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RESEARCH ARTICLE

Molecular Detection of Multi-drug Resistant *Acinetobacter baumannii* from Patients Wounds

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Abstract

150 swabs were collected from patient's wounds and hospital units, population of this study included both genders with different ages in AL-Imamein AL-Kadhimein Medical City in Baghdad. The collected swabs were cultured using appropriate media and tested biochemically in order to find out the profile of bacteria that colonize patient's wounds and hospital units. The results of culture revealed that, the most common isolated bacteria were, *Staphylococcus aureus* followed by *Pseudomonas aeruginosa and A.baumannii*. The isolates of *A. baumannii* was tested for antibiotic susceptibility, the results showed that, clinical and environmental isolates were (100%) resist to Ceftriaxone, Ceftazidime Ampicillin–Sulbactam, and Cefepime. Metallo Beta Lactamase (MBL) genes as *bla* IMP gene and *bla* VIM were detected by Molecular method (PCR) in 12 clinical and environmental isolates of *A.baumannii*. The result revealed that among all isolates (12) *only bla* IMP gene was detected in 4 isolates while the reminder isolates (8) of *A.baumannii* have no amplified bands for neither *bla* VIM nor *bla* IMP. It was concluded that, *A.baumannii* responsible for many nosocomial infections, and these bacteria were high resistant to different types of antibiotics.

Introduction

Wound infection is a harmful interaction between the patient and the pathogen, it's caused by the growth and spread of microorganisms, usually bacteria, that can delay healing, these infections remain the major global problem leading to many complications and causing high rates of death among hospitalized patients [1, 2]. Wounds may infect by microorganisms which derived from the host normal flora and the environment of the hospital [3, 4]. According to data from various medical records, the epidemiology of the pathogens of wounds is represented by; *Pseudomonas aeruginosa Staphylococcus aureus*, *Escherichia coli*, and *Acinetobacter* spp. [4, 5, 6].

In Iraq, a study by Al- Kaisse *et al*. (2015) to burn patient's wounds and burn units, bacterial isolates revealed: *Ps.aeruginosa* was the most common isolate followed by *S.aureus*, *K.pneumoniae*, *E. coli*, *Ps.putida*, *E.aerogene*, *A.baumannii* and *P.mirabilis* [7]. Ghaima, 2016, in his study showed that 96 (20.2 %) of 476 isolates were obtained from burns and wounds from different hospitals in Karkh and Resafa of Baghdad city were identified as Oxacillin resistant *A.baumannii* [8]. *A.baumannii* is a common gram-negative lactose non-fermenter opportunistic bacteria associated with nosocomial infections, especially in immune-compromised patients in an intensive care unit (ICU), soldiers with infected wounds, surgical wound, and soft tissue infections, also diabetic Foot ulcer [9]. The global major concern now a day is the increasing rates of antibiotic resistance among bacteria worldwide.

It was revealed that the most common resistance mechanism in *Acinetobacter* spp. is by The production of Metallo ß-lactamase enzymes [8, 10]. MBL genes are either present on the bacterial chromosome or on mobile genetic elements like plasmids and transposons, these genes have the ability to spread between various strains of bacteria [11].

There are many types of acquired MBL enzymes; these enzymes are; (KPC), (IMP), (SPM), (VIM), (GIM)), and (NDM) [12, 13]. This study aimed to estimate the incidence of multi-drug resistant *A.baumannii* isolates obtained from clinical and environmental samples by molecular method.

Materials and Methods

This study was conducted on 150 clinical and environmental swabs, 75 clinical swabs collected from wounds of patients with AL-Imamein AL- Kadhimein Medical City. The population of this study included four age groups of both genders, the ages were confined between (6-75) years. 75 environmental swabs were taken from different sites of surgical units and patient wards include: gloves, beds, floors, walls and normal saline of washing. The collected swabs were identified by API 20 E kit after culturing on Blood and MacConkey agar. Antimicrobial susceptibility testing was done by disc diffusion method using Muller-Hinton agar.

In this study 13 antibiotics were used, 7 of them were extended-spectrum beta-lactamases (ESBLs), and six antibiotics were non ESBLs represented by Aminoglycosid and Fluoroquinolone. Genomic DNA was extracted from the culture of *A.baumannii* isolated of wound's patients and hospital units' samples, and the presence of ESBL genes examined through polymerase chain reactions (PCR) by specific primers to detect (*bla*VIM) and (*bla*IMP) genes (Table 1).

