Review Article

Beta-Lactamases in *Acinetobacter baumannii* clinical isolates recovered from humans in Iraq

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Abstract

Antibiotic resistance is a problem of deep scientific concern both in hospital and community settings. Production of extended-spectrum b-lactamases (ESβLs) is a significant resistance-mechanism that impedes the antimicrobial treatment of infections caused by Enterobacteriaceae and is a serious threat to the currently available antibiotic armory. Metallo-β-lactamases have emerged worldwide as a major source of acquired broad-spectrum β-lactam resistance. Multidrug resistant *A. baumannii*, is now recognized to be of great clinical significance. Many reports relay to the spread of *A. baumannii* in the hospital settings which leads to enhanced nosocomial outbreaks associated with high death rates. Our review will focus on the evolution and the emergence of multidrug resistance *A. baumannii* in Iraq as well as the types of the beta lactamase genes in relation with the predominant ESBL-genes circulating Iraqi's hospitals. Therefore, we have analysed the results from Iraq during the last 15 years. This review showed the predominance of bla_{CTXM} gene group in *A. baumannii* in Iraq. We conclude that the spread of CTX-M group in Iraq reflects the global emergence of CTX-M β-lactamases in *A. baumannii* isolates.

Keywords: *Acinetobacter*; Multi-drug resistance; β-lactamases

Introduction

Acinetobacter baumannii is Gram-negative, strictly aerobic, non fastidious, non-fermenting, non-motile, oxidase -negative, catalase-positive bacteria with a DNA G + C content of 39% to 47% (Howard et al., 2012).

Acinetobacter is a heterogeneous group of bacteria that are typically free living saprophytes, found almost everywhere, commonly distributed in the environment. However, different species of the genus are generally associated with various habitats e.g. soil, human, sewage, water, foods and animals (Jung and Park, 2015). *A. baumannii* is an emerging bacterial pathogen responsible for widespread and persistent outbreaks among hospitalized patients. *A. baumannii* infections are difficult to control since many epidemic strains can resist several classes of antimicrobial agents and disinfectants and are able to contaminate abiotic surfaces of the hospital environment, including the medical equipment (Giannouli et al., 2013). *A. baumannii* most problematic nosocomial pathogens that mainly is considered as one of the affect critically ill patients in intensive care units (Kempf et al., 2012). The present review analyses the growing burden of antibiotic resistance among *A. baumannii* in Iraq, focusing on the b-lactamases enzymes production, and on the b-lactamase genes and their ability to be transferred.

Bacteriological studies on the antibiotic resistance in *A. baumannii* in Iraq are relatively new; the first published paper appeared in 2001. Since then, more data have been made available and the real situation of antibiotic resistance in *A. baumannii* in hospitals is alarming.

Epidemiology and Pathogenesis

Acinetobacter baumannii is now recognized as causing a wide range of severe nosocomial infections, including skin and soft tissue infections, urinary tract infection, wound infections and secondary meningitis(McConnell et al., 2013).Ventilator-associated pneumonia is a frequent nosocomial infection in critically ill patients, with a high mortality rate,reaching up to 33–50% (Chaari et al., 2013) .

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A. baumannii can also cause community-acquired infections, including pneumonia and bacteraemia. Other possible community-acquired infections include ocular infections, and endocarditis (Falagas et al., 2007).

Most reports of community-acquired pneumonia have occurred during the summer months in tropical and subtropical climates (Antunes et al., 2014).

The most common sources of bloodstream infections caused by *A.baumannii* are lower respiratory tract infections and intravascular devices (Jung et al., 2010), although urinary tract infections and wound infections have also been reported as foci of infection. 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 Afghanistan and Iraq (McConnell et al., 2013) .*A. 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 percentage of multidrug resistance and the poor penetration of some antimicrobial agents into burn sites, these infections can be extremely challenging for clinicians. Recent studies reporting high incidences of *A. baumannii* infection in burn units have underscored the importance of *A. baumannii* in this patient population (Keen et al., 2010).

