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Coexistence of the *bla*IMP and *bla*SIM Genes in Clinical Isolates of *Acinetobacterbaumannii*IN Babylon Hospitals-Iraq

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Abstract : Carbapenem antibiotics assume a basic parts in the treatment of serious nosocomial diseases brought on by microorganisms with decreased susceptibility to different antimicrobials. Shockingly , the prevalence of carbapenem- resistant bacteria has all the earmarks of being expanding and treatment choices for infections brought on via carbapenem-resistance bacteria are constrained and connected with high death rates.
This study was directed to decide the event of carbapenemases (blaIMP and blaSIM) producing*A. baumannii*isolates acquired from Babylon hospitals. Isolates were recognized by biochemical tests and more affirmed utilizing API 20E system. Carbapenem susceptibility was measured by utilizing disks diffusion test. Phenotypic identification of carbapenemase was performed utilizing the imipenem-EDTA disk and modifiedHodge tests. At that point isolateswere subjected to monoplex PCR focusing on *bla*IMP and *bla*SIM qualities. Ten (0.76%) *A. baumannii*isolateswere recuperated from clinical specimens. One (10%) isolatewas observed to be imipenem and meropenem resistant (MIC > 512 µg/ml). Six isolates(60%) gave positive result with the imipenem-EDTA double disk synergy test and modified Hodge test. PCR tests indicated five isolates(50%) were harbored *bla*IMP genes and six (60%) isolateswere harbored *bla*SIM genes. The present discoveries uncovered to rise of blaIMP and blaSIM carbapenemase producing*A. baumannii* clinical disengages in Babylon hospitals.

Introduction

Over the past 6 decades, antibiotics played a significant role in limiting the spread of infectious diseases, and consequently, improving their prognosis and reducing mortality [1]. The most disturbing issues experienced amid this period are the microorganism's capacity to amass differing resistance mechanisms and the development of strains that are resistant to all commercially accessible antibiotics combined with the absence of new antimicrobial agents[2]. This has brought about a restricted choice of antimicrobial agents for treatment of multidrug resistant isolates of *A. baumannii*. The most activeagents *in vitro* against the multidrug resistant *A. baumannii*(MDRAB) are the polymixins-polymixin B and polymixin E (Colistin) and tigecycline[3].

Multidrug resistant *A. baumannii*infections have a tendency to happen in immunosuppressed patients, in patients with serious underlying diseases, and in those subjected to invasive proceduresand treated with broad-spectrum antibiotics [4]. In this manner, infectionsdue to*A. baumannii* are as often as possible found in

intensive care units (ICUs)[1], where they are involved as the reason for ventilator-associated pneumonia (VAP), urinary tract infections, and bacteremia. *A. baumannii* likewise causes, but less as often as possible, complicated skin and soft tissue, abdominal, and central nervous system infections[5,6]. *A.baumannii* has turned into a noteworthy pathogen found in battle related injuries[7].

Materials and Methods

Isolation and Identification of A. baumannii :

A total of 1300 clinical specimens (included 588 burn swabs, 136 wound swabs, 50 from throat, 204 urine, 110 stool, 20 sputum, 162 blood, 15 ears and 15 eyes) were gathered from patients in Babylon Province hospitals more than one year time span beginning from March, 2014 to March, 2015. Isolates were recuperated from clinical specimens after culturing on MacConkey agar and incubated for overnight at 37°C, lactose non fermenting bacteria were sub-cultured and incubated for extra overnight. Suspected bacterial isolates which their cells are Gram negative coccobacillary or diplobacillus and negative to oxidase which further distinguished utilizing API20 E system.

Antimicrobial susceptibility testing:

Isolates were cultured on Mueller-Hinton agar and their susceptibilities to various antibiotic agents were tested by disk diffusion method as indicated by the Clinical and Laboratory Standard Institute's rules [8].

