The rising problem of *Acinetobacter baumannii* in the healthcare environment and the growing research into novel treatment solutions to combat it.

**Abstract**

*Acinetobacter baumannii* is an opportunistic pathogen which has grown to international attention over the last 15 years due to its rapid infiltration of the healthcare environment becoming a common nosocomial infective agent. Whilst also gaining fame through the infection of soldiers wounds returning from the Iraq and Afghanistan conflicts, providing it the nickname ‘iraqibacter’. It is now a highly multi drug resistant pathogen which has had reports of bacteria resistant to all current antibiotics. This review will look at the virulence factors behind its success and the struggling treatments currently being used and investigating where the scientific community are taking the field of research for new and effective treatments against this pathogen.

**Introduction**

*Acinetobacter baumannii* is a gram negative bacillus that is a pleomorphic, non- motile, non-fermenting, opportunistic pathogen that has risen to international significance in the last 15 years. [1-4] It is a major cause of nosocomial infections and has a particularly high incidence among patients who are immunocompromised. [4,5] It frequently causes outbreaks in hospitals worldwide and is reportedly responsible for over 7,000 hospital- acquired infections and 500 deaths in the United States alone yearly. [4,6-11] Generally *A baumannii* infections manifest in the form of respiratory tract infections, urinary tract infections, meningitis, endocarditis, wound infection, bacteremia and pneumonia. [4,10-15]) It has recently been reported however, of cases of necrotising fasciitis in severe cases of wound infection. [16] Normally associated with aquatic environments, it has gained notoriety recently due to its prevalence in infections of injured service personnel from the recent desert conflicts of Iraq and Afghanistan, gaining it the nickname of ‘iraqibacter’. [9,2] Due to its prolific biofilm producing virulence factors it causes major problems in healthcare settings where it can adhere and remain alive for days on surfaces such a polystyrene, glass and metal. [17-23] Confounding this is its many other virulence factors which help the bacteria avoid or negate the immune system of the host, and or induce apoptosis or necrosis, increasing the significance of the infection.[24]

Antibiotic resistance ,especially multi drug and pan resistance, has furthered the international concern around the *A baumannii* as traditional treatment options become increasingly ineffective. In recent years the marked rise if multidrug resistant strains of the bacterium have lead to its designation as a red alert human pathogen [6] and the Centres for disease control and prevention (CDC) classifying a *baumannii* as a serious threat to hospitals. [11] With antibiotic resistance being identified as one of the three most important problems facing human health. [25] However the international attention provided to a *baumannii* has lead to research and novel treatment concepts that hope to bring about an effective solution to the problem from novel vaccine concepts [3] to the use of chelating agents to disrupt biofilm formation and bacterial colonisation of abiotic surfaces. [1].

In this review paper we will discuss the virulence factors that make this opportunistic pathogen so successful and dangerous, specifically antibiotic resistance and biofilm formation and discuss some of the treatment options available to fight it.

**Virulence Mechanisms**

*A baumannii* is a notoriously effective organism at surviving in both hostile environments and surfaces, as well as avoiding host defences/ immune system. This ability is down to *a baumannii’s* many virulence factors.

Biofilm formation in *A. baumannii* is likely to be one of the most important virulence factors in its ability to survive on abiotic and biotic surfaces for substantial periods of time. [26] This will aid in its ability and success in nonsarcomial transmission and host infection. Biofilm formation is highly prevalent in the *A.baumannii* species with some studies suggesting it is three times more likely to form a biofilm than any other member of the *Acinetobacter* genus. A biofilm is generally a collection or colony of bacteria embedded in a self produced extracellular matrix made of polysaccharides and proteins. [26] Biofilms allow increased resistance to antibiotics, phagocytosis and both the
A further benefit of biofilm formation for bacterial infection is quorum sensing within the biofilm which often leads to the activation of virulence factors within the constituent bacteria. [26] A number of factors influence the ability of a baumannii to produce biofilms. The production of OmpA (outer membrane protein A) is a highly important mechanism for the initial adhesion of the bacteria to biotic surfaces such as epithelial cells of the larynx, bronchus and Alveoli.[18,27,28] Although the mechanism behind OmpA ability to increase adhesion has not been well investigated it is believed that fibronectin in the cell membrane of host cells is a major target of binding [29]. The importance of pili in later stages of biofilm formation is another factor improving a.baumannii’s ability to form biofilms. The pili present on the outside of the bacterial cell are generally of two forms short pili structures and long Filamentous extensions, as shown in figure 1. These long filaments have been observed in binding between bacteria in biofilm formation and vary widely in length between a baumannii strains. [30] The short pili however were observed in a 2009 study to be important in binding of the cell to respiratory epithelial cells and therefore performs a similar function to the OmpA pores discussed earlier. [31] This variation is believed to be a large contributing factor to the difference between the biofilm forming abilities of the various strains of a.baumannii.

