DETECTION OF PLASMID– MEDIATED OQXA AND OQXB GENES IN CLINICAL ISOLATES OF ACINETOBACTER BAUMANII TAKEN FROM IRAQI PNEUMONIC PATIENTS

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Abstract -
Horizontal gene transfer plays a fundamental role in getting new traits like antibiotic resistance which is associated with dramatic increase in infection incidence, patients' morbidity and mortality rates. The present study investigated the possible role of plasmid- mediated multiple drug resistance trait via OqxA and OqxB in Acinetobacter baumannii. The study included 48 VITEK- identified MDR A. baumannii isolates taken from pneumonic Iraqi patients. Each isolate was examined to the presence of plasmid(s) using 0.7% agaros gel electrophoresis. Positive isolates were subjected for genetic detection of plasmid-located efflux genes, OqxA and OqxB using polymerase chain reaction technique. Results revealed the presence of mega-plasmid in all the studied isolates. Results also showed that 45.8% of isolates were positive for OqxA gene, 12.5% were positive for OqxB gene, and 12.5% of isolates were positive for both genes. On the other hand, 29.1% detected to be negative for both genes. Those results refer to the significant role of horizontal gene transfer represented by plasmid in acquisition of multiple drug resistance trait in MDR- A.baumannii. To the far of our knowledge, it is for the first time to detect plasmid-mediated OqxA and OqxB genes in MDR A. baumannii, where, OqxA gene looks to be superior to OqxB in exhibiting multiple drug resistance in A. baumannii.

Keywords - MDR A. baumannii, mega-plasmid, plasmid-mediated OqxA, plasmidmediated OqxB.

I. INTRODUCTION

According to the WHO reports, the multiple drug resistance (MDR) phenomenon is considered as one of the most substantial aspects facing human health [1] A. baumannii was reported by the World Health organization (WHO) on the top of pathogen list that requires urgent treatment. Its potential ability to acquire resistance to traditional antibiotics, alongside with its inherent virulence factors has created a deadly combination culminated in plaguing the hospitals with this successive pathogen [2] .Infection with MDR A. baumannii had usually associated with long hospital stays and high mortality rates [3] .

Several factors had led to the evolution of multiple drug resistance (MDR) A.baumannii including, misuse of antibiotics, imprecise compliance with physician prescription, genomic equipment with mobile genetic elements and the presence of extrachromosomal elements which potentially mediates horizontal gene transfer, ultimately leading to acquisition of new traits including but not limited to acquisition of drug resistance [4]and [5].

A. baumannii is characterized by its open genome with overabundance of mobile genetic elements like plasmids, islands, transposons and integrons. Such genomic constituent equips the bacteria with a pivotal ability to evolve and in particular the acquisition of MDR[6].

Since the last two decades, A.baumannii, has been a notable pathogen causing a nosocomial infections and associated with critically ill patients expressed by sever wounds and burn infections, meningitis, endocarditis, skin and soft tissues infection, UTI, bacteremia and ventilator associated pneumonia among whom the mortality rate may reach up to 35% [7]. Efflux pump liberate antibiotics and toxic substances from the cell that will result in increasing the minimum inhibitory concentration. Efflux pumps compartments have been detected in almost all bacteria species and manifested by acquisition of both intrinsic and extrinsic antimicrobial resistance by lowering the intracellular antibiotic concentration. Efflux pump genes are mostly located on the bacterial chromosome, however plasmid- mediated efflux genes were further detected , such as Tet, MexCD and QacBIII [8] .

Several mobile genetic elements have been characterized in A.baumannii, different size of conjugative and mobilizable plasmids, insertion sequence (IS), transposons (Tn), integrons and transposable bacteriophages. Among them, plasmids and integrons are mostly contributed to MDR trait[9].

In A.baumannii, five major classes of efflux pumps had been characterized: Major facilitator (MF), Multidrug and toxic efflux (MATE), Resistance-nodulation cell division (RND), Small multidrug resistance (SMR) and ATP- binding Cassette (ABC) [10] and [2].

All the mentioned efflux pumps utilize the proton motive power as energy source to conduct their function, with exception of ABC superfamily that

utilize ATP-hydrolysis for substances transportation. From those five transporters super-families, the NRD is the most important one in A.baumannii, due to its extrusion activity to a broad range of antimicrobial agents and antiseptics[2] .

II. MATERIALS AND METHODS

2.1. Study design and bacterial isolates:

Forty eight A.baumannii isolates were obtained from clinical samples (sputum and bronchioalveolarlavage) of pneumonic patients admitted to the Respiratory Care Unit (RCU)/ Al-Imamain Al-Kadhimain Medical City, from September 2019 till February 2020. The preliminary identification of bacteria was done depending on the conventional bacteriological methods and biochemical tests. Further, bacterial identification was confirmed by VITEK system. Bacterial isolates were cultured in Lauria Bertani broth, from which the plasmids were extracted. For storage, isolates stored in -80 in nutrient broth with 20% glycerol.

