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β-LACTAMASE IN *ACINETOBACTER SPECIES*: A GLOBAL THREAT

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ABSTRACT

Acinetobacter species are ubiquitous in nature. The members of genus *Acinetobacter* causes nosocomial infections. *Acinetobacter spp*. has emerged as globally challenging pathogen due to multiple antibiotic resistant. Major concern is that it shows resistance to all available β-lactam antibiotics classes such as carbapenems and third-generation cephalosporins. *Acinetobacter spp.* can survive on both natural and hospital environment, especially in intensive care unit and have been identified in military camps and predominantly isolated from burns and wounds unit. It is demonstrated that mobile genetic elements are responsible for the clonality spreads. Indeed, new antibiotics are much needed for the treatment since there are only limited options left for the clinicians. This review details about the nature, spread, clinical significance, detection method, current antibiotic regimen and highlights its public health threat.

KEYWORDS

Acinetobacter spp., β-lactamase, and current antibiotic resistances.

INTRODUCTION

In the present century nosocomial infections have become an important health threat issue because of the antibiotic resistance shown by bacteria[1]. The prevalence of antibiotic resistance among the pathogens is the reason for leading global threat. In general, β-lactamase are enzymes produced by certain bacteria to defend itself from the β-lactam antibiotics and β-lactam inhibitors. The modes of resistance shown by these bacteria are by hydrolyzing the β-lactam ring. More than 340 β-lactamase enzymes have been identified till date [2, 3]. The β-lactamase has created a challenge for clinicians, microbiologists and medical scientists. Over the past 30 years *Acinetobacter species* prevails as a clinically important organism by posing multiple drug resistance to the β-lactam antibiotics. However, one opportunistic species *Acinetobacter baumannii* attracted the close attention among the clinicians, scientists and microbiologists, which increased nosocomial infection around the world. Infections have been implicated in varieties like ventilator-associated pneumonia, bactereamia, meningitis, skin and soft issue infections, UT infection [4]. In U.S the surveillance data indicates that 5 to 10% ICU patients acquired pneumonia because of *A.baumannii* [5]. *Acinetobacter* have unique colonization ability to cause infection and it can colonize on any surface. This colonizing feature has created difficulty in the treatment [6]. *Acinetobacter spp.* has been showing resistance towards all antibiotic classes and this has become a huge crisis towards the human health-care and encountering this genera have become unstoppable. Mostly the infected patients and carriers serve as reservoirs for the transmission of these species [7, 8]. Patients in ICU generally acquire these nosocomial infections due to ill, weak immune system, and spreads through patient–to-patient, cross-contamination and it is likely that antibiotic selective pressure as a reason for this spread of several drug resistant genes [1, 9]. *A .baumannii* holds the second position next to *pseudomonas.* Its involvement in causing hospital acquired infections and raising the threat to intensive care unit have been reported[10].

TAXONOMY:

In 1911, a Dutch Microbiologist named Beijerinck isolated the organism from the soil and named it as micrococcus calcoaceticus. Until 1971 the genus was not identified[11]. The taxonomy of the *Acinetobacter* genus was developed recently. Morphological appearance of *Acinetobacter* is non-fermentative, Gram-negative coccobacilli with a DNA G+C content of 39-47%, non-motile, catalase positive and oxidase negative, aerobic and normal growth temperature is 20° C to 30° C [10]. Good growth occurs on solid media and in sheep blood agar at 37°C and colonies appears as non-pigmented. The taxonomic classification is complicated and confusing till date. There is significant taxonomic modification occuring from the family Neisseriaceae to Moraxallaceae based on DNA/DNA hybridization. It is more difficult to identify and distinguish the species between *A.baumannii, Acinetobacter calcoaceticus* and the unnamed Genospecies 3, 13TU (sensu Tjernberg and ursing) hence, they termed it as *A.baumannii* and *A. calcoaceticus* complex (abc). These complexes were associated with hospital

acquired infection [12, 13]. Species belonging to the genus *Acinetobacter* are listed below:

Acinetobacter baumannii, *Acinetobacter baylyi*, *Acinetobacter bouvetii*, *Acinetobacter calcoaceticus*, *Acinetobacter gerneri*, *Acinetobacter haemolyticus*, *Acinetobacter johnsonii*, *Acinetobacter junii*,*Acinetobacter lwoffi*, *Acinetobacter parvus*, *Acinetobacter radioresistens*, *Acinetobacter schindleri*, *Acinetobacter soli*, *Acinetobacter tandoii*, *Acinetobacter tjernbergiae*, *Acinetobacter towneri*,*Acinetobacter ursingii*, *Acinetobacter venetianus*.

