

Phenotypic and Genotypic Characterization of Acinetobacter Infection in Intensive Care Units in EgyptMarwa M. Shalaby¹, Mina S. Meseedah¹, Asmaa M. Shahin¹, Amany M. Abdelwahaab², Soheir S. Maklad²¹Microbiology & Immunology Departments, Faculty of Medicine, Tanta University, Tanta, Egypt²Microbiology & Immunology Departments, Faculty of Medicine, Al-Azhar University, Cairo, Egypt
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Abstract: *Acinetobacter baumannii* is a multidrug resistant organism associated with nosocomial infections particularly in intensive care units. This study was carried out to investigate the biotype, resist type and genotype of *A. baumannii* isolated from different ICU patients at Tanta University Hospital using pulsed field gel electrophoresis (PFGE) to monitor outbreaks and spread of different clones and determine their relatedness with that isolates from ICU environment and Hospital Care Workers (HCW). Twenty four *A. baumannii* isolates were studied; 20 of them were mostly (70.8%) isolated from respiratory specimens of patients, 3 were from ICU environment and only one isolate from a HCW. Antibiogram analysis showed that the isolates were fully (100%) resistant to piperacillin, ceftriaxone, Cefoperazone and Aztreonam. Resistance to other antibiotics were 95.8%, 91.7%, 83.3%, 75%, 70.8%, 66.7% for Cefotaxime, Netilmicin, Tobramycin, Cefoperazone-sulbactam, Piperacillin-Tazobactam, Ciprofloxacin respectively Among isolates; 3 biotypes, 9 resist type and 10 distinct genotypes were identified, with predominance of PFGE clone E (37.5%). Some environmental isolates had identical resistance and PFGE profiles and were closely related to an isolate from a HCW. Cluster analysis showed that there was a persistent endemic clone in Tanta Hospital ICUs. Conclusion: survival and circulating some clonally related *A. baumannii* were identified among patients and different ICU environment and HCW, which were probably selected because of their resistance to the majority of antimicrobial agents. These data provided a better understanding of *A. baumannii* epidemiology within hospitals, possible source and route of transmission and the resistance pattern in order to implement a more strict prevention programs and improve antimicrobial therapy.

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1. Introduction

Acinetobacter baumannii is a well-recognized opportunistic pathogen that gives rise to nosocomial infections such as ventilator-associated pneumonia, urinary tract, cardiovascular, wound and meningitis especially in the intensive care units (1).

Large outbreaks involving multidrug resistant Acinetobacter strains have been reported worldwide (2) and are difficult to control because this pathogen easily spreads and persists in hospital setting, favoring the transmission between patients either via human reservoirs or via inanimate materials (3). The occurrence of *A. baumannii* outbreak is facilitated by tolerance to desiccation and UV radiation and also multidrug resistance contributing to the maintenance of this organism in the hospital setting. The epidemiology of Acinetobacter is complex with the co-existence of epidemic and endemic infections which is favored by the selection pressure of antimicrobials (4).

The increasing rates of resistance of *A. baumannii* to the major antimicrobial drugs makes identification and control of hospital outbreaks mandatory (5). Appropriate identification of *A.*

baumannii and discrimination among isolates during an outbreak could lead to a better understanding of the mode of spread and thus enabling better control of the outbreak and help in optimal selection of empirical and subsequent target therapy (6).

Pulsed Field Gel Electrophoresis of macro restriction fragments of *A. baumannii* is regarded as the gold standard for epidemiological typing since it is discriminatory and reproducible typing method that accurately characterizes strains responsible for a possible outbreak, evaluates their persistence and identifies routes of transmission (7).

The aim of this work was to investigate the biotype, resist type and genotype of *A. baumannii* isolated from different ICU patients at Tanta University Hospital using pulsed field gel electrophoresis (PFGE), to monitor outbreaks and spread of different clones and determine their relatedness with isolates from ICU environment and Health Care Workers (HCW).

2. Patients Materials and Methods

Patients and Isolates:

During one year study 24 *A. baumannii* isolates were identified. Twenty isolates were recovered from 129 patients with different ages, admitted to different ICUs in Pediatrics (PICU), Chest, Internal Medicine, Burn ward and Neurology Departments at Tanta University Hospital. In addition, 4 *A. baumannii* were recovered from 22 hospital environment and (HCW).

