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Microbiology

NOSOCOMIAL INFECTIONS AND ANTIMICROBIAL RESISTANCE DUE TO *ACINETOBACTER BAUMANNII*

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KEYWORDS :

INTRODUCTION:

Nosocomial infections or hospital associated infection occur worldwide and mainly affect developing countries. These infections are the major causes of high mortality and increased morbidity among hospitalized patients. It is estimated that more than 75% mortality of patients are directly or indirectly related to nosocomial infections[1].

The most common causative agents of nosocomial gram-negative infections are members of the family Enterobacteriaceae. In contrast, non lactose fermenting organisms, most prominently, *Pseudomonas* and *Acinetobacter* species, as well as less common organisms *Stenotrophomonas, Burkholderia*, and *Achromobacter* species[2].

Emergence of nosocomial infection by *Acinetibacter baumannii*

Among the *Acinetobacter* genomic species, *Acinetobacter baumannii* is recognized as species most frequently isolated from patients. *A.baumannii* have been isolated from various types of opportunistic infections[3,4,5,6]. The true frequency of nosocomial infection caused by *A.baumannii* is not easy to assess, partly because the isolation of these organisms from clinical specimens may not necessarily reflect a true infection but, it could be colonizer[7].

The rate of Acinetobacter infection has constantly increasing. In 2002 Irebue et al. reported 4.6%, Joly guillow 2005 reported 9% and in 2013 Nwadik et al reported 14% of isolates were Acinetobacter[3,8,9]. During the study period however, from March 1, 2003, to May 31, 2004, the rate of Acinetobacter isolation increased 3-fold[10]. In 2017, we reported 10% of ICU associated infection due to *A.baumannii*.

Thus, *Abaumannii* is emerging as an increasingly important multiple drug resistant pathogen, spreading in hospitals, and causing severe adverse outcomes. It has become a leading nosocomial pathogen in many hospitals.

Phenotypic Identification of *A.baumannii*

A.baumannii are non-fermenting Gram-negative coccobacilli, non-motile, strict aerobic, oxidase negative and catalase positive. Its cells are often found in diploid formation or in clusters of variable length[11].

The morphology of *Acinetobacter* spp is variable in Gram stained human clinical specimens and thus cannot be used to differentiate *Acinetobacter* from other causes of common nosocomial infections. *A.baumannii* are non-lactose fermenting bacteria, however they partially ferment lactose on MacConkey agar. All the species of the *Acinetobacter* genus grow well on MacConkey agar. *Acinetobacter baumannii* are strict aerobes and grow well on nutrient agar.

Its colonies are pale yellow to grayish white, like coliforms, on solid media and are sometimes strongly mucoid at 37°C. Good growth also occurs at high temperature. Occasional strains may require methionine for growth on minimal medium[12]. Nitrates are not reduced to nitrites in complex medium (exceptions occur). Acid is produced from D-glucose by most strains. Strains which produce acid from D-glucose also produce p-xylosidase. y-Glutamyltransferase is produced by most strains. Horse or sheep blood is not hemolyzed; gelatin is not hydrolyzed. Citrate (Simmons) is utilized by prototrophic strains. Auxotrophic strains utilize citrate when the medium is supplemented with a growth factor. Histamine is not utilized. The G+C content of the DNA is 40 to 43 mol%. Isolated from human clinical specimens or the natural environment. Most Acinetobacter strains isolated from human patients belong to this species. The type strain is strain CIP 70.34 (= ATCC 19606)[13].

Genotypic Identification of *A.baumannii*

Taxonomy of the genus *Acinetobacter* has revealed 23 validly named species and a number of DNA-DNA hybridization groups (genomic species) with provisional designations[14]. However, the identification of *Acinetobacter* at species level is not very easy task. The need for identification of *Acinetobacter* spp. to species level in routine clinical practice has long been debated. From a clinical and infection control perspective, however, it is mandatory to distinguish between the *A. baumannii* group and *Acinetobacter* spp. outside the *A. baumannii* group, since the latter organisms are only it shows quite variable that hindered progress understanding the pathogenic role of *Acinetobacter*[15]. Thus, precise identification of *Acinetobacter* species would be significant in epidemiology, and infection control policies [16]. Since 1986, the taxonomy of the genus *Acinetobacter* was reassessed; molecular methods provided the necessary tools to identify *Acinetobacter* at the species level. Detailed studies of the epidemiology of the different members of this genus became possible using genotyping methods[17]. There have been different molecular methods developed to identify of *Acinetobacter* spp.[18].

