversity in beta-lactam antibiotics and enzymes capable of hydrolysing them is found.

Nowadays, the most important lactamases are extended spectrum beta-lactamases (ESBLs) and carbapenemases, which when present can result in resistance against all known beta-lactam antibiotics [Blair (2015)]. These names are based on the antibiotics they can hydrolyse, but a more structured characterization is based on sequence homology, leading to four Ambler classes (A-D).

Class A, C and D all work via a similar mechanism, which uses a serine that gets covalently bound to the antibiotic, forming an acyl-enzyme intermediate, before hydrolysis. Class B beta-lactamases use a divalent metal ion such as Zinc to catalyse the hydrolysis [Nowak (2016)]. Besides the beta-lactam antibiotics also lactamase inhibitors have been developed. These inhibitors form a covalent acyl enzyme intermediate but cannot be hydrolysed resulting in enzyme inactivation. As a consequence class B beta-lactamases are unaffected by these inhibitors [Hsu (2017)].

A. baumannnii possesses two intrinsic beta-lactmases, which are usually expressed at low levels, but can be overexpressed after genetic mutations. The first is AmpC, belonging to Ambler Class C, can contribute to broad-spectrum resistance and are insensitive for a commonly used inhibitor [Lee (2017)]. One category of beta-lactam antibiotics that are invulnerable against AmpC are carbapenems [Queenan (2010)].

The other is the carbapenem hydrolysing class D lactamase, which are also called oxacillanases (or OXA), OXA-52. Overexpression of the native OXA-52 is together with expression of other acquired oxacillanases one of the most important mechanisms for carbapenem resistance [Lee (2017)].

Acquired class A carbapenemases are also found in *A. baumannii,* but much less frequently than OXAs. A more problematic development is the acquisition of class-lactamases, because these enzymes have a very broad substrate range and are not inhibited by beta-lactamase inhibitors. [Hsu (2017)] Luckily, these enzymes are not as wide-spread as the oxacillanes. In the review article of Lee *et al.* a list of all beta-lactamases that are reported in *A. baumannii* can be found.

3.2: Group-transfer reactions

Another class of antibiotics that is often modified are aminoglycosides. In an Iranian study some form of aminoglycoside resistance was observed in 95,6% of 87 clinical isolates. This resistance was correlated to presence of several antibiotic modifying enzymes [Sheikhalizadeh (2017)].
Based on the catalysed reaction three groups of aminoglycoside modifying enzymes can be distinguished: acetyltransferases, phosphotransferases and nucleotidyltransferases. All three types of enzymes are observed in *A. baumannii* [Sheikhalizadeh (2017)]. Reasons for the importance of group transfer reactions in aminoglycoside resistance could be the high number of exposed NH_2 and OH groups and the requirement for the drug to be able to bind inside the active site of RNA-transcriptase.

Other enzymes working according to these mechanisms are a chloramphenicol acetyltransferase (Cat), a macrolide modifying enzyme (mph) and a rifampicin ADP-ribosylating transferase (arr-2) [Poirreil (2011)].

4) Alteration of target structure

Besides altering the antibiotic itself bacteria can also change the target of the antibiotic. These changes should prevent binding or recognition by the antibiotic while the target preserves its functionality. Blair *et al.* divide this group into two main mechanisms: change of the target after DNA-mutations and protection of the target by other proteins (**Figure 5**). However since both mechanisms contribute only in a minor way to the resistance in *A. baumannii* they have been put together in this chapter.

4.1: Mutation of target

Antibiotic targets that can be modified by mutation can either be proteins, e.g. penicillin binding proteins, or RNA molecules, such as for example ribosomal RNA. These mutations can arise from either intrinsic point mutations, which are carried on linearly, or by acquiring a homologous gene via horizontal gene transfer.

