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ACINETOBACTER BAUMANNII: AN EMERGING UNIQUE MULTI-DRUG RESISTANT PATHOGEN

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ABSTRACT
Acinetobacter baumannii complex emerged as a nosocomial opportunistic pathogen in intensive care unit probably as a consequence at least in part, of increasing use of broad spectrum antibiotics in hospital. It causes outbreaks of infection and health care–associated infections, including bacteremia, pneumonia, meningitis, urinary tract infection, and wound infection. Ventilator-associated pneumonia and bloodstream infections are most common, and mortality rates can reach 35%. Dealing with multi-drug resistant *Acinetobacter baumannii* is a great challenge for physician and clinical microbiologists not only due to its ability to survive in a hospital milieu but also because of the increase of mortality and morbidity associated with this pathogen. The changing trend in antibiotic susceptibility pattern from sensitive to resistance to commonly used antimicrobial agents creates problem in management of infections caused by *A. baumannii*. Polymyxins show reliable antimicrobial activity against *A. baumannii* isolates. Available clinical reports, although consisting of small-sized studies, support their effectiveness and mitigate previous concerns for toxicity. Minocycline, and particularly its derivative, tigecycline, have shown high antimicrobial activity against *A. baumannii*, though relevant clinical evidence is still scarce. This review summarizes the recent advances, with particular focus on evolutionary and genomic aspects, virulence factors, pathogenesis, antibiotic resistance, therapeutic regiments of *Acinetobacter baumannii* and also proposes new avenues of research.

KEY WORDS: *Acinetobacter baumannii*, Polymyxin, nosocomial, MDR.

INTRODUCTION
Acinetobacter baumannii has emerged as leading opportunistic pathogen and nosocomial pathogen worldwide.[1] It is responsible for variety of infection especially in ICU patients and the emergence of multidrug-resistant (MDR) strains.

In the clinical environment, *Acinetobacter baumannii* and its close relative (genomic species 3 and 13TU), together forming the `*A. baumannii* complex` are the genomic species of greatest clinical importance, together accounting for the vast majority of infections and hospital outbreaks.[7]

The different types of infections include pneumonia (both hospital and community acquired), septicaemia, endocarditis, skin and soft tissue infections, urinary tract infections, and meningitis. In most cases, it is thought that infections are acquired after exposure to *A. baumannii* that persists on contaminated hospital equipment or by contact with healthcare personnel that have been exposed to the organism through contact with a colonized patient.[2]

The risk factors for acquiring Acinetobacter infection include hospitalization, especially in Intensive Care Units (ICUs), poor general health status, and the performance of mechanical ventilation, cardiovascular or respiratory failure, previous antimicrobial therapy, and the presence of central venous or urinary catheters.[8] Commonly associated with aquatic environments,[4] it has been shown to colonize the skin as well as being isolated in high numbers from the respiratory and oropharynx secretions of infected invididuals.[5] A. baumannii has a high incidence among immunocompromised individuals, particularly those who have experienced a prolonged (> 90 days) hospital stay.[3]

In recent years, it has been designated as a ``red alert`` human pathogen, generating alarm among the medical fraternity, arising largely from its extensive antibiotic resistance spectrum.[6] The World Health Organization

(WHO) has recently identified antimicrobial resistance as one of the three most important problems facing human health.[9] The most common and serious MDR pathogens have been encompassed with the acronym ``ESKAPE,`` standing *Enterococcus faecium, Staphylococcus aureus, Klebsiella pneumoniae, Acinetobacter baumannii, Pseudomonas aeruginosa* and *Enterobacter* spp.[10]

Member of the genus *Acinetobacter* first began to be recognised as significant nosocomial pathogens during the early 1970s. In early in vitro studies, most clinical isolates were susceptible to commonly used antimicrobial agents, such as ampicillin, gentamicin, chloramphenicol and nalidixic acid, so that the infections caused by these organisms could be treated relatively easily.[11] However multidrug resistance clinical isolates of *Acinetobacter* spp have been reported increasingly during the last two decades, almost certainly as consequence of extensive use of potent broad-spectrum antimicrobial agents in hospitals throughout the world. The treatment of these infections is hampered by the rapid rise in prevalence of *A. baumannii* strains that are resistant to almost all available antibiotics, including β-lactams, fluoroquinolones, tetracyclines and aminoglycosides.[12] In these multidrug resistant (MDR) strains, colistin (also known as Polymyxin E) is often the only remaining treatment[13], although colistin-resistant clinical isolates have already been reported.[14]

HISTORICAL BACKGROUND

The history of the genus *Acinetobacter* dates back to the early 20th century, in 1911, when Beijerinick, a Dutch microbiologist, described an organism named *Micrococcus calcoaceticus* that was isolated from soil by enrichment in calcium acetate containing minimal medium.[15] Originally described as *Micrococcus calcoaceticus*, the genus *Acinetobacter* (coming from the Greek ``akinetos,`` meaning motionless) was proposed some 43 years later by Brisou and Prevot[16] to differentiate it from the motile organisms within the genus *Achromobacter*. The genus *Acinetobacter* was widely accepted by 1968 after Baumann et al. published a comprehensive study of organisms which concluded that *Acinetobacter belonged* to a single genus and could not be further sub-classified into different species based on phenotypical characteristics.[17]

Table – 1: Historical milestones

YEAR	INVENTION
1911	M. Beijernick, a Dutch microbiologist first isolated the organism named *Micrococcus calcoaceticus* (now recognized as *Acinetobacter*) from soil using minimal media enriched with calcium acetate.
1968	Brisou & Prevot, was first proposed the name *Acinetobacter* (Greek word Akinetos → motionless) to separate the nonmotile from the motile microorganisms within the genus *Achromobacter*.
1971	Sub-committee on the Taxonomy of Moraxella & Allied bacteria officially acknowledge the genus *Acinetobacter* based on Baumann`s 1968 publication.
1974	The genus *Acinetobacter* was listed with a single species *Acinetobacter calcoaceticus* in Bergey`s Manual of Systemic Bacteriology.

In 1971, the sub-committee on the Taxonomy of Moraxella and Allied Bacteria officially acknowledged the genus *Acinetobacter* based on the results of Baumann`s 1968 publication.[18] In the 1974 edition of *Bergey`s Manual of Systemic Bacteriology*, the genus *Acinetobacter* was listed with the description of a single species, *Acinetobacter calcoaceticus*.[19] 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*s were able to acidify glucose whereas others were not.[20]

TAXONOMY

The genus *Acinetobacter*, as currently defined, comprises Gram-negative, strictly aerobic, non-fermenting, non-fastidious, non-motile, catalase-positive, oxidase-negative bacteria with a DNA G + C content of 39% to 47%.[12] Based on more recent taxonomic data, it was proposed that members of the genus *Acinetobacter* should be classified in the new family *Moraxelaceae* within the order *Gammaproteobacteria*, which includes the genera *Moraxella, Acinetobacter, Psychrobacter*, and related organisms.[21] A major breakthrough in the long and complicated history of the genus was achieved in 1986 by Bouvet and Grimont, who-based on DNA-DNA hybridization studies-distinguish 12 DNA (hybridization) groups or genospecies, some of which are given formal species names, including *A. baumannii, A. calcoaceticus, A. haemolyticus, A. jhonsonii, A. junii*, and *A. lwoffii*.[22] In 1989, Tjernberg and Ursing described three additional DNA groups coded 13 through 15;[23] concurrently, Bouvet and Jeanjean described five DNA groups of proteolytic *Acinetobacter* species that they number 13

through 17.[24] More recently, 10 additional *Acinetobacter* species were described, including 3 species of human origin, *A. parvus, A. schindleri, and A. ursingii* [25] 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*.[26]

Table – 2 : Genospecies of *Acinetobacter* spp.

NAME	GENOMIC SPECIES	INVENTOR
A. calcoaceticus	1	In 1986 Bouvet & Grimont who based on DNA – DNA hybridization studies - distinguish 12 DNA (hybridization) groups or genospecies.
A. baumannii	2	
A. pittii	3	
A. haemolyticus	4	
A. junii	5	
--	6	
A. jhonsonii	7	
A. lwofii	8	
--	9	
--	10	
--	11	
A. radioresistens	12	
A. nosocomialis	13TU	In 1989 Tjernberg & Ursing described 3 additional DNA groups
--	14TU	
--	15TU	
--	13BJ	Bouvet & Jeanjean described 5 DNA groups of proteolytic Acinetobacter.
--	14BJ	
--	15BJ	
--	16BJ	
--	17BJ	

Acinetobacter genomic species 3 and 13TU, which were recently renamed *Acinetobacter pitti* sp. nov., and *Acinetobacter nosocomialis* sp. nov., respectively.[27] Four species of *Acinetobacter* i.e., *Acinetobacter baumannii, Acinetobacter calcoaceticus, Acinetobacter pittii* and *Acinetobacter nosocomialis* are saccharolytic and are very closely related & difficult to distinguish from each other by phenotypic properties and therefore been proposed to refer to these species as the *Acinetobacter calcoaceticus – Acinetobacter baumannii* complex.[28] However this group of organisms comprises not only the three most clinical relevant species that have been implicated in the vast majority of both community-acquired and nosocomial infections, i.e., *A. baumannii, A. pittii* and *A. nosocomialis,* 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.[12]

Table –3: Recently identified *Acinetobacter* spp.

