CHAPTER-2

REVIEW OF LITERATURE

2.1. HISTORICAL PERSPECTIVE

The history of the genus Acinetobacter dates back to the early 20th century, in 1911, when Beijerinck, a Dutch microbiologist, described an organism named Micrococcus calcoaceticus that was isolated from soil by enrichment in calcium acetate containing minimal medium [21].

The current genus designation, Acinetobacter (from the Greek ε [akinetos], i.e., nonmotile), was initially proposed by Brisou and Prevot in 1954 to separate the nonmotile from the motile microorganisms within the genus Achromobacter [22]. In the 1974 edition of Bergey’s Manual of Systematic Bacteriology, the genus Acinetobacter was listed, with the description of a single species, A. calcoaceticus (the type strain for both the genus and the species is A. calcoaceticus ATCC 23055). In the “Approved List of Bacterial Names,” in contrast, two different species, A. calcoaceticus and A. lwoffii, were included, based on the observation that some Acinetobacter were able to acidify glucose whereas others were not [23].

2.2. CURRENT TAXONOMY

The genus Acinetobacter, as currently defined, comprises Gram negative, strictly aerobic, nonfermenting, nonfastidious, nonmotile, catalase positive, and oxidase negative bacteria with a DNA GC content of 39% to 47%. Based on more recent taxonomic data, it was proposed that members of the genus Acinetobacter should be classified in the new family Moraxellaceae within the order Gammaproteobacteria, which includes the genera Moraxella, Acinetobacter, Psychrobacter, and related organisms. A major breakthrough in the long and
complicated history of the genus was achieved in 1986 by Bouvet and Grimont, who based on DNA hybridization studies distinguished 12 DNA (hybridization) groups or genomospecies, some of which were given formal species names, including *A. baumannii*, *A. calcoaceticus*, *A. haemolyticus*, *A. johnsonii*, *A. junii*, and *A. lwoffii*. Work done by Bouvet and Jeanjean, Tjernberg and Ursing, and Nishimura et al., resulted in the description of further Acinetobacter genomic species, including the named species *A. radioresistens*, which corresponds to Acinetobacter genomic species 12 described previously by Bouvet and Grimont. Some of the independently described (genomic) species turned out to be synonyms, e.g., *A. lwoffii* and Acinetobacter genomic species 9 or Acinetobacter genomic species 14, described by Bouvet and Jeanjean (14BJ), and Acinetobacter genomic species 13, described by Tjernberg and Ursing (13TU). More recently, 10 additional Acinetobacter species were described, including 3 species of human origin, *A. parvus*, *A. schindleri*, and *A. ursingii* and 7 species isolated from activated sludge (recovered from sewage plants), namely, *A. baylyi*, *A. bouvetii*, *A. grimontii*, *A. tjernbergiae*, *A. towneri*, *A. tandoii*, and *A. gerneri*, increasing the actual number of validly described (genomic) species to 31, of which 17 have been given valid species names.

Four of the above described species, i.e., *A. calcoaceticus*, *A. baumannii*, Acinetobacter genomic species 3, and Acinetobacter genomic species 13TU, are very closely related and difficult to distinguish from each other by phenotypic properties. It has therefore been proposed to refer to these species as the *A. calcoaceticus*, *A. baumannii* complex. However, this group of organisms comprises not only the three most clinically relevant species that have been implicated in the vast majority of both community acquired and nosocomial infections, i.e., *A. baumannii*, Acinetobacter genomic species 3, and Acinetobacter genomic species 13TU, but also an environmental species, *A. calcoaceticus*, that has frequently been recovered from soil and water but has, to our knowledge, never been implicated in serious clinical disease. Therefore, since it is the environmental species that has given its name to the complex, the designation *A. calcoaceticus*, *A. baumannii* complex may be misleading and not appropriate if used in a clinical context [1].
2.3. NATURAL HABITAT

Members of the genus Acinetobacter are considered ubiquitous organisms, since Acinetobacter can be recovered after enrichment culture from virtually all samples obtained from soil or surface water [24]. These earlier findings have contributed to the common misconception that *A. baumannii* is also ubiquitous in nature [25]. In fact, not all species of the genus Acinetobacter have their natural habitat in the environment. However, a systematic study to investigate the natural occurrence of the various Acinetobacter species in the environment has never been performed.

Acinetobacter species indeed seem to be distributed widely in nature i.e., *A. calcoaceticus* is found in water and soil and on vegetables. *A. baumannii* infections that have been observed in tropical climates [26, 27] may be associated with an environmental source. Acinetobacter genomic species 13TU was found on human skin in Hong Kong but not in Europe. Also, it has not been identified in the inanimate environment. Thus, the natural habitats of both *A. baumannii* and Acinetobacter genomic species 13TU vary with geographical locations hence remain to be defined.