Predominant pathogenic species of *Acinetobacter* are listed below:

A. baumannii and A. calcoaceticus (abc), A. *baumannii, A*. *lwoffi,* A. *Johnsonii,* A. *Ursingii,*A. *schindleri*[20]

EPIDEMIOLOGY:

These opportunistic pathogens of *Acinetobacter species* have been globally important nosocomial infection. Some of the reports have suggested that isolates from various countries like Europe, North American, Argentina, Brazil, China, Taiwan, Hong Kong, Japan, Korea, Iran and Afghanistan spreads during the outbreak[4]. This Infection depends on the rate of bacteria carried by the patient and on the persistence of organism in the environment. Recent studies have demonstrated that 19 %to 54 % of *Acinetobacter* infection has been associated with the mortality rates. In addition, the pathogen was also reported in 2004 Asian tsunami disaster [14]. *A.lwoffii* is the second clinically isolated species among *Acinetobacter* members[15]. These *Acinetobacter spp* can be isolated from numerous sources like water, soil, sewage, spoiled foods and animals. It can also survive in both moist and dry surface and can be isolated from hospital environment such as respiratory tract patients, throat infection patients and some of medical equipments[16]. To note, there is some interesting information that fruits and vegetables carries the organism and serve as a vector that involves in spreading these pathogens mostly in hospital environments. Studies reported that 41% of patients in ICU are infected through fruits and vegetables carried organism because of their inhabitant nature. *Acinetobacter* can colonize in aquatic conditions due to hydrophilic nature[17]. Studies have reported that 12% *Acinetobacter. Spp* shows resistance to the standard antibiotics, 80% to aminoglycosides and ciprofloxacin, 70% to Ceftazidime, 20-30% to β-lactam and β-lactam inhibitor. In 2004, 3fold raise of MDR has been reported by Livermore DM[18]. In contrast to hospital environment, it exists as a commanals on normal healthy skin and mucus parts of human. Another study suggested that *Acinetobacter spp.* remains as normal flora in healthy humans up to 42.5% and it increased upto 75% in hospital orientated patients; mostly patients with burns, wounds, malignant disease surgeries; patient with weak immune system are highly prone to infection when compared to normal healthy people[11]. These outbreaks are reliantly more in South Korea, Turkey, Spain and Europe [19]. Species of *Acinetobacter* that causes various diseases are given in table 1.

Table1
***Acinetobacter Spp.* infection [1]**

Acinetobacter spp.	Isolated From Disease
A.Haemolyticu	Endocarditic
A. Genospecies 3	Bacteraemia
A .junii	Sepsis in neonatal ICU, paediatric oncologist unit.
A.johnsonii	Cather-Related blood Stream Infection.
A.lwoffii	Meningitis,Peritonitis, Endocarditis,Endophthalmitis

CLASSIFICATION:

Currently, Molecular and Functional classification scheme for β-lactamase are widely in use. This classification is based on the increasing nature of β-lactamase, inactivation properties of the β-Lactamase against β-Lactam antibiotics, their biochemical properties, function and molecular structure of the enzyme[1]. The molecular classification of β-lactamases is based on the nucleotide and amino acid sequences and divided into four major classes A, B, C and D [21]. In 2010, Bush and Jacoby presented the functional classification of the β-lactamase. This was based on the antimicrobial substrate profile, enzyme inhibition profile, enzyme net charge, hydrolysis rate and on the inactivation properties of the β-lactamase inhibitors clavulanic acid, sulbactam and tazobactam and represented into 4 major groups 1 ,2, 3 and 4, and subgroups (a-f). According to these two classifications, ESBL belongs to Class A β-lactamase and 2be subgroup. Carbapenemase enzyme family belongs to 2df and class D. Most predominant MBL enzyme family belongs to 3a subgroups and class B[22].