Virulence factors

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) .*A. baumannii* OmpA bind to the host epithelia and mitochondria, once bound to the mitochondria, OmpA induce mitochondrial dysfunction and causes the mitochondria to swell (Howard et al., 2012), This is followed by the release of cytochrome c which leads to the apoptosome formation. These reactions all contribute to apoptosis of the cell. OmpA, being the most abundant surface protein on the pathogen, is also involved the formation of biofilms and complement resistance (Gaddy and Actis, 2009).

Lipopolysaccharide derived from *A.baumannii* has been shown to be a potent inducer of pro-inflammatory cytokine expression in human monocytes, the ability of *A. baumannii* to stimulate an inflammatory response in human monocytes is likely to be a significant contributor to the pathogenesis of infection (Gordon and Wareham, 2010). The *A. baumannii* MDR phenotype seems to play an important role in the remarkable capacity of the bacteria to persist and spread in the hospital environment, together with its ability to colonize both biotic and abiotic surfaces and to grow as biofilm (Longo et al ., 2014). The capacity of *A. baumannii* to persist in the environment maybe due to its ability to form biofilms on both biologic and abiotic surfaces. Biofilm formation is also a mechanism of pathogenesis in device-related infections and provides a source of repeated transmission by prolonging survival on inanimate objects (Lewis, 2010). Under harsh environmental conditions, *A. baumannii* cells deep in the biofilm can undergo dormancy, becoming metabolically inactive and robust to environmental stress. The multiple antimicrobial resistance mechanisms found in *A. baumannii* also may play a role in its environmental survival (Greene et al., 2015).

Bacteria in a biofilm, as a structural community, are enclosed in a polymeric matrix constituting a protective mechanism to resist in harsh environments and during host infection. These bacteria become more resistant to antibiotic stressors, antibiotics or cleaning and therefore this biofilm structure represents an important virulence factor (Espinal et al., 2012).

Multi Drug Resistance

The continuous emergence, worldwide spread, and increasing rate of bacteria resistant to multiple antibiotic are becoming major problem to public health (Hong et al., 2015). Multidrug and pandrug resistant bacterial infections are closely associated with prolonged hospitalization, high mortality, multiple morbidities, and increased cost due to the limited antibiotic therapeutic options for infected patients (Garner et al., 2015).

Healthcare-associated infections, especially with multi drug resistant G-ve bacteria are an emerging threats in infection control(Wellington et al ., 2013). Muti drug resistant G-ve is responsible for wide rang infections and have a significant impact on mortality and morbidity. The spread of these bacteria as well as the spread of resistance genes is an emerging public health issue (Reichel et al., 2014).

β-Lactamases Enzymes

Beta-lactamase production is most frequently suspected in a Gram-negative bacterial isolate that demonstrates resistance to β -lactam antibiotic. The most common mechanism is the production of enzymes that modify or degrade the antibiotic before it can reach the appropriate target site. In this case, the beta-lactamase family of enzymes degrade β-lactam antibiotics and are found widely disseminated amongst Gram-negative and Gram-positive bacteria (Wilke et al., 2005).

The Ambler system classifies β-lactamases based on their

amino acid sequence into four main classes – A, B, C, and D. Class A, C, and D enzymes all harbor a serine residue at the active site, whereas the active site of class B enzymes harbors a zinc residue. Class A β-lactamases are plasmide –borne or chromosomal, and preferentially hydrolyse penicillins (e.g.SHV-1, TEM-1, KPC). Class B encompasses the metallo-β-lactamases, which hydrolyse carbapenems, cephalosporins and penicillins (e.g.VIM-1and IMP-1). Class C β-lactamases are the chromosomally-encoded AmpC enzymes, and class D β-lactamases encompass the oxacillin-hydrolysing β-lactamases (e.g. OXA-1) (Harris, 2014).

