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# Dissemination of Class 1, 2 Genes in Extensive Drug Resistant (XDR) Acinetobacterbaumannii Isolated from Clinical Speciemens in Babylon Province, Iraq

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***Abstract---*** *Six hundred samples from Al-Hilla Teaching Hospital and Mirjan Medical City in Babylon Province were composed through the period from October 2018 to February 2019. In addition to the Vitek2 method, twenty A.baumannii isolates were collected as unpolluted and main crops after samples were recognized using morphological properties and biomedical tests. Twenty A.baumannii isolates, as calculated by the Vitek 2 method. Results indicated that entirely isolates were resistant to at least 3 groups of antibiotics, therefore these isolates were expressed as being (MDR) 20(100 percent) multidrug resistant. Of which 15 isolates (75%) is marke d as XDR However,0(0%) for A.baumannii isolates were not resistant to all existing agents in all the classes of antibiotics tested and therefore PDR isolates cannot be considered. Two sets of primers were examined in all the isolates for the identification of Class 1, 2 Integron resistance genes. Showed positive results 6(30 percent) for Int1 and Int2 4(20 percent) of investigated genes.*

***Keywords---*** *A.baumannii, XDR MDR, Clinical isolates*

## I. INTRODUCTION

*Acinetobacter baumannii*are (Gm-ve), aerobic coccobacilli, nonmotile, negative to oxidase ,non-fermentative, that are abundant in environment and usually establish within the hospital situation producing a diversity of opportunistic nosocomial infections .The bacteria can be isolated from soil water, environment and from skin of human (Al-Ajeeli, 2016). The morpho logy 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-fermenting lactose, though they partly lactose ferment on agar of Mac C onkey. Wholly species of the *Acinetobacter* genus mature well on agar of MacConkey excepting for *A. lwoffii*.

One of the most interesting features of *A.baumannii*is the ease by which it can acquire resistance to various antibiotics. Mutations, new gene acquisitions, up-regulating expression of existing genes (should those be genes of drug-destroying enzymes or efflux pumps), or losing them, like in the case of porins, all may contribute to the broad spectrum resistance of these strains. As a result of the rapid acquisition of resistance genes to different and multiple classes of antibiotics, several drugs have already been removed from treatment. Options for Infections of *A.baumannii* such as penicillin, cephalosporin, aminoglycoside, quinolone and tetracycline (Al-Samaree and Al-Khafaji, 2016). Integrons are transportable bacterial genetic elements that serve as rally stages for catching genes

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then joining site-specific recombination mechanisms into exogenous and promoterless open reading frames (ORFs) and converting them into functional genes (Mazel, 2006).The word integron was initially invented to define the community of seemingly transportable elements and these elements are usually connected with transposons, conjugative plasmids, confirming their distribution through bacterial species (Cambray *et al*., 2011). Integrons seem to show an important role in transforming antibiotic resistance for the reason that they can bind, integrate and express gene cassettes which encode resistance to anti biotics (Phongpaichit *et al*., 2011).

## II. MATERIALS AND METHODS

### *Isolation and Characterization*

The bacterial isolates have been recovered from patients admitted to Al-Hilla Teaching Hospital and Mirjan Medical City, Babylon, Iraq. Wholly (20) isolates were exposed for isolation and purification to typical bacteriological culturing processes on blood and MacConkey agar plates for 24-48 hours at 37oC. The isolates were confirmed by Viteck 2 compact system (Biomérieux) (Macfaddin, 2000: Lahiri, and Purai, 2004).

### *Molecular Methods*

### *DNA Extraction and Amplifications*

Genomic DNA was mined from bacteria by rendering Favor Prep TM Genomic DNA Mini Kit to the industrialist's (Favorgen / Taiwan) protocols. The extracted DNA was kept at 2-8 oC. In training, primers for the oligo-nucleotides were used. The primer were used by conventional PCR thermocyclers to detect antibiotic resistance. As in table (1) these primers were given by (Alpha business, Canada).PCR master mix reaction was set by PCR Master Mix kit (Bioneer / Korea) and used as directed by the client.

