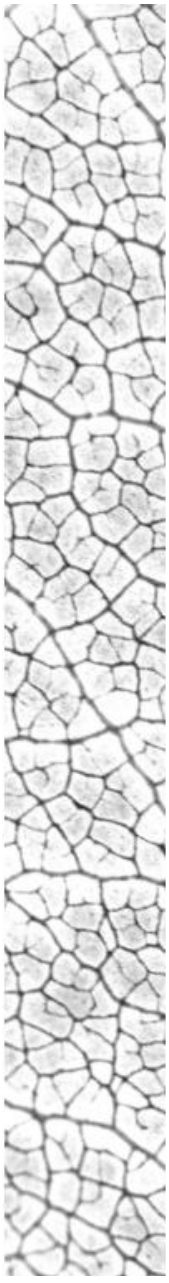

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Relationship between multi-drug resistance and extended-spectrum β-latamase genes of *Acinetobacter baumannii* in different wards at Basra Hospitals

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Abstract

Background: The present study was aimed for determination the prevalence of Extended- Spectrum β- Lactamase (ESBL) genes from Acinetobacter baumannii isolates obtained from hospital-acquired infection at Basra hospitals and to determine the relationship ESBL genes with Multidrug Resistance (MDR) among these A. baumannii isolates. **Methodology:** A total 120 samples were collected from burn, urine, Intensive Care Unit (ICU) and pediatric wards at different Basra hospitals and these were identified depending on standard microbiological methods with VITEK-2 system. The high percentages were (31.5%) isolates of Staphylococcus aureus followed by pseudomonas aeruginosa (27.6%) and Acinetobacter baumannii (25.3%). **Results:** Furthermore, antimicrobial susceptibility test and Multi-Drug Resistance of A. baumannii were achieved according to CLSI method. It has been found that A. baumanii had high resistance that Cefotaxime (94%), Imipenem (91%), Cefoxitin (85%), Merpenem (82%), Gentamicin (76%), Aztreonam (73%), Ciprofloxacin (70%) and Ampicillin (61%). On the other hand, high percentage (84.6%) of multidrug resistance was observed in A. baumannii. In addition, phenotypic expression of ESBL- A. baumannii was demonstrated by Double Disc Synergy Test (DDST) method in (73%) isolates of MDR- A. baumanii . ESBL genes (bla- TEM, bla- SHV, bla- CTX) of these isolates were detected by Polymerase Chain Reaction (PCR) assay. The most frequent genes of A. baumannii were bla- TEM genes 21(64%), followed by bla-SHV genes 17(52%) and bla- CTX genes 14(42%). **Conclusion:** From this study, we concluded that significant relationship can be occurred between MDR isolates of A. baumannii with ESBL genes among environment of Basra hospitals.

Keywords: *Multidrug Resistance,* ESBL genes, Acinetobacter baumannii, Hospitals

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INTRODUCTION

Acinetobacter genes are Gram-negative bacteria and many species of these bacteria have ability to cause human diseases, for exampies: pneumonia and wound in fections (Tuwaij, 2014). *Phenotypic and Molecular Characterization of β-Lactamase Producing Serratia spp. Isolates in Najaf Hospitals* (Doctoral dissertation, Ph. D. thesis. College of Science, Kufa University). Whereas the percentage about 80% for these diseases were Acinetobacter baumannii . A. baumannii can spread among hospitalized patients because they have mony virulence factors, antibiotics resistance efflux pumps and iron acquisition mechanisms (Slama, 2008). In last three decades, multiple drug resistance (MDR) isolates were appeared as hospital acquired infection in many countries (Perez, et al. 2007). A. baumannii is a well-know pathogenical bacteria in hospital along with Pseudomonas aeruginosa (McConnell, Actis, & Pachón, 2013). Acinetobucter banmannii is considered as worldwide infection in various wantries during the last three decades and these resistance was encoded by two genes. These genes are included lactamase encoded enzymes and blaTEM, blaCTX. These genes gave this bacteria resistance in the wide variety of environments (Zarrilli et al., 2009). In addition may researches are recorded that Acinetobacter spp. have other genes for resistance in the hospital environment: OXA-48, OXA-181, SHV, balOXA-23,24,51 and methylase and ESBI genes. A. baumannii produce biofilm by fimbria on the surface it with exopolysaccharide and convert it to super bug on the cell wall Nordmann, Naas, & Poirel, 2011). Chen et al.,2012). Therefore, the purpose of the present study was to investigate antibiotics susceptibility of A.