Different clinical samples were obtained from patients according to the clinical underlying diseases involving endotracheal aspirate (ETA), sputum suction, burn wound, swabs, blood and urine samples. Different environmental samples were obtained from bed sides tables, dressing tables and pillows and also from nail bed of HCW.

According to CDC guidelines, the epidemiological, clinical and demographic data of patients were recorded. They included age, gender, days of hospital sitting, types of infection, medical comorbidities, major risk factors such as urinary catheters, intravenous catheterization and mechanical ventilation, age >55, multiple isolates, mechanical ventilation, previous antibiotics, co-infection and comorbidity.

Methodology:

I. Microbiological isolation and identification of *Acinetobacter* from pathological samples:

All specimens were processed and identified according to the standard procedures. Isolation and identification were achieved by: Gram stained smears and cultures on Brain heart infusion agar (Oxoid), 5% Blood agar and MacConkey's agar with crystal violet (Britania, Argentine), aerobically incubated for 24 hours at 37°C, 41°C and 44°C (9) at three plates. The obtained suspected colonies were investigated by film stained by Gram stain and by Biotyping.

II. Biotyping using API 20 NE system (BioMer'eux)

This system is used for phenotypic identification of *A. baumannii* to the species level. It contains 25 conventional and assimilation tests (10).

III. Antimicrobial susceptibility testing and Antibiogram typing (resist typing):

Antimicrobial susceptibility of *A. baumannii* isolates was investigated by the standard disk-diffusion method following the criteria of the clinical and laboratory standards institute (CLSI, 2011). Twenty antibiotic disks (Oxoid) were used. They were Piperacillin; PRL(100 mg), Ampicillin-sulbactam; SAM (10/10mg), Piperacillin-tazobactam; TZP (75/10mg), Cephoperazone-sulbactam; SCF (75/30mg), Cefotaxime; CTX (30mg), vcCeftriaxone; CRO (30mg), Ceftazidime; CAZ (30mg), Meropenem; MEM (10mg), Imipenem; IPM (10mg), Gentamicin; CN (10mg), Amikacin; AK (30mg),

Tobramycin; TOB (10mg), Netilmicin; NET (30mg), Tetracycline; TE (30mg), Ciprofloxacin; CIP (5mg), Levofloxacin; LEV (5mg), Trimethoprim-sulfamethoxazole; SXT (1.25/23.75mg) and Colistin; CT (10mg)(11).

IV. Genotyping of *A. baumannii* isolates by pulsed field gel electrophoresis (PFGE) and Dendrogram analysis:

Genotyping of *Acinetobacter* isolates was performed by PFGE at Naval Medical Research Unit 3 (NAMRU -3), Cairo using the Bio-RAD CHEF Mapper Electrophoresis unit and Bio-RAD gel documentation system with UV transilluminator (Bio-RAD laboratories, Nazareth, Belgium number 170-3670 and 170-8171 respectively).

Bacterial genome was cut with Apa-I restriction enzyme (RE) for *Acinetobacter* (New England Biolabs#01145) and Xba-I restriction enzyme for *Salmonella braenderup* as molecular standard (Roche # 674257). PFGE is able to determine the length of DNA fragments in relation to other samples and can give an estimate of the length of pieces by comparing their position in gel relative to a ladder; molecular size standard which is Xba-1 (digested *S. braenderup* H 9812, size range 33-1.135 KB (12). Preparation of PFGE agarose plugs, lysis of bacterial cells in plugs, cutting DNA by RE in plugs, casting the gel and loading of plug slices and electrophoresis run were followed as instructed by the protocol of (13).

Data analysis:

PFGE patterns were interpreted in ethidium bromide stained gel and analyzed using the gel documentation system according to the criteria suggested by (14), with difference of six bands or less used to define strain relatedness. Isolates corresponding to $\geq 87\%$ clustering threshold were considered to belong to the PFGE pattern (15). PFGE-generated DNA profiles were entered into the BioNumerics software package, version 3.0 (Applied Maths, Sint-Martens-Latem, Belgium). Cluster analysis and Dendrogram was performed by the unweighted pair group method with mathematical averaging (UPGMA), and DNA relatedness was calculated by using the band-based Dice coefficient with a tolerance setting of 1.5% band tolerance and 1.5% optimization setting for the whole profile (15).

V. Statistical analysis:

Statistical presentation and analysis of the present study was conducted, using the mean, standard error, student t-test, paired t-test, Chi-square by SPSSV17, software, correlation between variables was evaluated using Pearson's correlation coefficient (16).