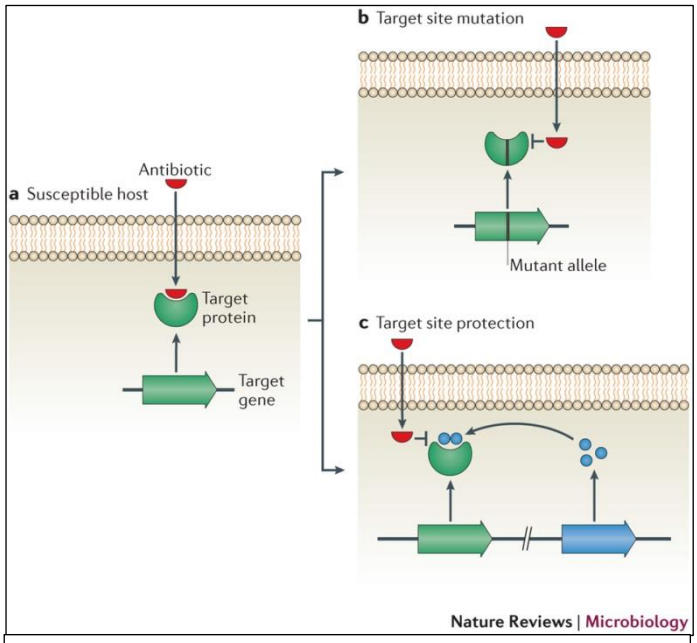

Figure 5: Target site changes.
"a | A susceptible host in which an antibiotic is able to bind tightly to its specific target and exert an inhibitory effect. b | Mutation of the target site (…) results in a functional target with reduced affinity for the antibiotic, which does not bind efficiently and therefore has a reduced or negligible effect. c | Modification of the target by addition of a chemical group can also prevent antibiotic binding without altering the primary protein sequence of the target, which retains its activity." [Blair (2015)]

Point mutations in gyrase and topoisomerase IV are widely distributed among *A. baumannii* and they are the main factor in (fluoro) quinolone resistance [Poirel (2011)]. Several specific amino acids changes are known to contribute to the resistance phenotype, but other mutations may also affect the resistance phenotype [Lean (2016)]. Nucleotide mutations in the active site of RNA-polymerase were mentioned by Poirel *et al.* as an important mechanism for rifampicin resistance.

Finally mutations in penicillin binding proteins (PBP's) are a well-known mechanism against beta-lactam antibiotics. PBP's synthesize peptidoglycan and usually multiple PBP's are expressed with different affinities for different beta lactams. Although this is an important mechanism for e.g. *S. pneumonia* [Blair (2015)], the contribution in *A. baumannii* seems to be only minor [Nowak (2016)].

4.2: Target protection

Antibiotic targets can be protected by other proteins in two ways, which are both found in *A. baumannii.* In the first mechanism, shown in figure 5, a secondary protein is synthesized which shields the target from the antibiotic. Another way of target protection is by modification of the target (not shown in figure 5). For this way a secondary enzyme is transcribed which modifies the target making it unrecognizable for the antibiotic.

The only example of the first mechanism found in *A. baumannii* that works via this mechanism is TetM. According to Poirel *et al.* TetM is widespread and Arenz *et al.* showed how it binds to the ribosome and repels tetracycline antibiotics from their binding site.

An example of the second mechanism is methylation of 16SRNA by ArmA. This was found by Sheikhalizadeh *et al.* to be an important factor in aminoglycoside resistance. This mechanism has a complementary function to the aminoglycoside modifying enzymes since they both result in steric inhibition of the antibiotic.