Table 1: Primers used in this Study

| Genes | Sequence | References |
|---|---|---|
| *Int1* | F 5'-GGTGTGGCGGGCTTCGTG-3'<br>R 5'-GCATCCTCGGTTTTCTGG-3' | (Peymani*et al.*, 2012) |
| *Int2* | F5'-CACGGATATGCGACAAAAAGGT-3'<br>R5-'GTAGCAAACGAGTGACGAAATG-3' | |

## III. RESULTS

### *Antibiotic profile of A. baumannii Isolates by Vitek 2 System*

Twenty A.baumannii isolates, as calculated by the Vitek 2 method, displayed diverse designs of resistance to various antibiotics as showing the highest resistance to penicillins with a resistance rate of 20(100%) isolates. For cephalosporin antibiotics, advanced resistance was also identified with 20(100%) of cefepime resistant isolates, Cefoxitin, Ceftazidime, Ceftriaxone, and 17(85%) of cefepime resistant isolates. For the carbapenemantibiotics, imipenem, meropenem displayed the high resistant rate 20(100%) isolates Aminoglycosides variable resistance to Amikacin 11(55%) isolates. Gentamicin resistant 16 (80%). Tobramy cine resistant 20 (100%)I solates. as shown in figure(1).

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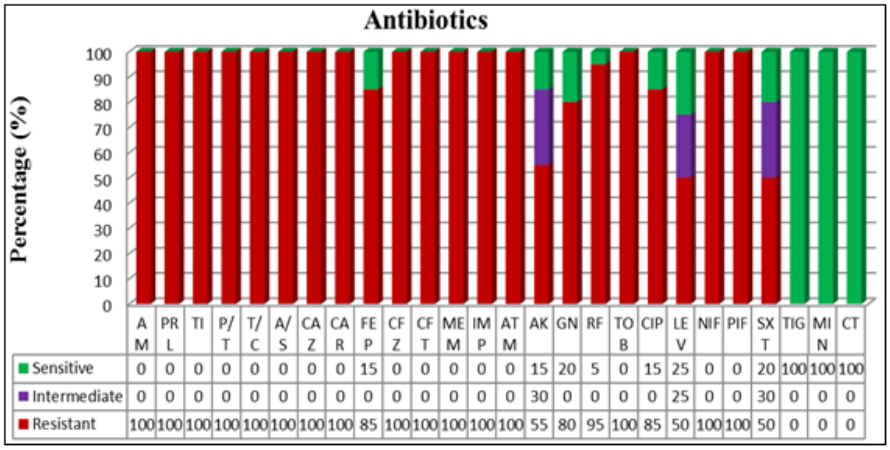


Figure 1: Antibiotic Profile for 20 isolates of A. baumannii by Vitek 2 System

AM, Ampicillin; PRL, Piperacillin; Ti,Ticarcillin; P/T, Pipracillin/Tazobactam; T/C, Ticarcillin/Clavulanic Acid; A/S, Ampicillin/Sulbactam; CAZ, Ceftazidime; CARCeftriaxone; FEP, Cefepime; CFZ, Cefazolin; CFT, Cefotxin; MEM, Meropenem; IMP, Imipenem; ATM, Aztreonam; AK, Amikacin; CN, Gantamicin; RF, Rifampicin; TOB, Tobramycine; CIP, Ciprofloxacin; LEV, Levofloxacin; NIF, Nitrofurantoin; PEF, Pefloxacin; SXT, Trimethoprim-Sulfamethoxazole; TIG, Tigecycline; MIN, Minocycline; CT, Colstin.

***Molecular Detection of Class 1, 2 Integron Genes in Clinical Isolates of A. baumannii***

Two sets of primers were examined in all the isolates for the identification of Class 1, 2 Integron resistance genes. Results are shown in figures (2and 3).Showed positive results 6(30 percent) for *Int1* and *Int2* 4(20 percent) of investigated genes, my study also revealed that integron of class 1, 2 was significantly associated with antibiotic resistance, including aminoglycosides, fluoroquinolones and compounds of betalactam. In other studies at Tabriz's Imam Reza hospital, Iran measurable percentage of (Peymani *et al*., 2012), two were detected in the current *A.baumannii* isolates, with positive MDR pattern. Seventy four (92.5%) of MDR isolates were found to have the class 1 integron, Class 2 integron gene was not found in this study. In other studies detection of class 1integron in *Acinetobacter baumannii* isolates collected from nine hospitals in Turkey (ÇİÇek *et al*., 2013),the *Int1*gene were detected in (38.7% ) of the total isolates and no *Int2*integron was detected.