MIC determination:

Contingent upon producer's guidelines the antibiotic stripes (E-test) were applied to the agar surface , the antibiotics promptly diffuses into the encompassing medium in high to low concentration from one end of the strip to the next. The gradient stays stable after dispersion, and the zone of inhibition made takes the form of oval (Liofilchemmanufacture). Furthermore microbroth dilution method was done.

Imipenem-EDTA double disks synergy test:

Screening for metallo β-lactamases (MBL) was performed utilizing disks containing 1900 µg of EDTA in addition to 10 µg of imipenem disks were placed on the inoculated plates containing Muller Hinton agar. An expansion of ≥ 17 mm in zone diameter in the presence 1900 µg of EDTA contrasted with imipenem alone showed the presence of a MBL [9].

Modified Hodge test:

Imipenem was utilized for carbapenemase detection as described by Lee and his colleagues [10]. Positive test has a clover leaf-like indentation of *E. coli* Top-10 growing along the test organism growth streak inside the imipenem disk diffusion zone.

Genotypic recognition of blaIMP and blaSIMgenes :

DNA was extricated from the isolates by utilizing genomic extraction mini kit according to the manufacture instructions (Bioneer company, Korea). To amplify the genes encoding carbapenemases, a monoplex-PCR was run utilizing the primers of *bla*IMP gene (587bp: F/"5 AAG TTA ACG GGT GGG GC-3'and R/'5-AGT GAT GCG TCT CCA GCT TC - 3') as outlined by AL-Harmoosh[11], and primers of *bla*SIM gene (1138 bp: F/"5-AGA TAG TAA ATT TTA TAG - 3'and R/'5-CTC TAA CGC TAA TAG - 3') were described by Senkyrikova and his colleagues [12].

Amplification was performed in a 20 µl volume as recommended by PromegaMaster mix instruction. PCR amplifications were carried out on a thermal cycler (Prime, England). The cycling conditions for amplification of *bla*IMP gene were: initial denaturation of 94°C for 3 min and 30 cycles of 30 sec at 94° C, 1 min at 56° C, and 40 sec at 72°C, followed by 3 min at 72°C and for *bla*IMP gene were as a follow: initial denaturation of 94°C for 3 min and 40 cycles of 30 sec at 94° C, 30 sec at 50° C, and 40 sec at 72°C, followed by 3 min at 72°C . Amplified productswere distinguished by agarose gel electrophoresis in 1% Tris-borate-

EDTA (TBE) agarose (Promega, USA) and staining with ethidium bromide. The electrophoresis result was identified by utilizing gel documentation system (Claver, England).

Results

Isolation and Identification of A. baumannii :

Depending on the biochemical tests and API20E system it has beenable to isolate and identify of 10 (0.76%) isolates as *A. baumannii* from the 1300 clinical samples (Table 1).

Antimicrobial susceptibility testing :

As determined by disk-diffusion method, every one of the *A. baumannii*isolates showed distinctive pattern of resistanceto various antibioticagents (Fig.1), exhibiting highest resistance to penicillins (carbenicillin and ampicillin) with resistance rate of (100%), while 3(30%) of resistance were resistant to piperacillin.

High resistance rates were watched for each of amoxicillin/clavulanicacid and aztreonam (80%) , (70%) for cefepime , (60%) for each of ceftazidime and cefotaxime

The results likewise revealed that were high resistance rates for each of tobramycine, and gentamicin (70%) and moderate to amikacin (50 %).

The isolates demonstrated low resistance rates for the carbapenem antibiotic agents, imipenem , meropenem and ertapenem (10%). The percentage of resistance rate of the remaining antibiotic agents were as the following : (80%) for chloramphenicol, followed by colistin sulfate with (70%) , polymyxin B (50%) , trimethoprim-sulfamethoxazole (50%), (40%) for quinolones, (ciprofloxacin) and (20%) to each of tetracycline and doxycycline. Results revealed that all tested isolates were resistant at least of three classes of antibiotics, so that these isolates were considered to be multidrug resistant.