Table 1: The Sequence Forward and Reverse Primers of *bla* VIM and *bla* IMP genes in *Acinetobacter baumannii* isolates

Primer Name	Sequence→5' 3'	Detected Gene	Product Size (bp)
bla VIM-F	TTTGGTCGCATATCGCAACG	*bla*VIM	500bp
bla VIM-R	CCATTCAGCCAGATCGGCAT		
bla IMP-F	GTTTATGTTCATACWTCG	bla_{IMP}	432bp
bla IMP-R	GGTTTAAYAAAACAACCAC		

Statistical Analysis System

Statistical Analysis System program was used for analysis of data Chi-square(x2), it was used to significant compare between percentage in this study.

Results and Discussion

The wound infections remain the major global problem leading to many complications and causing high rates of death among hospitalized patients.It was observed that most of the patients who had wound infection at age ranged 21-35 year, and this was in agreement with other study in Iraq, in Diyala Al-Azawi, (2013) found that, most of wounds infection occurred in individuals of (20-39) years old [14].Also another study by Chandra *et al.* (2014) showed that, the age group (31-40) year had most affected by wound infection.

These results may be attributed to most of those persons in working age (active age), so they were more exposed to accidents during their work. Other explanations may be because some of them are soldiers, so they may exposure to fire or shotgun [15].

With US military treatment facilities in Iraq, these bacteria were named as "Iraqibacter", and has quickly become one of the most troublesome pathogens for health care centers [16].This study showed that distribution of wound's patients, according to gender revealed 66 (88%) males and 9 (12%) females. Wounds were more common in males in comparison to that in females, which belong to, that male exposure to accident more than females depending on the nature and the site of their work especially the soldiers. Table (2) summarized the causes of wounds.

Table 2: Patients Distribution According to Causes of wounds

Patients wounds (age)	Causes of wounds			Total No. (%)	Chi-square (χ^2)
	Soldier's injuries No. (%)	Diabetic Miletus No.(%)	Other causes No (%)		
< 20 years	0(0%)	1 (1.3%)	6 (6.78%)	7(9.3%)	2.382 NS
≥ 20 years	39 (52%)	25 (33.3%)	4(5.3%)	68(90.7%)	9.815 **
Total	39 (52%)	26 (34.7%)	10(13.3%)	75 (100%)	9.072 **
Chi-square (χ^2)	12.077 **	9.658 **	4.129 *	14.957 **	---
* (P<0.05), ** (P<0.01).					

Bacterial Diagnosis

Clinical Isolates

The obtained swabs from wound patients were cultured on MacConkey and blood agar. The results of bacterial culture clarified that, 57 (76%) with highly significant ($P \leq 0.01$), gave positive result for culture while 18 (24%) were negative. The negative culture (18 cases) may be due to either patients on antibiotic or the swabs were obtained from the new wound before contamination. Different bacterial species of Gram positive and negative were identified, the objected study showed that positive culture were (43) single bacterial isolates while the remainder (14) were with mixed bacterial growth (Table, 3).

Table 3: Clinical Isolates from Patient's wounds

Pathogens isolation	Number and frequency		Total No.	Percentage (%)
	Single Isolates	Mixed Isolates		
Staphylococcus aureus	18	5	23	30.7
Pseudomonas aeruginosa	8	3	11	14.7
Acinetobacter baumannii	8	0	8	10.6
Staphylococcus epidermidis	3	1	4	5.3
Escherichia coli	1	3	4	5.3
Proteus mirabilis	2	1	3	4
Enterobacter cloacae	2	0	2	2.7
Klebsiella pneumonia	1	1	2	2.7
Total No.	43	14	57	76%
Chi-square (χ^2)	---	---	---	8.492 **
** (P<0.01).				