The functional classification scheme, originally proposed in 1995 by Bush et al., divides b-lactamases into three groups with various subgroups. Typically, Group 1 enzymes are the chromosomal cephalosporinases represented in Ambler class C, and Group 3 enzymes are the metallo-enzymes represented in Ambler class B. Group 2 enzymes are organized into 12 subgroups from 2a to 2f. ESβLs are classifid under Group 2be in the functional classifiation scheme (Akinci and Vahaboglu, 2010).

Extended Spectrum β-Lactamase

Extended-spectrum beta-lactamases (ESβLs)-producing Gram-negative bacteria have become one of the major threat in terms of nosocomial infections in human medicine (Ewers et al., 2011). The production of extended spectrum-beta- lactamase has mainly been documented for Enterobacteriaceae spp., and it confers resistance to the majority of the commonly used beta lactam antibiotics, including Third generation cephalosporins. However, the main therapeutic burden results from the multidrug phenotype of these bacteria which is caused by a frequent genetic linkage with other resistance mechanisms. This confers additional resistance to other antibiotic classes including aminoglycosides and fluoroquinolones, which may result in therapeutic failures and possibly life-threatening microbial infections (Hunter et al., 2010). Extended-spectrum β-lactam antibiotics resistance is mainly caused by extended-spectrum β-lactamases such as bla_{SHV}, bla_{TEM} and bla_{CTX-M} (Cullik et al., 2010).

The resistance acquired by ESβL producing bacteria deliver from genetic point mutation and it is an unsolved, growing problem. In the near future both microbiologist and clinicians will be battling against extended spectrum – beta- lactamase producing strains due to their dynamic evolution and epidemiology in averting and controlling them (Rawat and Nair, 2010).

Bradford revealed that extended spectrum – beta- lactamase are undergoing continuous mutations, causing the development of new enzymes showing expanded substrate profiles. Currently, over 150 extended spectrum– beta- lactamase have been described in a worldwide distribution. The genes encoded for ESβLs are mostly plasmid encoded, and most ESβLs can be divided into 3 genotypes: TEM, SHV and CTX-M (Kumar and Kumar, 2016).

Sulphydryl variable (SHV)-type b-lactamases

SHV-1 was the first described SHV enzyme, encoded by chromosomally-encoded bla_{SHV-1} in isolates of the genus *Klebsiella*. bla_{SHV-1} was later shown to be plasmid-borne, with the ability of transferring the resistance phenotype to other bacterial strains. Its presence on plasmids has lead to the world-wide dissemination of genes for bla_{SHV}, which can now be found in many Enterobacteriaceae species (Bradford, 2001).

The bla_{SHV-1} gene has undergone evolution since its discovery, to yield over a >170 enzyme variants, including extended spectrum enzymes. SHV β-lactamases are broad spectrum enzymes, conferring activity against narrow-spectrum cephalosporins and penicillins (Harris, 2014). SHV-extended spectrum –beta- lactamase arise predominantly from a single nucleotide mutation in the bla_{SHV} gene, resulting in a glycine to serine amino acid substitution at codon 238 (G238S). This substitution increases the size of the active site of the enzyme, allowing hydrolysis of the third generation cephalosporin cefotaxime .An additional glutamic acid to lysine amino acid substitution at codon 240 (E240K) further increases the SHV-extended spectrum –beta- lactamase spectrum of the third generation cephalosporin CTX. An additional glutamic acid to lysine amino acid substitution at codon 240 (E240K) further increases the SHV-ESβL spectrum of activity, allowing hydrolysis of the third-generation cephalosporin ceftazidime (Huletsky et al., 1993).

TEM-type b-lactamases

First instance of a plasmid- mediated β- lactamase was reported from ampicillin resistant *E. coli* isolate obtained from specimen of urine from a woman named Temoniera in Greece in 1965. This enzyme was designated as TEM-1. Within a decade this enzyme had spread to different species of Enterobacteriaceae as well as to *Haemophilus influenza* and *Neisseria gonnorheae* .In 1969, TEM-2, acloslely related beta-lactamase was found in a *P. aeruginosa* isolate in Great Britain. All bla_{TEM} genes are found on complete or partial versions of Tn3, Tn1, Tn2 or their variants (Bailey et al., 2011). The chromosomal origin of bla_{TEM} genes remains unknown, with bla_{TEM} predominantly observed as a plasmid-borne gene, although bla_{TEM} has been encoded by chromosome of different bacterial species (Chouchani et al ., 2007).