NAME	ORIGIN
Acinetobacter parvus	Human origin
Acinetobacter schindleri	
Acinetobacter ursingii	
Acinetobacter baylyi	Activated sludge (recovered from sewage plants)
Acinetobacter bouvetii	
Acinetobacter tjernbergiae	
Acinetobacter towneri	
Acinetobacter tandoii	
Acinetobacter gerneri	

BIOLOGICAL CHARACTERISTICS

Morphological features: *Acinetobacter* may be identified presumptively to the genus level as gram-negative, catalase-positive, and oxidase-negative. During periods of rapid growth (exponential phase), the organisms typically appear bacillary to coccobacillary measuring 1 – 1.5 by 1.5 – 2.5 micron in size.

Gram staining morphology **:** After Gram straining they appear as Gram negative coccobacillary cells often appearing as diplococci. This similarity in appearance to *Neisseria gonorrhoeae* led to the archaic taxonomic genes designation ``*Mima*`` (to mimic). The organisms are often difficult to de-stain and may therefore be misidentified as either gram-negative or gram-positive cocci, (hence the former designation *Miae*).

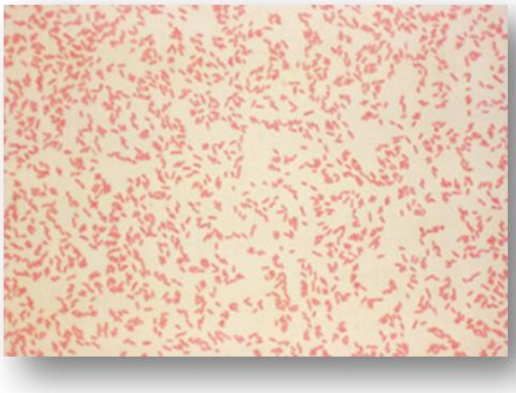
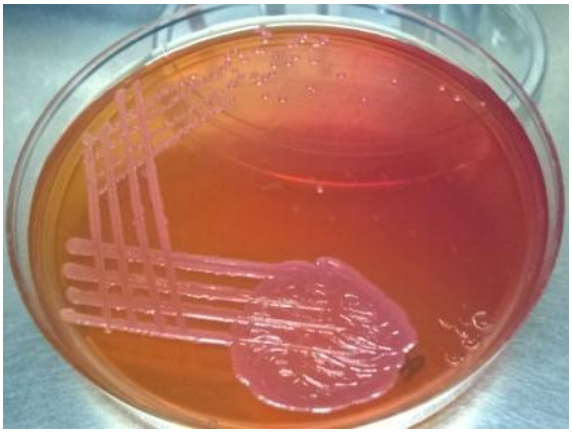


Fig. 1 : Gram negative coccobacilli

Cultural characteristics : Colony characters provide presumptive identification of the causative organisms. Though it is not a confirmatory way to diagnose the organism but can give idea which helps us to determine the right pathway to detect the organism. *A. baumannii* strains grow well on usual culture medium that are routinely used in clinical microbiology laboratories and produce colonies after overnight incubation at 37^0C.

Nutrient Broth
(Shows pellicle formation after overnight incubation)

Blood Agar
(Colonies are 0.5 – 2 mm in diameter, translucent to opaque, and it never shows pigmentation)



Macconkey agar
(Colonies are small pink to light lavender colour)
Fig. 2 : Colony characters of *Acinetobacter baumannii*

Biochemical properties : *A. baumannii* strains presented a large metabolic activity. They had the capacity to produce acid from glucose, xylose, galactose, manose, rhamnose and lactose. The production of acid from maltose and urea test is variable reactions. All strains were positive to Simon's citrate. The negative reactions: the acid production from manitol and sucrose, esculin hydrolysis, H_2S on TSI, Nitrate Reduction, Methyl Red and Voges-Proskauer.[29]

Table –4 : Biochemical properties of *Acinetobacter baumannii*

Test, substrate	***A. baumannii***
Fermentative or Oxidative	Oxidative
Catalase	Positive
Oxidase	Negative
Acid from Glucose	Positive
Xylose	Positive
Manitol	Negative
Sucrose	Negative
Galactose	Positive
Manose	Positive

Rhamnose	Positive
Lactose	Negative
Maltose	Variable
TSI	K/K
H_2S on TSI	Negtive
Simon citrate	Positive
Urease	Variable
Nitrate reduction	Negative
Methyl Red	Negative
Voges – Proskauer	Negative

Species Identification : Species identification with manual and semi automated commercial identification systems that are currently used in diagnostic microbiology, such as the API 20NE, Vitek 2, Phoenix and MicroScan WalkAway systems, remains problematic.[30] This is due to limited database content and substrate used for bacterial species identification has not been tailored specifically to identify *Acinetobacter*. In particular, the three clinically relevant members of *Acinetobacter calcoaceticus – Acinetobacter baumannii* Complex such as *A. baumannii*, *Acinetobacter* genomic species 3 & *Acinetobacter* genomic species 13TU are uniformly identified as *A. baumannii* by the most widely used identification systems. In referring to these species, it therefore seems appropriate to use the term *A. baumannii* group instead of *Acinetobacter calcoaceticus – Acinetobacter baumannii* Complex. This reflects *A. baumannii*, *Acinetobacter* genomic species 3 & *Acinetobacter* genomic species 13TU share important clinical and epidemiological characteristics and also eliminates the confusion resulting from inclusion of an environmental species *A. calcoaceticus.*

MOTILITY

Acinetobacter baumannii is a Gram-negative nosocomial pathogen that has been described as non-motile.[31] The genus name *Acinetobacter* derives from the Greek word *Akineto*, meaning motionless. However *A. baumannii* does display surface associated motility; first reported in isolates belonging *A.calcoaceticus – baumannii* complex in the 1970s. *A. baumannii* appears to be capable of both *Twitching* & *Swarming* like surface motility, probably via two distinct mechanisms.

Twitching motility is a flagella-independent form of bacterial translocation over moist surfaces. It occurs by the extension, tethering, and then retraction of *polar type IV pili***,** which operate in a manner similar to a grappling hook.[32]The term twitching motility was first coined by Lautrop in 1961[33] to describe flagella-independent surface motility in *Acinetobacter calcoaceticus*. Twitching motility is mediated by type IV pili located at one or both poles of the cell[34] and is distinct from swimming motility (such as in *Escherichia coli* and *P. aeruginosa*), which is mediated by the rotation of Unipolar flagella, and from swarming motility (such as in *Proteus mirabilis*), which is mediated by peritrichous flagella.

Recently, a form of motility in *A. baumannii* was reported that is decreased in the presence of light.[35] *Acinetobacter baumannii* senses and responds to blue light. Motility were observed only when bacterial cells were incubated in darkness. This is due to the production of light-sensing photoreceptors, with those harbouring a blue-light-sensing-using flavin (BLUF); light, oxygen, or voltage (LOV); or photoactive yellow protein (PYP) domain being the most prevalent, as predicted by in silico analysis of bacterial genomes.[36]

BIOFILM FORMATION

The *A.baumannii* MDR (Multi Drug Resistance) phenotype seems to play an important role in the remarkable capacity of the microorganism to persist and spread in the hospital environment, together with its ability to colonize both biotic and abiotic surfaces and to grow as biofilm.[37] Because of the presence of dormant cells, uncommonly found in other Gram-negative bacteria, the environmental persistence of *A. baumannii* fits the reported ability of some clinical isolates to survive for a long time on abiotic surfaces under desiccated conditions.[38] It is becoming evident that biofilm-forming ability can be considered one of the main virulence factors common to a large number of *A. baumannii* clinical isolates.[39]

Recently evidence has shown that biofilm formation at the solid-liquid interface is at least three times higher in *A. baumannii* than in the other *Acinetobacter* species, giving rise to a thick pellicle clearly visible on the top of broth culture.[40] As far as adhesion on abiotic surfaces is concerned, numerous studies have revealed a high propensity of *A. baumannii* clinical isolates to form biofilm on different substrata, such as glass or plastic.[39,41] The ability of *A.baumannii* to grow as biofilm on abiotic surfaces plays an important role in causing nosocomial infections, due to the surface colonization of hospital equipment and catheters, central venous catheters (CVCs), endotracheal tubes etc.[42] The biofilm formation of *A. baumannii* has been reported to be under the control of several factors, including the presence of antibiotic resistance genes, growth conditions and cell density.[43] The adhesion of *A. baumannii* to both biotic surfaces, such as bronchial epithelial cells, and to plastic surfaces is enhanced by the presence and expression of the bla_{PER-1} gene.[44]

NATURAL HABITAT

Member of the genus *Acinetobacter* are consider ubiquitous in nature that they can be recovered from almost all soil and surface water samples.[45] These earlier findings have contributed to the common misconception that *A. baumannii* is also ubiquitous in nature.[46] While not all *Acinetobacters* find their habitat in the natural environment, a thorough and systematic study to investigate the natural occurrence of the various *Acinetobacter* species in the environment has yet to be performed.[12] Equally misleading is the concept that *A. baumannii* is normal component of the human flora.