The results demonstrated that, *S.aureus* was the commonest isolate (23 isolates, 30.7%) followed by *Ps.aeruginosa* (11isolates, 14.6%). *A.baumannii* (8 isolates, 10.6 %), both *E.coli* and *S.epidermidis* were 4 isolates for each (5.3 %), *P.mirabilis* (3isolates 4%), and *K.pneumonia* and *E.cloacae* both were the lowest isolated microorganisms which only 2 isolates (2.7%) for each

Environmental Isolates

Seventy five environmental swabs were revealed that, 24(32%) of swabs gave a positive result for bacterial growth with significant differences ($P \leq 0.05$), 17 of them appeared as a single colony while the bacterial growth for other 7swabs gave mixed colonies. It was noticed that the percentage of positive result (32%) of bacterial growth of environmental sample was less than clinical sample (76%). 51(68%) of environmental swabs gave negative results for bacterial growth and this result more than that of clinical swabs which was 18(24%). The *Ps.aeruginosa* culture was the predominant and the number of isolates reached to 7(9.3%) followed by 4(5.3%) isolates for each *A baumannii* and *S. aureus*. Both of *E.coli* and *E.cloacae* were 3 isolates (4%) while *K.pneumoniae* 2 isolates (2.6%) and the least isolated microorganism was *P.mirabilis* (1 isolates 1.3 %) as shown in Table (5).

Table 5: Environmental Isolates from hospital Units

Isolate	Number and frequency		Total No.	Percentag (%)
	Single Isolates	Mixed Isolates		
Pseudomonas aeruginosa	3	1	7	9.3
Acinetobacter baumannii	4	-	4	5.3
Staphylococcus aureus	3	1	4	5.3
Escherichia coli	2	1	3	4
Enterobacter cloacae	3	-	3	4
Klebsiella pneumonia	2	-	2	2.7
Proteus mirabilis	-	1	1	1.3
Total No.	17	4	21	28%
Chi-square (χ^2)	---	---	---	5.028 *
* (P<0.05).				

The final results of culturing 150 samples isolated from different types of patients wounds and patient units clarified that, 81(54%) samples were observed to have bacterial growth (positive samples), while 69 (46%) samples showed that no growth on media and represented negative samples. The bacteria cultured from both patients' wounds and units revealed that *S. aureus* was the most common bacterial isolates and represented 27(18%), While *Ps.aeruginosa* 18(12%), followed by *A.baumannii* 12(8%), *E. coli* 7(4.7%) *E. cloacae* 5(3.3%), and 4(2.7%) were for *S. epidermidis*, *K. pneumonia* and *P. mirabilis*

Diagnosis of *Acinetobacter baumannii*

Twelve *A.baumannii* were identified which appeared under microscope as gram-negative coccobacilli occurred in pairs or short chains. On MacConkey agar colonies of this bacterium appeared pale colonies because this bacterium unable to ferment the lactose, also gave negative results for oxidase test while catalase test was positive. API-20E system has used to confirm *A.baumannii* diagnosis. All of the colony morphology, Gram stain reaction, microscopically examination, came in according with that mentioned by Jawetz *et al*. (2013) [17]. The WHO categorized carbapenem-resistant

Acinetobacter baumannii, as 1: critical of priority pathogens list for R and D of new antibiotics priority [18]. *A.baumannii* are very important opportunistic pathogens of health care centers, they are capable of survival on inanimate objects for extended period which promote their persistence in hospitals [19].Also they have been isolated from the hands of nurses causing nosocomial infections, They can be isolated from burns, wounds urinary tract, and soft tissue infections causing death of patients [20, 21].

Antibiotic Susceptibility Test for *Acinetobacter baumannii*

The antimicrobial susceptibility test was implemented on 12 isolates of *A.baumannii* using 13 types of antibiotic disks, seven antibiotics were Extended-spectrum beta-lactamases (ESBLs) represented by, Ceftazidime, Ceftriaxone, Ampicillin-sulbactam, Aztreonam, Imipenem, Cefepime and Piperacillin and other 6 antibiotics were non ESBLs represented by Aminoglycoside (Amikacin, Gentamicin), Trimethoprim sulfa-methoxazol and Fluoroquinolone (Ciprofloxacin, Doxycycline and Levofloxacin), by the disc diffusion method.

The antibiogram for all *A.baumannii* isolated in this study (clinical and environmental) revealed (100%) resist to Ceftriaxone, Cefepime, Ampicillin-sulbactam and ceftazidime. The resistance of *A.baumannii* isolates against Aztreonam reached to 91.66%, while Gentamycin was 75% and 58.33% for both of Amikacin and Trimethoprim sulfamethoxazole, also resistance to both Levofloxacin and Piperacillin reached 50%, whereas low resistance appeared against Ciprofloxacin and Doxycycline and reached to 41% and 33.33% respectively. Most isolates appeared high sensitivity against Imipenem and reached to 75% of the total 12 isolates of *A.baumannii*.