2.4. PATHOGENESIS OF ACINETOBACTER INFECTIONS

*Table 2.1. Predisposing factors [36]:*

<table>
<thead>
<tr>
<th>Predisposing factors</th>
<th>Causes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Immuno suppression</td>
<td>Diabetes Mellitus</td>
</tr>
<tr>
<td></td>
<td>Cancer</td>
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<td></td>
<td>Steroids</td>
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<td></td>
<td>Transplantation</td>
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<tr>
<td>Trauma</td>
<td>Gunshot, knife wounds, punctures</td>
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<tr>
<td></td>
<td>Surgery</td>
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<tr>
<td></td>
<td>Burns</td>
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<tr>
<td>Foreign body implantation</td>
<td>Catheters; urinary or blood</td>
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<tr>
<td></td>
<td>Prosthetic devices; joints, valves</td>
</tr>
<tr>
<td></td>
<td>Corneal implants</td>
</tr>
<tr>
<td>Infused fluids</td>
<td>Dialysate</td>
</tr>
<tr>
<td></td>
<td>Saline irrigations</td>
</tr>
</tbody>
</table>
Infection caused by Acinetobacter spp:

Predisposing factors to colonization / infection of Acinetobacter:

Prolonged respiratory therapy and prior therapy with antibiotics such as cephalosporins and aminoglycosides are predisposing factor to colonization/infection in ICU stay. Several conclusions regarding colonization in hospitalised patients can be drawn from the published studies.

- A high rate of colonization can be found in debilitated hospitalized patients.

- The predominant site of colonization is the skin; other sites especially respiratory or digestive tract may be involved on certain occasions.

- The observed discrepancies between carriage rate for outpatients and hospitalized patients suggests that infecting or colonising organisms in hospital acquired infections may derive more often from cross transmission or hospital environmental sources.

- Acinetobacter spp. has unique characteristics that favour persistence in the hospital environment. However they are ubiquitous organisms that can be isolated readily from non clinical sources such as soil, drinking water, sewage and food stuffs.

- The dissemination and persistence of Acinetobacter spp. in the hospital environment accounts for the reservoir of infection especially during outbreaks.

Materials used for respiratory therapy or support, ventricular drainage catheters, intravascular catheters, dialysis fluid bottles and medical equipments may therefore become contaminated both by the patients themselves and by staff during handling. The latter should be considered during outbreaks of infections.
Acinetobacter spp accounts for 1-3% of all nosocomial infections with the principal sites and types of infection including respiratory tract infections, bacteraemia, urinary tract infection, surgical wounds, meningitis and skin or eye infection. Hospitalization in ICU and previous administration of antibiotics are associated with higher incidence of the infections [28]. Tubes, catheters, and artificial devices serve as portals of entry in these patients.

**Respiratory infection:**

Acinetobacter spp accounts for 3-5% of nosocomial pneumonia [28]. They play a special role in nosocomial pneumonia for the subset of ICU patients requiring mechanical ventilation [29]. Several factors have been identified to increase risk of pneumonia or colonization of the lower respiratory tract by Acinetobacter spp. They include advanced age, chronic lung disease, immune suppression, surgery, use of antimicrobial agents, presence of invasive devices such as endotracheal and gastric tubes and the type of respiratory equipment [30, 31].

Crude mortality rates of 30-75% have been reported for nosocomial Acinetobacter pneumonia but it is difficult to establish whether such critically ill patients would have survived if nosocomial pneumonia had not occurred [30].

**Bacteremia:**

Acinetobacter spp. may be found either as a single pathogen or as part of polymicrobial bacteraemia. Acinetobacter is the most common species in most adult patients [32]. Immunocompromised patients with malignant disease, trauma or burns seem to be more prone for such infections. Mortality attributable to Acinetobacter infection was 27%. However, prognosis of the patients is determined by the underlying disease. Previous antibiotic treatment may be responsible for selection of resistant strains [33].
Meningitis:

Secondary meningitis is the predominant form of Acinetobacter meningitis, although sporadic cases of primary meningitis have been reported, particularly following neurosurgical procedures or head trauma. All most all the cases are caused probably by \textit{A. baumannii} [34]. Risk factors include the presence of a continuous connection between the ventricles and external environment e.g. ventriculostomy or a CSF fistula. Ventricular catheter for more than 5 days is an important risk factor [35, 36].

Urinary tract infection:

Nosocomial urinary tract infection is infrequently caused by Acinetobacter spp. It occurs most commonly in elderly debilitated patients, inpatients confined to ICUs, and in patients with permanent indwelling urinary catheters. Most patients (80%) tend to be men, perhaps reflecting the higher prevalence of indwelling urinary catheters in this population as a result of prostatic enlargement [28].

Traumatic Battlefield and Other Wounds:

\textit{A. baumannii} may occasionally cause skin/soft tissue infections outside of the military population. The organism caused 2.1% of ICU acquired skin/soft tissue infections in one assessment [37]. It is a well known pathogen in burn units and may be difficult to eradicate from such patients. \textit{A. baumannii} is commonly isolated from wounds of combat casualties from Iraq or Afghanistan [38, 39, 40, 41].

2.5. ANTIBIOTIC RESISTANCE

Numerous reports documented high rates of multiple antibiotic resistance found in Acinetobacter spp [42]. This results in therapeutic problems in treating patients with nosocomial infections in ICUs [43].

Until early 1970s, nosocomial Acinetobacter infections could be treated successfully with gentamicin, monochyline, nalidixic acid, ampicillin or carbenicillin, either as single agent or in antibiotic combinations. Many
Acinetobacter are now resistant to clinically achievable levels of most commonly used antibacterial drugs, including penicillins, cephalosporins, most aminoglycosides, chloramphenicol and tetracyclines. Partial susceptibility remains, for relatively new antibiotics, such as broad spectrum cephalosporins (cefotaxime, ceftazidime), imipenem, tobramycin, amikacin and fluoroquinolones.

**Mechanism of resistance**

- **Amp-C Cephalosporinase**

  Resistance is mediated by several enzymes which include:

  Chromosomally encoded cephalosporinase (AmpC type).