3. Results:

Distribution and source of clinical samples (Table 1)

During one year study 24 *A. baumannii* isolates were identified. Twenty isolates (15.5%) were recovered from 129 patients with different ages and sex presented with one or more underlying diseases which is an important risk factor for multidrug resistant (MDR) *Acinetobacter* infection. The most frequent co-morbidity was cerebrovascular diseases (45%) followed by poly-trauma (15%), cardiac diseases, respiratory disorders and acute renal failure (10%) for each. *A. baumannii* were most commonly isolated from PICU (37.5%) followed by Chest ICU (33.3%) and were mostly isolated from end tracheal samples (50%), sputum suction tip (20.8%) and blood,

urine, burn (4.2%) for each. All patients were receiving one or more antibiotics at time of sampling (cephalosporin, B-Lactam combination (35%) for each, carapenam (25%) and ciprofloxacin (59%), the duration of hospital stay before sampling ranged from 3-13 days (more than 7 days in 70%). The risk factors for infection of hospitalized patients with multidrug resistant *A. baumannii* were mainly duration of hospital stay > 7 days, previous antibiotic therapy, use of invasive devices (ventilator, urinary or central venous catheter. Mechanical ventilators were the most common devices associated with *Acinetobacter* infection. Four *A. baumannii* isolates (18.2%) were recovered from 22 environmental and HCW, from PICU environment and one from a nail bed of 3 HCWs from the same PICU (Table 1).

Table 1: Distribution and source of the clinical isolates

	Sample	<i>A. baumannii</i> isolates	Source
-No of patient samples - Underlying diseases	129	20 (15.5%) -Cerebrovascular 9(45%) - Polytrauma 3(15%) - Acute renal failure 2(10%) -Cardiac disease 2 (10%) -Respiratory 2 (10%) -Endocrine 2 (10%)	- PICU (37.5%) - Chest ICU (33.3%) -Neurology ICU (16.7%) - Medical ICU (8.4%) - Burn wound (4.2%)
-No of Environment and HCW samples -Source of the samples	22	4 (18.2%) -3 PICU environment -1 HCW nail bed	PICU (100%)

Biotyping results of *Acinetobacter* isolates (Fig 1):

All of the 24 isolates were identified as Acb-complex using API 20 NE. Three biotypes were identified. The most common biotype was biotype 1 (66.7%) (P<0.01) profile number 0041073, followed by biotype 2 (29.7%) profile number 0041473. The least common one was biotype 3 (4.2%) profile number oo41051.

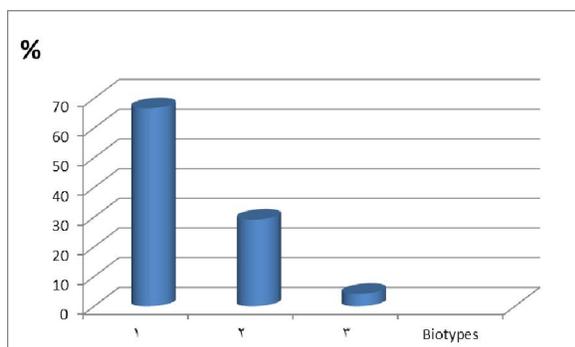


Fig (1): Biotypes of *Acinetobacter* isolates using API 20 NE.

Antibiotic susceptibility and (Resist typing) of the studied *Acinetobacter* isolates (table 2)

High resistance rates to most used antibiotics can be clearly noticed with 100% resistance rate to Piperacillin, Ceftriaxone, Cefoperazone and Aztreonam. Resistance to other antibiotics were 95.8% for Ceftazidime, Amikacin and Sulfamethoxazole-trimethoprim, 91.7% for Netilmicin, 83.3 % for Tobramycin, 75 % for Cefoperazone-sulbactam and Ampicillin-sulbactam, 70.8% for Piperacillin-tazobactam. Most isolates were sensitive to Colistin (87.5%), (table 2).

Antibiogram typing profiles (resist typing) showed differences in susceptibility among isolates. Nine different resist type profiles could be identified from 24 *Acinetobacter* isolates according to antibiotic susceptibility testing results. Resist type 1 was the most common type (20.8%) that showed resistance to 5 antibiotics (IPM, MEM, CIP, LEV, CN) together with resist type VI (20.8%) that showed resistance to 2 antibiotics (IPM, CT), followed by resist type V (16.7%) that showed resistance to 4 antibiotics (TE, CN, CT, LEV), resist type VII (12.3%) which showed resistance to all antibiotics (pan resistance), resist type II (8.3%) showed resistance to 7 antibiotics (SAM, TOP, CN, NET, IPM, MEM, LEV),

also resist type IX (8.3%) showed resistance to one antibiotic (CN). The least common types were II, IV,

VIII (4.2%), (table 3).