VIRULENCE FACTOR:

A.baumannii infection mechanism was unclear. Virulence factors are limited and with low invasive potential. There are many factors that can influence in causing infection likely to be susceptible the host and increase the virulence potential of the organism. Infection depends on the immune system of host. The pathogencity is due to the presence of lipopolysaccharide (LPS) on outer membranes of *A.baumannii* and production of polysaccharide capsule and it renders the cell surface change and make more hydrophilic and so that it can enhance the virulence. In addition, virulence factor can induce infection in host by means of biofilm formation, lipase production can damage the lipids present in the tissues and elaboration of siderophores to scavenge iron that enhances the infection especially by novel catechol-based siderophores [23].

MECHANISM OF RESISTANCE BY ENZYME:

Acinetobacter are generally characterized as “naturally transformable” due to genetic exchange that encodes the antibiotic

resistance[12]. *Acinetobacter spp.* are resistant to all classes' β-lactam antibiotics with significant development to resistance acquired through plasmids transfer, transposons, and integrons[11]. Through the enzymatic drug modification these bacteria acquire resistance against the selected antibiotics. β-lactamase enzymes that can modify the antibacterial drugs or those activated and chemically transform into another form[24]. These resistant enzymes act by catalyzing process in two steps. In the first step, an active serine residue forms a covalent bond with the antibiotics by clearing the β-lactamase ring and in second step molecular hydrolysis of water takes place. The antibiotics-enzymes complex finally regenerates the active β-lactamase and releases the inactive antibiotics[5]. Presence of ISABA1 element increases the AmpC genes expression. Interpedently genome sequences analysis revealed several large genomic islands like AbaR, R_2, R_3 and R_5 associated with the multidrug resistant gene encoded by *Acinetobacter* that are acquired by transformation from other Gram negative species. Class D oxacillinase plays a specific resistance action in *Acinetobacter.* OXA-51 is an important gene present worldwide and also acts as genetic marker in identification of the organism to the species level. An OXA-51 enzyme carries an insertion element ISABA1 upstream of gene[25]. A mobile genetic element carries the antibiotics resistance genes that resist the activity of an antibiotic (drugs). Generally, mobile elements like plasmid, transposons and integrons are responsible for resistance. Transposons are discrete pieces of DNA located in the chromosome or on plasmids that are flanked by terminal inverted or direct repeats and carry the genes needed for movement to different locations within a bacterial cell and are responsible for turning genes on or off [26]. In addition whole genome comparisons have revealed the presence of several transposons in resistance island and indicate that structures were for the island dynamics [27]. Resistance genes within the transposons were stable and important in rapid transfer to other foreign resistance genes[27,28,29]. Integrons are gene expression elements that accumulate promoter less open reading frames (ORF's) known as gene cassettes. More than 40 different antibiotics resistance genes have been identified. In these units a mosaic genomic island carries multiple integrons isolated in *A.baumannii* Fournier et al. An 86kb genomic region was identified during genome sequencing of an epidemic MDR *A. baumannii* with a 26% mortality rate. The region contains 88 predicted ORFs. There are 45 antibiotic resistance genes located in 86Kb region and this area is known as hotspot due to the presence of antibiotic resistance genes[30]. The mode of resistance to β-lactam antibiotics in *A.baumannii* due to over expression of chromosome or production of plasmid-encoded class A, B, D β-lactam. *A.baumannii* produces variety of ESBL such as TEM, SHV, CTX-M, PER-1 and VEB-1 types around the world and PER-1 type is the most predominant in India. The mechanisms of β-lactam resistance include inaccessibility of the drugs to their target alterations and/or inactivation of the drugs by β-lactamase[30].

Class A β-Lactamases:

The First ESBL was isolated from Europe in 1980's and subsequently in U.S. Newly evolved ESBL enzymes have a greater ability to degrade all β-lactam antibiotics which lead to the emerging multiple drug resistance and these play a vital role for choosing the antimicrobial therapy for treatment. ESBL producing organisms increased due to prolonged exposure to the antibiotic, long term stay in ICU, illness such as immunocomprised patient and poor catheterisation[31].