  Recently, insertion sequences (ISs) have been found (IS Aba1, IS 1135) which cause increase production of the chromosomal β-lactamase of *A. baumannii* (a 1200-bp sequence)

  Other β-lactamase: In addition to the class C cephalosporinase discussed, other β-lactamases have been reported in Acinetobacter these include,

  a. TEM-1 type β-lactamases,
  
  b. SHV type - β lactamases,
  
  c. CTX-M type β-lactamases,
  
  d. PER-1 β-lactamases,
  
  e. VEB-1 β-lactamases,

  Although they are important, it is difficult to assess their impact on resistance in the presence of the AmpC cephalosporinase [44].

- **Serine and metallo-β-lactamases (carbapenemases)**

  The most problematic recent occurrence is the emergence of numerous OXA enzymes in Acinetobacter that confer β-lactam resistance [45]. The use of
carbapenems to treat Acinetobacter infection has also resulted in outbreaks of infection with carbapenem resistant Acinetobacter species. The first description of a serine carbapenemase in Acinetobacter was:

a. ARI-1 (OXA-23) [46]. Although OXA carbapenemases may not robustly hydrolyze imipenem, their presence in an organism that may have an IS element that acts as a promoter can result in imipenem resistance [47].

b. IMP type and VIM type β-lactamase VIM-2 β-lactamases detected in Acinetobacter isolates from Korea confer significant levels of resistance to carbapenems [48]. IMP type metallo-β-lactamases (IMP-1, -2, -5, -6, and 11) are also being reported with increasing frequency [48].

- **Outer membrane protein (OMP [porin]) changes**

  By reduction of transport into the periplasmic space via changes in porins or OMPs, access to penicillin binding proteins is reduced. With less β-lactam entering the periplasmic space, the weak enzymatic activity of the β-lactamase is amplified.

  Many outbreaks of infection with imipenem resistant A. baumannii are due to porin loss. Carbapenem resistant isolates of A. baumannii had reduced expression of 47-, 44-, and 37-kD OMPs.

- **Aminoglycoside-modifying enzymes (AMEs)**

  Resistance to aminoglycoside by AMEs is also a major unwelcome feature in the multidrug resistant phenotype of Acinetobacter [49].

  All 3 types of AMEs namely acetylating, adenylating, and phosphorylating have been identified in A. baumannii.
- **Quinolone resistance**

  Molecular analysis of a collection of quinolones resistant *A. baumannii* isolates revealed that mutations in both in gyrA and parC are responsible for quinolone resistance.

- **Efflux pump**

  The natural role of efflux is to remove chemicals that could potentially disorganize the cytoplasmic membrane; however, from the point of view of antibiotic resistance, efflux pumps have a potent ability to actively expel β-lactams, quinolones, and sometimes even aminoglycosides. Efflux pumps usually have 3 components: the pump itself, which lies in the cytoplasmic membrane; an exit portal (porin channels traversing the outer membrane); and a linker lipoprotein between the two. An RND type efflux pump has been described in *A. baumannii* [50]. By inactivation of adeB, it was shown that this efflux pump was responsible for aminoglycoside resistance and was involved in resistance to quinolones, tetracyclines, chloramphenicol, erythromycin, and trimethoprim.

**Carbapenem**

In the last few years, resistance to antibacterial drugs has been increasing in Acinetobacter spp, posing substantial treatment challenge in the future [51]. Carbapenems have potent activity against Acinetobacter spp. and are usually the drugs of choice against multidrug resistant *A. baumannii* isolates.

Acinetobacter spp. may develop resistance to carbapenems through various mechanisms, including class B and D carbapenemase production, decreased permeability, altered penicillin binding proteins, and rarely, over expression of efflux pumps[52].
Carbapenem resistance is also attributed to various causes such as reduced expression of outer membrane protein (29 kDa, 33-36 kDa) [52], Metallo-β-lactamase, AmpC β-lactamase and several other carbapenemases.

Table 2.2. Classification of carbapenemases:

<table>
<thead>
<tr>
<th>Classification</th>
<th>Enzyme</th>
<th>Bacteria</th>
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<tbody>
<tr>
<td>Class A</td>
<td>KPC,SME,IMI,NMC,GES</td>
<td>Enterobacteriaceae (rare reports in P.aeruginosa)</td>
</tr>
<tr>
<td></td>
<td>PER,SFO,SFC,KBC</td>
<td></td>
</tr>
<tr>
<td>Class B</td>
<td>IMP,VIM,GIM,SPM,</td>
<td>P.aeruginosa, Acinetobacter,</td>
</tr>
<tr>
<td></td>
<td>SIM,NDM</td>
<td>Enterobacteriaceae</td>
</tr>
<tr>
<td>Class D</td>
<td>OXA</td>
<td>Acinetobacter</td>
</tr>
</tbody>
</table>

Of the β-lactamases, those with carbapenemase activity are most concerning and include the serine oxacillinases (Ambler class D OXA type) and the metallo-β-lactamases (MBLs) (Ambler class B) [53, 54, 55]. Thus far, the Ambler class A carbapenemases (KPC, GES, SME, NMC, and IMI) have not been described for A. baumannii. The first identified OXA type enzyme with carbapenem hydrolyzing activity was from a clinical A. baumannii strain isolated in 1985 from Edinburgh, Scotland [56]. This plasmid encoded resistance determinant (initially named ARI-1) was found to be transferable, and the gene was later sequenced and named blaOXA-23 [57]. This enzyme type now contributes to carbapenem resistance in A. baumannii globally. OXA-27 and OXA-49 are closely related enzymes that make up the blaOXA-23 gene cluster in A. baumannii [58,59].