Acinetobacters are part of the human skin flora. In an epidemiological survey performed to investigate the colonization of human skin and mucous membranes with *Acinetobacter* species, upto 43% of nonhospitalzed individuals were found to be colonized with these organisms. The most frequently isolated species were *A. lwoffii, A. johnsonii, A. junii* and *Acinetobacter* genomic species 3.[47] In contrast, *A. baumannii*, the most important nosocomial *Acinetobacter* species, was found only rarely on human skin (0.5%)[48] & (3%)[47] and in human faeces (0.8%).[49] Interestingly *A. baumannii* was recovered 22% of body lice sampled from homeless people, suggesting another potentially important reservoir for the pathogen.[50]

On the basis of ecology, epidemiology, and antibiotic phenotype of different isolates, Towner proposed the existence of three major *Acinetobacter* populations.[51] One of them, which consists mainly of *A. baumannii* and closely related members of the *A. baumannii* complex, is represented by strains isolated from medical environments and equipment, medical personnel, and hospitalized patients. In general these isolates tend to be resistant to multiple antibiotics. The second population is represented by strains that can be found in human and animal skin flora as well as in spoiled food samples. Members of this group include *Acinetobacter johnsonii, Acinetobacter lwoffii,* and *Acinetobacter radioresistens*. The last group includes antibiotic-sensitive isolates obtained from environmental sources such as soil and wastewater samples and mainly comprises *Acinetobacter calcoaceticus* and *A. johnsonii*.

CLINICAL MANIFESTATION

Infections associated with *Acinetobacters* include ventilator-associated pneumonia, skin and soft-tissue infections, wound infections, urinary tract infections, secondary meningitis and blood stream infections.[52] Sporadic cases of conjunctivitis, osteomyelitis and synovitis have also been reported.[53] The risk factors for acquiring *Acinetobacter* infection include hospitalization, especially in intensive care units (ICUs), poor general health status, the performance of mechanical ventilation, cardiovascular or respiratory failure, previous antimicrobial therapy, and the presence of central venous or urinary catheters.[54]

Hospital – acquired pneumonia : Hospital-acquired pneumonia represents the most common clinical manifestation of *A. baumannii* infection. These infections occur most typically in patients receiving mechanical ventilation in the intensive care setting. It is thought that ventilator associated pneumonia caused by *A. baumannii* results from colonization of the airway via environmental exposure, which is followed by the development of pneumonia.[55] *Acinetobacter* species are a common cause of late-onset VAP, which occurs more than five to seven days after admission to the hospital, and such species are associated with a higher mortality rate than are other bacteria.[56] The crude mortality rate of ventilator associated pneumonia caused by *A. baumannii* has been reported to be between 40% and 70%.[57]

Community – acquired pneumonia : Community acquired pneumonia caused by *A. baumannii* much less frequent than nosocomial infection.[58] The disease most typically occurs during the rainy season among people with a history of alcohol abuse and may sometimes require admission to an ICU.[59] It is characterized by a fulminant clinical course, secondary bloodstream infection, and mortality rate of 40 to 60%.[58]

Bloodstream infections : *Acinetobacter* was a more common cause of ICU-acquired bloodstream infection than of non-ICU ward infection.[60] Risk factors associated with acquiring *A. baumannii* bloodstream infections include immunosuppression, ventilator use associated with respiratory failure, previous antibiotic therapy, colonization with *A.baumannii* and invasive procedures.[61] Crude mortality rates for *A. baumannii* bloodstream infections have been reported to be between 28%[62] and 43%.[60]

Burn infections : Burn infection can be especially problematic as it can delay wound healing and lead to failure of skin grafts, and wound site colonization can progress to infection of the underlying tissue and subsequent systemic spread of the bacteria.[63] *Acinetobacter baumannii* is an important cause of burn infections, although it can be difficult to differentiate between infection and colonization of burn sites. Because of the high rates of multidrug resistance and the poor penetration of some antibiotics into burn sites, these infections can be extremely challenging for clinicians. The prevalence of *A. baumannii* burn site infection likely varies considerably depending on institution and geographic location.

Traumatic battlefield and other wounds : *Acinetobacter* is a well documented pathogen of burns units and is difficult to treat in patients with severe burns.[63] *Acinetobacter baumannii* is commonly isolated from wounds of combat casualties from Iraq or Afghanistan.[64] It was the most commonly isolated organism in one assessment of combat victims with open tibial fractures.[65]

Soft tissue infections : Soft tissue infections caused by *A. baumannii* have emerged as a significant problem in military personnel sustaining war-related trauma in Iraq and Afghanistan.[66] Skin and soft tissue infections related to war injury can produce cellulitis and necrotising fasciitis which require surgical debridement in addition to antibiotic therapy.[66]

Meningitis : *Acinetobacter baumannii* is an increasingly important cause of meningitis, with the majority of cases occurring in patients recovering from neurosurgical procedures.[67] Although rare cases of community – acquired *A. baumannii* meningitis have been reported.[68]

Clinical features of *A. baumannii* meningitis are consistence with those of bacterial meningitis caused by other organisms and include fever, altered consciousness, headache, and seizure.[69]

Osteomyelitis : Osteomyelitis caused by *A. baumannii* occurs predominantly in military personnel sustaining war-related trauma and has become as a significant problem in U.S. military operations in Iraq and Afghanistan.[70]

Urinary Tract Infection : *Acinetobacter baumannii* is an occasional cause of UTI, being responsible for just 1.6% of ICU acquired UTIs in one study.[71] Typically, the organism is associated with catheter-associated infection or colonization.

In addition to the above-mentioned infections, *A. baumannii* is an infrequent cause of endocarditis. Individual case reports have described *A. baumannii* endocarditis associated with prosthetic valves[72] and intravascular catheters.[73]

PATHOGENESIS – VIRULENCE POTENTIAL

Despite extensive research into the virulence potential of this emerging pathogen, little is still known about its true pathogenic potential or virulence repertoire. While it is believed that several factors may contribute to the virulence potential of *A. baumannii* one factor in particular, OmpA, a member of the Outer membrane proteins (OMPs), has been determined to contribute significantly to the disease causing potential of the pathogen.[74] *A. baumannii* OmpA bind to the host epithelia and mitochondria, one bound to the mitochondria, OmpA induces mitochondrial dysfunction and causes the mitochondria to swell. This is followed by the release of cytochrome c, a heme protein, which leads to the formation of apoptosome. These reactions all contribute to apoptosis of the cell. OmpA may also facilitate the persistence and survival of *A. baumannii by* assisting biofilm formation and surface motility.[75]

Acinetobacter baumannii LPS contains a lipid a moiety, the carbohydrate core, and the repetitive O-antigen. The role of LPS in *A. baumannii* pathogenesis was recently investigated using a mutant lacking the LpsB glycotransferase that results in a highly truncated LPS glycoform containing only two carbohydrate residues bound to lipid A.[76] The mutant showed decreased resistance to human serum and decreased survival in a rat model of soft tissue infection compared with the isogenic parent strain, indicating a role for the surface carbohydrate residues of LPS in pathogenesis. It has also been demonstrated that CD14 and Toll-like receptor 4 play a role in clearing *A. baumannii* from the lung through detection of LPS[77] suggesting that *A. baumannii* LPS activates the innate immune response.