Table (2): Antibiotic susceptibility of the studied Acinetobacter isolates.

Antibiotic	R	S	I
	N (%)	N (%)	N (%)
Piperacillin (PRL)	24 (100%)	-	-
Ampicillin-sulbactam (SAM)	18 (75%)	1 (4.2%)	5 (20.8%)
Tobramycin (TOB)	20 (83.3%)	2 (8.3%)	2 (8.3%)
Gentamicin (GN)	10 (41.7%)	13 (54.2%)	1 (4.2%)
Amikacin (AK)	23 (95.8%)	1 (4.2%)	-
Netilmicin (NET)	22 (91.7%)	2 (8.3%)	-
Piperacillin-tazobactam (TZP)	17 (70.8%)	-	7 (29.2%)
Tetracycline (TE)	12 (50%)	4 (16.7%)	8 (33.3%)
Trimethoprim-Sulfamethoxazole (SXT)	23 (95.8%)	-	1 (4.2%)
Colistin (CT)	3 (12.5%)	6 (25%)	15 (62.5%)
Ceftriaxone (CRO)	24 (100%)	-	-
Cefotaxime (CTX)	23 (95.8%)	-	1 (4.2%)
Ceftazidime (CAZ)	23 (95.8%)	1 (4.2%)	-
Cefoperazone (CEP)	24 (100%)	-	-
Imipenem (IPM)	9 (37.5%)	14 (58.3%)	1 (4.2%)
Meropenem (MEM)	10 (41.7%)	14 (58.3%)	-
Ciprofloxacin (CIP)	16 (66.7%)	5 (20.8%)	3 (12.5%)
Levofloxacin (LEV)	11 (45.8%)	11 (45.8%)	2 (8.3%)
Aztreonam (ATM)	24 (100%)	-	-
Cefoperazone-sulbactam (SCF)	18 (75%)	1 (4.2%)	5 (20.8%)

*R=resistant, S=sensitive, I= intermediate sensitive. NB: Intermediate sensitive is considered as resistant.

Table (3): Antibiotyping (resist typing) of the studied Acinetobacter isolates according to antibiotic susceptibility tests results.

Resistotype	No	Resistant to	%
I	5	IPM, MEM, CIP, CN, LEV	20.8
II	2	SAM, TOB, CN, NET, IPM, MEM, LEV	8.3
III	1	CAZ, IPM, MEM	4.2
IV	1	IPM, MEM, SXT	4.2
V	4	TE, CN, CT, LEV	16.7
VI	5	IPM, CT	20.8
VII	3	-----	12.5
VIII	1	CT, SCF	4.2
IX	2	CN	8.3
Total	24		100
P value	0.01		

Genotyping profiles of the studied Acinetobacter isolates by PFGE

PFGE typing of the 24 *A.baumannii* isolates yielded 10 distinct clusters named from A to j which were subdivided into 8 subtypes. Isolates clustered in the same genotype showed similarity $\geq 87\%$. Two types C and D were possibly related; they gave 2-3 different bands probably part of an outbreak. PFGE type E was the prominent type (37.5%) including 9 closely related isolates **Fig (2)**.

The three isolates of genotype F were indistinguishable; part of outbreak &3 strains of genotype E were also indistinguishable and closely related to E5. All of them might be parts of outbreak strains from VAP outbreak in Chest ICU, **Fig (3)**. Two isolates of E3 genotype were indistinguishable and were closely related to isolates of E2, E1. All of them were belonging to genotype E and were part of an undetected outbreak in PICU. Two isolates of genotype B were from PICU environmental samples

and were indistinguishable & were closely related to genotype B1 that was isolated from a HCW in PICU.