Figure1: Images of both short and long filamentous pili. Image was taken using SEM (scanning electron microscopy) of Acinetobacter cells. [30]

Of further importance in biofilm formation, as suggested by the name, is the production of BAP’s (biofilm associated proteins). A 2012 study showed that BAP proteins are highly important in forming the 3D tower like structure in biofilms as well as developing the internal water channels essential for quorum sensing. [32] Furthermore a study carried out by Goh et al in 2013 showed that 90% of acinetobacter baumannii strains tested expressed the 17kb bap gene , making bap
proteins arguably the most prevalent biofilm forming mechanism in the acinetobacter family. [33] BlaPER-1 is a gene found in acinetobacter and other gram negative bacteria believed to be highly important in biofilm formation. [4] However there seems to be some dispute within the literature as to wether it is in fact beneficial in cell surface adhesion rather than biofilm production. [4,34] Despite Strong relationships being reported by Lee et al [4], two further papers by Rao et al [34] and Sechi et al [35] disagree showing no significant difference in biofilm forming ability of their bacteria with the blaPER-1 gene or not. However unlike Roa and Sechi, the experiment carried out by Lee et al all adjusted the environmental conditions during the bacterial adherence assay to be more humid and anaerobic to simulate the environment within a host. [4] Therefore it could be surmised that the blaPER-1 gene is more important or becomes important in host infection rather than on abiotic surfaces.

One of the major constituent parts of the biofilm not yet spoken about is extracellular polymeric substances (EPS). The EPS is an extracellular matrix of biomolecules, primarily polysaccharides, proteins and extracellular DNA which provides a protective barrier to the bacteria housed within the biofilm. [36,37] Polysaccharides within the biofilm make the biggest contribution to the properties expressed by the biofilm. for instance the polysaccharide make up can result in significant quantities of water being held to avoid dehydration or act as binding molecules which intercept host molecules such as antibodies trying to enter the biofilm and stop them reaching the bacteria therefore providing defence against the host immune system. [36,38,39] Virulence factors associated with biofilm production and discussed above are summarised in figure 2.

<table>
<thead>
<tr>
<th>Virulence Factor</th>
<th>Virulence proteins</th>
<th>Protein function</th>
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<tbody>
<tr>
<td>Biofilm Formation</td>
<td>Bap</td>
<td>Biofilm maintenance &amp; Cellular adhesion</td>
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<td></td>
<td>OmpA</td>
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<td>Various</td>
<td>Pili production</td>
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<td>Extracellular polysaccharide substance (Various)</td>
<td>Protection from environment</td>
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**Figure 2:** Table summarising the virulence factors associated with biofilm production and maintenance.

Although Biofilm formation is by far the most influential and important virulence factor in a baumannii's success as a pathogen a number of other factors are also important in aiding the bacteria's success.

One such virulence factor is host cell invasion in order to avoid the host immune system. A number of proteins are involved in cellular invasion particularly members of the Omp protein family (OmpA, OMP33-36) and phospholipase D. [28,40-42] Omp33-36 has also been shown to be responsible for promoting apoptosis of host cells as well as interfering with autophagy. [41,43,44] OmpA and H binding protein are also believed to be involved in resistance to host compliment. This was demonstrated in a study which looked at the affect of transferring a attenuated version of the OmpA gene and looking at its affect on bacterial ability to evade the compliment system. [45] The results showed a 1000 fold decrease in bacterial numbers in bacteria with attenuated OmpA gene. [46]