The structure of the capsular polysaccharides from two clinical isolates of *A. baumannii* were recently reported revealing a linear aminopolysaccharide consisting of three carbohydrate residues in one strain and a branched pentasaccharide in the other.[78] In addition to LPS the capsular polysaccharide has also been identified as a pathogenicity factor in *A. baumannii.* The capsular polysaccharide appears to play an important role in protecting bacteria from the host innate immune response.

Bacterial phospholipases are lipolytic enzymes that catalyze the cleavage of phospholipids. These enzymes are thought to contribute to the pathogenesis of gram negative bacteria by aiding in the lysis of host cells, via cleavage of phospholipids present in the host cell membrane, and by degrading phospholipids present at mucosal barriers to facilitate bacterial invasion. The key proteins that have been shown to contribute to *A.baumannii* virulence include phospholipases D and C. While phospholipases D is important for resistance to human serum, epithelial cell evasion and pathogenesis,[79] phospholipases C enhances toxicity to epithelial cells.[80]

Penicillin-binding proteins (PBPs) are most commonly associated with binding to and inactivating β-lactam antibiotics. However, PBPs also participate in the final steps of the biosynthesis of the peptidoglycan layer and thus contribute to bacterial cell stability.[81] An A. *baumannii* mutant with a transposon insertion in the *pbpG* gene, which encodes the putative low molecular-weight penicillin-binding protein PBP7/8, demonstrated reduced growth on ascities plates.[82]

Outer membrane vesicles (OMVs) are vesicles secreted from the outer membrane of various gram negative bacteria and consist of outer membrane and periplasmic proteins, phospholipids, and LPS. OMVs have been reported to participate in bacterial virulence by delivering virulence factors to the interior of host cells, to facilitate horizontal gene transfer, and to protect the bacterial from the host immune response.[83] Multiple stains of *A. baumannii* secrete OMVs during growth in vitro. A proteomic analysis of purified *A. baumannii* OMVs identified the OmpA protein as well as putative proteases and putative haemolysis as potential virulence factors. Moreover, OMVs could deliver OmpA to the interior of eukaryotic cells and induce cell death; exposure of cells to OMVs isolated from an *ompA*-deficient strain did not produce cell death.[84]

ANTIBIOTIC RESISTANCE

Acinetobacter baumannii is an encapsulated gram negative coccobacilli containing proteins, namely porins and efflux channels, on the outer cell membrane, which mainly contribute to their resistance mechanisms.[85] However as compared to other gram negative bacteria, it has fewer and smaller porin channels, which thereby decrease its cell permeability and increase its antibiotic resistance.[86] It was also discovered that the cell wall of the bacteria changes according to the environmental conditions, thus causing an increase in its thickness when

it is placed in a very dry conditions, thereby again providing extra resistance at high temperature also.[87] The irrational use of antibiotics in the ICU set up and the various bacterial mechanisms of resistance contribute to summation of resistance function for this untreatable, risky microorganism.[88] Risk factors[88] for colonization or infection with multidrug-drug resistant *A. baumannii* are.

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

There is rising concern about antimicrobial resistance among *Acinetobacter* species since the past decade.[89] Presence of porin channels, efflux mechanisms and the non static behaviour of the bacteria in the hot and humid conditions equip the species with extensive antimicrobial resistance.[90] *A. baumannii* labelled as MDR-Ab when it is resistant to more than two of the following five classes of antibiotics – [12]

1. Antipseudomonal cephalosporins (ceftazidime or cefepime);
2. Antipseudomonas carbapenems (imipenem or meropenem);
3. Ampicilin/Sulbactam;
4. Fluoroquinolones (ciprofloxacin or levoflaxacin);
5. Aminoglycosides (gentamicin,tobramycin or amikacin).

Carbapenem resistance : In the past years, carbapenems were considered as the most important agents or the treatment of infections caused by MDR *A. baumannii*. Carbapenem resistant *A. baumannii* (CRAB) is now emerging as a potential threat[91] and it is usually resistant to almost all antimicrobial classes except colistin and tigecycline, which have shown some promise against the this organism.[91] The presence of porin channels and other outer membrane proteins helps in the delivery of the drugs into the target proteins, for their antibiotic action. Unluckily, the porin channels are smaller and lesser in the *A. baumannii* strains, which prevent the entry of the drug molecules, which confer the resistance which is seen in case of carbapenems.[92] The most important mechanism of carbapenem resistance in *A. baumannii* is enzyme inactivation by the production of beta-lactamases, which hydrolyze the carbapenems. These hydrolyzing enzymes include metallo-β-lactamases (which have been sporadically reported in some parts) and class D β-lactamases (widespread). The main gene clusters responsible for this resistance are blaOXA-23-, blaOXA-24/40-, and blaOXA-58-like gene clusters. They are identified either in the chromosome or in plasmids of *A. baumannii* strains.[93] Another mechanism of reduced susceptibility to carbapenems are .

- ➢ Altered pencillin-binding proteins and porins
- ➢ Upregulation of the efflux system

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

Fluoroquinolones resistance : Fluoroquinolone resistance in *A. baumannii* is primarily due to mutations resulting in substitutions in the fluoroquinolone-resistance determining regions (QRDRs) of the target enzymes DNA gyrase and DNA topoisomerase IV.[90] Subunits of DNA gyrase and DNA topoisomerase IV are encoded by the genes *gyrA* and *parC*, respectively. The most common amino acid codon mutations that lead to fluoroquinolone resistance in *A. baumannii* occur at Ser 83 and Gly 81 within *gyrA*, and Ser 80 and Glu 84 within *parC*.[94] Clinically significant resistance to fluoroquinolones may be achieved with only a single mutation in *gyrA*.[95] However double amino acid substitutions in *gyrA* and *parC* genes are necessary for higher level resistance.[94] There has not been evidence of mutations in *parC* without a concurrent mutation in *gyrA*, which suggests that DNA topoisomerase IV could be a complementary target for fluoroquinolones.[96]

Another mechanism of fluoroquinolone resistance involves chromosomally encoded efflux systems that decrease drug concentrations within the bacterial cell.[97] Studies have shown a reversal of multidrug resistance in *A. baumannii* isolates in the presence of efflux pump inhibitors.[95] In *A. baumannii* the formerly mentioned RND type efflux pump (AdeABC) has been identified, and mutations in a 2-step regulator (AdeR) and sensor (AdeS) system lead to increased expression and increased efflux.[90] In some cases a single point mutation in a regulatory gene can lead to increased expression.[98] Intracellular accumulation of fluoroquinolones may also be limited by decreased production of outer membrane proteins.[97] These mutations alter membrane permeability, leading to inhibition of drug influx.[95, 97]

Aminoglycosides resistance : There are several known mechanisms of resistance employed by *A. baumannii* that affect aminoglycosides. The most prevalent mechanisms are the aminoglycoside-modifying enzymes (AMEs), specifically acetyltransferases, nucleotidyltransferases, and phosphotransferases.[12] This process results in deactivation of the aminoglycoside-modifying hydroxyl or amino groups, effectively reducing their affinity for the target binding site.[99] These AMEs are often found within class 1 integrons and can be located on either plasmids or chromosomes, which house gene cassettes, facilitating the development of resistance.[100] The presence of an AME in an organism can cause selective resistance to a specific aminoglycoside while other aminglycosides remain viable in high-level resistance across all aminoglycosides.

Another resistance mechanism is the production of 16S rRNA methylase (*armA, rmtA, rmtB, rmtC* and *rmtD).*[127] This mechanism alters the target binding site

for aminoglycosides with the 30S ribosomal subunit, but unlike AMEs, it result in high level resistance across all aminoglycosides.

Polymyxin-E (Colistin) resistance : Polymyxins are proposed to exert their antibacterial effect on Gram-negative bacteria via a two – step mechanisms – Initial binding to and permeabilization of the outer membrane followed by destabilization of the cytoplasmic membrane.[101] While the exact mechanism of bacterial killing is not clearly defined, a critical first step in the action of Polymyxins is the electrostatic interaction between the positively charged peptide and negatively charged lipid A, the endotoxic component of lipopolysaccharide (LPS).[102] Modification of lipid A, a component of LPS, with the addition of 4-amino-4-deoxy-l-arabinose (Ara4N) or/and phosphoethanolamine is considered to be the mechanism of colistin resistance in Gram-negative pathogens, such as *Salmonella* enteric and *Pseudomonas aeruginosa*.[103] This addition removes the negative charge of lipid A, thus lowering the affinity of positively charged colistin. However Ara4N biosynthesis and attachment genes are not present in *A. baumannii*, which suggests that Ara4N modification of lipid A is not suitable to explain colistin resistance in *A. baumannii*.[104] Around the key target of colistin, lipid A, there are currently two main hypotheses of the resistance mechanism.