Table (4): Characterization of different *A.baumannii* genotypes

PFGE cluster	Subtypes	Relatedness	Source of isolates	No of isolates	%
A	A1	Possibly related	Neurology ICU	2 -1 A -1 A1	8.3%
B	B1	Closely related	2 B from PICU environment Indistinguishable B1 from PICU & HCW	3 - 2B - 1B1	12.5%
C	C1	Closely related	Neurology ICU	2	8.3%
D	-	-	PICU environment	1	4.2%
E	E1,E2,E3,E4,E5 2E3 + E2,E1, E	Indistinguishable Closely related	Chest ICU Outbreak (3E+1E5) Undetectable Outbreak in PICU	9 -3 E -2E2 -1E1 -1E3 -1E4 -1E5	37.5%
F	-	Indistinguishable	Chest ICU outbreak	3	12.5%
G	-	-	-	1	4.2%
H	-	-	-	1	4.2%
I	-	-	-	1	4.2%
J	-	-	-	1	4.2%

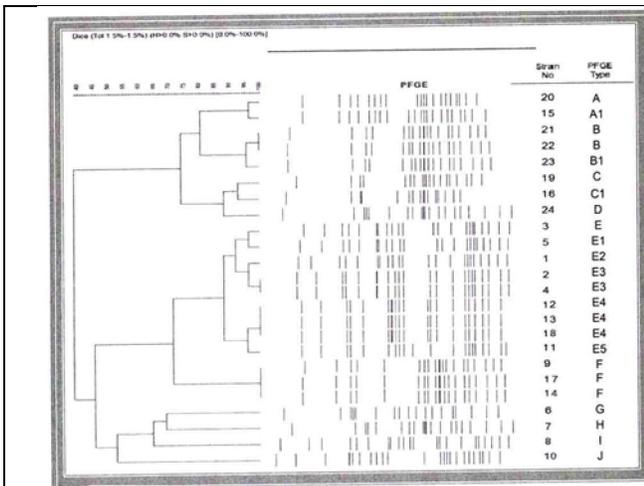


Figure (4): Dendrogram analysis of the 24 *Acinetobacter* isolates

PFGE typing of the 24 *Acinetobacter* strains yielded 10 distinct clusters named from A to J which were further subdivided into 8 subtypes (A1, B1, C1, E1, E2, E3, E4, and E5). Isolates clustered in the same genotype showed similarity $\geq 87\%$. Two types (C and D) were possibly related. PFGE type E was the predominate type (37.5%) including 9 closely related isolates.

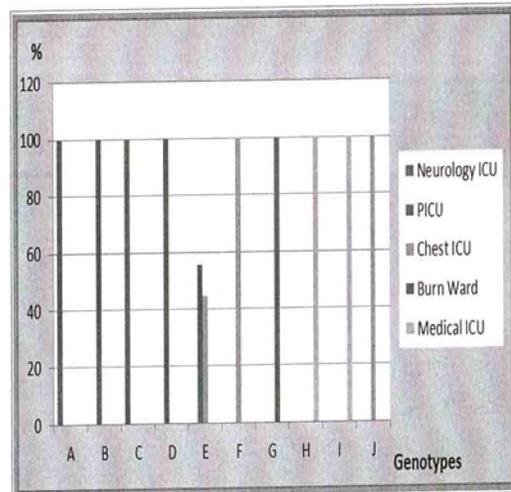
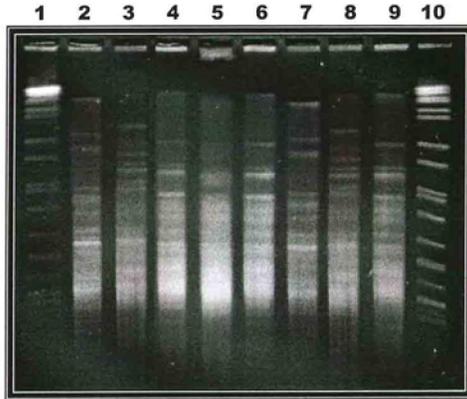


Figure (5): The correlation PFGE types and sources of *Acinetobacter*

This figure shows that most of the clonally related strains were isolated from the same place which may indicate endemicity of these strains. However, the predominant genotype (E) was circulating in different places and it could be isolated from PICU and Chest ICU.



PFGE profiles of *Acinetobacter* isolates no. 19,20,21,22,23,16,15 and 24 (lanes 2 to 9) digested with ApaI. Lanes 1 and 8 show XbaI digested *Salmonella braenderup* (H9812) as reference size markers (size range 33 to 1,135 kb). Strains no.21 and 22 (from PICU environmental samples) were indistinguishable (genotype B) and they were closely related to no.23 (B1) that was isolated from a HCW in PICU. Strains no. 19 and 16, that were isolated from Neurology ICU, were closely related (C and C1 respectively) and both were possibly related to no. 24 (from PICU environment)(D). Strains no 20 and 15 were possibly related (both from Neurology ICU)(A and A1 respectively).