However, most work on the development of molecular methods has been dedicated to developing methods for distinguishing the individual genomic species. The "gold standard" method is DNA-DNA hybridization[19], but this technique is rather laborious and is normally used only in special situations in reference laboratories. Consequently, many research groups have concentrated on the development of alternative molecular methods for distinguishing individual genomic species.

As an alternative to direct sequence-based identification, a range of more rapid molecular fingerprinting methods have been developed for distinguishing individual genomic species, with varying degrees of success. These methods can be divided into those based on structural features, such as outer-membrane protein patterns[20], and those based on nucleic acid analysis. The most widely used techniques amongst the latter group include amplified fragment length polymorphism (AFLP) analysis[21], amplified rDNA restriction analysis(ARDRA)[17] ribotyping[22], tDNA spacer fingerprinting[23], 16S-23S spacer analysis[24], and 16S rDNA sequencing[25].

A.baumannii has a naturally occurring carbapenemase gene intrinsic to this species[26,27]. The first report of this gene described bla_{OXA-51} [28], but since then a large number of closely related variants have been found [26,27,28] and we have referred to them collectively as "$bla_{OXA-51-like}$" genes. Carbapenem resistance has only been associated with these genes when the insertion sequence IS*Aba1* is upstream[27] and is not an indicator of whether an isolate has such a gene. Although it is clear that $bla_{OXA-51-like}$ genes are present in at least the vast majority of isolates of *A. baumannii*, there has been some debate as to whether they are present in all isolates of this species[29,30]. If they are consistently found and are also unique to this species, then their detection could provide a simple and convenient method of identifying *A. baumannii* which could more easily be carried out than current definitive methods.

Risk factors for developing *A.baumannii* infections

A.baumannii can survive on various surfaces within hospital settings, including catheters and other medical equipments. Thus environmental contamination is an important source of infection as pathogens are spread directly from surfaces or through poor hand hygiene of healthcare workers to patients[31]. The main risk factors of *Acinetobacter* infections are invasive procedure e.g. catheterization, mechanical ventilation, nasogastric tube,[32,33] another major risk factors are prolonged hospital stay, ICU stay, widespread use of broad

spectrum antibiotics or previous use of third generation cephalosporins[34].

The risk factors within the ICU's concern the immunosuppressed patients, high invasive procedures and patients who suffered from previous sepsis[35]. Other risk factors include inappropriate empirical treatment[36] and in case of emergency Surgical procedures may contributing to the spread of *A.baumannii*, however Villers et.al. found the previous use of fluoroquinolones as a main risk factor[37].

CLINICAL MANIFESTATIONS OF *A. BAUMANNII* INFECTIONS

Acinetobacter are a heterogenous group of organisms that have emerged as significant nosocomial pathogens mainly affecting patients with impaired host defenses in the intensive care unit setting. Members of the genus Acinetobacter, particularly *A. baumannii*, are implicated in a wide spectrum of infections, including nosocomial BSI, nosocomial pneumonia, secondary meningitis, skin and soft tissue infections, and urinary tract infections[38]. The primary pathogenic role of *A.baumannii* is undoubtedly to cause hospital-acquired infections, mainly among patients at intensive care units (ICU) [39]. *Acinetobacter* spp. has been implicated in ventilator-associated pneumonia, catheter related blood stream infections, urinary tract infections, cerebrospinal-shunt-related meningitis, and wound infections [39].

Hospital acquired pneumonia

Pneumonia, mainly ventilator-associated pneumonia (VAP), is the most commonly identified clinical manifestation of *A. baumannii*[3,40]. Currently, these bacteria represent one of the most important agents causing ventilator-associated pneumonia (VAP) along with other gram positive and gram negative bacteria includes *S. aureus, P.aureginosa* and Enterobacteriaceae[41,42,43]. There are several factors which contribute to acinetobacter associated nosocomial pneumonia, such as head injury, neurosurgery, acute respiratory distress syndrome, aspiration, previous antibiotic therapy, deficiencies in the effectuation of infection control rules, and prolonged hospital stay[42].

This could be attributed to the colonization of airways by *A. baumannii* which can develop into true infection upon prolonged hospital admission and extensive antibiotics administration.