The first is the loss of LPS hypothesis proposed by Moffatt et al.[105] and Henry et al.[106] Initially they found inactivation of lipid A biosynthesis gene *lpxA, lpxC* or *lpxD* – resulting in complete loss of LPS production in *A. baumanii*. The strains loss of LPS was tested to be colistin resistance.[105] They further found insertion sequence ISAba11 in either *lpxA* or *lpxC*, resulting in the complete loss of LPS production and a high level of colistin resistance.[105] in response to total LPS loss, *A. baumannii* alters the expression of critical transport and biosynthesis systems associated with modulating the composition and structure of the bacterial surface.[106] An LPS deficient colistin-resistant strain with a less negative charge might be the reason for a loss of affinity to colistin.[107]

The second is the PmrAB two-component system-mediated hypothesis. It was first proposed by Adams et al.[104] in 2009. By comparing the DNA sequence of genes encoding PmrA and PmrB between colistin-susceptible and resistant strains, they showed that mutations in the genes *pmrA* and *pmrB* are linked to colistin resistance in *A. baumannii*. There results indicate that increased expression of PmrAB system is essential for colistin resistance in *A. baumannii*, but amino acid alterations might not be essential for resistance.[108]

THERAPEUTIC STRATEGIES

As determined by the infectious Disease Society of America, *A. baumannii* is one of the ``red alert`` pathogens that greatly threaten the utility of our current antibacterial armamentarium.[109] Prior to the 1970s, it was possible to treat *Acinetobacter* infections with a range of antibiotics, including aminoglycosides, β-lactams, and tetracyclines.[110] However, resistance to all known antibiotics has now emerged in *A. baumannii*,[111] thus leaving the majority of today`s clinicians in unfamiliar territory. *Acinetobacter baumannii* acquired antibiotic mechanisms, includes degradation enzymes against β-lactams, modification enzymes against aminoglycosides, altered binding sites for quinolones, and a variety of efflux mechanisms and changes in outer membrane proteins.[12] Given the rapid and extensive development of antibiotic resistance, several attempts have been made to develop alternative control strategies for dealing with *A. baumannii* including, but not limited to the following.

Bacteriophage : Recently renewed interest in the area of antibacterial phage therapy has gained some traction.[112] Due to the high specificity of phage and their ability to work quickly, bacteriophage therapy is being re-examined as an alternative treatment to help counteract the phenomenon of antibiotic resistance.[113] Indeed, a recent study by Yang et al.[114] has resulted in the isolation and characterization of the virulent AB1 bacteriophage which has been shown to be effective against *A. baumannii* and as such represents a novel therapeutic of some potential.

Bacterial gene transfer therapy : The design and delivery of vectors containing bactericidal genes that can be introduced into recipient pathogenic organisms by conjugation using attenuated donor cell is referred to as bactericidal gene transfer therapy. Using this approach, Shankar et al.[115] have shows that mice treated with a single dose of 10^{10} CFU of donor cells containing bactericidal genes had lower levels of *A. baumannii* in burn wounds compared with untreated mice.

Radioimmunotherapy: Radioimmunotherapy can target microorganisms as quickly and efficiently as cancer cells.[116] This approach takes advantage of the specificity of antigen-antibody interactions to deliver radionuclides that emanate lethal doses of cytotoxic radiation directly to the target cell. Given that previous studies have already described the development of antibodies against *A. baumannii*[117] the application of radioimmunotherapy as a novel therapeutic strategy for *A. baumannii* is a definite possibility.

Photodynamic therapy : It involves the combination of nontoxic photosensitizers with oxygen and visible to produce reactive oxygen species that oxidize biomolecules thereby killing cells.[118] The use of photodynamic therapy to treat localized bacterial infections generally involves the topical application of a photosensitizers into the infected tissue, followed by illumination with red (or near-infrared) which is capable of penetrating the infected tissue.[119]

Nanoparticle technology : Nitric oxide has been shown to exhibit potent antimicrobial activity as well as playing an important role in modulating immunity[120] and regulating wound healing.[121]

CONCLUSION

In conclusion, *A. baumannii* is an important opportunistic and emerging pathogen that may lead to serious nosocomial infections. *A. baumannii* and its close relatives have become major cause of hospital-acquired infections primarily because of the remarkable ability of these bacteria to survive and spread in the hospital environment and to rapidly acquire resistance determinants to a wide range of antibacterial agents. The evidence suggests that hospital acquired *A. baumannii* infections prolong the lengths of hospital stays and subsequent health care costs. However, the direct effects of *A. baumannii* on mortality appear less well defined. Presently colistin is the only therapeutic option for most of the patients. Epidemic spread of MDR strains among patients in hospitals, particularly in ICUs, has been observed frequently with infected patients disseminating large numbers of these organisms into their environment. The problem is then compounded by the long-term survival of these organisms on numerous surfaces and inanimate objects, and by their high degree of resistance to drying, disinfectants and antibiotics. Consequently, once endemic, these organisms are extremely difficult to eradicate from a particular healthcare unit. Other special features of the genus *Acinetobacter* include the fact that, perhaps by accident, it has evolved a range of its own special resistance genes (particularly carbapenemases), as well as the capacity to over-express them in response to antibiotic challenges. It has also owned molecular mechanisms to capture resistance genes from other organisms, and has developed a range of expression mechanisms (e.g. provision of promoters on insertion sequences) that enables foreign resistance genes to be expressed. With the increasing clinical importance of *A. baumannii* and the emergence of MDR strains, new and novel therapeutics is required to control this pathogen.

REFERENCES

1. Choi JY, Park YS, Kim CO, Park YS, Yoon HJ, Shin SY, et al. Mortality risk factors of : *Acibetobacter baumannii* bacteraemia. Intern Med J., 2005; 35: 599-603
2. Rodriguez-Bano J, Garcia L, Ramirez E et al. Long term control of hospital-wide, endemic multidrug-resistant *Acinetobacter baumannii* through a comprehensive ``bundle`` approach. Am J Infect Control., 2009; 37: 715-722.
3. Montefour K, Frieden J, Hurt S, Helmich C, Headly D, Martin M, et al. *Acinetobacter baumannii:* an emerging multidrug-resistant pathogen in critical care; Crit Care Nurse., 2008; 28: 15-25.
4. Turton JF, Kaufmann ME, Gill MJ, Pike R, Scott PT, Fishbain J, et. al. Comparison of Acinetobacter baumannii isolates from the United Kingdom and the United States that were associated with repatriated casualties of the Iraq conflict. J. Clin Microbiol., 2006; 44: 2630-4.
5. Sebeny PJ, Riddle MS, Petersen K. *Acinetobacter baumannii* skin and soft-tissue infection associated with war trauma. Clin Infect Dis., 2008; 47: 444-9.
6. Cerqueira GM, Peleg AY. Insights into Acinetobacter baumannii pathogenicity. IUBMB Life 2011; 63:1055-60.
7. Van Looveren M, Goosens H, ARPAC Steering Group. Antimicrobial resistance of Acinetobacter spp. in Europe. Clin Microbial Infect., 2004; 10: 684-704.
8. M. A. R Popescu, S. Dumitru, S.E. Soare et.al. Phenotypic and genotypic characterization of antibiotic resistance patterns in *Acinetobacter baumannii* strains isolated in a Romanian hospital. Farmacia, 2010; 58: 3.
9. Bassetti M, Ginocchio F, Mikulska M. New treatment options against gram-negative organisms. Crit Care., 2011; 15: 215;
10. Rice LB. Federal funding for the study of antimicrobial resistance in nosocomial pathogens; no ESKAPE. J Infect Dis., 2008; 197: 1079-81;
11. Bergogne-Berezin E. Resistance of Acinetobacter spp. to antimicrobials – overview of clinical resistance patterns and therapeutic problems. CRC Press., 1996; 133-183.
12. Peleg AY, Seifert H. Peterson DL. *Acinetobacter baumannii*: emergence of a successful pathogen. Clin microbial Rev., 2008; 21: 538-82.
13. Li, J., R. L. Nation, J. D. Turnidge, R. W. Milne, K. Coulthard, C. R. Rayner, and D. L. Paterson. Colistin: the re-emerging antibiotic for multidrug resistant Gram negative bacterial infections. Lancet Infect. Dis., 2006; 6: 589-601.
14. Gales, A. C., R. N. Jones, and H. S. Sader. Global assessment of the antimicrobial activity of polymyxin B against 54731 clinical isolates of Gram negative bacilli: report from the SENTRY Antimicrobial Surveillance Programme (2001-2004). Clin. Microbiol. Infect., 2006; 12: 315-321.
15. Beijerinck, M. 1911. Pigmenten als oxydatieproducten gevormd door bacterien.Versl. Koninklijke Akad. Wetensch. Amsterdam., 1911; 19: 1092-1103.
16. Brisou J, Prevot AR. (Studies on bacterial taxonomy. X. The revision of species under acromobacter group). Ann Inst Pastuer (Paris)., 1954; 86: 722-8.
17. Baumann P. Doudoroff M, Stanier RY. A study of the Moraxella group. II. Oxidative-negative species (genus Acinetobacter). J Bacteriol., 1968; 95: 1520-41.
18. Lessel EF. International Committee on Nomenclature of Bacteria Subcommittee on the Taxonomy of Moraxella and Allied Bacteria; Minutes of the Meeting, 11 August 1970. Room Constitution C, Maria-Isabel Hotel, Mexico City, Mexico. Int J Syst Bacteriol., 1971; 21: 213-4.