Two other acquired OXA type gene clusters with carbapenemase activity have been described, including the blaOXA-24-like (encoding OXA-24, -25, -26, and -40) and the blaOXA-58-like carbapenemase genes. The crystal structure of OXA-24 was recently described and provides important insights for future drug development toward this emerging class of carbapenemases [60]. blaOXA-58 was identified more recently and, similar to blaOXA-23, is often plasmid mediated [61], which may explain its widespread distribution [62, 63, 64]. blaOXA-58 has also been identified in A. junii from Romania and Australia [65]. The final gene cluster, blaOXA-51-like genes (encoding OXA-51, -64, -65, -66, -68, -69, -70, -71, -78, -79, -80, and -82), is unique in that it is naturally occurring in
A. baumannii, hence its chromosomal location and prevalence [66, 67, 68, 69, 70, 71]. Similar to other class D enzymes, its product has a greater affinity for imipenem than for meropenem [72]. Its role in carbapenem resistance appears to be related to the presence of ISAbal [69]. In the absence of this element, cloning studies suggest a minimal effect on carbapenem susceptibility, even in the presence of an over expressed multidrug efflux pump (AdeABC) [67].

Given the multiplicity of β-lactam resistance mechanisms in A. baumannii [53], the contributions of the acquired carbapenem hydrolyzing oxacillinases to carbapenem resistance are often difficult to determine. This issue has been addressed by Heritier et al., who studied the changes in susceptibility profiles of both natural and recombinant plasmids containing blaoXA-23, blaoXA-40 (only a recombinant plasmid, as no natural plasmid was identified), and blaoXA-58 in different host backgrounds. blaoXA-23 and blaoXA-40 appeared to produce higher MICs of imipenem than did blaoXA-58, and all blaoXA genes produced higher MICs of imipenem in the presence of an over expressed AdeABC efflux pump. Inactivation of the blaoXA-40 gene led to susceptibility to carbapenems, and resistance was restored with complementation. Interestingly, the natural plasmids containing blaoXA-23 and blaoXA-58, extracted from clinical isolates, produced significantly greater levels of resistance to carbapenems than did their respective recombinant plasmids in similar host backgrounds. This discrepancy is most likely due to the presence of IS elements in the natural plasmids. The importance of IS elements for carbapenem resistance due to oxacillinases in A. baumannii has only recently been appreciated [73]. These elements provide two main functions. First they encode a transposase and therefore are mobile. Second, they can contain promoter regions that lead to over expression of downstream resistance determinants. Most commonly, these elements have been described in association with blaoXA-23 [74] and blaoXA-58 [75, 76, 77], but they may also promote carbapenem resistance in association with blaoXA-51 [79]. Interestingly, certain IS elements, especially ISAbal, appear relatively unique to A. baumannii. As described in this section, IS elements are also important for the expression of resistance to other antibiotics in A. baumannii [78]. Despite MBLs being less commonly identified in A. baumannii than the OXA type carbapenemases, their hydrolytic activities toward carbapenems are significantly more potent
These enzymes have the capability of hydrolyzing all β-lactams (including carbapenems) except the monobactam aztreonam, which may assist in laboratory detection. Of the five MBL groups described to date, only three have been identified in *A. baumannii*, including IMP [80,81,82,83], VIM [84], and SIM [85] types. Several geographic regions, such as Spain, Singapore, Greece, and Australia, have shown the presence of both OXA and MBL type enzymes in the same strains [86]. Unlike the OXA type enzymes, MBLs are most commonly found within integrons, which are specialized genetic structures that facilitate the acquisition and expression (via a common promoter) of resistance determinants. Most acquired MBL genes in *A. baumannii* have been found within class 1 integrons, often containing an array of resistance gene cassettes, especially those encoding aminoglycoside modifying enzymes [87]. Not surprisingly, *A. baumannii* strains carrying integrons have been found to be significantly more drug resistant than strains without integrons. The clinical significance of this unique genetic structure is that overuse of one antimicrobial may lead to overexpression of multiple resistance determinants as a consequence of a common promoter. In isolation, integrons are not mobile and therefore are embedded within plasmids or transposons that act as the genetic vehicles for resistance dissemination.

Non enzymatic mechanisms: β-lactam resistance, including carbapenem resistance, has also been ascribed to non enzymatic mechanisms, including changes in outer membrane proteins (OMPs) [88], multidrug efflux pumps [89], and alterations in the affinity or expression of penicillin binding proteins [90, 91]. Relative to other gram negative pathogens, very little is known about the outer membrane porins of *A. baumannii*. Recently, the loss of a 29 kDa protein, also known as CarO, was shown to be associated with imipenem and meropenem resistance [92, 93]. This protein belongs to a novel family of OMPs found only in members of the Moraxellaceae family of the class Gammaproteobacteria (124). No specific imipenem binding site was found in CarO [94], indicating that this porin forms non-specific channels. The loss of the CarO porin in imipenem resistant *A. baumannii* appears secondary to CarO gene disruption by distinct insertion elements [94]. Clinical outbreaks of carbapenem resistant *A. baumannii* due to porin loss, including reduced expression of 47-, 44-, and 37-kDa OMPs in *A. baumannii*
strains endemic to New York City [95] and reduced expression of 22- and 33- kDa OMPs in association with OXA-24 in Spain [96], have been described.