Gene	Primer sequence	Product size	Reference
Oqx-A	F:5-GCG TCT CGG GAT ACA TTG AT-3	482 bp	[12]
	R:5-GGC GAG GTT TTG ATA GTG GA-3		
Oqx-B	F:5-CTG GGC TTC TCG CTG AAT AC-3	498 bp	[12]
	R:5-CAG GTA CAC CGC AAA CAC TG-3		

2.2. Antimicrobial susceptibility test:

Antibiotic susceptibility test was carried out by conventional method, Kirby- Bauer method as prescribed by Clinical and Laboratory Standard Institute (CLSI) guidelines 201811 .Briefly, bacterial inoculums equivalent to 0.5 McFarland standard was streaked by lawn streaking technique (heavy streaking) on Muller-Hinton agar plates and tested against different classes of antibiotics, including: Aztronem, Cefotaxime, Ceftriaxone and Ceftazidime, Gentamicin, Tobramycin, Ciprofloxacin and Meropinem, Impenim, Levofloxacin, Amikacin, Tigacycline and Colistin, each antibiotic was loaded on wafer disc. Inhibition zone was calculated in millimeters. The antibiotic susceptibility of each isolate was further confirmed by VITEK system.

2.3. lasmid extraction and molecular detection of OqxA and OqxB genes:

Bacterium inoculum was cultured in 1 ml Lauria-Bertani broth, incubated at 37 °C for 24 hrs. Plasmid was extracted according to the manufacturer instructions using (Wizard Plus SV Minipreps DNA purification system/ Promega/ USA) kit. In the final step, elution buffer was minimized to 35 µl. For plasmid identification, 5µl of plasmid extract and 2µl loading dye (Promega/ USA), were resolved on 0.7% gel electrophoresis, run at 70 Voltage / 2 hrs., the plasmid molecular weight was determined using a ladder ranging from 10200- 500 bp from (Bioneer/Republic of Korea). Samples identified to possess plasmids were further examined by polymerase chain reaction (PCR) technique (PCR Express/Hybaid/USA), to detect the presence of OqxA and OqxB genes, using the following primers:

The PCR thermo-cycling condition was performed as follows:

Gene	Initial denaturation	Denaturation	Annealing	Extension	Final Extension	Number of cycles
Oqx-A	95C°/5 min	95C°/1 min	56C°/1min	72C°/1min	72C°/3min	30 cycles
Oqx-B	95C°/5 min	95C°/1 min	57C°/1min	72C°/1min	72C°/3min	30 cycles

The PCR products were further identified by 2% gel electrophoresis, run at 70 voltage for 1:30 hrs., using a ladder ranging 2000- 100 bp (Bioneer/Republic of Korea).

2.4. Data analysis:

Data were described as frequency and percentage.

III. RESULTS AND DISCUSSION

3.1. Antimicrobial susceptibility:

The results of antimicrobial susceptibility test showed that all isolates (100%) were multidrug resistance, this percentage in agreement with the study done by Sura et. al, 2019 [13]. The frequency of resistance ranged from high resistance to Aztronem (90.8 %) , Cefotaxime (87.3%), Ceftriaxone (87.1 %) and Ceftazidime (84.1) respectively. Moderate resistance to Gentamicin (73.3%), Tobramycin (70%), Ciprofloxacin (69%) and Meropinem (55.2%) and low rate of resistance to Impenim (46.7%), Levofloxacin (45.2%), Amikacin (43%), Tigacycline (41.3%) and Colistin (27%). The current study showed that the lowest resistant was to Colistin, this result agreed with other study reported that (97%) of the isolates were sensitive to Colistin[14] .

3.2. Plasmids detection in A. baumannii isolates:

Results of 0.7% Agarose gel electrophoresis revealed that all MDRA.baumannii isolates under investigation possessed plasmids more than 10200 bp (Figure-1).

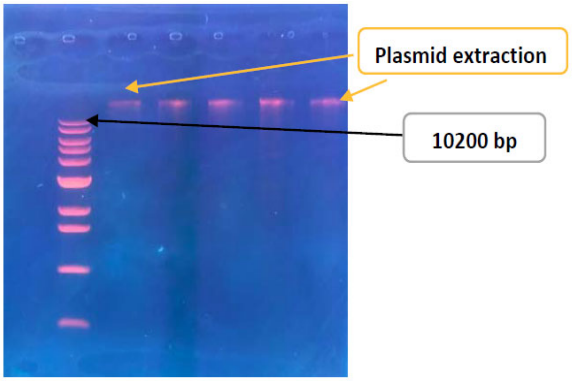


Fig.1: Gel electrophoresis of plasmids extracted from A. baumannii isolates analyzed on 0.7% agarose, appeared in size more than 10200 bp.