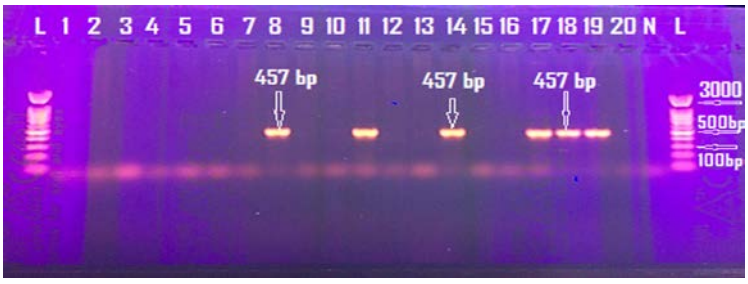


Figure 2: Gel electrophoresis (1.5 agarose, 70 volt for 60-120min) for the product Int1gene (amplified size 457 bp) using A.baumannii isolate DNA template .Lane (L), molecular size marker with DNA (100-bp Ladder). Lanes (8,11.14. 17,18 and 19) show positive results. Lanes (1,2,3,4,5,6,7.9,10,12,13,15,16 and 20) show negative results. Lane (N), Controlling negative.

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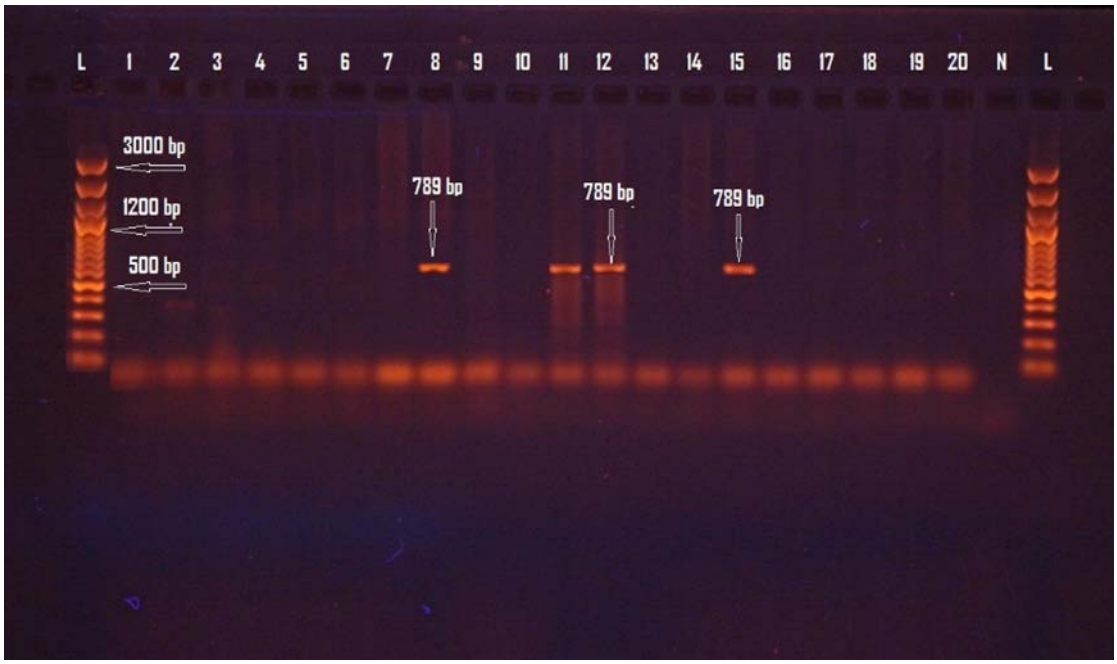


Figure 3: Gel electrophoresis (1.5 agarose, 70 volt for 60-120 min) for the Int2 gene product (amplified size 789 bp) using A.baumanniiisolate DNA template. Lane (L), molecular scale marker of DNA (100-bp Ladder). Lanes (6,11.12 and 15) show positive results. Negative results are shown in lanes (1,2,3,4,5,7, 8,9,10,13,14 ,16,17, 18,19 and 20). Lane (N), Controlling negative.

## IV. DISCUSSION

In fact four chief machines that can due to resistance to β-lactam antibiotics by β-lactamases, they inhibit the growth of sensitive bacteria by inactivating enzymes in the bacterial cell membrane, which are involved in cell synthesis in the third phase. It is during this stage that linear peptidoglycan strands are entangled in a fishnet like polymer that surrounds the bacterial cell and provides osmotic stability in the patients' hypertonic mileage. Alteration in target affinity, reduced uptake and over expression of efflux mechanisms (Zowawi *et al*, 2015).