NDM-1 which stands for New Delhi metallo-β-lactamases actually refers not to a single bacterial species but to a transmissible genetic element encoding multiple resistance genes that was initially isolated from a strain of *Klebsiella pneumoniae* obtained from a patient who acquired the organism in New Delhi, India. To confirm the plasmid location of the NDM-1 gene, the plasmid DNA bands from the S1-PFGE were excised from the gel and used as DNA templates for PCR. Carbapenem resistance in *A. baumannii* has been sporadically attributed to the production of IMP-type metallo-β-lactamases (MBLs) and OXA-type carbapenemases. Metallo beta lactamases (MBL) are enzymes that have wide spread of activity and they confer a high level of resistance to all β-lactams including carbapenem, except aztreonam. MBLs require divalent zinc ion for their enzymatic activity which is not diminished by serine β-lactamase inhibitors like sulbactam, tazobactam, clavulanic acid etc but is inhibited by metal chelators like EDTA and thiol based compounds such as 2-mercaptopropionic acid, 10-phenanthroline, calcium dipicholinate etc [97]. This MBL production is typically associated with resistance to aminoglycosides and fluoroquinolones further compromising therapeutic option. Several phenotypic tests have been developed for MBL detection, such as the

- E-test
- Double-disk synergy tests
- Combined disk (CD) assay
- The Modified Hodge test

### 2.6. THERAPY OF ACINETOBACTER INFECTION

Prior to the 1970s, it was possible to treat Acinetobacter infections with a range of antibiotics, including aminoglycosides, β-lactams, and tetracyclines [98]. However, resistance to all known antibiotics has now emerged in *A. baumannii* [99,100], thus leaving the majority of today’s clinicians in unfamiliar territory.
Compounding the problem is the large number of pharmaceutical companies that have abandoned antibiotic drug discovery and development, driven primarily by the risks of poor financial returns relative to those for more lucrative classes of drugs [100,101]. The dearth of antibiotics, especially for gram negative organisms, has recently stimulated attention from major research and governing bodies [102]. Unfortunately, at this stage, very little is in the therapeutic pipeline [103] and the new agents with activity against gram negative organisms are all modifications of existing classes. Novel antibiotic targets and mechanisms of action are urgently required.

Given the current therapeutic environment, optimizing the use of existing antimicrobials is critical. To achieve this goal, a thorough understanding of the pharmacokinetic and pharmacodynamic parameters that predict maximal drug efficacy yet minimize the evolution of drug resistance, as well as evidence based approach to therapeutic strategies for highly drug resistant strains, is required. The following section concentrates on the available in vitro, animal, and human data to assist the reader in the management of infections due to highly drug resistant A. baumannii.

Acinetobacter spp. tend to be resistant to a variety of antibiotics and very few of the major antibiotics are now reliably effective for the treatment of Acinetobacter infections. Due to geographic variations in the antibiotic resistance it is recommended that any antibiotic should be used only after extensive invitro susceptibility testing has been performed. Ticarcillin often combined with sulbactam, ceftazidime or imipenem may be useful. Aminoglycosides can sometimes be used successfully in combinations with an effective β-lactam and other combination of a β- lactam with a fluoroquinolone has been proposed.

Over use of imipenem has been associated with the report of several outbreaks caused by carbapenem resistant strains, often leaving polymyxins and sulbactam as the only antibiotics with invitro activity against these organisms. The sulbactam ampicillin combination has been used clinically with encouraging results.
2.7. PREVENTION AND CONTROL

Acinetobacter has emerged as important nosocomial pathogens and is capable of rapid adaptation to the hospital environment. Persistence of Acinetobacter spp. in the contaminated medical equipment like respiratory equipment provides opportunity for contamination of patients and staff, leading to long term outbreaks. Therefore emphasis of initial control measures should be on strict isolation of infected or colonized patients to limit dissemination of outbreak strains in the environment

- To prevent cross transmission via the hands of the staff strict hand washing policy and practice should be enforced

- As substantial contamination is found in the vicinity of infected or colonized patients, good housekeeping practices should be enforced. Complete disinfection of the unit and medical equipment must be done to prevent an outbreak

- Increased use of antibiotics favours the emergence and spread of Acinetobacter spp. Multiresistant Acinetobacter spp. are likely to be selected in the hospital in response to increasing antibiotic pressure. Control of antibiotic usage is therefore important part of preventive measures against the emergence of epidemic Acinetobacter infection.

