

Phenotypic Characterization of Extended-Spectrum Beta-Lactamases and Metallo-Beta-Lactamase of Multi Drug Resistant *Acinetobacter baumannii* Causing Nosocomial Infections in Erbil City

Sakar B. Smile*, Kamal I. AL-OTRACHI

Department of Medical Microbiology, College of Medicine, Hawler Medical University, Erbil, IRAQ

*Correspondent author email: sakarsb.bio@gmail.com

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Abstract

Background: Resistance to broad-spectrum beta-lactams, mediated by extended-spectrum beta-lactamases, and metallo-beta-lactamase enzymes, is an increasing problem worldwide. The main aim is to study phenotypic characterization of extended-spectrum beta-lactamases and metallo-beta-lactamase multidrug resistant *Acinetobacter baumannii* in Erbil City. Materials and Methods: A total of 112 *Acinetobacter baumannii* bacteria were isolated from patients of all age groups from clinical specimens sputum, blood, pus, wound swab, urine and body fluids (Pleural fluid and cerebrospinal fluid). Samples were collected from different medical wards and intensive care unit departments of hospitals in Erbil City for a period of one year from March 2018-to-March 2019. Isolates were tested for the presence of extended-spectrum beta-lactamases and metallo-beta-lactamase. Detection of extended-spectrum beta-lactamases was done by the combined disk diffusion method, while metallo-beta-lactamase was detected by meropenem and imipenem combined with ethylenediaminetetraacetic acid disk method. Results: Twenty-five percent (28) *Acinetobacter baumannii* isolates were positive for extended-spectrum beta-lactamases, while 100 % (112) were metallo-beta-lactamase producers. Conclusion: *Acinetobacter baumannii* is becoming a global medical challenge due to the emergence of multi-drug resistance. Newer beta lactamase is a matter of concern as they are developing rapidly and lead to treatment failure. Carbapenems are known to be effective therapeutic agents for *Acinetobacter baumannii* infections and its resistance limits the use to polymyxins and colistin. Several new medicines are still in research and combination of drug therapy is being used in hospitals together to treat multidrug resistant *Acinetobacter baumannii* infections.

Key words: *Acinetobacter baumannii*, beta-lactamases, extended-spectrum beta-lactamases, metallo-beta-lactamase.

Introduction

Acinetobacter baumannii (*A. baumannii*) is an opportunistic pathogen, with the following characteristics of being Gram-negative, oxidase-negative, non-fermentative, non-motile coccobacilli and has broad range of antibiotic resistance. The bacterium is prevalent in most places especially in hospitals and other health care institutes [1]. High morbidity and mortality are the characteristics of nosocomial infections caused by *A. baumannii*, which

included urinary tract, skin and soft tissue infections, pneumonia and bacteremia especially in patients with severe health conditions [2].

During the last two decades, the advent and widespread distribution of bacterial infections resistant to beta-lactams, especially to 3rd generation of cephalosporins and carbapenems, has become a globally significant problem [3]. Carbapenems were once the mainstay of therapy, which are no longer effective in

controlling the infections caused by this organism. Though these drugs are still active against the vast majority of *A. baumannii* strains worldwide, the clinical utility of this class of antimicrobial is increasingly being jeopardized by the emergence of both enzymatic and membrane-based mechanisms of resistance. Increasing number of metallo-beta-lactamase (MBLs) in *A. baumannii* is an ominous development in the global appearance of resistance to beta-lactams (β -lactams) [4]. Beta-lactamases act on many cephalosporins, carbapenems, penicillins and monobactams. MBLs also mentioned as Class B beta-lactamases act on cephalosporins, penicillins and carbapenems but not on monobactams. MBLs have zinc as metal ion which is related to cysteine or histidine residue and it reacts with the carbonyl group of the amide bond of penicillins, cephalosporins and carbapenems [5].

Described, carbapenem resistance can be due to developed carbapenemase production. Carbapenem resistance is mainly due to either reduced levels of drug accumulation or increased expression levels of the pump efflux. Other risk factors responsible for colonization and infection with MBL producers include age of patient, duration of hospitalization, underlying diseases like diabetes, tumours or overcrowding in the hospital wards [6].

Outbreaks of infection caused by strains of *A. baumannii* resistant to multiple antibiotic classes, including carbapenems, are a serious concern in many specialized hospital units, including intensive care units (ICUs). The foremost implication of infection with carbapenem-resistant *A. baumannii* is the need to use "last-line" antibiotics such as colistin, polymyxin B, or Tigecycline [7].