19. Lautrop, H. 1974. Bergey`s manual of determinative bacteriology. Williams & Wilkins Co., Baltimore, MD.
20. Skerman, V. B. D., V. McGowan, and P. H. A. Sneath. 1980. Approved list of bacterial names. Int. J. Syst. Bacteriol., 1980; 30: 225-420.
21. Rossau, R., A. van landschoot., M. Gillis, and J.de Ley. Taxonomy of *Moraxellaceae* fam.nov., a new bacterial family to accommodate the genera *Moraxella, Acinetobacter,*and *Psychrobacter* and related organisms. 1991. Int J Syst. Bacteriol., 41: 310-319.
22. Bouvet, P. J., and P. A. Grimont. 1986. Taxonomy of the genus Acinetobacter with the recognition of *Acinetobacter baumannii* sp. Nov., *Acinetobacter haemolyticus* sp. nov., *Acinetobacter johnsonii* sp. nov. And *Acinetobactr junii* sp. nov., and emended description of *Acinetobacter calcoaceticus* and *Acinetobacrer lwoffii*. Int. J. Syst. Bacteriol., 1986; 36: 228-240.
23. Tjernberg I, Ursing J. Clinical strains of Acinetobacter classified by DNA-DNA hybridization. APMIS., 1989; 97: 595-605.
24. Bouvet PJM, Jeanjean S. Delineation of new proteolytic genomic species of the genus Acinetobacter. Res Microbiol., 1989; 140: 291-299.
25. Nemec A., T. De Baere, I. Tjernberg, M. Vaneechoutte, T. J. van der Reijden, and L. Dijkshoorn. 2001. *Acinetobacter ursingii* sp.nov. and *Acinetobacter schindleri* sp. nov., isolated from human clinical specimens. Int. J. Syst. Evol. Microbiol., 2001; 51: 1891-1899.
26. Carr, E. L., P. Kampfer, B. K. Patel, V. Gurtler,and R. J. Seviour. 2003. Seven novel species of Acinetobacter isolated from activated sludge. Int. J. Syst. Evol. Microbiol., 2003; 53: 953-963.
27. Nemec Krizova L, Maixnerova M et al. Genotypic and phenotypic characterization of the *Acinetobacter calcoaceticus-Acinetobacter baumannii* complex with the proposal of *Acinetobacter pittii* sp. nov. (formerly *Acinetobacter* genomic species 3) and *Acinetobacter nosocomialis* sp. nov. (formerly *Acinetobacter* genomic species 13TU).2011.Res Microbiol., 2011; 162: 393-404.
28. Gerner-Smidt, P., I. Tjernberg, and J. Ursing. 1991. Reliability of phenotypic tests for identification of Acinetobacter species. J. Clin. Microbiol., 1991; 29: 277-282.
29. Constantiniu S., Romaniuc A., Iancu S L., Filimon R., Tarasi I. Cultural and Biochemical characteristics of Acinetobacter spp. strains isolated from hospital units. The Journal of preventive medicine., 2004; 12(3-4): 35-42.
30. Bernards, A.T., J. van der Toorn, C. P. van Boven , and L. Dijkshoorn. 1996. Evaluation of the ability of a commercial system to identy Acinetobacter genomic species. Eur. J.Clin. Microbiol. Infect. Dis., 1996; 15: 303-308.
31. Bergogne-Berezin, E, & Towner, K. J. 1996. *Acinetobacter* spp. as nosocomial pathogens: microbiological, clinical, and epidemiological features. Clin Microbiol Rev., 1996; 9: 148-165.
32. John S. Mattick. Type IV Pili and Twitching motility.2002. Annu.Rev.microbiol., 2002; 56: 289-314.
33. Lautrop H. *Bacterium antiratum* transferred to the genus *Cytophaga*, Int. Bull. Bacteriol. Nomencl., 11: 107-8.
34. Bradely DE. A function of Pseudomonas aeruginosa PAO pili : twitching motility.1980. Can J. microbial., 26: 146-54.
35. Mussi, M. A., Gaddy, J. A., Cabruja, M., Arivett, B. A., Viale, A. M., Rasia, R. & Actis, L. A. 2010. The opportunistic human pathogen *Acinetobacter baumannii* senses and responds to light. J. Bacteriol., 2010; 192: 6336-6345.
36. Losi, A., and W. Gartner. Bacterial bilin and flavin-binding photoreceptors. 2008. Photochem Photobiol. Sci., 2008; 7: 1168-1178.
37. Gurung J., Khyriem A.B., Banik A., Lyngdoh A.V., Choudhury B., Bhattacharyya P. 2013. Association of biofilm production with multidrug resistance among clinical isolates of *Acinetobacter baumannii* and *Pseudomonas aeruginosa* from intensive care unit. Indian J. Crit. Care Med., 2013; 17: 214-218.
38. Gayoso C. M., Mateos J. M., Mendez J. A., Fernandez Puente p., Rumbo C., Tomas M., Martinez de Ilarduya O., Bou G. 2013. Molecular mechanisms involved in the response to desiccation st_{ress} and persistence in *Acinetobacter baumannii*. J. Proteome Res. Dec 17 (Equb ahead of print).
39. Eijkelkamp B. A., Stroeher U. H., Hassan K. A., Papadimitrious M. S., Paulsen I. T., Brown M. H. 2011. Adherence and motility characteristics of clinical *Acinetobacter baumannii* isolates. FEMS. Microbiol. Lett., 2011; 323: 44-51.
40. Marti S., Rodriguez-Bano J., Cate;-Ferreira M.,Jouennet T., Vila J., Seifert H., De E. 2011. Biofilm formation at the solid-liquid and air-liquid interfaces by *Acinetobacter* species. BMC. Res. Notes. 4, 5.
41. Tomaras, A. P., Dorsey C. W., Edelmann R. E., Actis L. A. Attachment to and biofilm formation on abiotic surfaces by *Acinetobactr baumannii*: Involvement of a novel chaperone-usher pili assembly system. Microbiology., 2003; 149: 3473-3484.
42. Djeribi R., Bouchloukh W., Jouenne T., Menaa B. 2012. Characterization of bacterial biofilms formed on urinary catheters. Am. J. Infect. Control., 2012; 40: 854-859.
43. Gaddy J. A., Actis L.A. Regulation of *Acinetobacter baumannii* biofilm formation. Future Microbiol., 2009; 4: 273-278.
44. Lee H. W., Koh Y. M., Kim J. C., Lee J. C., Lee Y. C., Seol S. Y., Cho D. T., Kim J. 2008. Capacity of multidrug-resistance clinical isolates of *Acinetobacter baumannii* to form biofilm and adhere to epithelial cell surfaces. Clin. Microbiol. Infect., 2008; 14: 49-54.