Infection control perspective

There are three major factors possibly contributing to the persistence of A. baumannii in the hospital environment, i.e., resistance to major antimicrobial drugs, resistance to desiccation, and resistance to disinfectants. Resistance to antibiotics may provide certain A. baumannii strains with a selective advantage in an environment, such as the modern ICU, where microorganisms are confronted with extensive exposure to antimicrobials. Several researchers have observed that resistance rates in epidemic A. baumannii strains are significantly higher than those in sporadic A. baumannii strains [104,105,106]. Resistance to the fluoroquinolones in
particular was associated with epidemic behaviour [104]. The recently observed increase in carbapenem resistant *A. baumannii* strains was associated almost exclusively with hospital outbreaks [107,108]. It has been suggested that any clinical *A. baumannii* isolate with resistance to multiple antibiotics indicates a potential nosocomial outbreak strain [109]. It has also been shown that *A. baumannii* strains survive desiccation far better than other Acinetobacter species, such as *A. johnsonii*, *A. junii*, and *A. lwoffii* [110,111]. This together with their greater susceptibility to commonly used antimicrobials, may explain why Acinetobacter strains belonging to these species have been implicated only very rarely in hospital outbreaks. The majority of *A. baumannii* strains had survival times that were considerably longer than those found for *Escherichia coli* and other Enterobacteriaceae but similar to those observed for *Staphylococcus aureus*. These observations, as well as the previously suggested airborne spread of Acinetobacter spp. in hospital wards [112,113], may explain the occurrence of repeated outbreaks after incomplete disinfection of contaminated dry surfaces. Prolonged survival of *A. baumannii* in a clinical setting, i.e., on patients’ bed rails, has been found to be associated with an ongoing outbreak in an ICU and illustrates that dry vectors can be secondary reservoirs where *A. baumannii* can survive [114]. Wisplinghoff et al. recently compared the in vitro activities of various disinfectants, such as propanol, mecetronium ethylsulfate, polyvinyl pyrrolidone iodine, triclosan, and chlorhexidine, against sporadic and epidemic *A. baumannii* strains by using a broth macrodilution method [115]. They concluded that resistance to currently used disinfectants is probably not a major factor favouring the epidemic spread of *A. baumannii*, since all disinfectants inhibited growth of all *A. baumannii* isolates when concentrations and contact times recommended by the respective manufacturer were used. However, with most of the disinfectants tested, a substantial number of viable bacteria remained if contact times were 30 s or if diluted agents were used, as may occur in day to day clinical practice. No significant differences in susceptibility between outbreak related and sporadic strains were observed under these conditions. Minor deviations from the recommended procedures leading to decreased concentrations or exposure times may play a role in nosocomial cross transmission, but larger studies using additional methods would be required to confirm these findings.
2.8. MAGNITUDE OF THE PROBLEM

Development to antibiotic resistance continues to be a heavy burden on economy and on life expectancy of patients in both developed and developing countries. Before 1970s, Acinetobacter infections were mostly postsurgical urinary tract infections in patients hospitalised in surgical units. The significant improvement in resuscitation techniques during the last 30 years has changed the types of infection caused by Acinetobacter.

Since 1980s, Acinetobacter has spread rapidly among patients in intensive care units. Today, Acinetobacter accounts for 9% of nosocomial infections, with most Acinetobacter infections involving the respiratory tract, due to emergence of multi drug resistance isolates.

Resistance to many antimicrobial agents belonging to different classes and increased antimicrobial resistance of this organism makes its treatment difficult. Although carbapenems are known to be one of the very few antimicrobial agents showing consistent activity against Acinetobacter, the emergence of carbapenem resistant Acinetobacter strains leaves the drug out of utility. Carbapenem resistance in Acinetobacter occurs by β-lactamase production like metallo-β-lactamases (MBL) or oxacillinases, porin loss or modification of penicillin binding proteins, but it is mainly attributed to production of carbapenemases.

Hence early detection of carbapenem resistance in Acinetobacter which is essential to prevent nosocomial infection and dissemination of organism.

2.9. VIRULENCE FACTORS

Although Acinetobacter spp. are considered to be relatively low grade pathogens, characteristics that may enhance the virulence of strains involved in infections include;

- The presence of a polysaccharide capsule formed of L-rhamnose, D-glucose, D-glucuronic acid and D-mannose, which makes the surface of strains more hydrophilic.
• The property of adhesion to human epithelial cells in the presence of fimbriae and capsular polysaccharide.

• The production of enzymes which may damage tissue lipids.

• The potentially toxic role of the lipopolysaccharide component of cell wall and the presence of lipid A.

Several virulence determinants involved in biofilm formation, iron acquisition, lipopolysaccharide (LPS) synthesis and resistance to the bactericidal activity of human serum, adherence, host cell invasion, and death have been reported in previous studies. While these presumably encompass just a minor fraction of elements involved in A. baumannii virulence, new approaches are needed to expand our understanding of the basic features of this organism which will ultimately be essential to control the spread of A. baumannii infections and to develop effective means to prevent and treat this harmful pathogen.

2.10. TRIMERIC AUTOTRANSPORTER

Pathogenic and non pathogenic bacteria decorate their cell surfaces with, or secrete into the extracellular environment, many different proteins. The expression of proteinaceous virulence factors directly or indirectly affects the host and thereby increases the pathogen’s ability to survive and multiply; however, it should be remembered that non pathogenic organisms also secrete proteins adaptive to their lifestyles.

In the case of Gram positive organisms, surface proteins generally follow the sec dependent pathway to cross the cell membrane and are either released into the extracellular environment or remain anchored by virtue of one of several peptide anchoring signals. Secretion in Gram negative bacteria, however, is constrained by the presence of the outer membrane, which necessitates more complex and specialized protein secretion pathways. Evolutionary pressures and bacterial economics, which dictate that it is easier to adopt and adapt mechanisms than to develop new mechanisms independently, have resulted in the development of a
limited number of Gram negative bacterial secretion systems. To date, five protein secretion pathways have been defined among the Gram negative bacteria. Type I secretion is exemplified by the secretion pathway defined for *Escherichia coli* hemolysin (HlyA); here, accessory proteins are needed to construct a channel through the periplasm. The secretion of pullulanase (PulA) from *Klebsiella oxytoca* is the best studied example of type II secretion, which requires the action of 14 additional accessory proteins associated with various regions of the cell envelope. The type III secretion pathway requires a complex apparatus of proteins, which forms a tightly regulated oligomeric structure spanning the inner and outer membranes. Type IV secretion is perhaps the least understood of the Gram negative bacterial secretion apparatuses. The type IV export process involves the coordinate action of at least nine proteins, which are variously associated with the inner and outer membrane and which are localized within the periplasm and cytoplasm. [116].