TEM-2 and TEM-1 are the accepted parenteral TEM β-

lactamase enzymes. TEM-2 is encoded by bla_{TEM-2} and TEM1 can be encoded by any of seven subtypes of bla_{TEM-1} ($bla_{TEM-1A, 1B, 1C, 1D, 1E, 1F, and 1G}$) (Pomba-Feria and Caniça, 2003).

CTX-M-type b-lactamases

The CTX-M-1 enzyme was first described on a highly cefotaxime-resistant *E. coli* strain isolated from an ear infection from a newborn child in Germany. Subsequently, the CTX-M-2 enzyme was investigated from cefotaxime-resistant *Salmonella enteric serovar Typhimurium* strains isolated from patients suffering from septicemia, meningitis or enteritis in Argentina (Akinci and Vahaboglu, 2010) . Since then, the CTX-M enzymes have formed a rapidly growing family of extended spectrum beta- lactamase distributed both over a wide geographic area and among a wide range of clinical bacteria, particularly members of the Enterobacteriaceae (Bonnet, 2004).

CTX-M b-lactamases are found exclusively in the functional group 2 (Bush and Jacoby, 2010) and thought to originate from chromosomal extended spectrum beta- lactamase genes found in *Kluyvera spp.*, an opportunistic pathogenic bacteria of the Enterobacteriaceae found in the environment (Bush and Jacoby, 2012) . The first proteins of CTX-M were discovered in the late 1980s and today more than 100 variants have been sequenced. Based on their amino acid sequences, they can be divided into five groups (CTX-M group 1, 2, 8, 9, and 25) (Shaikh et al., 2015).

OXA-type b-lactamases

Most OXA-type b-lactamases, so named because of their oxacillin-hydrolysing capabilities, do not hydrolyse extended-spectrum cephalosporins and are not regarded as extended spectrum beta lactamase. The exceptions to this rule are OXA-10 and OXA-13 to OXA-19 (Toleman et al., 2003).

Other ESBL type

PER-2, which shares 86% homology to PER-1, has been detected in S. *enterica serovar Typhimurium, K. pneumoniae, E. coli, Proteus mirabilis*, and *Vibrio cholerae* (Petroni et al., 2002). GES-1 was initially described in a *K. pneumoniae* isolate from a neonatal patient just transferred to France from French Guiana , other unusual enzymes having ESBL have also been described (e.g. BES-1, CME-1, VE-B-1, PER, SFO-1, and GES-1) (Shaikh et al., 2015; Bradford, 2001).

Metallo Beta Lactamases

Metallo-β-lactamases have emerged worldwide as a major source of acquired broad-spectrum β-lactam resistance. They hydrolyze virtually all classes of β-lactams (except monobactams), including carbapenems, which often consider the last option for the treatment of infections with multi drug resistant G-ve bacteria (Scotta et al., 2011).

There are two dominant types of transferable MβLs among clinical isolates, VIM and IMP. Most of the VIM- and IMP-type MβL genes are present as gene cassettes inserted into integrons located on plasmids or on the chromosome. These integrons may be associated with transposon-like structures which may contribute to their variable location and spread (Scotta et al., 2011).

Carbapenems (meropenem, imipenem and ertapenem) have the broad spectrum of antibiotic among all beta-lactams and are primarily used to treat infections by aerobic G-ve bacteria. According to their dependency on divalent cations for enzyme activation, carbapenemases can be divided into metallo-carbapenemases (zinc-dependent class B) and non metallo -carbapenemases (zinc-independent classes A, C and D) (Jeon et al., 2014).