45. Baumann, P. 1968. Isolation of Acinetobacter from soil and water. J. Bactriol., 1968; 96: 39-42.
46. Fournier , P. E., and H. Richet. 2006. The epidemiology and control of *Acinetobactr baumannii* in health care facilities. Clin. Infect. Dis., 42: 692-699.
47. Seifert, H., L. Dijhshoorn, P. Gerner-Smidt, N. Pelzer, I. Tjernberg, and M. Vaneechouttee. 1997. Distribution of Acinetobacter species on human skin: comparison of phenotyupic and genotypic identification methods. J. Clin. Microbiol., 1997; 35: 2819-2825.
48. Berlau, J., H. Aucken, H. Malnick, andT. Pitt. 1999. Distribution of *Acinetobacter* species on skin of healthy humans. Eur. J. Clin. Microbial. Infect. Dis., 1999; 18: 179-183.
49. Dijkshoorn, L., E. van Aken, L. Shunburne, T. J. van der Reijden, A. T. Bernards, A. Nemec, and K. J. Towner. 2005. Prevalence of *Acinetobacter baumannii* and other *Acinetobacter* spp in faecal samples from non-hospitalised individuals. Clin. Microbiol. Infect., 2005; 11: 329-332.
50. La Scola, B., and D. Raoult. *Acinetobacter baumannii* in human body louse, Emerg, Infect. Dis., 2004; 10: 1671-1673.
51. Towner KJ. *Acinetobacter* : an old friend, but a new enemy. 2009. J Hospt Infect., 2009; 73: 355-363.
52. Bergongne-Berezin E. Resistance to *Acinetobacter* spp. to antimicrobials – overview of clinical resistance patterns and therapeutic problems. In: Bergongne-Berezin E, Joly – Guillou ML, Towner KJ, editors. *Acinetobacter*, microbiology, epidemiology, infections, management, Boca Raton: CRC Press., 1996; 133 – 183.
53. Glew RH, Moellering RC, Kunz LJ. Infections with *Acinetobacter calcoaceticus (Herellea vaginicola)*: Clinical and laboratory studies. Medicines., 1977; 56: 79-97.
54. Manuela-Anda RP., Silvia D., Simona ES., Gabriela B., Aurelian U., Manole C., Codruta V. Phenotypic and genotypic characterization of antibiotic resistance and patterns in *Acinetobacter baumabbii* strains isolated in a Romanian hospital. Farmacia., 2010; 58: 3.
55. Dijkshoorn L. Nemec A & Seifert H. An increasing threat in hospitals: multidrug resistant Acinetobacter baumannii. Nat Rev Microbiol., 2007; 5: 939-951.
56. Garnacho-Montero J, Oritz-Leyba C, Jimenez-jimenez FJ, Barrero-Almodovar AE, Garcia-Garmendia LJ, Bernabeu-Wittell M, et. al. Treatment of multidrug-resistant *Acinetobacter baumannii* Ventilator-Associated Pneumonia (VAP) with intravenous colistin: A comparison with imipenem-susceptible VAP. Clin. Infect Dis., 2003; 36: 111-18.
57. Garnacho J, Sole-Violan J, Sa-Borges M, Diaz E & Rello J. Clinical impact of pneumonia caused by *Acinetobacter baumannii* in intubated patients: a matched cohort study. 2003.Crit Care Med., 2003; 31: 2478-2482.
58. Leung WS, Chu CM Tsang KY, Lo FH & Ho PL. Fulminant community – acquired *Acinetobacter baumannii* pneumonia as a distinct clinical syndrome., 2006; Chest 129: 102-109.
59. Anstey NM, Currie BJ, Hassell M, palmer D, Dwyer B, Seifert H. Community acquired bacteremic *Acinetobacter* pneumonia in tropical Australia is caused by diverse strains of *Acinetobacter baumannii*, with carriage in the throat in at-risk groups. J. Clin Microbiol., 2002; 40: 685-6.
60. Wisplinghoff H, Bischoff T, Tallent SM, Seifert H, Wenzel RP & Edmond MB. Nosocomial bloodstream infections in US hospitals: analysis of 24,179 cases from a prospective nationwide surveillance study. 2004. Clin infect Dis., 2004; 39: 309-317.
61. Jung JY, Park MS, Kim SE et al. Risk factors for multidrug resistant *Acinetobacter baumannii* bacteraemia in patients with colonization in the intensive care unit. 2010 BMC Infect Dis 10: 228.dicotsof mortality.
62. Seifert H, Strate A and Pulverer G. Nosocomial bacteraemia due to Acinetobacter baumannii. Clinical features, epidemiology, and predictors of mortality.
63. Trottier V, Segura PG, Namias N, King D, Pizano LR & Schulman CI. Outcomes of *Acinetobacter baumannii* infection in critically ill burned patients. J Burn Care Res., 2007; 28: 248-254.
64. Yun H. C., C. K. Murray, S.A. Roop, D. R. Hospenthal, E. Gourdine, and D. P. Dooley. Bacteria recovered from patients admitted to a deployed U.S. military hospital in Baghdad, Iraq., 2006; Mil. Med. 171: 821-825.
65. Johnson, E. N., T. C. Burns, R. A. Hayda, D. R. Hospenthal, and C. K. Murray. Infectious complications of open type III tibial fractures among combat casualties. Clin. Infect. Dis., 2007; 45: 409-415.
66. Sebeny PJ, Riddle MS & Petersen K. *Acinetobacter baumannii* skin and soft-tissue infection associated with war trauma.2008. Clin Infect Dis., 2008; 47: 444-449.
67. Cascio A, Conti A, Sinardi L et al. Post-neurosurgical multidrug-resistant *Acinetobacter baumannii* meningitis successfully treated with intrathecal colistin. A new case and a systematic review of the literature.2010. Int J Infect Dis 14:e572-e579.
68. Ozaki T, Nishimura N, Arakawa Y et al. Community acquired *Acinetobacter baumannii* meningitis in a previously health 14 month-old boy. J Infect Chemother., 2009; 15: 322-324.
69. Rodriguez Guardado A, Blanco A, Asensi V et al. Multidrug-resistant *Acinetobacter* meningitis in neurosurgical patients with intra ventricular catheters: assessment of different treatments.2008. J Antimicrob Chemother., 2008; 61: 908-913.

70. Schafer JJ & Mangino JE. Multidrug-resistant *Acinetobacter baumannii* osteomyelitis from Iraq.2008. Emerg Infect Dis., 2008; 14: 512-514.
71. Gynes, R., and J.R. Edwards. Overview of nosocomial infections caused by gram-negative bacilli. Clin. Infect. Dis., 2005; 41: 848-854.
72. Olut AI & Erkek E. Early prosthetic valve endocarditis due to *Acinetobacter baumannii:* a case report and brief review of the literature.2005. Scand J Infect Dis., 2005; 37: 919-921.
73. Bhagan-Bruno S, Lather N & Fergus IV. *Acinetobacter* endocarditis presenting as a large right atrial mass : an atypical presentation. Echocardiography., 2010; 27: E39-E42.
74. Choi CH, Lee EY, Lee YC, Park TI, Kim HJ, Hyun SH, et al. Outer membrane protein 38 of *Acinetobacter baumannii* localizes to the mitochondria and induces apoptosis of epithelial cells. 2005. Cell Microbiol., 2005; 7: 1127-38.
75. Gaddy JA, Actis LA. Regulation of *Acinetobacter baumannii biofilm* formation.2009. Future Microbiol., 2009; 4: 273-8.
76. Luke NR, Sauberan SL, Russo TA et al. Identification and characterization of a glycosyltransferase involved in *Acinetobacter baumannii* lipopolysaccharide core biosynthesis.2010.Infect Immun., 2010; 78: 2017-2023.
77. Knapp S, Wireland CW, Florquir S et al. Differential roles of CD14 and toll-like receptors 4 and 2 in murine *Acinetobacter pneumonia.*, 2006.Am J Respir Crit Care Med., 2006; 173: 122-129.
78. Fregolino E, Gargiulo V, Lanzetta R, Parrilli M, Holst O & Castro CD. Identification and structural determination of the capsular polysaccharides from two *Acinetobacter baumannii* clinical isolates, MGI and SMAL., 2011; Carbohydr Res., 2011; 346: 973-977.
79. Jacobs AC, Hood I, Boyd KL, Olson PD, Morrison JM, Carson S et al. Inactivation of phospholipases D diminishes *Acinetobacter baumannii* pathogenesis., 2010;78: 1952-62.
80. Camarena L, Bruno V, Euskirchen G, Poggio S, Synder M. Molecular mechanisms of ethanol induced pathogenesis revealed by RNA-sequencing., PLoS Pathog., 2010; 6:e 1000834.
81. Sauvage E, Kerff F, Terrak M, Ayala JA & Charlier P. The penicillin-binding proteins: structure and role in peptidoglycan biosynthesis. FEMS. Microbiol Rev., 2008; 32: 234-258.
82. Russo TA, Benan JM, Olson R, MacDonald U, Luke NR, Gill SR & Campagnari AA. Rat pneumonia and soft tissue infection models for the study of *Acinetobacter baumannii* biology. Infect Immun., 2008; 76: 3577-3586.
83. Ellis TN & Kuehn MJ. Virulence and immunomodulatory roles of bacterial outer membrane vesicles.2010.Microbiol Mol Biol Rev., 2010; 74: 81-94.
84. Jin JS, Kwon SO, Moon DC, Gurung M, Lee JH, Kim SI & Lee JC. *Acinetobactr baumannii* secretes Cytotoxic outer membrane protein A via outer membrane vesicles. 2011.PLoS ONE., 2011; 6: e 17027.
85. van Looveren M, Goossens H; ARPAC Steering Group.``Antimicrobial resistance of Acinetobacter spp. in Europe.`` Clinical Microbiology and Infection., 2004; 10(8): 684 – 704.
86. Vila J, Marti S, Sanchez - Cespedes J. ``Porins, efflux pumps and multidrug resistance in Acinetobacter baumannii.`` Journal of Antimicrobial Chemotherapy., 2007; 59(6): 1210 – 5.
87. Houang ET, Sormunen RT, lai L, Chan CY, Leong AS. ``Effect of desiccation on the ultra structural appearances of *Acinetobacter baumannii* and *Acinetobacter lwoffii*.`` Journal of Antimicrobial Chemotherapy., 2007; 59(6): 1210 – 5.
88. Hujer KM, Hujer AM, Hulten EA, Bajaksouzian S, Adams JM, Donskey CS, et al. Analysis of antibiotic resistance genes in multidrug-resistant *Acinetobacter* sp. isolates from military and civilian patients treated at the Walter Reed Army Medical Centre.2006. Antimicrobiol Agents and Chemotherapy., 2006; 50(12): 4114-123
89. Lockhart SR, Abramson MA, Beekmann SE, et al. Antimicrobial resistance among gram negatrive bacilli as causes of infections in intensive care unit patients in the United States between. J Clin Microbiol., 1993 and 2004.2007; 45: 3352-359.
90. Bonomo RA, Szabo D. Mechanism of multidrug resistance in Acinetobacter species and pseudomonas aeruginosa. Clin Infect Dis., 2006; 43(Suppl 2): 49 – 56.
91. Zarrilli R, Giannouli M, Tomasone F, Triassi m, Tsakris A. Carbapenem resistance in Acinetobacter baumannii; the molecular epidemic features of an emerging problem in health care facilities. J Infect Dev Ctries., 2009; 3(5): 335 – 41.
92. Mussi MA, Limansky AS Viale AM. Acquisition of resistance to carbapenems in multidrug-resistant clinical strains of *Acinetobacter baumannii*: natural insertional inactivation of a gene encoding a member of a novel family of beta-barrel outer membrane proteins. Antimicrob Agents Chemother., 2005; 49: 1432 – 440.
93. Poirel L, Nordmann P. Carbapenem resistance in Acinetobacter baumannii; mechanisms and epidemiology. Clin Microbiol Infect., 2006; 12: 826 – 36.
94. Hamouda A, Amyes SG. Novel gyrA and parC point mutations in two strains of *Acinetobacter baumannii* resistant to ciprofloxacin.2004. J Antimicrob Chemother., 2004; 54: 695-6.
95. Spence RP, Towner KJ. Frequencies and mechanisms of resistance to moxifloxacin in nosocomial isolates of *Acinetobacter baumannii.*2003. J Antimicrob Chemother., 2003; 51: 177-80