**Autotransporters**

Given the intricate nature of the other secretion systems described above, the apparent simplicity of the autotransporter (or type V) secretion mechanism is astonishing. Proteins secreted by this mechanism possess an amino terminal signal sequence (with features required for passage through the sec translocon), a passenger domain and a carboxy terminal \(\beta\) domain (Figure.2.1.A). Although proteins secreted by the type II and type V pathways utilize the sec apparatus to traverse the inner membrane, they differ in how they pass through the outer membrane. Proteins secreted by the type V mechanism mediate their own translocation (hence the apt term autotransporter) across the outer membrane by virtue of their \(\beta\) domains; the \(\beta\) domain forms a pore in the outer membrane through which the passenger domain of the molecule is translocated to the cell surface. By contrast, proteins secreted by the type II secretion system require additional proteins for their secretion across the outer membrane [116].
The nomenclature for secretion systems has not always been as clear as this discussion implies; for many years, debate has continued about the nomenclature used to delimit protein secretion pathways. As each new extracellular or outer membrane protein is identified, it is expediently slotted into one or another category based on homology to defined systems or on a defined strategy. Yet, until recently, little was being done to redress the inconsistencies that have arisen by parcelling the proteins into such convenient categories. Such is the case with all proteins secreted across the inner membrane via the sec dependent mechanism. It has been suggested that all such proteins should be classified in the type II pathway and that steps involved in outer membrane secretion should represent different terminal branches of the pathway. However, the application of this rule presents its own problems. For example, autotransporters are generally secreted by the typical sec dependent system, which would make them a terminal branch of the general secretory apparatus. However, a handful of autotransporters have rather unusual extended signal sequences, and these proteins might utilize pathways other than sec. After inner membrane translocation, the passenger domain inserts into an outer membrane pore formed by a β barrel type structure (the so called β domain of the autotransporters) and passes through this pore to the surface of the bacterium. At the surface it can
undergo further proteolytic processing to achieve its physiological role. However, despite the similarities, some differences do exist between the classical and unlinked autotransporters. Most obviously, of course, the passenger protein and the pore forming β domain of the unlinked autotransporters are translated as two separate proteins, whereas the true autotransporters are produced as a single polypeptide. In addition, the pore forming unit of the unlinked autotransporters is predicted to consist of a 19 stranded β-barrel structure, whereas the β domain of the majority of autotransporters is predicted to comprise 14 strands.

The autotransporter family of proteins contains more than 700 members and is the largest family of gram negative bacterial extracellular proteins [117,118]. These proteins have a variety of effector functions, including adherence, invasion, proteolysis, cytotoxicity, serum resistance, and cell to cell spread, among others [119]. All autotransporter proteins share a common domain organization comprising an N-terminal signal peptide, an internal effector domain (also designated a passenger domain), and a C-terminal translocator domain (also designated a β domain). The process of autotransporter secretion begins with export across the inner membrane through a sec dependent process, initiated by the signal peptide. Subsequently, the translocator domain inserts into the outer membrane and forms a β-barrel structure. Ultimately, the passenger domain is extruded across the outer membrane and presented on the bacterial surface. (Figure 2.1 B)

**Differences between the translocator domains of conventional and trimeric autotransporters:**

The cardinal feature of autotransporter proteins is the presence of an outer membrane C terminal translocator domain that is capable of transporting a fused passenger domain from the periplasm to the bacterial surface. Examination of deletion mutants and chimeric constructs established that the translocator domain in conventional autotransporters is relatively uniform in size and consists of 300 amino acids [120,121]. Secondary structure predictions suggest that the translocator domain in these proteins contains 14 transmembrane anti parallel β-strands and a transmembrane α-helix and forms a β barrel with a central hydrophilic channel [120].
In recent work, Oomen et al. solved the crystal structure of the translocator domain from the *Neisseria meningitidis* NalP conventional autotransporter and found a monomeric $\beta$ barrel containing 12 transmembrane $\beta$-strands and a central pore, with a transmembrane $\alpha$-helix spanning the pore.

In contrast to observations with conventional auto transporters, studies of the YadA adhesin revealed that the C terminal 70 amino acids were sufficient for translocating an N terminal FLAG epitope across the outer membrane [122]. Similarly, experiments with the Hia adhesin demonstrated that the final 76 amino acids were capable of presenting a functional heterologous passenger domain on the bacterial surface [123]. Further biochemical analysis established that the C-terminus of both YadA and Hia formed heat resistant sodium dodecyl sulfate (SDS) resistant trimers in the outer membrane [122,123], raising the possibility that the short translocator domains function by oligomerizing. Given that the final 70 amino acids of YadA and the final 76 amino acids of Hia are predicted to contain four $\beta$ strands, we was proposed that the functional translocator is trimeric and contains four $\beta$ strands from each subunit in the trimer, resulting in a 12 stranded $\beta$ barrel reminiscent of *Escherichia coli* TolC and E. coli $\alpha$-hemolysin. Thus, the translocator domain appears to be a 12 stranded $\beta$-barrel with a single central channel in both conventional autotransporters and trimeric autotransporters, but the composition of the $\beta$-barrel is different, with $\beta$-strands from a single subunit in conventional autotransporters and from three subunits in trimeric autotransporters. Notably, work on *Neisseria gonorrhoeae* IgA1 protease raises the possibility that in some conventional autotransporters 6–10 $\beta$-barrels assemble to form a common channel.