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Table 1. Primers sequences of three genes of ESBL- A. baumannii

Gene	Nucleotide sequences (3-5)	Product size(bp)	Anneal. ℃	Ref.
bla TEM	F-CAGCGGTAAGATCCTTGAGA R- ACTCCCCCGTCGTGTAGATAA	800	45	[12]
bla SHV	F- GGCCGCGTAGGCATGATAGA R- CCCGGCGATTTGCTGATTTC	868	53	
bla CTX	F- AACCGTCACGCTGTTGTTAG R-TTAGGCGTGGTGAAGTAAG	594	53	

baumannii at Basra- hospitals and determine the genes responsible for extended- spectrum beta- lactamases (ESBL) production.

MATERIAL AND METHODS

Sample Collection

Atotal of 120 samples were collected from burn, urine, Intensive Care Unit (ICU) swabs and pediatric swabs at different hospitals.

Isolation and Identification of *Acinetobacter baumannii*

All samples were cultured on Blood Agar, MacConkey Agar and CHROM agar Acinetobacter for isolation of *Acinetobacter baumannii* , incubated for 24h at 37℃. In addition, VITEK-2 identification system tests were used according to the manufacture instructions (Constantiniu, Romaniuc, Iancu, Filimon, & Taraşi, 2004. Wayne, 2011).

Antibiotic Sensitivity Testing

Disk Diffusion Method was used for antibiotic sensitivity test through Kirby- Bauer antibiotic testing according to clinical and Laboratory Standards Institute (CLSI) using eight antibiotics (Bauer, 1966. Antibiotics were as follows: Imipenem (IPM, 10µg), Meropenem (MEM, 10 µg), Gentamycin (GM, 10 µg), Ampicillin (AMP, 10 µg), Ciprofloxacin (CP, 5 µg), Cefoxitin (CEF, 30 µg), Cefotaxime (CEF, 30 µg) and Aztreonam (AZT, 30 µg).

Determine of Multiple Drug Resistance of A. baumannii

All isolates of *A. baumannii* was determined as Multiple Drug Resistanse (MDR) according (Wayne, 2011). Any isolate was resistance for three different antibiotics classes was considered as MDR isolate.

Primary Phenotypic Detection of ESBL- A. baumannii

All isolates of MDR- *A. baumannii* were selected for primary detection of Extended- Spectrum- β-Lactamases (ESBL) enzymes bases on [10]. In this method, all isolates of MDR- *A. baumannii* was tested against three antibiotics as following: Ceftriaxone 30 µg, Cefotaxime 30 µg and Ceftazidime 30 µg and any bacterial isolate appearing zone inhibition by ≤ 25mm, ≤ 27mm, ≤ 22mm respectively was represented as ESBL isolate.

Contirmatory Phenotypic Detection of ESBL- A. baumannii

Table 2. Numbers and percentages of the bacterial species

Bacterial species	n= 130 isolates No. (%)
Staphylococcus aureus	41(31.5%)
Pseudomonas aeruginosa	36(27.6%)
Acinetobacter baumannii	33(25.3%)
E. coli	13(10%)
Enterobacter aerogenes	7(5.3%)

Double Disc Synergy Test (DDST) was done for contirmative of the primary phenotypic detection by ESBL- *A. baumannii*. According to this test: bacterial suspension was adjusted to 0.5 Mc-Ferland standard tube. Amoxicillin 10µg + Clavulanic acid 20µg was placed in the center of Muller- Hinton Agar plate, around three sides of Ceftriaxone 30µg, Cefotaxime 30µg and Ceftazidime 30µg were placed with distance of 15mm to ceter of Amoxicillin 10µg + Clavulanic acid 20µg. In addition, these plates were incubated at 37℃ for 24h. Any inhibition zone was considered as positive result (Sarojamma, & Ramakrishna, 2011; Okebiorun, & Jatto, 2017).

Extraction of DNA

The total DNA of all isolates of ESBL- *A. baumannii* were extracted by the DNA extraction kit (Bioneer Company Korea) according to the manufacture recommendation.

Detection of ESBL- genes by PCR Method

Three primer sequences for three genes of ESBL- *A. baumannii* were used in the current study as shown in (**Table 1**). Polymerase Chain Reaction (PCR) assay was performed for dection of genes *bla TEM*, *bla SHV* and *bla CTX*. The PCR products were analyzed electrophoretically on 1% agarose gel at 100 volt for 45-60 min in TBE containing ethidium bromide and the results were examined under UV radiation (Ahanjan, Kholdi, & Rafiei, 2014).