PFGE profiles of *Acinetobacter* isolates no. 9, 10, 11, 12,13,14,17 and 18 (lanes 2 to 9) digested with ApaI. Lanes 1 and 8 show XbaI digested *Salmonella braenderup* (H9812) as reference size markers (size range 33 to 1,135 kb). Strains no.9, 14, and 17 were indistinguishable (genotype F). Also, strains no.12 and 13, and 18 were indistinguishable (E4) and were closely related to 11(E5) and all of them might be outbreak strains from VAP outbreak in Chest ICU.

Table (5): The relationship between epidemiologic data and the three typing methods (biotyping, resistotyping, genotyping) of *A. baumannii*.

Isolate no	Sample source	Sample type	Biotype	Resistotype	Genotype
20	Neurology ICU	Sputum suction tip	2	VI	A
15	Neurology ICU	Sputum suction tip	1	VI	A1
21	PICU	Environment	1	V	B
22	PICU	Environment	1	V	B
23	PICU	Environment	2	V	B1
19	Neurology ICU	Sputum suction tip	1	IX	C
16	Neurology ICU	Sputum suction tip	1	IX	C1
24	PICU	Environment	3	V	D
3	PICU	ETA	1	I	E
5	PICU	ETA	1	I	E1
1	PICU	ETA	1	I	E2
2	PICU	ETA	1	I	E3
4	PICU	ETA	1	I	E3
12	Chest ICU	Sputum suction tip	1	VII	E4
13	Chest ICU	ETA	1	VII	E4
18	Chest ICU	ETA	1	VII	E4
11	Chest ICU	ETA	1	VIII	E5
9	Chest ICU	ETA	2	VI	F
17	Chest ICU	ETA	2	VI	F
14	Chest ICU	ETA	2	VI	F
6	Burn Ward	Wound swab	1	II	G
7	Medical ICU	Urine	1	III	H
8	Medical ICU	Blood	2	IV	I
10	Chest ICU	ETA	2	II	J

In addition two isolates C,C1 were isolated from neurology ICU that were closely related were also possibly related to genotype D isolate that was isolated from PICU environment. Isolates of genotype A, A1 were from neurology ICU & were possibly related (Fig 4,5) (table 4).

Correlation between epidemiologic data and the three typing methods (biotyping, resist typing, genotyping) of *A.baumannii*.

Comparison between the 3 typing methods used during the study with each other as well as with epidemiological data including sources of the samples revealed that most of the clonally related isolates were isolated from the same place which may indicate endemicity of these isolates. However, the predominant genotype (E) including 9 isolates (37.5%) of isolates was circulating in different places since it could be isolated from PICU and Chest ICU. On the other hand different genotypes could be isolated from the same place. Two of the 3 environmental isolates were indistinguishable (genotype B) and they were closely related to the isolate recovered from a nurse in the same PICU (B1). As regards to the most common resist type (I and VI), isolates of resist type I (20.8%) were restricted to the same PICU. However, isolates of resist type VI (20.8%) were not restricted to a specific IC U, **Table (5)**.

4. Discussion:

Acinetobacter spp. especially *A.baumannii* is an emerging cause of health care associated infections, especially among clinically compromised patients admitted to ICUs (17). Most of the *A. baumannii* isolates are resistant to multiple antimicrobial agents, with increasing resistance to penicillins, β -lactams, amino glycosides, fluoroquinolones and carbapenems, which were the drugs of choice for treatment of the infection (18). *A. baumannii* is able to acquire antibiotic resistance genes and survive for days both in the hospital environment and on the hands of healthcare workers (HCWs), which could lead to possible transmission and the persistence of endemic *A. baumannii* strains in hospitals (19).

Macro restriction analysis using pulsed-field gel electrophoresis (PFGE) is considered the standard molecular method for epidemiological analyses of *A. baumannii*. Documentation of Antibiogram and DNA fingerprinting data of *A. baumannii* isolates is important to determine the prevalence of these isolates within the hospital and their transmission in outbreaks in order to provide better outbreak control and effectively manage the patients' infections (19).

The aim of this work was to investigate the biotype, resist type and genotype of *A. baumannii* isolated from different ICU patients at Tanta University Hospital using pulsed field gel electrophoresis (PFGE) to monitor outbreaks and spread of different clones and denote their relatedness with that isolates from ICU environment and Hospital Care Workers (HCW).