**Antibiotic resistance**

Antibiotic resistance is a growing problem commonly seen in *a baumannii* in the clinical world. [24] There are a number of mechanisms observed and studied that cause or aid antibiotic resistance in *A. baumannii*. [7] B lactam’s are some of the most over-arching mechanisms, affecting cephalosporins, carbapenems & sulbactam effectiveness. [47-49] Acinetobacter-derived cephalosporinase (ADC) AmpC B-lactamase is the largest problem for cephalosporins as it is capable of resistance to even ceftazidime and cefepime, third and fourth generation antibiotics respectively. ADC B- lactamase can also produce extended spectrum B-lactamase (ESBL) which...
can further increase antibiotic resistance. [50-52]  
Carbapenems are also affected by B-lactamases, specifically metallo-B-lactamase NDM-1. Traditionally found in the enterobacter family, it has been reported in A baumannii since 2011. [55,56] A.baumannii naturally has an intrinsic production of carbapenemase, fortunately normally at low levels. [53,54] The acquisition of plasmids containing stronger promoters of Carbapenemase has lead to a sharp increase in resistance. Most notorious for providing this stronger promotion are the OXA- group B-Lactamase genes, of which five hold significant clinical relevance. [57]  
Further affecting Sulbactam effectiveness in A baumannii is the decrease in Penicillin binding protein (PBP) expression. [49,58]  
Aminoglycosides are another family of antibiotic which have been slowly loosing their effectiveness. Resistance to aminoglycosides has traditionally been found in the form of aminoglycoside-modifying enzymes which affect the binding site on the 16s and 30s ribosomal subunits and inhibited binding of the antibiotic. [59] Highly prevalent in Worldwide Clone 2 strains are aminoglycoside-resistance mechanism A (ArmA) A.Baumannii. This resistance mechanism shows high resistance to amikacin, gentromycin and tobamicin. This mechanism is an RNA methyltransferase and functions by methylating a guanine residue within the aminoglycoside-binding site A inhibiting binding of aminoglycosides.  
Lastly and most concerning is the growing reports of colistin resistance. Although research is needed in this area it has been observed that resistance can be caused by a decrease or complete loss of lipopolysaccharide A which colistin binds to. [60,61] However research published by Moffatt et al. has only been able to show this in mainly laboratory isolates and not from clinical isolates enough to determine that this is causing the resistance we are seeing in the clinical population. [61]  

Current treatment options.

Current treatment options are universally made up of traditional antibiotics use with the growing attainment of resistance to many or even most antibiotics. In A.Baumannii treatments with these antibiotics are becoming more and more redundant. [24] Carbapenems have been the go to antibiotic for Acinetobacter infections due to their efficacy and very good safety profile. [24]  
Recently however this class of antibiotic has had to be replaced by other less commonly used antibiotics, due to growing resistance among the genus. [7]  
Sulbactam has had limited results and therefore has generally not been prescribed [7] However a number of studies have shown promising results when Sulbactam was used in conjunction with other antibiotic treatments such as Ampicillin or Cefoperazone. [62] Although this showed promising results at the beginning of the century a number of studies have shown an increasing growth of resistance as its use became more wide spread within the US, ultimately leading to decreases in seseptability from 89% to 40% from 2003 to 2008 respectively. [63] More recently attempts have been made to combine Sulbactam with Polymyxins, most notably colistin.  
Colistin has shown promise with dealing with A. Baumannii infections with one study showing a favourable outcome for 60% of patients suffering with ventilator associated pneumonia. However the pharmacodynamics and kinetics of colistin are still poorly understood and therefore treatment with colistin can have poor side affects. Prolonged use of Colistin has been shown to cause nephrotoxicity in patients, although this is usually reversible it does bring into question the safety of using this drug in certain populations. [65] Colistin although one of the last remaining effective antibiotics to A baumannii infections it has limited use due to its affects. Most notably to those with history of renal impairment or decreased function and the elderly. [66-68] With the alarming emergence of resistance to Colistin (as high as 3.2% as reported by Gales et al in 2006) it has been recommended to be provided at loading dose as to limit the growth of resistance to one of the last resort antibiotics. [64] Coupled with the waining effectiveness of traditional treatment and growing concern of safety it is highly important that new replacements be found, as certain patient groups are becoming untreatable due to the strong side effects of the remaining treatments as mentioned previously.
Future Treatment

With the growing failure of tradition treatments to successfully treat *A. baumannii* infections (predominantly antibiotics such as carbapenems) a wide variety of novel treatments and concepts have been developed and researched.