The main aim is to study phenotypic characterization of extended-spectrum beta-lactamases (ESBL) and MBL multidrug resistant *A. baumannii* in Erbil City.

Materials and Methodologies

A total of 112 *A. baumannii* bacteria were isolated from patients of all age groups from clinical specimen's Sputum, Blood, Pus, wound swab, Urine and Body fluids (Pleural fluid and cerebrospinal fluid) collected from

different medical wards and ICU departments of hospitals in Erbil City for a period of one year from March 2018-to-March 2019.

Samples were inoculated on Blood agar (BD BBL, USA) and MacConkey agar (BD BBL, USA). isolates were identified by using standard microbiological methods like; Gram stain and biochemical tests such as; catalase test, oxidase test, coagulase test. Then, Species identification was done by VITEK 2 compact (bioMérieux, France). Antimicrobial susceptibility of isolated *A. baumannii* were tested by modified Kirby-Bauer disc diffusion method as per the recommendation of Clinical and Laboratory Standard Institute (CLSI, 2015) and VITEK 2 compact as per guideline [8]. Isolates showed multidrug resistant (MDR) were further tested for MBL production. MDR was defined as resistance to two or more drugs or drug classes of therapeutic relevance. *A. baumannii* ATCC19606 was used as quality control strains. VITEK 2 compact – identification and sensitivity testing confirmation were done by (GN card and AST N326). This study was conducted according to the ethical committing at Hawler Medical University.

Beta-lactamase test

The Cefinase disc (BD BBL, USA) was used to detect beta-lactamase enzyme among all *A. baumannii* isolates. It was impregnated with the chromogenic cephalosporin, Nitrocefin. This compound exhibits a very rapid color change from yellow to red as the amide bond in the β -lactams ring is hydrolyzed by a beta-lactamase. When a bacterium produces this enzyme in significant quantities, the yellow-colored disc turns red in the area where the isolate is smeared on disc. *Staphylococcus aureus* (ATCC 29213) were used as Positive control [9].

Antimicrobial Susceptibility Testing

Antimicrobial susceptibility testing was performed for 19 different therapeutically relevant antibiotics by the Kirby–Bauer disc diffusion method on Mueller-Hinton agar (MHA) (BD BBL, USA) according to Clinical Laboratory Standards Institute guidelines [8] and VITEK 2 compact (AST N326).

Antimicrobial agents including imipenem (IPM: 10 µg), meropenem (MEM: 10 µg), ceftazidime (CAZ: 30 µg), cefotaxime (CTX: 30 µg), cefepime (FEP: 30 µg), ciprofloxacin (CIP: 5 µg), Levofloxacin(LVX: 5 µg), piperacillin (PIP, 100 µg), Piperacillin-tazobactam(TZP: 100/10 µg), Netilmicin (NET: 10 µg), Tobramycin(NN: 10 µg), Amikacin (AN: 30 µg), Gentamicin (GN: 10 µg), Tetracycline (TE: 30 µg), Trimethoprim/sulfamethoxazole (SXT: 1.25/23.75 µg), Tigecycline (TIG: 15 µg), Polymexin B (TE: 30 µg), and Colistin (CL, 10 µg) were placed on an inoculated media. Then, the media were incubated for 18-24 h at 35 °C [8]. The isolates were tested against antibiotics showing resistance to at least three categories of drugs i.e. penicillins and cephalosporins, fluoroquinolones, and aminoglycosides that were considered as a multi-drug resistant [10].

ESBL Phenotypic Detection of Isolates:

Identification of ESBLs was performed for the isolates by double-disk synergy test (DDST). The isolates were examined for the inhibition zone of ceftazidime (30 µg), cefotaxime (30 µg), ceftazidime (30 µg) + clavulanic acid (10 µg) and cefotaxime (30 µg) +clavulanic (10 µg) on Muller Hinton agar. Pairs of disks (ceftazidime with ceftazidime/clavulanic acid and cefotaxime with cefotaxime /clavulanic) were placed on Muller-Hinton agar with 20 mm space between them. ESBL test was considered positive if the inhibition zone diameter in presence of clavulanic acid was ≥ 5 mm larger than that without it [11].