Class A carbapenemases, which include the KPC, SME, IMI and NMC-A families and some GES enzymes, have most frequently been discovered in isolates from Enterobacteriaceae and in species such as *P. aeruginosa* (Lee and Lee, 2010; Thomson, 2010). These enzymes are inhibited by clavulanate, except for some KPC-type enzyme(s) such as KPC-2, and hydrolyze cephalosporins or penicillins more efficiently than carbapenems. Class B carbapenemases, which include the IMP and VIM families as well as SPM-1, have previously been detected in strains of *P. aeruginosa*,*A. baumannii* and members of the Enterobacteriaceae family (Thomson, 2010).

Class D carbapenemases belong to the OXA family and were identified in *A.cinetobacter* clinical isolates; they poorly hydrolyze carbapenems and are weakly inhibited by clavulanate (Thomson, 2010). Recently, class C carbapenemases that can hydrolyze carbapenems, including CMY-2, DHA-1 and ACT-1, have been identified in Enterobacteriaceae. These carbapenemases are plasmid-mediated class C –β lactamases that exhibit catalytic activity for imipenem (Mammeri et al., 2010). In addition, CMY-10 revealed catalytic activity for imipenem (Jeon et al., 2014).

A group of metallo-β-lactamases are encoded chromosomally mainly in environmental bacteria or opportunistic pathogens, such as GOB1 from *Elizabeth kingia meningeseptica*, BcII from *Bacillus cereus* and L1 from *Stenotrophomonas maltophilia*. However, the clinically relevant metallo-β-lactamases are encoded in mobile genetic elements and include VIMs, IMPs and the more recently emerged NDMs (New Delhi Metallo-β-lactamase) (Yong et al., 2009).

Though different inhibitors have been tested *in vitro*, there

is no clinical drug able to inhibit any of the metallo-β-lactamases. These metallo-β-lactamases show an extended substrate spectrum, including not only carbapenems, but also penicillins and the last-generation cephalosporins. Though they do not hydrolyze aztreonam (Meini et al., 2014).

Bacteria harboring the bla_{NDM-1} gene invariably carry resistance to other classes of antibiotics, including trimethoprim/ sulfamethoxazole, fluoroquinolones and aminoglycosides (Rogers et al., 2013).

The first acquired MβL, IMP-1, was reported in *Serratia marcescens* in Japan in 1991. Since then, MβLs have been described worldwide. Endemicity of IMP- and VIM -type enzymes has been reported in Greece, Japan and Taiwan. although outbreaks and single reports of IMP and VIM producers have been reported in many other countries (Queenan and Bush, 2007). These enzymes hydrolyze all β-lactams except aztreonam. Their activity is inhibited by EDTA but not by clavulanate (Nordmann et al., 2011).

AmpC b-lactamases

Another large group of broad-spectrum b-lactamases are the AmpC enzymes, which are typically encoded on the chromosome of many Gram-negative bacteria, including Citrobacter, Serratia and Enterobacter species, where its expression is usually inducible (Jacoby, 2009).

Beta- Lactamases in *A. baumannii* Isolates Recovered from Humans in Iraq

The detection of MDR *A. baumannii* has been increasingly reported in the last years in Iraq, Since 2006, antibiotic resistance *A. baumannii* has been continuing to appear in Iraq. Indeed, Al-khafaji (2006) have reported a high frequency of *A. baumannii* isolates from clinical samples (44.11 % recovered from wounds and 17.64 % from sputum), isolates were completely sensitive to imipenem while they were 100% resistant to ciprofloxacin, aztreonam and cephalothin. Also, they found that the resistance percentage to tobramycin and tetracycline were 82.35% and 100% respectivel. Al -Karkhe (2001) observed that 17 *A. baumannii* isolates were collected from patients in some hospitals in Baghdad.

Another study carried out in 2007 reported that *A. baumannii* clinical isolates showed 100% sensitivity to meropenem results obtained by Musafer (2007), she noticed that percentage of isolation of A.baumanni in wounds samples was (47.61 %).

Results of study carried out in 2010 found that *A. baumannii* clinical isolates developed 100% resistance to cefotaxime, ceftazidime, ceftriaxone, 95.45% to cefepime, chloramphenicol, aztronam and 40.90% to imipenem (Al-Mash'hadani, 2010).