96. Valentine SC, Contreras D, Tan S et al. Phenotypic and molecular characterization of *Acinetobacter baumannii* clinical isolates from nosocomial outbreaks in Los Angeles County, California.2008. J Clin microbial., 2008; 46: 2499-507.
97. Van looveren M, Goossens H. Antimicrobial resistance of *Acinetobacter spp.* in Europe., 2004; 10: 684-704.
98. Perez F, Huzer KM, Deer PN, Decker BK, Rather, Bonomo RA. Global challenge of multidrug-resistant *Acinetobacter baumannii*., 2007; 51: 3471-84.
99. Smith CA, Baker EN. Aminoglycoside antibiotic resistance by enzymatic dactivation.2002. Curr Drug Targets Infect Disord., 2002; 2: 143-60.
100. Yamane K, Wachino J, Doi Y, Kurokawa H, Arakawa Y. Global spread of multiple aminoglycoside resistance genes.2005. Emerg Infect dis., 2005; 11: 951-3.
101. Wiese, A., T. Gutsmann, and U. Seydel. Towards antibacterial strategies; studies on the mechanisms of interaction between antibacterial peptides and model membranes. J. Endotoxin Res., 2003; 9: 67 – 84.
102. Clausell, A,. M. Garcia-Subirats, M. Pujol, M. A. Busquets, F. Rabanal, and Y. Cajal. Gram – negative outer and inner membrane models; insertion of cyclic cationic lipopeptides. J. Phys. Chem. B., 2007; 111: 551 – 563
103. Ratetz CR, Whitfield C. Lipopolysaccharide endotoxins.2002. Annu Rev Biochem., 2002; 71: 635-700.
104. Adams MD, Nickel GC, Bajakasouzian S et al. Resistance to colistin in *Acinetobacter baumannii* associated with mutations in the PmrAB two component system.2009. Antimicrob Agents Chemother., 2009; 53: 3628-34.
105. Moffatt JH, Harper M. Harrison P et al. Resistance to colistin in *Acinetobacter baumannii* is mediated by complete loss of lipopolysaccharide production.2010. Antimicrob Agents Chemother., 2010; 54: 4971-7.
106. Henry R, Vithanage N, Harrison p et al. Colistin-resistant, lipopolysaccharide-deficient *Acinetobacter baumannii*. 2012.Antimicrob Agents Chemother., 2012; 56: 59-69.
107. Soon RL, Nation RL, Cockram S et al. Different surface charge of colistin-susceptible and resistant *Acinetobacter baumannii* cells measured with zeta potential as a function of growth phase and colistin treatment.2011. J Antimicrob Chemother., 2011; 66: 126-33.
108. Park K, Choi JY, Shin D et al. Correlation between over expression and amino acid substitution of the PmrAB locus and colistin resistance in *Acinetobacter baumannii.* 2011.J Antimicrob Agents., 2011; 37: 525-30.
109. Talbot, G. H., J. Bradley, J.E. Edwards, Jr., D. Gilbert et al. Bad bugs need drugs: an update on the development pipeline from the Antimicrobial Availability Task Force of the infectious Disease Society of America.2006. Clin Infect. Dis., 2006; 42: 657-668.
110. Bergogne-Berezin, E,. and K. J. Towner. *Acinetobacter* spp as nosocomial pathogens: microbiological, clinical and epidemiological features.1996. Clin. Microbial. Rev., 1996; 9: 148-165.
111. Falagas, M. E., and I. A. Bliziotis. Pandrug-resistant gram-negative bacteria: the dawn of the post-antibiotic era? .2001.Int. J. Antimicrob. Agents. 29:630-636.
112. Coates AR, Hu Y. Novel approaches to developing new antibiotics for bacterial infections.2007. br. J Pharmacol., 2007; 152: 1147-54.
113. Poole K. Overcoming multidrug resistance in gram negative bacteria.2003. Curr Opin Investig Drugs., 2003; 4: 128-39.
114. Yang H, Liang L, Lin S, Jia S. Isolation and characterization of a virulent bacteriophage AB1 of *Acinetobacter baumannii*.2010.BMC Microbiol., 2010; 10: 131.
115. Shankar R, He LK, Szilagyi A, Muthu K,Gamelli RI. Et al. A novel antibacterial gene transfer treatment of multidrug-resistant *Acinetobacter baumannii*-induced burn sepsis.2007. J. Burn Care Res., 2007; 28: 6-12.
116. Mihu MR, Martinez LR. Novel therapies for treatment of multi-drug resistant *Acinetobacter baumannii* skin infections.2011. Virulence., 2011; 2: 97-102.
117. McConnell MJ, Dominguez-Herrera J,Smani Y et al. Vaccination with outer membrane complexes elicits rapid protective immunity to multidrug-resistant *Acinetobacter baumannii.*, 2011; 79: 518-26.
118. Castano AP, Mroz P, Wu MX, Hamblin MR. Photodynamic therapy plus low-dose cyclophosphamide generates antitumor immunity in a mouse model.2008.Proc Natl Acad Sci USA., 2008; 105: 5495-500.
119. Demidova TN, Hamblin MR. Photodynamic therapy targeted to pathogens.2004. Int. J Immunopathol Pharmacol., 2004; 17: 245-54.
120. Mowbray M, Tan XJ, Wheatley PS, Rossi AG, MorrisRE, Weller RB. Topically applied nitric oxide induces T-lymphocyte infiltration in human skin, but minimal inflammation.2008. J Invest Dermatol., 2008; 128: 352-60.
121. Soneja A, Drews M, Malinski T. Role of nitric oxide, nitroxidative and oxidative stress in would healing.2005. Pharmacol Rep., 2005; 57(Suppl): 108-19.