TEM-1 was the first plasmid-mediated β-lactamase isolated from *E.coli* in 1965 and named after the patient Temoniera. TEM-1 β-

lactamase is present worldwide. *Klebsiella pneumonia* carried a novel plasmid mediated β-lactamase in 1984 and was isolated in France. More than 100 TEM type β-lactamase have been reported so far. TEM-1 and TEM-2 are derivatives of TEM type ESBL and share more similarities[32]. The new ESBL enzymes developed had expanded the substrate profile due to the continuous mutation. Main ESBL producing pathogens are *Enterobacteriacea* such as *E.coli, K. pneumonia, k.oxytoca, P.mirabilis, Enterobacter, salmonella spp.* Nonfermenter Gram negative bacilli *A. baumannii, p. aeruginosa.* These narrow-spectrum enzymes can inactivate benzylpenicillins. TEM 1 and TEM 2 are active against aminopenicillins; SCO-1 against penicillins and CARB-5 inactive carboxpenicillins have been reported. Many β-lactamase types belong to it. It has the ability to hydrolyze the wide spectrum of β-lactam antibiotics classes. These can activate the benzylpenicillins, cephalosporins, monobactams[1]. Apart from *Enterobacteriacea and P.aeruginosa*, ESBL have been reported in *A.baumannii* such as TEM-1, TEM-2, and CARB-5. These are clavulanic acid inhibited penicillinase[32]. PER-1 was the first ESBL isolated from *A.baumannii* in Turkey. This PER-1 ESBL is present throughout the world. PER-1 can show resistance to Penicillins and extended spectrum cephalosporins[1]. In 2003, the second most important ESBL found in *A.baumannii* was VEB-1 (Vietnamese extended spectrum β- lactamase) which was identified in France. Identical VEB-1 strains have been isolated from Belgium. ESBL in *Acinetobacter* may be either chromosomal or plasmid mediated. These ESBL type β-lactamase have been detected worldwide: 40% of ESBL have been detected in Turkey and 54 % ESBL isolated in Korea[33]. CTX-M-2 type ESBL has the ability to hydrolyze Cefotaxime and Ceftriaxone. CTX-M-2 in *A.baumannii* has been reported in Japan and CTX-M-43 identified in Bolivia. TEM-92, TEM-116 plasmid encoded genes in *A.baumannii* have been reported from Netherland. SHV-12 and SHV-5 chromosome encoded genes are also identified and reported. Along with these types, RTG-4, novel ESBL isolated in *A.baumannii* from France has been reported[33].

Class B β-Lactamase:

Metallo β-lactamase gradually increased due to their ability to inactivate β-lactam antibiotic like carbapenems which are widely used antibiotics against *A.baumannii.* Imipenem (N -formimidoyl thienamycin) and meropenem are the two commonly used carbapenem agents. MBL established several built-in antibiotic resistance mechanisms. These MBL's potential to extend the horizontal through gene transfer have been described[34]. Classifications of Metallo-β-lactamases were based on the presence of metal (iron or zinc) element in their active site. These enzymes have the ability to inactivate wide group of antibiotics like penicillins, cephalosporins, carbapenem. So far, five main types of MBLs have been reported throughout the world namely IMP, VIM, SPM, GIM and SIM[35]. These IMP MBLs group of enzymes is mostly detected as part in class 1 integrons. Variety of IMP variants 1, 2, 4, 5, 6 and 11 has been reported globally. IMP MBL was first described in *P.aeruginosa* which was isolated in Japan. IMP -1 have been reported from around Europe. Report from England also show IMP-1 in both *A.baumannii* and *A.junii.* The blaIMP-1 have been reported from Korean hospital. IMP1 isolated from *A.baumannii* and the gene identified in two different plasmid within integrons was reported from Taiwan and IMP4 was also detected from Hong Kong and Korea. Next predominant type VIM-1 (Verona integrons-encoded MBL) was identified *A. baumannii* from Greece[36, 37]. The VIM β-lactamase shares more than 40% amino acid similarity with the IMP enzyme. SIM-1 (Seoul

imipenemase) has been found in *A.baumannii*[37]. Recent novel SIM type MBL have been reported from tertiary care hospital in Seoul. Korea shows 17 % of *Acinetobacter spp* and 96% identical to either blaIMP-1 or blaVIM-2 allele. These belong to subclasses B1[34, 37].Four B1 subclasses enzymes with accession numbers are given in table 2. Mostly, these enzymes exhibit low resistance to imipenem and meropenem and genes of blaSIM located in class 1 integron. GIM-1 has been reported in Germany [35].