Ventilator-Associated Pneumonia (VAP) is the most common hospital acquired respiratory tract infection, developed in patients who have been mechanically ventilated for duration of more than 48 hours. Ventilator is a device to assist respiration, through a tracheostomy or by endotracheal intubation[44]. VAP is classified and termed as Early Onset (development of VAP ≤ 96 hours of MV) and Late Onset (development of VAP more than 96 hours)[45]. Intubation is the most important risk factor of VAP along with this, position of patients, severity of illness are also the risk factors for the development of pneumonia among hospitalized patients[46].

Comorbidity like hypertension, diabetes and other systemic diseases also influence development of VAP[47,48]. VAP can be identified by using a combination of chest X-ray, clinical and laboratory criteria. The Clinical Pulmonary Infection Scoring (CPIS) system helps in diagnosing. Prevention and control of health care associated pneumonia is documented in Guidelines for Prevention of Healthcare-Associated Pneumonia, 2003[49]. This Guideline strongly recommends that surveillance should be conducted to facilitate identification of trends and for inter-hospital comparisons.

Blood stream infections and sepsis

Bloodstream infections (BSIs), particularly sepsis caused by *Acinetobacter* is associated with high mortality[40,50]. The characteristics of Acinetobacter BSI have been described by researchers from various parts of the world[51,52,53,54]. Since Acinetobacter species are ubiquitous organisms and most species represent contaminants rather than true pathogens, species identification of all BSI isolates should be attempted. While BSI rates as high as 8.4 percent have been reported for Acinetobacter species[55], *A. baumannii* accounted for about 1–2 percent of BSI in recent studies[56,57], still being among the ten most prevalent causes of BSI. The crude mortality of *A. baumannii* BSI may be as high as 52 percent[34,54,58], but was 34 percent in nosocomial BSI in unselected patient populations[57]. Others reported that *A. baumannii* BSI is not associated with a significantly increased mortality rate in critically ill patients[59]. Nosocomial BSI is often catheter-related or secondary to respiratory or urinary tract infections, as well as wound infections, particularly in burn patients.

The most common sources of bacteremia are infected intravascular and respiratory catheters. Bacteremia due to *A. baumannii* can be secondary infection to pneumonia, and can also result from central-venous line catheters, which act as a main route for dissemination of organisms into the blood stream[60]. Surgical wounds, burns and infections of the urinary tract can be a sources of bacteremia in lower extent, while in 21–70% of the cases the origin remains unknown[61]. In a study performed on paediatric cancer, *Acinetobacter* accounted for 6.7% of the total bloodstream infections in one centre[62].

Central line associated bloodstream infections (CLA-BSI) results from bacterial colonisation that may lead to significant clinical problem. Therefore routine evaluations of BSI infections are necessary to control infections.

Wound infections

A.baummanii is also causes of hospital associated infection in burn patients and surgery patients[63], but the infection caused is often less severe. This bacterium gained a bad reputation among soldiers injured during the Iraqi war and it was named "*Iraqibacter*" due to clones spreading from Iraq to American military hospitals in Germany and US[3,64]. Such infections are not associated with high mortality rates but may be a source of bacteremia and sepsis if inappropriate therapy is given.

Another infection caused by *Acinetobacter* spp. is the secondary meningitis being the predominant form of *Acinetobacter* meningitis[65]. Until the year 1967, there were about 60 reports of incidents of *Acinetobacter* meningitis, most of which were community acquired. However, since 1979, the vast majority of cases have been nosocomial infections, with almost all caused probably by *Acinetobacter baumannii*. The mortality rates from different series were ranged from 20 to 27%. In most cases, majority patients have been adult men and had undergone lumbar punctures, myelography, ventriculography, or other neurosurgical procedures, although one patient had posttraumatic otorrhea without intervention[66].

Other manifestations

A. baumannii also caused urinary tract infection which mainly assocaed to indwelling Foley catheters[40]. These infections are usually benign and occur more frequently in rehabilitation centers than in ICUs. Nosocomial meningitis is a not infrequent manifestation of *Acinetobacter* infection [67,68].

The discovery and development of antibiotics was undoubtedly one of the greatest advances of modern medicine. Unfortunately the emergence of antibiotic resistance bacteria is threatening the effectiveness of many antimicrobials, which has increased the hospital stay of the patients which in turn causes economic burden.