On the basis of BLAST analysis, at least 43 additional Gram negative bacterial proteins have a C-terminus with significant sequence. Among these proteins, the Eib family, Omp100, DsrA, EmaA, Hsf, Hag, UspA2, UspA2H, NadA, NhhA and XadA have all been characterized to some extent. All possess the features of autotransporter proteins, and all, except possibly XadA, are capable of oligomerizing [124,125,126,127], suggesting that they are Hia like YadA like autotransporters. Phylogenetic comparison of the C-terminal 300 amino acids of a random selection of conventional autotransporter proteins and of all Hia like YadA
like autotransporters containing a pfam 03895 domain demonstrated that these two
groups of proteins form completely separate clusters, providing strong evidence that
the two groups are different phylogenetically.

The mechanism of surface localization of trimeric autotransporters:

By analogy to other Gram negative bacterial extracellular proteins, it was
hypothesized that the passenger domains in trimeric autotransporters are translocated
across the outer membrane through a pore, in this case a pore formed by the trimeric
β-barrel. In considering the mechanism of translocation, it is noteworthy that the
crystal structures of the Hia primary binding domain and the YadA collagen binding
domain are trimers [128] , indicating that the passenger domains in trimeric auto
transporters have a trimeric architecture and that all three passenger domains
connected to a single trimeric β-barrel are translocated across the outer membrane.
Physical constraints argue that trimerization of the passenger domains cannot occur
in the periplasm, in particular because a folded trimer would be unable to cross the
pore formed by a fused trimeric β-barrel. Accordingly, the passenger domains must
be translocated across the pore as separate polypeptides, probably unfolded or
partially unfolded. If the process of translocation begins at the junction between the
passenger domain and the translocator domain, with the C-terminal end of the
passenger domain being secreted first, a hairpin loop would be formed across the
outer membrane. In this model, the pore in the trimeric β-barrel must be large enough
to accommodate four to six unfolded polypeptides, depending on whether the three
subunits are translocated sequentially or simultaneously. However, if the process of
translocation begins with the N terminus of the passenger domain being secreted
first, the pore in the trimeric β-barrel must be large enough to accommodate three
unfolded polypeptides (one per subunit by the time all three passenger domains have
been translocated). All of these possibilities require the pore size of trimeric auto
transporters to be larger than the NalP pore, which is 1.25 nm and is large enough for
only two unfolded polypeptides [129]. Once all three subunits have been
translocated, it is possible that the free energy associated with trimer formation
provides the basis for protein folding and achievement of native confirmation,
perhaps explaining why trimeric autotransporters lack a BrkA like intra molecular
chaperone domain. Recent work suggests that the Omp85 outer membrane protein might play a role in translocation of the passenger domain of conventional autotransporters. Whether the same is true for trimeric autotransporters remains unclear.

![Type V Secretion System: Autotransporter Family](image)

Figure 2.2. Type V Secretion System: Autotransporter Family

Previous work carried out has shown that *A. baumannii* synthesizes the surface polysaccharide poly-N-acetyl(1-6)-glucosamine (PNAG) and that synthesis of this polysaccharide was both well conserved among clinical isolates and played a critical role in *A. baumannii* biofilm formation [20]. Moreover, it was demonstrated that PNAG was a target for opsonic and protective antibodies in two models of *A. baumannii* infection: pneumonia and bacteremia in immunocompetent mice [130]. To gain greater insight into *A. baumannii* virulence factors, an ORF was found in *A. baumannii* ATCC 17978, A1S_1032 that codes for a protein belonging to the trimeric autotransporter (TA) family, which was termed the Acinetobacter trimeric autotransporter, or Ata (Figure 2.3). TAs encompass a large family of proteins
produced by many Gram negative bacteria that contain a C-terminal domain that is believed to form the trimeric $\beta$-barrel that allows for the transport of the N-terminal passenger domain to the bacterial cell surface. These proteins form lollipop shaped surface projections on the bacterial surface and have been extensively studied as vaccine candidates against multiple pathogens. Two relevant examples of TAs as vaccine components include the *Neisseria meningitidis* NadA autotransporter (AT) which is undergoing phase III clinical evaluation against serogroup B meningococcal disease, and the conventional AT pertactin, produced by *Bordetella pertussis* strains, that is a component of four out of the five pertussis vaccines currently licensed for use in the United States. It was identified, in addition to PNAG, Ata that is also important for biofilm formation, binding to extracellular matrix/basal membrane (ECM/BM) proteins, and the adhesion of *A. baumannii* cells to collagen type IV, as well as for the survival of *A. baumannii* in a murine model of infection [131]. PCR analysis of 75 clinical strains of *A. baumannii* obtained from various geographical locations and types of infections revealed that 44/75 (58.6%) of the strains were positive for *ata* by PCR, 43 of which (56.3%) produced variable but mostly high levels of surface Ata, as detected by flow cytometry [131].

![Figure 2.3. Structure of Acinetobacter trimeric autotransporter](image)

The present study was conducted in two parts. Part-I: Evaluation of drug resistance in *Acinetobacter* (with special reference to carbapenemases genes such as OXA-23, OXA-58 and NDM-1). Part II: Evaluation of virulence factor in
Acinetobacter where the functional properties of antibodies raised to recombinant Ata protein was evaluated and specifically tested for their anti-adhesive, opsonophagocytic, and bactericidal activity in vitro and their protective efficacy in murine models of pneumonia in both immunocompetent and neutropenic mice.
PART-I

EVALUATION OF DRUG RESISTANCE IN ACINETOBACTER