This study showed that the alarming increase of infections was caused by multi-drug resistant bacteria and it was noticed that Ciprofloxacin reported as the second most effective drug against *A. baumannii* with sensitivity reached to 58.33%. Chandra *et al*. (2014) referred that from total 250 samples were collected from the surgical site infected, *S.aureus* and *Acinetobacter* spp. were the highest MDR [15]. Antibiotics act selectively against bacteria and different antimicrobial agents have distinctive modes of action against various microorganisms, these antibiotics can be administered topically or systemically [22].

The carbapenem including (Imipenem, Meropenem, Doripene and Ertapenem) are still the first choice for treatment of serious infections with ESBL-producing Gram-negative bacilli. It has been reported that the ESBL-producing Gram-negative bacilli are still susceptible to these drugs [23].

The emergence of carbapenemase-producing Gram-negative bacteria show serious need for hygiene measures and preventive strategies to limit their spread. Although the conjugative plasmids in gram-negative bacteria play an important role in the rapid dissemination of carbapenemase genes, other mobile genetic elements, such as (insertion sequences, integrons, and transposons) are involved in the acquisition of these resistances [24].

Molecular Detection of MBLs Genes (*bla* VIM and *bla* IMP) in *Acinetobacter baumannii*. Isolates

In this study DNA extracted from 12 isolates of *A. baumannii* by DNA extraction kit, the result reveled that purity was (1.5-1.8) and concentration of extracted DNA was (50-100ng). The DNA was detected by gel electrophoresis which appeared as single and clear bands (Figure, 1).

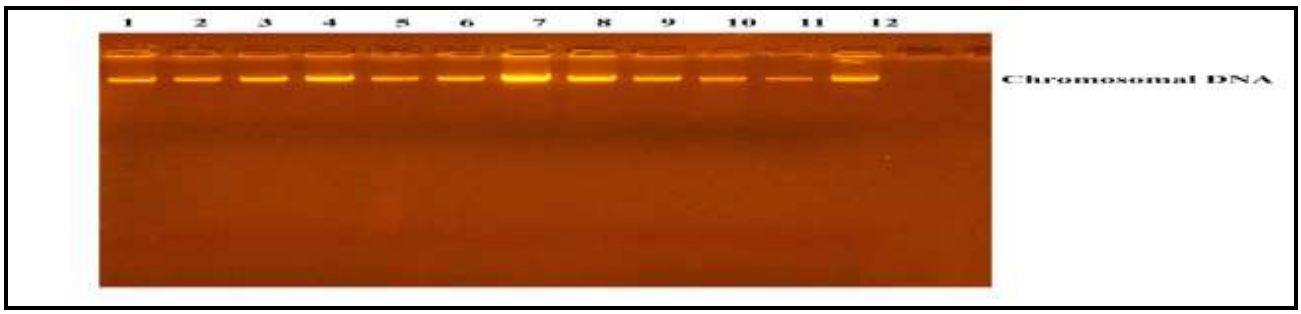

Figure 1 : Electrophoresis of extracted DNA of *Acinetobacter baumannii* isolates on 1% agarose (70 vol/ 30 min) to check purity and integrity

PCR Analysis

Resistance genes, *bla*VIM and *bla* IMP gene which responsible for carbapenemase-resistant antibiotic were detected by PCR technique in 12 *A.baumannii* isolates, the result of amplification showed no bands detected of *bla*VIM genes in both clinical and environmental isolates of *A. baumannii*, while only 4 isolates were carried *bla* IMP, (Figure, 2). The distribution of *bla* gene in clinical and environmental *A.baumannii* isolates were clarified in Table (6).