MECHANISMS OF ANTIBIOTIC RESISTANCE

One of the most interesting features of *A.baumannii* is the ease by which it can acquire resistance to various antibiotics. Numerous reports in the medical and scientific literature have documented the high rates of antibiotic resistance found in *Acinetobacter* spp [69,70,71]. Frequent multiple antibiotic resistance exhibited by nosocomial *Acinetobacters* and the resulting therapeutic problems involved in treating patients with nosocomial infections in ICUs is becoming a serious problem worldwide. Antibiotic resistant can be intrinsic and acquired.

Intrinsic resistance is defined as inherent or innate (not acquired) antimicrobial resistance, which is reflected in wild-type antimicrobial patterns of all or almost all representatives of a species. Intrinsic resistance is so common that susceptibility testing is unnecessary. *A.baumannii* is intrinsically resistant to ampicillin, ampicillin-clavulanic acid, aztreonam, ertapenem, trimethoprim, chloramphenicol and fosfomycin [12,72].

Acquired resistance occurs when microorganisms become resistant to particular antimicrobial agent to which it was previously susceptible. Acquired resistance occurs due to the mutation of genes responsible for normal physiological processes and cellular structures. It may also occur from the acquisition of foreign resistance genes or from a combination of these two mechanisms.

Mechanism of resistance of antibiotics can be enzymatic and non enzymatic.

NON-ENZYMATIC MECHANISMS

Decreased permeability across the cell wall

Bacteria modify their cell membrane porin channels; either in their frequency, size or selectivity; thereby preventing the antimicrobials from entering into the cell. For example, porins form channels that allow transport of molecules across the outer membrane and play a significant role in *A.baumannii* virulence. Because porins affect membrane permeability, they also play a significant role in the mechanism of resistance. Reduced expression of some porins, including CarO, Omp22-33, Omp33-36, Omp37, Omp43, Omp44, and Omp47, is associated with carbapenem resistance in *A.baumannii*[73,74,75,76,77]. Loss of Omp29 in *A.baumannii* producing OXA-51-like or OXA-23-like carbapenemases results in increased imipenem resistance[78,79]. Aztreonam, chloramphenicol, and nalidixic acid resistance is associated with OmpA[80]. One study showed that OmpA and CarO physically interact with OXA-23 carbapenemase, and these interactions are associated with antibiotic resistance[81]. These results provide a novel view to increase understanding of bacterial antibiotic resistance mechanisms

Role of efflux pumps

Bacteria possess efflux pump which mediate expulsion of drugs from the cell, soon after their entry, thereby preventing the intracellular accumulation of drugs. Efflux pumps are associated with resistance against many different classes of antibiotics, such as imipenem[82] and tigecycline[83], in *A.baumannii*. Reversal of antibiotic resistance by efflux pump inhibitors, such as 1-(1-naphthylmethyl)-piperazine and carbonyl cyanide 3-chlorophenyl-hydrazone, supports the importance of efflux pumps in *A.baumannii* antibiotic resistance[84]. Four categories of efflux pumps, such as the resistance-nodulation-division superfamily, the multidrug and toxic compound extrusion family, the major facilitator superfamily, and the small multidrug resistance family transporters, are related to antimicrobial resistance in *A. baumannii*[85].

Alteration of Target Sites

Modifications in antibiotic target sites for antibiotics can induce antibiotic resistance in A. baumannii. In the absence of other known resistance mechanisms, only overexpression of altered PBPs with a low affinity for imipenem induce imipenem resistance[86]. Quinolone resistance is associated with modifications in GyrA (one subunit of DNA gyrase) and ParC (one subunit of topoisomerase IV) in epidemiologically unrelated *A. baumannii* isolates[87].

ENZYMATIC MECHANISMS

Production of enzyme which destroy the function of antibiotics. Enzyme such as:

Aminoglycoside modifying enzymes

Enzymes like acetyltransferases, adenyltransferases and phosphotransferases, destroy the structure of aminoglycosides. Several reports show that many MDR *A.baumannii* isolates produce a combination of aminoglycoside-modifying enzymes[88,89]. A study from China identified a MDR *A. baumannii* strain carrying four aminoglycoside-modifying enzymes[90]. Another study from Greece reported that all *A.baumannii* strains contain aminoglycoside-modifying enzymes[91], indicating the high prevalence of these enzymes in *A.baumannii*.