Twenty (15.5%) isolates were recovered from 129 patients admitted to Tanta University Hospitals and 4 isolates (18.2%) were recovered from 22 hospital environmental and HCWs screening samples. The mean age of the patients was ranged from 1 – 85 years with equal males (50%) and females (50%) distribution. Phenotypic identification of *Acinetobacter* to the species level was performed using API 20NE to determine the biotypes of the isolates, all of the 24 isolates were identified as *Acbcomplex* this result was similar to the study of who (10) reported that identification of isolates of *A. baumannii* based upon growth at 37° C, 41° C, and 44° C along with acid production from glucose could be very useful.

In the present study, the most frequently identified predisposing factors were cerebrovascular diseases (45%) and Polytrauma (15%), the length of hospital stay before sampling range from 3-13 days and almost all of the patients (95%) had undergone one or more invasive procedures such as mechanical ventilation (60%), urinary catheterization (30%), or central venous catheter (5%). This might have been the mode of *A. baumannii* patient-to-patient transmission for the patients in the same ICU in this study. These results showed concordance with that of (7) and (20). The data suggesting that invasive diagnostic and therapeutic procedures used in hospital ICUs predispose subjects to severe infections with *A. baumannii* (21) Main factor in our ICUs remains the lack of support and implementation of prevention and control policies. Single use devices were reused due to limited budget. Suction catheters for aspiration of respiratory tract were amongst most used equipment in this group.

One or more antibiotic was prescribed empirically to all patients (100%). In this study the most common antibiotics prescribed to the patients at time of sampling were the 3rd and 4th generation cephalosporin (35%), β -Lactam combinations (35%), and carapenam (25%). This could explain the high resistance rates to these antibiotics among the studied isolates. This finding was supported by several studies that reported the impact of implementing antibiotic restriction policies on the incidence of resistant Gram negative microorganisms (22). found a significant increase in *A. baumannii*, including an outbreak of

carbapenam resistant strains after increasing the carbapenam use.

There are global reports of MDR *A. baumannii* strains which often spread, causing outbreaks throughout entire cities, countries, and continents (7).

It is not surprising that all of *Acinetobacter* isolates during this study were MDR. This may be attributed to the selection of MDR strains by extensive and unwise use of antibiotics in our hospitals. Isolates showed 100% resistance rate to Piperacillin, Ceftriaxone, Cefoperazone and Aztreonam. Resistance to other antibiotics were 95.8% for Cefotaxime, Ceftazidime, Amikacin, and Sulfamethoxazole trimethoprim, 91.7% for Netilmicin, 83.3 % for Tobramycin, 75% for Cefoperazone-sulbactam and Ampicillin-sulbactam. Several studies in Egypt, Saudi Arabia, Bahrain, United Arab Emirates, and Qatar reported MDR *A. baumannii* (23) (24) (25) (26).

These results are somewhat coordinate with that of (27) who tested the drug susceptibility of 23 *Acinetobacter* isolates and reported resistance rates of 100% to Imipenem, Amikacin, and 3rd generation cephalosporin, 82.6% to Tobramycin, 73.9% to tetracyclins, 69.6% to ciprofloxacin, and 17.4% to Colistin. On the other hand, the least resistance rate was observed with Colistin (12.5%) that could be explained by decreased prescription of this drug. Similar observations were also noted by (28) who reported that susceptibility was attributed to decreased prescriptions.

It has become clear that most clinical isolates of *A. baumannii* belong to groups of closely related strains, referred to as clones, which spread geographically at the national or international level (29). Although certain clones present with widespread dissemination, isolates of *A. baumannii* from hospitals in the same country, or even from within a single hospital, show significant genetic diversity (30).

PFGE restriction analysis of chromosomal bacterial DNA has been used with excellent results in epidemiological studies of numerous *A. baumannii* outbreaks, and is currently regarded as the reference standard for epidemiological typing. It was also useful for determining the involvement of closely related strains in different outbreaks (31).