Aminoglycoside resistance is too a main threat that tips to resistant phenotypes presentation susceptibility to aminoglycoside antibiotics for example (gentamycin, amikicin and tobromycin) in *Acinetobacte*. Wholly 3types of enzyme-modifying aminoglycosides known were establish in *A.baumannii*. These enzymes are the O-phosphorus transferases (APH) and O-nucleotidyl transferases (ANT) that catalyze hydroxyl groups' nucleotidylation (adenylation) and phosphorylation, and finally the N-acetyl transferases (AAC) that catalyze amino groups ' acetylation thus making antibiotics (Moniri *et al*., 2010;Raheem *et al*.,2019).

Integron has many antibiotic resistance gene cassettes encoding resistance to a wide range of antibiotics in *Acinetobacter spp*. (Peymani*et al*., 201 2).Nevertheless, these nagetive integron isolate were also reistance to antibiotic, antibiotic resistance genes could be acquired from these isolates through chromosomal encoded enzymes or other mobile componentts. Most of the positive integrin isolates were reistance to aminoglycoside compounds. It could be explained by the fact that many gene cassettes which confer aminoglycoside resistance are carried on

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class1,2.The study also demonstrated a high rate of resistance to several types of betalacta m compounds in integron-positive strains suggesting the carriage of several beta-lactamase encoding genes by Class 1,2 integrom and carbapenemase OXA-types Metallo-beta-lactamase enzymes may have led to carbapaenem resistance, extended to cephalosporins, most penicillins, and penicillin-beta-lactamase inhibitor compounds, results showed a no correlation between the existence of Class 1,2 integron and fluoroquinolone resistance. Maybe because resistance to fluoroquinolone compounds is a result of chromosomal point mutations (Mahdian *et al*., 2015).

Integrons are transportable bacterial genetic elements that serve as rally stages for catching genes then joining site-specific recombination mechanisms into exogenous and promoterless open reading frames (ORFs) and converting them into functional genes (Mazel, 2006).The word integron was initially invented to define the community of seemingly transportable elements and these elements are usually connected with transposons, conjugative plasmids, confirming their distribution through bacterial species (Cambray *et al*., 2011).Integrons seem to show an important role in transforming antibiotic resistance for the reason that they can bind, integrate and express gene cassettes which encode resistance to anti biotics (Phongpaichit *et al*.,2011).Wholly recognized integrons have three efficient constituents essential for the obtaining of exogenous genes: I an integrase (intI) coding gene going to the family tyrosine recombinase which intermediates recombination; (ii) a main recombination site (attI); and (iii) an outward-oriented promoter (PC) for the appearance of integ rated/captured genes; Cassette integrations happen primarily at the ProximalattIsite, confirming perfect appearance of recently captured cassettes by bringing them under the PC promoter's control (Cambray *et al*., 2011).Established on the encoded sequence of IntI proteins, the integrons were classified into diverse classes (Chowdhury *et al*., 2013).To date, nine diverse kinds of integrons, from classes 1 to 8 and an unidentified class have been identified (Abraham, 2011), and of which classes 1, 2 and 3 are greatest clinically relevant because they relate mainly to genes of antibiotic resistance. These are mainly related with genes of antibiotic resistant and are also known as the integrons of mobile resistance (MRI) (Chowdhury *et al.*, 2013). Clinically, Class 1 integrons are greatest predominant, found in clinical Gram-negative isolate surveys of 22 per cent to 59 percent. These have furthermore recently started appearing in (Gm +ve) bacteria. Class 1 and 2 integrons were specified in UPEC and related to the carriage of multidrug resistance (MDR) (Abraham., 2011)

## V. CONCLUSION

This results showed (XDR) *Acinetobacter baumannii*, high rate resistance to Penicillins, Carbapenem and Cephalosporin antibiotics with rate of resistance of 20(100%) isolates, and 17(85%) resistant to Cefepime. Aminoglycosides variable resistance to Amikacin 11(55%) isolates. Gentamicin resistant 16(80%).Tobramycin resistant 20 (100%) isolates. The results revealed that all 20 examined isolates of *A. baumannii* had Class 1, 2 Integron resistance genes. With positive results 6(30 percent) for *Int1* and *Int2* 4(20 percent).

## VI. ETHICAL APPROVAL

Scientific Research Study Ethical Commission by ethical approval of ministries of ecological / health and higher education / scientific research in Iraq together.

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