β- lactamase enzyme

The enzymatic inactivation by beta-lactamases is the most common strategy adopted by the bacteria. The first evidence of enzymatic inactivation of penicillin came in 1940, even before the antibiotic was used in therapeutics. Abraham and Chain were able to demonstrate an enzyme in *E.coli* that hydrolyzed penicillin; they named it "penicillinase" [92]. Beta-lactamase is a broader name given to bacterial enzymes that hydrolyze various beta-lactam antibiotics. This breaks down the β lactum rings, there by inactivating the β lactam antibiotics.

β -lactam substrate bind to the active site of the beta-lactamase, and form a non-covalent complex. This is reversible step. The serine radical in the active site mounts a nucleophilic attack on the carbonyl leading to high-energy tetrahedral acylation intermediate. Protonation of the beta-lactam Nitrogen and cleavage of C-N bond results in opening up of the beta lactam ring and the intermediate then transitions into a lower-energy covalent acyl-enzyme complex. An activated water molecule then attacks the covalent complex leading to high-energy tetrahedral deacylation intermediate. Hydrolysis of the bond between the beta-lactam carbonyl and the oxygen of the serine is then hydrolyzed, which regenerates the enzyme and releases the inactive beta-lactam molecule[93].

The β-lactamase genes (*bla*) are found either chromosomally or on mobile elements commonly located within integrons, i.e. genetic elements with an integrase gene (*int*) capable of acquiring and expressing multiple resistance genes, facilitating their spread[94].

Classification of β-lactamases can be defined according to two properties, functional and molecular [Fig 1].

Functional classification: Previously classification of beta-lactamase enzymes was done by biochemical analysis, isoelectric point, substrate hydrolysis, enzyme kinetics and inhibition profiles of enzyme. After several revisions, currently functional classification divides beta-lactamases into four functional groups. The group 2 has several subgroups that are differentiated according to group-specific substrate or inhibitor profile. In this classification, carbapenemases fall under group 2f, 2df and 3[95].

Molecular classification: Ambler and others have classified beta-lactamases according to the amino acid sequences into four groups (A-D). Although this classification correlates well with the functional scheme, it lacks in the details concerning the enzymatic activity of the beta-lactamase. Molecular classes A and D enzyme are serine based which contain serine in their active site while molecular group B are metallo-beta-lactamases which contains zinc in their active sites[95].

Many genera of gram negative bacteria including *A.baumannii* possess a naturally occurring, chromosomally mediated β-lactamase & also some are plasmid mediated β-lactamases. The enzymes are thought to have evolved from penicillin binding proteins.

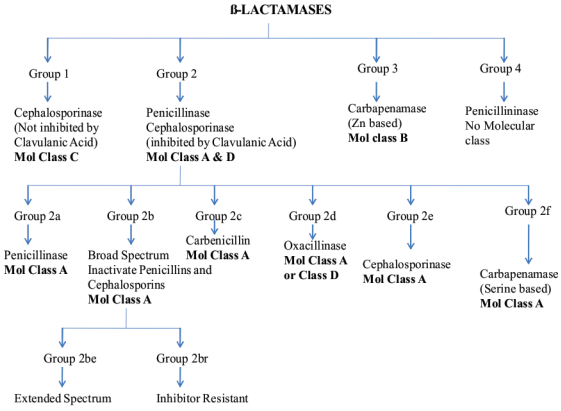

Fig 1 Classification of β-lactamases

Molecular Class A β-lactamases or Subgroup 2be enzymes that are able to hydrolyze penicillins, early cephalosporins and one or more of the third generation cephalosporins or monobactams. Because of their extend activity against generation cephalosporins, they are called Extended Spectrum Beta-lactamase (ESBLs). This development was likely to be the selective pressure exerted by β-lactam producing soil organisms found in the environment. In 1960s, the first plasmid mediated β-lactamase TEM-1 was described. Another common plasmid mediated β-lactamase is SHV-1[96]. Originally these groups had no broad-spectrum activities, but it gradually developed due to point mutations affecting the active site of the enzymes. Since 1980 more than 340 variants of theses enzymes have been observed [97]. Even though the *bla*SHV-like genes are mainly found in Enterobacteriaceae, some reports show their presence in non fermentors like *A. baumannii* and *P.aeruginosa* isolates [97,98,99]. During the last few decades CTX-M like cefotaximases has started to emerge and currently they are the most important ESBLs in enteric bacteria [97]. Whereas CTX-M enzymes have been rarely identified in non-fermenters, a few *A.baumannii* isolates producing variants of CTX-M have been identified in different parts of the world [97,100]. They are sensitive to β-lactamase inhibitors i.e. clavulanic acid, sulbactum and tazobactum. They confer resistance to a broad spectrum of cephalosporins

including 3rd and 4th generation drugs. The TEM- and SHV- like enzymes are the most commonly observed enzymes among ESBLs.