Phenotypic detection of MBL

For determination of phenotypic MBL production among the bacterial isolates a recently prepared bacterial suspension adjusted to 0.5 McFarland was streaked for confluent growth on a Mueller Hinton agar plate using a swab. Five microliters of ethylenediaminetetraacetic acid (0.35M EDTA) solution were added into a paper disk 6 mm diameter and dried without overfull. The disks were placed at the center of the plate. Ten

micrograms of meropenem, meropenem with EDTA, imipenem, and imipenem with EDTA disks were placed at a distance of 10 mm from the center, and the plate was incubated at 37°C for 16–18 h. Disks with EDTA alone were served as the negative control. The presence of zone around the antibiotics containing EDTA disks would indicate a MBL producer. We consider an isolate to be MBL positive if the zone of inhibition is larger than 2 mm when EDTA is added to the meropenem and imipenem disks. The test was repeated three times [12, 13].

Results

Over all 112 *A. baumannii* isolates were identified based on of colonial morphology on culture media, some biochemical tests and were confirmed by VITEK 2 Compact. Sputum samples (61, 54.5%) were the main predominant source of *A. baumannii*, the second largest source were blood samples (20, 17.9%), the third followed by wound swab (17, 15.2%), then CSF (5, 4.5%), C.V.Line (4, 3.6%), Pus (3, 2.7%), urine (1, 0.9%) and pleural fluid (1, 0.9%) as shown in (Figure 1).

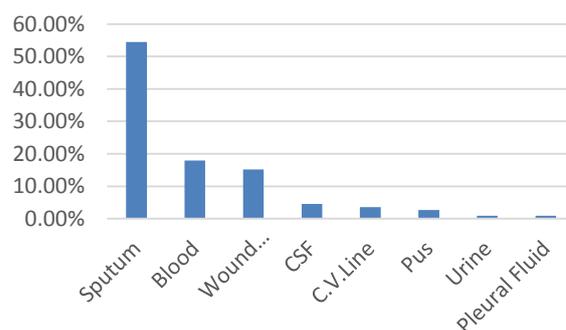


Figure 1: Distributions and percentage of infection sites of the *A. baumannii* isolates.

Beta-lactamase test:

All 112 *A. baumannii* isolates were tested to detect Beta-lactamase enzyme, and they were positive.

Antibiotic Susceptibility test (AST)

We found that all isolates were resistant with varied rates to (cefotaxime, ciprofloxacin, levofloxacin, amikacin, gentamicin,

piperacillin, piperacillin-tazobuctum, trimethoprim-sulfamethoxazole, Imipenem and meropenem). One hundred and ten (98.2%) of the isolates were resistant to Ceftazidime while the number of isolates and their resistance rate were (109, 97.3%) Cefepime and Tetracycline, (100, 89.3%) Tobramicin, (76, 67.9%) Netilmicin, (3, 2.7 %) Tigecycline respectively. Isolates were sensitive to Colistin and Polymixin B, furthered, all (112, 100%) (figure 2).

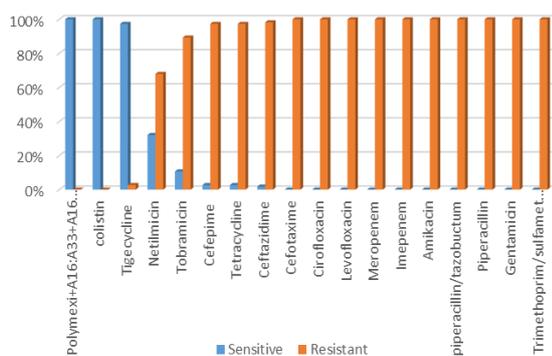


Figure 2: Percentage of antibiotic resistance among *A. baumannii*.

ESBL Phenotypic Detection of isolates:

Phenotypic identification of ESBL producing isolates have been carried out using DDST screening method. From a total of 112 samples, (28, 25%) *A. baumannii* isolates identified to be ESBL enzyme producers.

MBL Phenotypic detection of isolates:

All *A. baumannii* isolates were found to be MBL producers (figure 3).

Discussion

Infections caused by strains of *A. baumannii* resistant to multiple antibiotic groups, as well as carbapenems, are a heavy concern in few specialized hospital units and ICUs. The foremost implication of infection with carbapenem-resistant *A. baumannii* is "last-line" antibiotics like Tigecycline, colistin and polymixin B. *A. baumannii* infections tend to occur more frequently in patients on broad spectrum antibiotics, immune-compromised individuals and with underlying diseases and those exposed to invasive procedures [14].