Statistical Analysis

Data were analyzed by using Statistical Package for Social Science (SPSS) software version-21 (USA). The results were interpreted depending on (the Chi-square and Fisher tests) and statistical significance result was regarded as P-value ≤ 0.05.

RESULTS

During this study period, a total of 120 samples were collected from burn, urine, ICU and pediatric wards and these samples were identified depending on the VITEK-2 system. The study results were founded of the bacterial isolates as **Table 2**. Out of 120, 130 of different isolates were identified. The highest isolation

Fig. 1. A. baumannii isolates growing on CHROM agar

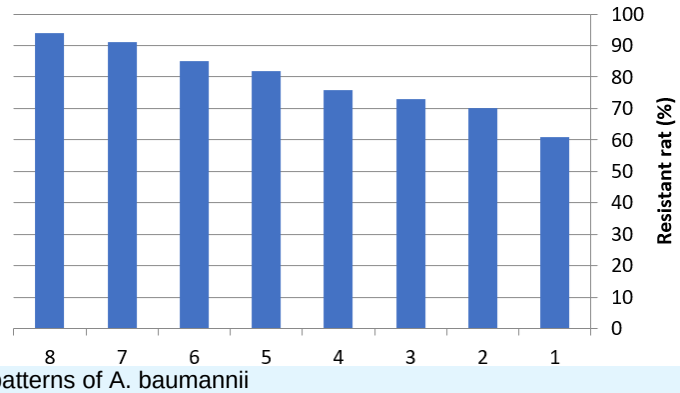

Fig. 2. Antibiotics resistant patterns of A. baumannii
1: Ampicillin; 2: Ciprofloxacin; 3: Aztreonam; 4: Gentamicin; 5: Meropenem; 6: Cefoxitin; 7: Imipenem; 8: Cefotaxine

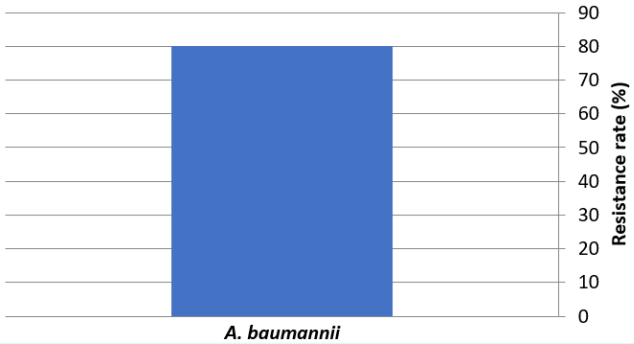

Fig. 3. Multidrug resistance (MDR) of A. baumannii isolates

percentage was Staphylococcus aureus (31.5%) followed by Pseudomonas aeruginosa (27.6%), Acinetobacter baumannii (25.3%), E. coli (10%) and Enterobacter aerogenes (5.3%).In the our study was demonstrated the prevalence of A. baumannii in different wards at Basra- hospitals, it was isolated in high percentage (25.3%) of all bacteriological specimens. In addition, the identification of A. baumannii was depended on culturing these isolates on CHROM agar and VITEK-2 system. A. baumannii isolates were appeared as bright red colonies after 24h and incubation at 37°C, as shown in **Fig. 1**. Furthermore, data in **Table 2** showed the majority of A. baumannii isolates. In this study, 33 isolates of A. baumannii were isolated from different samples of wards at Basra- hospitals. **Fig. 2** was showed that the most of bacterial isolates were highly resistant against several antibiotics especially third generation cephalosporins. It has been found that A. baumannii had high resistance to Cefotaxime 31(94%), Imipenem 30(91%), Cefoxitin 28(85%), Meropenem 27(82%), Gentamicin 25(76%), Aztreonam 24(73%) and Ciprofloxacin 23(70%) but low resistance to Ampicillin 20(61%). In **Fig. 3**, the results were showed highly incidences of multidrug resistance (MDR) of A. baumannii (84.6%), as shown in **Fig. 3**.