Together with a marked impermeability and the expression of multiple efflux systems, the plasticity of its genome enables the species to gather many resistance mechanisms, leading easily to multidrug resistance. Most of the time, acquired resistance to carboxy-penicillins, ureido-penicillins and third generation cephalosporins rests on the overproduction of the AmpC-type cephalosporinase. However, plasmid encoded several Extended spectrum β Lactamases (ESBL) have also been acquired by *A. baumannii* [96]. In both cases, imipenem and meropenem remain the drugs of choice.

Class B β-lactamases (often referred to metallo-β-lactamases (MBLs) require zinc as a divalent cation for their activity. They are capable of hydrolyzing all the β- lactam antibiotics except the aztreonam [101] and can be inhibited by EDTA or by other chelators [101]. They are often coded on plasmids as part of integrons, as well as on the chromosomes [101,102].

Class C β-lactamases are often referred to as AmpC-type enzymes. They were found in Gram-negative bacteria, conferring resistance to amino-pencillins, to first, second, and some representatives also to third generation cephalosporins [104]. Among *Haemophilus* and in some members of the Enterobacteriaceae family, as well as in acinetobacter the AmpC enzymes are typically chromosomally encoded, often repressed, expressed only when induced or de-repressed by mutations. Lately several plasmid-coded members have been spreading in various genera [105].

Class D β-lactamases are primarily oxacillinases hydrolyzing oxacillin in a more efficient manner than benzylpenicillin and having varying spectrum of activity beyond this group of drugs. They are the most wide-spread β-lactamases in *A. baumanni*. Only a few variants can hydrolyze the extended-spectrum cephalosporins and carbapenems, and even those that can do exhibit usually moderate activity against the extended spectrum of drugs.

In *A. baumannii*, the OXA-type class D β-lactamases are arranged into five families: the OXA-23-like, OXA-40-like, OXA-51-like, OXA-58-like and OXA-143-like groups, respectively [27].

CARBAPENEM HYDROLYSING ENZYME

More worrying are the emergence and dissemination of carbapenem-resistant clones since the end of the 1980s. Although carbapenem resistance can result from the over-expression of carbapenamase enzyme. Of note, the prevalence of such carbapenemase producing strains increases steadily from Northern to Southern European countries [106].

Carbapenem hydrolyzing enzymes represent the most versatile family of β-lactamases. Although known as "carbapenemases," many of these enzymes recognize almost all hydrolyzable β-lactams, and most are resilient against inhibition by β-lactamase inhibitors[94,107] Carbapenem hydrolyzing enzymes (also known as carbapenemases) are basically two types based on the reactivity of the enzymes. The first carbapenemases these enzymes were inhibited by EDTA, thereby named as metalloenzymes. Later work has described that all metallo-carbapenemases contain at least one zinc atom at the active site that serves to facilitate hydrolysis of a bicyclic β-lactam ring[108]. In the mid- to late 1980s, another set of carbapenem-hydrolyzing enzymes emerged[109], but EDTA did not inhibit their activity[110]. Subsequent studies showed that these enzymes utilized serine at their active sites and were inactivated by the β-lactamase inhibitors clavulanic acid and tazobactam[110]. Hence, Carbapenem hydrolyzing enzymes (also known as carbapenemases) are basically two types; serine carbapenemases and metallo-β-lactamases. Carbapenemases are mostly species-specific chromosomally and plasmid encoded enzymes[107].

MOLECULAR CLASS A CARBAPENEMASES

Molecular Class A carbapenemases of functional group 2f are serine based and has appeared sporadically in clinical isolates. Bacteria expressing these enzymes are characterized by reduced susceptibility to imipenem. MICs are increased from mild (e.g., imipenem MICs of >4 μg/ml) to fully resistant. There are 3 major families of class A serine carbapenemases, include the NMC, IMI, SME, and KPC enzymes. A fourth member of this class, the GES β-lactamases, was originally identified as an ESBL family. NMC, IMI and SME enzymes are chromosomal while KPC and GES enzymes are plasmid encoded [95].