Another example of that belongs to this category is LPS modification. The negatively charged LPS is targeted by cationic polymyxines, which result in destabilization of the outer membrane. Resistance can occur via two mechanisms that both modify the properties of the cell surface. The first is addition of phosphoethanolamine lipid A moiety of LPS, which makes it less negatively charged. This is caused by overexpression of pmrC after mutations in regulatory enzymes. The second mechanism is via mutations in lipid A biosynthesis genes that lead to complete loss of the LOS. [Lean (2016)]

5) Regulation

Antibiotic resistance mechanisms are often related to fitness costs and therefore it is important that expression of resistance mechanisms is tightly regulated. Moreover many systems need to be overexpressed to obtain the high levels of resistance observed in *A. baumannii.* Expression is regulated in different ways and variations in expression levels can have different causes. In this chapter a very short overview is given of several ways mechanisms that can lead to overexpression using a few examples found in *A. baumannii.*

The expression of multidrug efflux systems such as AdeABC is regulated by global stress responses mediated by the two component system (TCS). TCS is a ubiquitous signal transduction system among gram-negative bacteria which is composed of two proteins a sensor kinase and a transcription regulator. Knowledge about these systems is somewhat limited for *A. baumannii* but two systems have been linked to regulate efflux protein expression, namely AdeRS and baeSR. These systems regulate expression of hundreds of other genes as well, which can also be involved in antibiotic resistance [Kroger (2017)].

In contrast to the TCS drug specific expression regulation is also known. An example of this is the TetR gene which locally represses the transcription of TetA or TetB. Tetracycliine is able to bind the repressor resulting in a loss of activity [Vila (2007)].

ISAba1 is an insertion element found in *A. baumannii* that is also involved in expression of antibiotic protection mechanisms. This element carries a strong promotor and can cause overexpression of certain genes by 'hopping' in their promotor region. Among others overexpression of the native OXA-51 is known to be caused by this moving switch [Blair (2015)]. Insertion sequences can also cause deletions of genes as can be the case for CarO [Mussi (2005)].

The last mechanism that can lead to increased expression is mutation. Mutations in TCS AdeRS are a known mechanism that can lead to overexpression of AdeABC. Mutations in TCS PmrAB that lead to overexpression of PmrC is one of the two mechanism that leads to polymyxin resistance [Lean (2016)].

6) Discussion

All of the mechanisms preoposed by Blair *et al.* were found to be present in *A. baumannii.* The most important was the intrinsically low permeability for most antibiotics. This was caused by both constitutional expressed multidrug efflux pumps and the level of expression and types of porins. Mutations were found that lead to increasing levels of multidrug resistance by altered expression of these mechanisms. Drug specific resistance was further increased by a great diversity of systems such as narrow range efflux pumps, antibiotic modifying enzymes and expression of protecting proteins. Finally mutations in several key antibiotic targets made these targets inaccessible to some antibiotics.

The high prevalence of resistance against most antibiotics mentioned in Figure **1** can be contributed to all mechanisms mentioned above, but in particular to the low permeability. Overexpression of RND multidrug efflux pumps seems to be primary cause of increasing tigecycline resistance [Poirel (2011)]. The main reason for increasing carbapenem resistance seems to be the acquiring of new carbapenemases. The low occurrence of colistin resistance is due to the relatively low occurrence of the mutations in genes involved in LPS formation. However resistance against these peptides is rising [Na (2017)] and Mu *et al.* showed that colistin resistance was increased when grown under colistin pressure.

Another problem with colistin treatment is the high nephron- and neuro- toxicity [Wong (2017)], which is one of the main reasons that it is not widely used. The fact that it is not used often may also be the reason for the low occurrence of resistance against it.

6.1: Future research

The problems surrounding *A. baumannii* infections can be characterized into two groups. The first group consist of the infections caused by multidrug resistant strains that are still susceptible to some antibiotics. The second group are the extreme drug resistant or pan resistant strains. Although the most infections belong to the first group, the occurrence of the second group is growing. In the long run both groups would benefit from the development of new antibiotics, but in the short term the research focus should be different. In the remainder of the text first the short term solutions for both problems will be discussed, followed by a discussion over the development of new antibiotics.