Table (1): Distribution of bacterial isolates recovered from clinical specimens among various hospitals in Babylon Province.

Hospital's name	No. of samples	No. (%) of *Acinetobacter baumannii* isolates	No. (%) of other bacterial spp. isolates	No. (%) of no growth cultures
Al- Hillah Teaching Hospital	885	7 (0.8%)	710 (80%)	168 (18%)
Babylon Teaching Hospital for Maternity and Pediatric	415	3 (0.7%)	235 (56.6%)	177 (42.6%)
Total	1300	10 (0.76%)	945 (72.69%)	345 (26.5%)

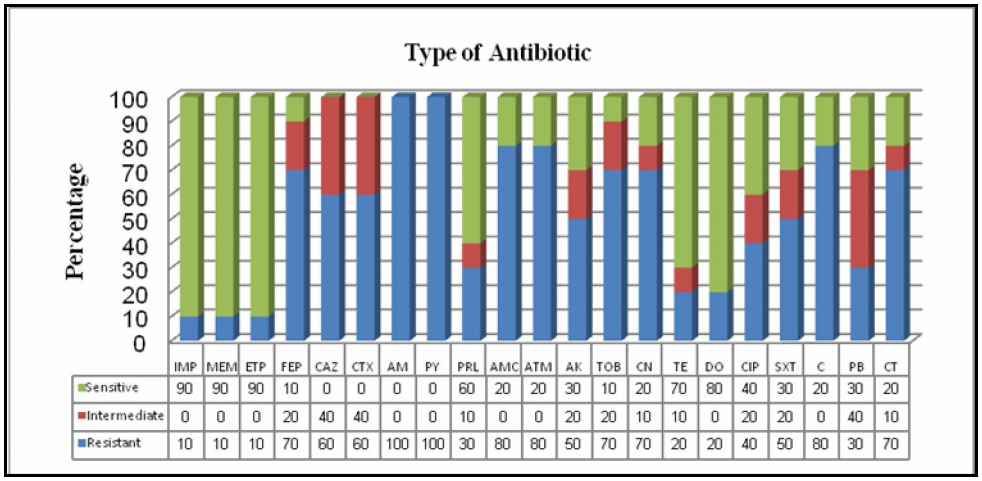

Figure (1) : Antibiotics susceptibility profile of *A. baumannii* isolates by disk diffusion method (n=10)

(IMP,Imipenem; MEM,Meropenem; ETP,Ertapenem; FEP,Cefepime; CAZ,Ceftazidime; CTX,Cefotaxime ; AM,Ampicillin ; PY,Carbenicillin ; PRL,Piperacillin ; AMC,Amoxi-clav; ATM,Aztreonam ; AK,Amikacin ; TOB,Tobramycine ; CN,Gantamicin ; TE,Tetracycline ; DO,Doxycycline ; CIP,Ciprofloxacin ; SXT,Trimethoprim-Sulfamethoxazole ; C,Chloramphenicol ; PB,Polymyxin B ; CT,Colistinsulphate).

MIC determination:

Table (2) revealed that *A. baumannii*isolates were resistant to imipenem, meropenem, and ertapenem with concentrations beyond values: 0.032 µg/ml - >512 µg/ml.

Table (2) : MIC of carbapenem antibiotics

Isolates No.	MIC (µg/ml)		
	IMP	MER	ERT
1	0.75	0.032	0.032
2	0.25	0.38	2
3	1	0.047	0.023
4	0.19	0.38	1.5
5	0.38	1.5	6
6	> 512	> 512	> 512
7	0.19	0.75	3
8	1	0.5	2
9	1.9	0.047	0.032
10	1	0.064	0.047

Phenotypic detection of carbapenem production:

1(10%) isolate exhibited upgrade of inhibition zone, with the imipenem-EDTA test whereas six isolates indicated positive results with modified Hodge test.