Despite the evidence and predominance role of plasmids in transmitting both virulence and antimicrobial resistance genes there was no study referred to the presence of plasmid-mediated Oqx-A and Oqx-B genes in A. baumannii and even the role of those genes in the acquisition of antimicrobial resistance trait. That motivated us to investigate the presence and possible role of plasmid- mediated RND-efflux pumps, both Oqx-A and Oqx-B in evolving the MDR trait in human clinical isolates using conventional PCR technique for detection.

Comparing to other studies conducted in other countries, the detected plasmid size in comparable to others in the same bacterial species worldwide. It was recorded before that A. baumannii harbor plasmids ranging in size from 2 kb to more than 100kb and that large plasmids usually encompass multiple antibiotic resistance genes and transposable elements comparing to the small ones that had led to overlook the small plasmids [6] and [15].

The presence of both small and big plasmids in A. baumannii, ranging from 4.7 Kb - 44.7 Kb was mentioned in a study conducted in Australia [5] . The globally disseminated A. baumannii strain, strain A297 (RUH875), was analyzed whole genome sequence and found to harbor three plasmids two of them 8.7 Kb carry tobramycin, kanamycin and gentamycin resistance genes, while the third one is 200 Kb carry resistance genes for streptomycin, sulphonamide and mercuric ions at a high frequency [16].

3.3. Molecular detection of OqxA and OqxB genes:

Conventional PCR technique was applied to detect Oqx-A and Oqx -B genes. PCR products for both genes primers were close to each other, the PCR product of Oqx-A gene primer was 482 bp comparing to 498 bp for Oqx-B. Both products appear close to 500 bp band of the ladder (Fig. 2).

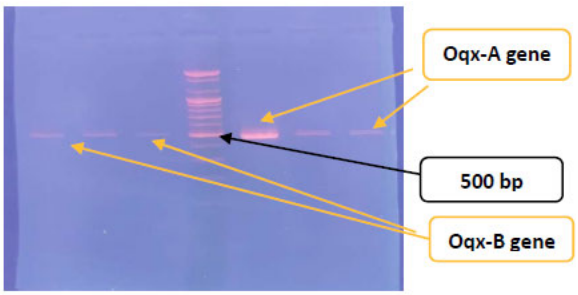


Fig.2: Detection of PCR products of OqxA and OqxB genes on 1% agarose gel electrophoresis.

Results showed the presence of Oqx-A gene in 22 out of 48 isolates (45.8%), OqxB gene was detected in 6 out of 48 isolates (12.5%), also 6 out of 48 isolates (12.5%) of the cases were positive for both OqxA and OqxB genes. While 14 isolates out of the 48 studied isolates (29.1%) were found to be negative for both genes (Table. 1).

Thus, it is revealed that Oqx-A Oqx-B genes present in about two third of the MDR isolates, pointing to the substantial role of those proteins in exhibiting the resistance trait against different antibiotic.

This speculation was supported by the fact that A.baumannii is featured to express a wide range of efflux pump transporters that enable them to exclude structurally different molecules including antibiotics from different classes out of bacteria, lowering their intracellular concentration and capacitate bacteria to survive and nourish[8].

As far as we know that those two genes were not identified before in A. baumannii plasmids. However, RND-OqxA and OqxB were first identified in conjugative plasmid in Escherichia coli isolated from swine manure, later they were further identified in different species of Enterobacteriaceae, including E.coli , K.pneumonia and Salmonella species, from different samples including humans, food animals and companion animals samples [17].

It is reported that Oqx-A and Oqx-B were identified on both chromosomal DNA and plasmids of K.pneumonia and enterobacteriaceae, flanked in both locations by IS26 like elements, conferring low-intermediate resistance to several antibiotics including tigecycline, quinolones and nitrofurantoin in addition to other disinfectants and detergents [8].

Moreover, those genes were further detected on plasmids of S. enterica. The results pointed to the presence of large plasmids in MDR- A.baumannii harbor efflux pump genes Oqx-A and Oqx-B. Those two genes provide bacteria with resistance trait, since they take charge of exodusing antimicrobial compounds and other toxic substances out of the bacterial cell and thus nourishing the multidrug resistance trait.

IV. CONCLUSION

It is concluded that acquisition of efflux pump (Oqx-A and Oqx-B) genes through horizontal gene transfer play a significant role in making A. baumannii a prosperous pathogen through improving its capacity to adapt the surrounding environment, this acquisition seems to be a potential agent in disseminating MDR A. baumannii in Iraq.

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