Table 2
Metallo-β-lactamase

Subclasses	Enzyme	Strain	Discovery in	Gene bank accession no.	Accession no.(protein)
Acquired B1	VIM-1	*A.baumannii*	1999	Y18050	CAE46717
	VIM-2	*A.baumannii*	2000	AF191564	AAK26253
	IMP-2	*A.baumannii*	2000	AB182996	BAD26594
	SIM-1	*A.baumannii*	2005	AY887066	AAX76774

Class C β-Lactamases:

These enzymes have the ability to hydrolyze pencillins and Caphalosporin but not cefepime or carbapenems. These are chromosomally encoded in A.baumannii. [1] Some early phylogenetic analysis studies have indicated that AmpC genes are closely related to the common β-lactamase ancestor gene which has been reported as ADC [*Acinetobacter*-derived caphalosporinases) from the Genbank. Presence of insertions sequence (IS) are small mobile genetic elements which are capable of inserting themselves at multiple sites in target molecule and these can provoke the mutation as a result of translocation and activate the adjacent genes .These IS element serves as switch to turn on in chromosomes bla_{ADC} genes[1,38].

Class D β-Lactamase:

The most common mechanism responsible for carbapenem resistance in *A. baumannii* was the production of carbapenem-hydrolyzing β-lactamase. The resistance in bacteria was mediated by any one of following enzymatic inactivation, active efflux of drugs, and modification of target sites. Three main acquired carbapenem-hydrolyzing class D oxacillinase (CHDL) gene clusters have been identified either in the chromosome or in plasmids of *A. baumannii* strains, represented by the *bla*OXA-23-, *bla*OXA-24/40-, and *bla*OXA-58-like genes[39]. There are 4 divided Phylogenetic subgroups, such as subgroups1 (OXA 23 Like) that contains the OXA23, 27, 49 β-lactamase, subgroups 2(OXA 24 like) that contains the OXA 24, 25,26, 40 and shares 60% amino acid a identify with respect to the subgroups1,subgroups 3 that contains the OXA 51 and 56% and 63% amino acid Subgroup 4-recently characterized OXA 58 enzymes that has 59% with subgroups 1 and 2)[1].

Carbapenem-hydrolyzing oxacillinase (CHDL'S) type OXA 23 type was

first identified in Scotland in 1995. More OXA type variants isolated from various parts of the world in the past and present. These OXA-24, OXA-25, OXA-40 type have been reported. OXA 24 and OXA 25 variants are found in Spain. There are predominant OXA types like OXA 26, OXA 40, and OXA 58 and these are isolated in Belgium, France, Toulouse (France) respectively. In some conditions, analogue of OXA 58 have been identified in Tunisia and it has been termed as OXA-97. These OXA type carbapenemase in *A.baumannii* OXA-23 was first isolated from Scotland. *Acinetobacter* resistant to imipenem (ARI-1) have been isolated in England, Brazil, Singapore, Korea, and China. OXA-58, a plasmid borne carbapenemase was reported from France, England, Argentina, Spain, Turkey, Romania, Austria, Greece, Scotland and Kuwait[36].

SURVEILLANCE

The surveillance system was useful for analyzing the collected data from various sources that helped to monitor the rate of change in prevalence of antibiotics resistant strain and persistence of antibiotic pattern[32]. Recent studies from India have reported the prevalence of Carbapenem resistant *Acinetobacter spp*. In 2003, Mumbai has published a report that was conducted during 1996-1998 which showed that 29% *Acinetobacter* isolates was resistant to imipenem. Subsequently, another study in tertiary health care center of North India, suggested that 18.5% of *Acinetobacter spp.* was resistant of Imipenem. Both *P.aeruginosa* and *Acinetobacter spp* have showed high resistance to imipenem about 17.32% and 22-16% to meropenem was reported from recent studies by AIIMS, New Delhi, India [40]. Other Surveillance studies of last ten years indicated an increased percentage of carbapenem-resistant isolates in Europe, North America and Latin America[41] and bla_{OXA-58} are frequently found in Europe, bla_{OXA-23} from Asian countries, South America and Europe has been reported. In 2005, isolates showing 87.5 % of carbapenem resistant MBL were reported from Chennai, India. Another study from India in 2008 have been reported the prevalence of MBL[42]. Irfan *et al.*, conducted a study in 2008 at Aga Khan University, Karachi, and it shows 96.6% of the carbapenem resistance in *Acinetobacter baumannii* were MBL producers [40]. This MDR *Acinetobacter spp*. becomes a serious infection to control in hospitals areas as well as problematic in choosing appropriate antibiotic therapy. All classes, places and location are given in table 3.