Genotypic detection of blaIMP and blaSIMgenes :

*bla*IMP genes were showed up in (50%) of *A. baumannii*isolates PCR products utilizing specific primers (Fig. 2), though, *bla*SIM qualities were showed up in (60%) of *A. baumannii*isolates PCR products utilizing specific primers. (Fig. 3). Consequently, table (3) shows the isolates that harbored *bla*IMP and *bla*SIM genes appeared as extensive drug resistant (XDR), which exhibited resistance to at least 5 classes of antibiotics were used in this study.

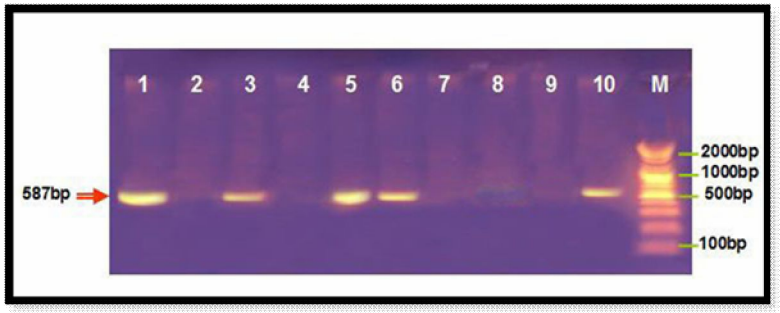

Figure (2):Agarose gel electrophoresis (1.5% agarose,70 volt for 1-2 hrs) for *bla*IMP gene product (amplified size 587 bp) using DNA template of *Acinetobacter baumannii* isolates. Lane (M), DNA molecular size marker (100- bp Ladder). Lanes (1, 3, 5, 6 and 10) of *A. baumannii*isolates show positive results. Lanes (2, 4, 7, 8 and 9) show negative results.

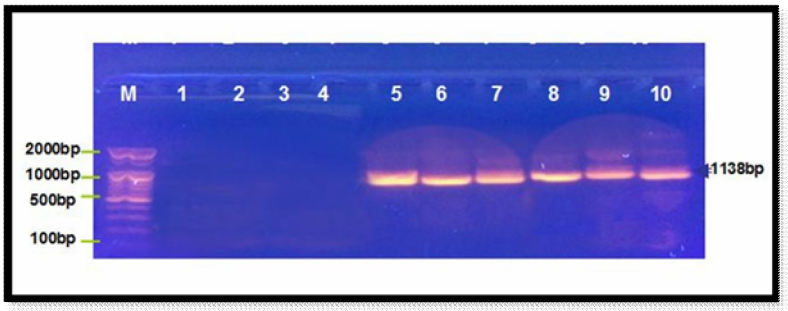

Figure (3):Agarose gel electrophoresis (1.5% agarose,70 volt for 1-2 hrs) for *bla*SIM gene product (amplified size 1138 bp) using DNA template of *Acinetobacter baumannii* isolates. Lane (M), DNA molecular size marker (100- bp Ladder). Lanes (5, 6, 7, 8, 9 and 10) of *A. baumannii* isolates show positive results. Lanes (1 , 2 , 3 and 4) show negative results.

Table (3) : Antibiotics resistant pattern of carbapenemase genes positive *A. baumannii*isolates.

Resistance pattern	No. (%) of *A. baumannii* isolates (N=10)	No. of antibiotics classes resisted
MDR	10 (100%)	3 or more
XDR	2 (20%)	9
	3 (30%)	7
	4 (40%)	6
	5 (50%)	5
PDR	1 (10%)	11

Discussion

The isolation rate of *Acinetobacter baumannii* appeared with low percentage (0.76%) accordingly to the biochemical tests and API20E.