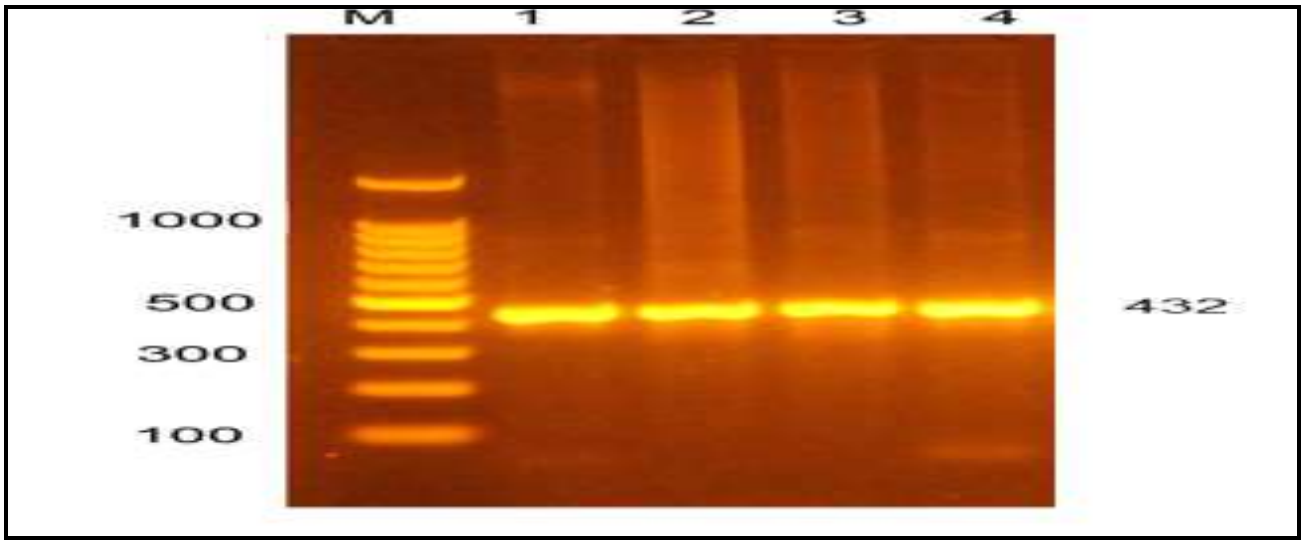

Figure 2: Gel Electrophoresis of PCR Product for Detection of MBLs *bla* IMP Gene (432) from *Acinetobacter baumannii* using 1% agarose for 90 min. at 70 V\Cm M: Marker DNA ladder Size (100bp). Lines 1-4 *Acinetobacter baumannii* isolates

Table 6: Distribution of *bla* Genes in Clinical and Environmental *A.baumannii* Isolates

Samples	No.*A.baumannii* isolates	*bla*VIM (500 bp)		Chi-square(χ^2)	*bla*IMP (432 bp)		Chi-square (χ^2)
		Positive	Negative		Positive	Negative	
PatientsWounds	8	0.0 %	8(66.7%)	11.49 **	4(33.3%)	4(33.3%)	0.00 NS
Units	4	0.0%	4(33.3%)	9.027 **	(0.0%)	4(33.3%)	9.026 **
Total	12	0(+)	12 (-)	15.00 **	4 (+)	8 (-)	9.751 **

** (P<0.01).

In this study it was found that among all (12) clinical and environmental isolates the *bla* IMP gene was detected in 4 isolates of *A.*b*aumannii*. The reminder (8) isolates of *A. baumannii* have no amplified bands for *bla* VIM, that means their resistance to antibiotics belongs to *bla* IMP genes were carried on the plasmid or this resistance belongs to other genes such as SPM-1, GIM-1, SIM-1 or NDM-1

Carbapenem-resistant *A.baumannii* that colonized gastrointestinal tract acts as a critical step before nosocomial infection especially in ICU [25]. Metallo beta lactamases (MBLs), like all ß-lactamases, can be divided into those that are normally chromosomally mediated and those that are encoded by transferable genes like plasmid [24].

Current study detected MBLs genes carried on chromosomal DNA, this was in agreement to some studies on MBLs, as the study of Mehul *et al*. (2011) detected 8(5%) isolates of MBL, were resistant to Imipenem, all these isolates were from surgical wards of patients suffering from burns, ulcer and abscess, from medical wards of patients suffering from pleural effusion and, from orthopedic patients of trochanteric fracture [26]. A study by Shanthi *et al*. (2012) on 179 clinically significant, carbapenem-resistant *Pseudomonas* and *Acinetobacter* spp. recovered from clinical specimens of patients hospitalized for 48 hours or more, they found that PCR amplified product of MBL genes *bla* VIM and *bla* IMP were detected in 92 (51.4%) isolates, 54 *A. baumannii* isolate carried the MBL gene [27].

Conclusion

It could be concluded that *A.baumannii is* one of the *m*ost common isolated pathogens from wounds and hospital environmental units, that appeared high resistance to wide range of effectively used antibiotic included ESBL antibiotics makes the treatment of the different types of wounds very difficult.

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