Table 3
β -lactamase identified in Acinetobacter spp

β -lactamase	Place	Location	Classes
PER-1	Turkey	Transposon	A
VEB-1	France, Belgium	Plasmid or chromosomal	A
CTX-M-2	Japan	Plasmid	A
CTX-M-73	Bolivia	Plasmid	A
TEM-92	Netherland	Plasmid or chromosomal	A
CHDC's OXA 23	Scotland	Plasmid	D
OXA 24, 25	Spain	Plasmid	D
OXA 26	Belgium	Plasmid	D
OXA 40	France	Plasmid	D

OXA 58	Toulouse, France	Plasmid	D
OXA 97(analogue to OXA 58)	Tunisia	Plasmid	D
IMP 1	Italy, Japan, South Korea	Class 1 integron	B
IMP2	Italy, Japan, South Korea	Class 1 integron	B
IMP 5	Portugal	Class 1 integron	B
IMP 6	Brazil	Class 1 integron	B
VIM1	Greece	Class 1 integron	B
VIM 2	South Korea	Class 1 integron	B
SIM 1	South Korea	Class 1 integron	B

TREATMENT AND CURRENT ANTIBIOTIC:

Screening the β-lactamases production indirectly helps the physician for selecting a suitable antibiotic therapy[43]. *Acinetobacter spp*. especially multiple drugs resistant isolated from *A.baumannii* is one of the current worries in antibiotic community. Reports from various data analyses indicated that ESBLs and MBLs enzymes from *Acinetobacter spp.* would receive major concern in future[44]. Genetic adaptability is one of the reasons for growing MDR strain. Carbapenems β-lactam antibiotics are the first choice of drug for the *A.baumannii* but developing resistance toward carbapenems have been reported in certain parts of world. Polymyxins, monocycline, tigecycline derivative show reliable resistance [44]. Currently, Colistin and polymyxins were in use to treat MDR strain. Unfortunately, colistin have been found to produce toxic character, which mainly effects the renal system, have been observed. Polymyxins (B and E) was abandoned during 1960'-1970' despite its nephrotoxicity and neurotoxicity. It was re-established again by decreasing dosage, modification in drug formulation and ventilation in ICU patient[45]. Onset progress of Tigecycline have been demonstrated through invitro testing and significantly developed as better antibiotics for the treatment. Doripenem is used against *Acinetobacter.spp*[46]. Combination drug therapies have been under investigation; dual or triple antibiotic combinations are recommended but with disadvantages reported. New glycyleycline antibiotic showing reasonably better invitro have been reported and clarifications are much needed regarding the antibiotics choices[44].

CONCLUSION

Today, *Acinetobacter species* gives more troubles to the clinicians. Regarding *A.baumannii, it has* emerged as a global threatening nosocomial infection with remarkable growth in resistance to the recent drugs. Studies demonstrate that colistin-resistance genes have been reported by Beno and Co-worker. Moreover, antibiotic lists became shorten for the future treatment. Major concern about *Acinetobacter spp.* Carbapenemase and Metallo β-lactamase are becoming worldwide. Rapid and accurate identification will help to detect these resistance genes. It is clinically important to know the mechanism of emerging multidrug resistance and to evaluate their dissemination and more important is to limit the emergence and spread of resistant organism and the appropriate

research work are much required. Recent studies indicate the significance of Phenotypic and genotypic methods which have become appropriate diagnostic procedure for the investigation and for therapeutic treatment. These researches will help to find a new set of antibiotics to the MDR organism.

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