High resistance rates were observed for most of the antimicrobials agents studied, including, penicillins, amoxicillin/clavulanic acid, aztreonam, and chloramphenicol. Antimicrobial resistance considerably restricts the available treatment options, especially resistance to carbapenem, which is considered to be the first option to treat severe infections due to *Acinetobacter* spp.[13]. El-Astal, mentioned that inappropriate and incorrect administration of antimicrobial agents and lack of appropriate infection control strategies may be the possible reasons behind increasing resistant rate of *A. baumannii* to common used antimicrobial drugs [14].

All *A. baumannii* isolates were screened by two phenotypic tests for carbapenemase production. The present study showed that (10%) of the isolates gave positive results by imipenem- EDTA disk test . Different studies which have used the IMP-EDTA to detect MBLs production in *A. baumannii* reported that (33%) of isolates have enhancement of inhibition zone, with the IMP-EDTA test[15]. However, there are four isolates which gave negative results with EDTA disk synergy test.

The most easily performed test for Carbapenemase detection is the modified Hodge's test, which has been found to be 100% sensitive for the detection of the carbapenemase[10]. Out of the 10 *A. baumannii* isolates which were enrolled in this study, 6 (60%) isolates were found to produce the carbapenemase enzyme by MHT and all the remaining isolates were found to be carbapenemase negative. In a previous local study, Alsehlawi and hiscolleagues reported that 4 (33.3%) of *A. baumannii* isolates recovered from Najaf hospitals were confirmed as carbapenemase producer using modified Hodge's test, whereas the same isolate gave negative result with imipenem-EDTA synergy test [15]. Another study from Croatia the Hodge test showed that 74% (72/97) of the *A. baumannii* isolates were positive for carbapenemase production [16] , whereas in a study from Pakistan has shown that 17 % of *A. baumannii* were positive for carbapenemase production by MHT [17].

Analysis of the genetic surroundings of the MBL-encoding genes identified in *A. baumannii* has revealed very similar structures, since the *bla*IMP, *bla*VIM or *bla*SIM genes are embedded in class-1 integron structures. In addition, the plasmid location of MBL genes explains their spread among *A. baumannii* and *P. aeruginosa* strains in specific areas, e.g., Italy and Korea [18].

Result from (Fig. 2) showed 5(50%) *A. baumannii* isolates had *bla*IMP positive results. This result agreement with similar Taiwanese study by Lin and his colleagues [19] who found that 1(20%) of *A. baumannii* isolates had *bla*IMP gene. In contrast, a study at Chinese hospital by Zhou and his colleagues [20] there is no detectable *bla*IMP genes in *A. baumannii* isolates.

*bla*SIM gene was appeared in 6(60%) of *A. baumannii* isolates (Fig. 3) . In contrast *bla*SIM gene was appeared in 7(3.3%) of *A. baumannii* isolates by Korean study [21] as well as there is no detectable *bla*SIM genes in *A. baumannii* isolates by Zhou, and his colleagues [20] in China.

As in the present study, such isolates (*bla*IMP and *bla*SIM positive *A. baumannii*) exhibit resistance to most antimicrobials that recommended by CLSI [8] and appeared to be extensive antibiotic resistance (XDR) (Table 3), this may creating a serious problem for choice of therapy[22], this results was more identical with the report of emergence XDR in *A. baumannii* isolates from patients in ICUs of Samsung Medical Center in Seoul, South Korea [23]. Hence, The occurrence of isolates contain *bla*IMP and *bla*SIM in Babylon Province hospitals may resulted from transfer of plasmid among resistant isolates rather than, several isolate may produce identical restriction pattern suggest the dissemination of *bla*IMP and *bla*SIM genes due to a clonal spread of resistant *A. baumannii* isolates [24].

Conclusion

Our study has demonstrated low spreading rate of multidrug resistant and *bla*IMP and *bla*SIM harbored *A. baumannii* isolates among patients with various infections. Sadly, numerous antibiotics endorsed to individuals are superfluous. As well as the overuse and misuse of antibiotics helps to produce drug-resistant bacteria.

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