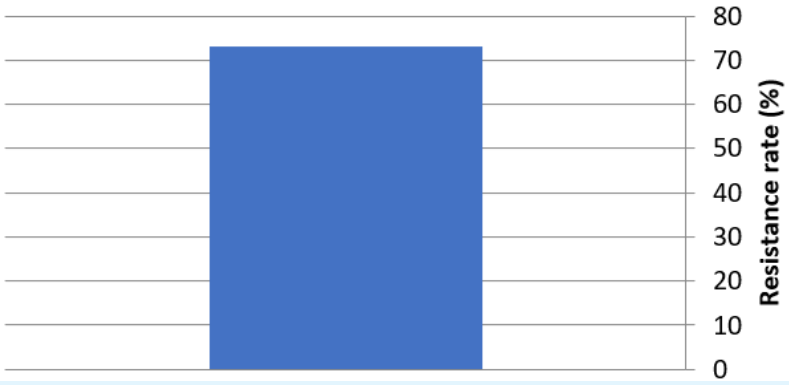

Fig. 4. Prevalence of ESBL- A. baumannii

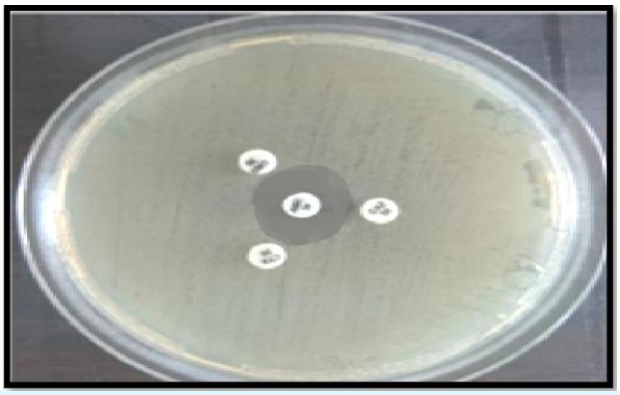

Fig. 5. Growth of ESBL- producing A. baumannii by DDST

The results as shown in **Figs. 4** and **5** showed that almost MDR isolates of A. baumannii (73%) were ESBL-producing bacteria. Out of 33 total isolates of A. baumannii were harbored for ESBL-genes. Twenty one (64%) isolates had bla- TEM genes, 17(52%) isolates had bla- SHV genes and 14(42%) isolates had bla- CTX genes, as shown in **Fig. 6**. PCR amplification of genes bla- TEM, bla- SHV and bla- CTX were determined in A. baumannii , as shown in **Fig. 7**.

DISCUSSION

The prevalence of bacterial contamination among several of Basra- hospital was the first study in our province. This study was included identification of the bacterial isolated in the burn wards, urine samples, ICU and pediatric wards. Compared with other studies, these results nearly in agree with the results obtained by (Ibraheem, Hindi, AL-Amedi, & Abd-AL-Ameer, 2009).in Baghdad hospitals- Iraq and it by (Jafari, & Karbasizade, 2014). in Hilla hospitals- Iraq. These bacterial species were represented as *S. aureus*, *Pseudomonas aeruginosa*, *Acinetobacter baumannii* , *E. coli* and *Enterobacter aerogenes* and those species were represented as hospital acquired infection. These infection is a pplied to any infection which are a result of treatment in a hospital or a health care service units and source of infecting organisms may be exogenous from another person or endogenous from the patient own flora (Ibraheem, Hindi, AL-Amedi, & Abd-AL-Ameer, 2009).Furthermore, the identification of *A. baumannii* was depended on culturing these isolates on CHROM agar. These isolates were appeared as bright red colonies after 24 h and incubation at 37◦C, as shown in **Fig. 1**. In addition this chromogenic medium is specific for detection of *A. baumannii* and MDR- *A. baumannii* isolates (Jafari, & Karbasizade, 2014).Indeed, another confirmatory tests were required by using VITEK-2 identification system with ID-GNB card because rate of accuracy of this system is about 98.6% for identification of bacterial isolates (Ligozzi, et al. 2002). In our study was showed the majority of *A. baumannii* isolates at different of Basra- hospitals, as shown in **Table 2**. Compared with other studies, this result was similar with studies of (Patwardhan, Dhakephalkar, Niphadkar, & Chopade, 2008). in Indina, (Abdullah, & Merza, 2019). in Iran. *A. baumannii* have high ability for survival on various surfaces including skin surfaces of human, Intensive Care Units, burn injury, catheters and many equipment in hospitals. These factors were considered as risk factors for prevalence of *A. baumannii* in several hospitals at different countries (Rezaei et al. 2018). *A. baumannii* is a major hospital acquired infections and it infected immunocompromised patients, patients in intensive care units and burn units (Tuwaij, 2014). Several mechanisms can induce drug resistance among nosocomial infections via plasmids and intagrons, but especially mechanism occur via ESBL genes. These