Their hydrolytic mechanism requires an active-site serine at position 70 in the Ambler numbering system for class A β –lactamases[111]. These organisms have the ability to hydrolyze a broad variety of β-lactams, including carbapenems, cephalosporins, penicillins, and aztreonam,and all are inhibited by clavulanate and tazobactam, placing them in the group 2f functional subgroup of β –lactamases[95].

MOLECULAR CLASS B CARBAPENEMASES

These are metallo-β-lactamases (MBLs), which are characterized by its resistance to all penicillins, cephalosporins, beta-lactamase inhibitors, and carbapenems but are susceptible to inhibition by aztreonam and metal ion chelators (EDTA), this activity can be reversed by adding Zn^{2+} ions [95]. The MBLs are divided into three subclasses (B1, B2, B3) based on primary amino acid sequence homology. These enzymes contains at least one Zn^{2+} ion in their active sites; B1 and B3 enzymes contain two Zn^{2+} ions where B2 enzymes contains only one Zn^{2+} ion. In fact, binding of another zinc ion decreases the activity of B2 enzymes. Zinc ions coordinate two water molecules that are necessary for hydrolysis. Zinc coordinating residues of B1 enzymes consists of two histidine and one cysteine. With class B2 enzymes, asparagine is found at the first position of the zinc binding motif HXHXD [112]. Most MBL genes (including VIM, IPM) are found as gene cassettes on class 1 integrons; few IMP genes are located on class 3 integrons. However, SPM-1 genes are not located on integrons or transposons. Mechanism of carbapenem hydrolysis is complex and varies from one MBL to another [95,111,112].

Chromosome borne MBLs

The first metallo-β-lactamases were detected in environmental and opportunistic pathogenic bacteria (*Bacillus cereus* (BCI, BCII), *Aeromonas spp* (CphA) and *Stenotrophomonas maltophilia* (L1)) as chromosomally encoded enzymes. Serine based beta-lactamses are produced by these microorganism. Chromosomal matello β lactamase was also found in few strains of *Bacteroides fragilis* (CcrA). The chromosomal OXA-51-like enzyme [113,102], this phenotype is mostly due to the acquisition of plasmid-borne OXA-23-like, IMP,VIM, SIM or, more recently, NDM-type carbapenemases [95].

Plasmid borne MBLs

Plasmid mediated MBLs have now attained significance because of its global spread. The majority of the mobile MBL genes are found as gene cassettes. These include bla_{IMP}, bla_{VIM}, bla_{GIM}, bla_{SIM}, and bla_{KMH}.

IMP: IMP-1 (for "active on imipenem"), located on a conjugative Plasmid. This enzyme hydrolyzed imipenem, penicillins, and extended-spectrum cephalosporins but not aztreonam. The hydrolytic activity was inhibited by EDTA and restored by the addition of Zn^{2+}. The first member of the IMP family found in Europe was in an *A.baumannii* isolate from Italy, which produced a related enzyme, IMP-2, as the first cassette on a class 1 integron[114]. Most of bla_{IMP} genes are harbored by class 1 integrons that are usually embedded in transposons and/or plasmids, footnoting their horizontal transfer and worldwide distribution. bla_{IMP}genes usually co-exist with other resistance genes, such as *aacA*, *catB,* and bla_{OXA}, resulting in multi-drug resistance. In Japan 1990 first Transferable carbapenem resistance gene was detected in a *P.aeruginosa*. After that it was subsequently reported in four *S.marcescens* isolates in Japan. IMP-2 was observed in *A.baumannii* in Italy. Currently, there are 37 known IMP types. While these are more commonly seen in *P.aeruginosa* and *A.baumannii* isolates [114].

VIM (for Verona integron encoded metallo-β-lactamase): This class 1 integron associated MBL was first observed in a *P. aeruginosa* isolate from Verona, Italy in 1997. The bla_{VIM-1} was embedded in a class 1 integron, which was located on the bacterial chromosome [95].VIM-2, the most important VIM-type MBL in clinical practice. VIM-2 was reported in a clinical isolate of *P.aeruginosa* from France. Currently there are 34 known VIM types. VIM-2 is the most dominant MBL across Europe [95]. The occurrence of VIM-producers has a geographical distribution pattern, with VIM-1 and VIM-4 found in Europe, VIM-3 in Taiwan, VIM-6 in Asia and VIM-7 in the USA, whereas VIM-2 is distributed worldwide [115].