In the current work, molecular typing using PFGE revealed the circulation of 10 distinct clusters named from A to J which were further subdivided into 8 subtypes (A1, B1, C1, E1, E2, E3, E4, and E5). Isolates clustered in the same genotype showed similarity $\geq 87\%$. Two types C and D were possibly related, genotype C isolated from Neurology ICU was possibly related to genotype D isolated from PICU. On the other hand different genotypes could be isolated from the same place. Most of the clonally related strains were isolated from the same place

which may indicate endemicity of these strains. However, the predominant genotype E (37.5%) was circulating in different places and contains 9 identical and closely related isolates and were isolated from PICU and Chest ICU. These results agree with that of (32) who carried out genotypic analysis of 66 *A. baumannii* strains by PFGE and revealed the circulation of 36 different PFGE types, of which type A and K accounted for 44% of the isolates.

In addition, the current study identified 4 distinct groups of strains, designated B (2 isolates), E3 (2 isolates), E4 (3 isolates), and F (3 isolates) that were involved in undetected outbreaks. These outbreak strains were also closely related to other strains that may be part of outbreaks. In spite of the limited number of isolates in this study we demonstrated that genotype B strains that were isolated from PICU environment were closely related to B1 (isolated from a HCW in the same PICU). This finding suggests the role of staff hand carriage in *A. baumannii* cross transmission. This emphasize the need for implementation of effective infection control policies in our hospitals especially in ICUs taking into consideration the epidemic features of multidrug resistant organisms in particular *A. baumannii* (33). Also recovered environmental strains, staff hands strains that were identical to isolates recovered from patients. Previously, (34) outbreak strains were clearly distinguished from epidemiologically unrelated strains showing highly distinct polymorphic PFGE profiles and they were easily distinguishable from one another and from the outbreak isolates. However, PFGE is laborious, time consuming, and requires technical experience. Other molecular typing techniques like MLST are further needed for establishing relatedness with clones that have spread globally (35).

The present study showed that clonally related strains can survive for a long time in hospitals and cause nosocomial infections. Although colonel relatedness among clone E strains continued for a long period, their drug susceptibility profiles were changeable. These results are very similar to that of (32) who also reported changeable drug susceptibility profiles within the same PFGE clones. Nonetheless, all of the remaining clones had identical susceptibility profiles.

Resist typing of isolates revealed 9 resist type designated in Latin numbers from (I to IX) Co-ordinance between resist typing and genotyping was 100% except for genotype E which was subdivided by resist typing.

Although most of the strains that were detected to be clonally related with PFGE and that were isolated on close dates presented similar antibiotic susceptibility patterns, yet this does not always the case since genotype or resist type of the strains

isolated from the same place was not always the same. This is in agreement with other previous data showing that sequential *A. baumannii* epidemics in the same ward were caused by different clones, one replacing the other in a well-defined temporal order (36).

The current study evaluated correlation between resist typing and genotyping that revealed that 3 strains of genotype were pan drug resistant, 5 strains were MDR being only sensitive to carpapenam, Gentamicin, ciprofloxacin, and Levofloxacin, and 1 strain was sensitive only to Colistin and Cefoperazone-sulbactam. The high resistance rate among the isolates of this clone might have been responsible for the high rate of infection caused by this strain during the study period. This result is also parallel with the previous reports stating that clonally related strains of *Acinetobacter* that differ insusceptibility patterns may coexist within a single hospital, dependent on the selective pressure related to antibiotic exposure (37).

Although carpapenam are still widely used for treatment of infections caused by *A. baumannii*, resistance to these antibiotics is reported increasingly worldwide, and this constitutes a major therapeutic problem (38). During the present study, we found that (41.7%) *A. baumannii* strains were resistant to at least one of the carpapenam. These carpapenam resistant strains were not restricted in a specific genotype; however, 40% of these strains belonged to the PFGE clone E. This finding is in agreement with previous data showing that the spread of carpapenam resistance in *A. baumannii* strains isolated from different hospitals was due to the acquisition of new epidemic clones (38) (32).

Conclusion

Survival and circulating some clonally related *A. baumannii* were identified among patients and different ICU environment including HCW, which were probably selected because of their resistance to the majority of antimicrobial agents. These data provided a better understanding of *A. baumannii* epidemiology within hospitals, possible source and route of transmission and the resistance pattern in order to implement a more strict prevention programs and improve antimicrobial therapy.

The current study proved that PFGE represents the gold standard of molecular typing for *Acinetobacter spp.* with very high discriminatory power thus can be useful in directing infection control efforts.

Recommendation

We suggest that large scale epidemiological studies over many years are required to study the epidemiology of *A. baumannii* in our hospitals. A strict

policy for the use of antibiotics is urgently needed. Implementation of effective surveillance programs and antibiotic cycling succeeded in controlling drug resistance in some countries.

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