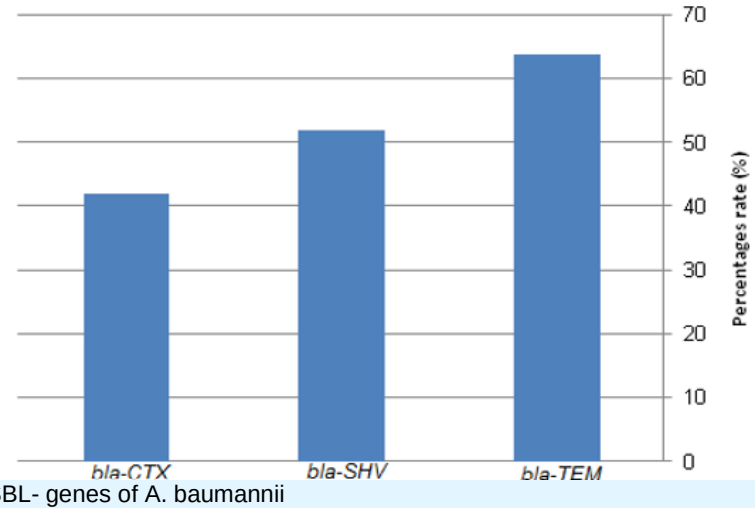

Fig. 6. Distribution of ESBL- genes of A. baumannii

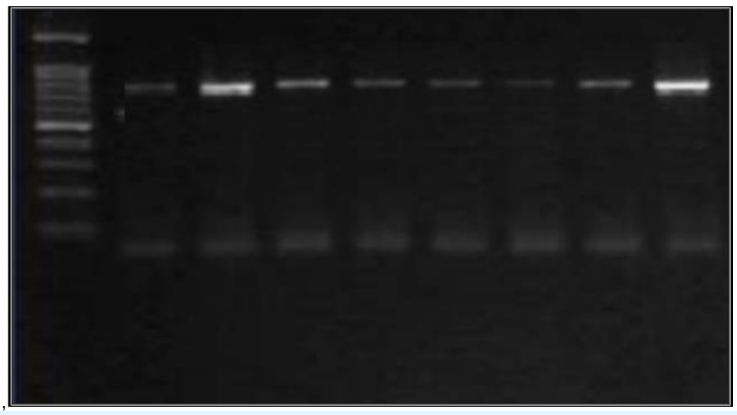

Fig. 7. PCR amplification of bla- TEM gene (800bp) in A. baumannii

genes cause expansion in the resistance percentages of antibiotics with production of multidrug resistance (MDR) isolates of *A. baumannii* (Safari, et al. 2015)The present study, *A. baumannii* isolates were high resistance to many antibiotics which include Cefotaxime (94%), Imipenem (91%), Cefoxitin (85%), Meropenem (82%), Gentamicin (76%), Aztreonam (73%), Ciprofloxacin (70%) and Ampicillin (61%). Furthermore, high prevalence of *A. baumannii* isolates were ESBL-producing. These results were similar with other studies in the different sites at several hospitals in Iraq (Tuwaij, 2014 Slama, 2008). Multidrug resistance of *A. baumannii* in the Intensive Care Units (ICU) was represented as therapeutic problems in several hospitals (Noori Karimi, Fallah et al. (2014). The study results were showed most isolates of *A. baumannii* that occurred by mutation toward class-A of β- lactamases (*bla-TEM*, *bla-SHV* and *bla-CTX* genes), for example: production of cefotaximases encoded by *bla-CTX* gene through ceftazidime (Nordmann, Naas, & Poirel, 2011). Ceftazidime resistance was selection in the present study to detect productional isolates of ESBL because it is substrate for ESBL enzymes encoded by TEM, SHV and CTX genes (McConnell, Actis, & Pachón, 2013). High rates of resistance of antibiotics for *A. baumannii* may be cause increasing in the morbidity, mortality and treatment costs in intensive care units at the last years (Ko, et al. 2007).

CONCLUSION

The present study was showed that highly percentages of *A. baumannii* isolates were considered as MDR. Furthermore, *A. baumannii* isolates produced *bla TEM*, *bla SHV*, *bla CTX* genes carried on plasmid in high percentages and frequency of ESBL- encoding genes show that these genes can be disseminates to another bacteria as the same or different species. In addition, the high frequency of MDR and ESBL-encoding genes may be due to overuse and inappropriate use of antibiotics in Basra- hospitals.

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