Results showed all isolates of *A. baumannii* were resistant 100% to nine antibiotics included, penicillin, ampicillin, cephalexin, carbenicillin, Amikacin, Ciprofloxacin, Gentamicin, tetracycline and amoxicillin. The results of metallo β lactamase (MBL) producing isolates test showed (10%) of *A. baumannii* produce MBL enzyme Majeed (2012). Al-Ajeeli (2013) revealed that most of the *A. baumannii* isolates were collected from wounds samples (41.17 %) , followed by sputum samples with percentage reached to (35.29 %) , that may belong to finding that respiratory system is more than other systems exposure to pathogens, especially when use the ventilator equipment because it participate in transmission of pathogen from patient to another , 3 isolates (17.64%) from urine and one isolate (5.88 %) from blood.

AL-Saleem (2013) recovered 128 isolates of *A. baumannii* from clinical and environmental specimens in a percentage of 6.05% and 5.42%, respectively, and she found the highest percentage (85%) of imipenem resistant *A. baumannii* (IRAB) isolates were isolated from blood disease (leukemia) department followed by ICU, RCU, Burns, surgical and other departments, respectively. All *A. baumannii* clinical isolates had 100% resistance to amoxicillin-clavulanic acid, cefepime, cefotaxime and rifampin, the highest resistance to aztreonam (97.39%), ceftriaxone (97.39%), ticarcillin-clavulanate (96.52%), chloramphenicol (95.65%), piperacillin (91.30%), cefalothin(91.03%), ceftazidime (89.57%), gentamicin (87.83%), trimethoprime-sulphamethoxazole (86.09%), ciprofloxacin (83.48%), amikacin (72.17%) and colistin (66.96%). Tobramycin and tetracycline recorded moderate resistance; 46.09% and 47.83%, respectively.

Al Marjani *et al*.(2013)revealed that 35.2% and 41.1% of *A. baumannii* isolates were produced ESBLs and MBLs respectively, and they found that all isolates of *A. baumannii* were carrying bla_{CTX} gene , and (42.8 %) of isolates were carry bla_{IMP1} gene. *bla* VIM-2 didn't appear in any isolate of ESBLs or MBLs producers. Al-kadmy et al. (2015) showed that percentage of bla_{TEM} gene 20%and bla_{CTX} gene 73.33% in *A. baumannii* isolates in Baghdad.

Al-Niaame et al. (2013) showed that among 34 clinical isolates, 12(35.29%) were *P. aeruginosa*, 7(20.58%) were *K. pneumonia* and 5(14.7%) were *A. baumannii,* and they reflected that multidrug resistance was significantly ($P<0.05$) higher in ESBL β-lactamase producers than non-ESBL and non- AmpC producers. Resistance to cefoxitin(30%) , and aztreonam (100 %) were observed in most of the ESBL producers isolates. A higher rate of AMPC β-lactamase (16.6%) production was seen among isolates from urine. maximum numbers AMPC production

(11.11%) *K. pneumoniae* followed by (5.55%) *E. coli* and 2 isolates only of *A. baumannii*. Al-Zubaidi(2013) recorded MDR *A. baumannii* from UTIs of Iraqi pregnant women.

Results of Mohammed (2015) in Diyala/Iraq showed highly resistance to cephalosporins and production of ESBLs among *A. baumannii.*

In 2016, Khadam and AL-Marjani (2016) recorded that all *A. baumannii* isolates have high level of resistance for imipenem ,cefotaxime , penicillin and tetracycline with percentage of (100%) , gentamycine and tobramycin with (87.5%) ,and all isolates were susceptible for colistin (100%), the presence of bla_{TEM} in (70%), bla_{CTXM} in (70%) and bla_{IMP} in (50%) of *A. baumannii* isolates.

Conclusion

We conclude that the spread of multidrug resistant *A. baumannii* isolates in Iraq. Research should focus on identifying novel agents with lower resistance.

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