In Asia, bla_{IMP} and bla_{VIM} are prevalent. bla_{IMP} is found mainly in Japan, Korea, China, Taiwan, and Iran [116,117,118]. In a study from India, bla_{VIM} type was the most common [119]. In a nation-wide survey 5 VIM variants were reported with VIM-2 being the most common. The others were

VIM-6, VIM-11, VIM-5 and VIM-18. There is limited data on the prevalence and distribution of metallo-beta-lactamases among Indian isolates. It has been detected in more than 23 species across 40 countries [111].

SPM-1 (for Sao Paulo metallo-β-lactamases) was identified in a *P. aeruginosa* isolate from Sao Paulo, Brazil. Genetic analysis has revealed that it is not a part of any integron but is associated with a new type of transposable structure. bla_{SPM-1} is a part of mobile pathogenicity island located on a plasmid[102,111].

GIM-1 (for German imipenemase) was first isolated from Germany in 2002. GIM had approximately 30% homology to VIM, 43% homology to IMPs, and 29% homology to SPM. bla_{GIM-1} was found on class 1 integron in a 22-kb nontransferable plasmid[102,116].

SIM-1 (for Seoul imipenemase) was first isolated from *P. aeruginosa* and *A. baumannii* isolates during a large scale screen of imipenem resistant isolates in Seoul. SIM-1 show 64 to 69% homology to the IMP family.

NDM-1 (for New Delhi metallo-β-lactamase) was first reported in 2009 from a *K. pneumonia* isolate obtained from a Swedish patient of Indian origin, who had received medical treatment in India. In July 2010, a team in New Delhi reported a cluster of three cases of *Acinetobacter baumannii* bearing bla_{NDM-1} that were found in the intensive care unit of a hospital in Chennai, India[120].

This gene located on a 180 kb plasmid, it expressed high level resistance to all penicillins, cephalosporins, aztreonam, cefoxitin, carbapenems and ciprofloxacin. It was susceptible only to colistin. This isolate also harboured an AmpC (CMY-4) gene, a novel erythromycin esterase gene (*ereC*) as well as genes coding for resistance to chloramphenicol and aminoglycosides. NDM-1 shares less identity with other MBLs. NDM-1 firmly binding to most cephalosporins and also to the penicillins but does not bind tightly to the carbapenems as tightly as IMP-1 or VIM-2. These possess shallow active-site groove with one or two divalent zinc ions, bordered by flexible loops [121] but In NDM-1, there is flexible hairpin loop moves over the zinc ion for hydrolysis and is later removed after the catalysis[122].

Class 1 integron was detected in the isolates, NDM gene was not carried in it or present near ISCR1 element [69,102]. Most other NDM-1 enzymes are susceptible to colistin and tigecycline[69].

Carbapenemase enzyme NDM-1 produced by certain strains of bacteria, and is able to inactivate all β-lactams antibiotics except aztreonam [121]. CMY-4 and CTX-M-15 β-lactamases expressed in most of the strains of NDM-1, which confer resistance to all β-lactams. Thus it provides resistance against all compounds that contain a beta-lactam ring such as penicillins, cephalosporins, and the carbapenems [122].

*bla*NDM-1 is plasmid encoded gene, it is located on a transmissible plasmid and its association leads to the extensive drug resistance which is exhibited by a majority of the NDM-1 producing *A.baumannii,* leaving only a limited therapeutic options [123]. Therefore, the NDM-1 hourbing *A.baumannii* are now being recognized as the world's newest "superbugs"[124]. There have been the increasing reports of NDM-1 producing strains from India and around the world [124,125].

OXA enzyme- OXA enzyme having carbapenemase properties was observed in 1985 in Scitland in an *A.baumannii.* Initially named ARI-1 (for Acinetobacter resistant to imipenem) and this enzyme was plasmid encoded. Sequencing the enzyme revealed that it belonged to OXA family of betalactamases. The enzyme was later renamed as OXA-23. Most of these enzymes are encountered in *Acinterobacter spp*. OXA carbapenemases were on the basis of amino acid homologies, they were sub-divided into nine major subgroups (1-9). OXA 23, 24 and 51 have been isolated from clinical isolates of *Acinetobacter spp*. OXA-50 has been observed as chromosomal enzymes in several *P.aeruginosa* strains. Similarly In several *A. baumannii* strains OXA-51 has been found as chromosomal encoded enzymes[95,102,111,113].

Thus, for developing countries like India, surveillance of antimicrobial resistance is essential for preventing the emergence and transmission of multidrug-resistant pathogens in healthcare facilities.

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