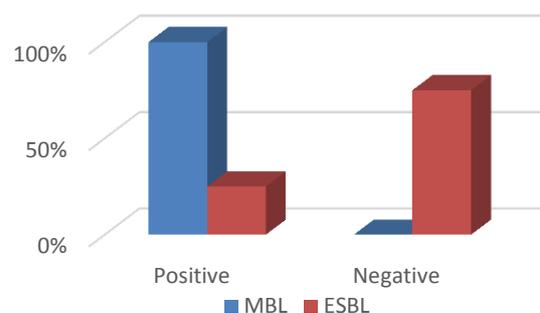


Figure 3: The percentage of ESBL and MBL producing *A. baumannii*

Beta-lactamases are grouped into 4 major molecular classes; A, B, C and D. A, C and D are referred as serine-beta-lactamases, whereas group B beta-lactamases are called MBL. Newer beta-lactamases that hydrolyse cephamycins, cephalosporins, monobactams and carbapenems are of increased concern as they limit therapeutic options leading to treatment failures and poor prognosis [15]. Different mechanisms contributing to resistance including; carbapenemase production, modification of penicillin-binding proteins (PBPs), loss of porins, and/or altered efflux pump activity [16].

In Our study, all isolates (100%) of *A. baumannii* were resistant to the majority of antibiotics (cefotaxime, ciprofloxacin, levofloxacin, amikacin, gentamicin, piperacillin, piperacillin-tazobuctum, trimethoprim-sulfamethoxazole, imipenem and meropenem), Followed by (110, 98.2%) of the isolates were resistant to Ceftazidime while Cefepime and Tetracycline (109, 97.3%), Tobramycin (100, 89.3%), Netilmicin (76, 67.9%), Tigecycline (3, 2.7 %) respectively. On the other hand, all (112, 100%) of *A. baumannii* isolates were sensitive to Colistin and Polymixin B. Our results agree with those mentioned by Hans *et al.* (2015), they found that all studied *A. baumannii* isolates obtained from Indian hospitals were resistant to gentamicin, erythromycin, trimethoprim/sulphamethaxole, piperacillin/tazobactam and ceftazidime. While they were sensitive to colistin and tigecycline [15]. In addition, our results agree with the reported results in Iran among MDR *A.*

baumannii in which 91.2% were resistant to ceftazidime [17].

In the present study, sputum samples (61, 54.5%) were the main source of *A. baumannii*, followed by (20, 17.9%) from blood samples, (17, 15.2%) wound swab, then (5, 4.5%) CSF, (4, 3.6%) C.V. Line, (3, 2.7%) pus, (1, 0.9%) urine and (1, 0.9%) pleural fluid. In previous study conducted in Coimbatore, South India isolation rate was found to be 73% from respiratory secretions [18]. In yet another study in Gurgaon, Haryana, 57.4% of the *A. baumannii* isolates were from respiratory secretions. 23.8% from blood and 2.5% from urine, an observation similar to ours, suggesting thereby that respiratory tract would be the most common site of isolation [19].

In the present study, (28, 25%) *A. baumannii* isolates were ESBL producers and (112, 100%) of isolates were MBL producers. Similarly, ESBL production was detected in (42, 28 %) of the isolates. In a study done by Sinha *et al.* (2007) dissimilarity, Phenotypic identification of ESBL producing isolates have been carried out using DDST screening method [20]. From a total of 100 samples, only seven (7%) *A. baumannii* isolates identified to be produced ESBL enzyme [21].

On another hand, 100% of meropenem and imepenem resistant strains were confirmed to be MBL producers. Contrary to our results, Muthusamy *et al.* (2012) detected 10% of the strains to be MBL producers in her study. In another study in South India, 14.8% of cases were MBL producers [22]. Noyal MJC *et al.* (2009) did a similar study in Pondicherry, South India and identified 6.5% MBL producers [23]. In a study done at AIIMS, New Delhi, 48.72% of *A. baumannii* strains were ascertained to be MBL- enzyme producers by the same method, thus implying rapid spread of resistance amongst this pathogen [24]. In another study done at India, found that 31.5% of *A. baumannii* were ESBL producers by the DDST and 14.4% were MBL producers by the combined disc diffusion test [25].

Conclusion

A. baumannii is becoming a global medical challenge due to the emergence of multi-drug resistance. Carbapenems are known to be effective therapeutic agents for *A. baumannii* infections and its resistance limits the use to polymyxins and colistin. Disappointingly, there are limited antibiotics for the treatment of infections caused by MDR *A. baumannii* on the horizon. Several new medicines are still in research and combination of drug therapy is being used in hospitals together to treat multidrug resistant *Acinetobacter baumannii* infections.

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