The first group is still susceptible to some antibiotics, so they should be treatable. But, due to the huge diversity, it can be hard to find the correct treatment for an individual infection and the problem is finding the drugs that work against the infecting organism in time. According to Wong *et*

al. susceptibility to carbapenems, sulbactam, tigecycline and collistin are most common. However, a quick and easy way to determine the susceptibility *a priori* is not available, because it is a multifactorial problem. Also the bacteria could become resistant to a certain antibiotic during the treatment [Wong (2017)]. Therefore more fundamental research into the mechanisms behind antibiotic resistance and the acquiring of new mechanisms is needed.

For the XDR and pan-resistant strains the current treatment options are limited. Besides developing new antibiotics, which will be discussed later, a relatively easy solution could be the co-administration of multiple antibiotics or inhibitors. This tactic is somewhat controversial since some research suggests that antibiotic resistance is developed more quickly. However multiple couples of antibiotics have shown good results for example administration of colistin with carbapenems or with tigecycline [Wong (2017)].

The functionality of co-administration of multiple antibiotics may be explained by the fitness costs of antibiotic resistance. If resistance against both antibiotics is realized via different mechanisms the fitness costs of both mechanisms could also work additively. For example Hua *et al.* showed that tigecycline resistant strains had a higher sensitivity to a beta-lactam antibiotic, which they contributed to reduced expression of a beta-lactamase. One of the reasons for the controversy is the lack of research that is being done towards usage of multiple antibiotics, so this should be an important subject to focus future research on. Also new combinations of antibiotics should be tried in future studies.

Administration of an antibiotic together with an enzyme inhibitor is less controversial, but also less useful for treatment of *A. baumannii* infections. The only usable inhibitors are beta-lactamase inhibitors and these inhibitors can be quite easily circumvented by using other (metallo) beta-lactamases. Therefore usage of these inhibitors might be useful in some infections, but definitely not for all strains.

6.2: New antibiotics

Development of new antibiotics is also as good possibility, although it might take longer than the options mentioned above. Recently Na *et al.* discovered a new group of quinole compounds that showed good results on *A. baumannii* strains that were fluoroquinole resistant. Mutations that result in resistance against other quinole compounds were observed in these strains, but they did not affect the activity of the new compounds

The compounds described above are found via screening many different compounds which is not the most efficient way to find new drugs. Therefore rational design of new drugs might be a better option to develop new drugs in the future. Of particular interest should be designing drugs in such a way that they can permeate into the cell well. The focus should on the permeability since this is the main basis of defence against almost all antibiotics and it is also quite conserved between different isolates.

The first possibility is to design drugs that can pass through porins of *A. baumannii.* Zahn *et al.* (2016) proposed to this for the OrpD like porin, since they described this protein as the major antibiotic transporting porin. This type of rational design requires intimate knowledge about the structure of the porins, which is given for several porins by Zahn *et al.*(2016). However not all outer membrane proteins are yet characterized so there might be other porins also suitable for drug transport.

Another possible inhibitor target would the RND-family efflux systems. Although there are several RND-type efflux systems they are all composed of similar components. Blocking these systems would increase the permeability for many drugs, which can result in clinical relevant levels of susceptibility

for these antibiotics. Due to the modularity of these efflux systems several targets can be targeted by the inhibitors. Already several molecules have been synthesised that are shown to block these efflux systems *in vitro* [Pages (2009)]. However, currently no efflux pump inhibitors are suitable for clinical use, as a consequence of their toxicity and side effects [Spengler (2017)].

Above the most important developments in antibiotic treatment are mentioned. However there also other treatment options that do not rely upon antibiotics for example the use of phages or vaccination. Since the focus of this review is upon antibiotic resistance these other possibilities are not discussed here, but in the review of Wong *et al.* several options are described in some detail.

For antibiotic based treatment I would suggest that for the near future combinational use of multiple antibiotics and inhibitors is the most beneficial. However in the long run research should focus upon characterizing the structures of the porins and efflux systems and developing antibiotics in such a way that they can circumvent these defence mechanisms, since the low permeability is the main defence mechanism.

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