Bactericidal gene transfer therapy is a promising novel field of treatment involving the use of bacterial vectors preferable attenuated cells to horizontally transfer genetic material to the pathogens present in the infection creating susceptibility to current treatment regimes or causing the bacteria to themselves breakdown under there own cellular mechanisms. [69] The difficulty with this treatment option is the need for the attenuated cells to come in direct contact with the pathogens resulting in practical treatment being mainly confined to surface wound infections rather than bacteamia or endocarditis where the attenuated cells were often not reaching the infection areas in great enough numbers to be effective.

Following similar lines of thought, the concept of bacteriophage therapy of bacterial infections has attracted renewed attention with the increase in MDR and improved understanding of bacteriophage and their manipulation. [70] bacteriophage treatment is a promising possibility due to bacteriophages specificity to bacterial antigens and their ability to take effect quickly within animal models. [71,72] With the discovery of the AB1 bacteriophage which is specific to a *baumannii* the possibility of using these virus’s as replacements for traditional antibiotics seems viable.

Radioimmunotherapy is a, as of yet un-explored treatment option. However studies have shown that it can be used to target microorganisms in the body just as well as cancer cells leading to optimism for this as a future treatment option. [73] It involves the production of radionuclides that act as antibodies to a *baumannii* and allow cytotoxic radiation direct to the bacteria, causing cell death with minimal haematological side affects. As studies have already shown the ability of scientists to create antibodies specific to a *baumannii* the practical application od radioimmunotherapy is a definite possibility. [74,75]

Nitric oxide or NO has been investigated as a possible treatment due to its known properties as an antimicrobial as well as wound healing and immunity [76-78] Mihu et al demonstrated in his study that NO could be used against a *baumannii* in cutaneous wounds. Not only was bacterial wound populations decreased but wound healing improved as well as decrease in collagen breakdown in the skin reducing risk of scarbing from the wound. [79]

A treatment only functional for skin wounds such as burns or cutaneous trauma is photodynamic therapy which involves the use of use of a photosensitiser combined with oxygen molecules which are activated by red or near infrared light to create reactive oxygen species toxic to the bacteria cells therefore killing them. This treatment is only functional in dermal injuries/ infections due to the need for the light to penetrate and reach the photosensitisers at the sight of infection. However most promising of all is the avenue of a vaccine to combat a *baumannii* infections. With ever increasing information and knowledge of bacterial genomes a study by Ming-Hsien et al has investigated the use of reverse vaccinology to find vaccine targets and therefore make an effective treatment [15,80-82] (insert table 2 from 3) A number of potential antigens for a vaccine were discovered in the study with three being deemed most suitable. These were OmpK, FKIB and Ompp1. When mouse models were used they found a considerable IgG response. Mice not vaccinated all died within 4 days post infection however with a combination vaccine using the three antigens the mice showed a 60% survival rate a very striking result. [3]

**Conclusion**

In conclusion *Acinetobacter baumannii* is a highly pathogenic bacteria which is an ever increasing problem in hospital acquired infections due to its propensity to colonise and survive on abiotic surfaces particularly medical equipment in hospitals. Combining this highly virulent bacteria with growing multi drug and pan resistance to antibiotics, leading to decreased effectiveness of current treatments. *A. baumannii* is a pathogen in need of urgent research looking into novel treatments, most notably vaccines which appear to be showing the most promise demonstrated in a 2015 study published in Human Vaccines & Immunotherapeutics. whilst there is some disagreement on how important certain virulence factors are in influencing certain characteristics such a the blaPER-1 gene in biofilm formation, it seems to be universally agreed in the literature that a *baumannii* possesses a wide range of virulence factors both for biofilm and platonic cells which...
contribute it its success as a opportunistic pathogen.

References

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