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ORIGINAL ARTICLE

FimH and *CsgA* Adhesion genes Among *Acinetobacter* spp. Isolates and their Relation to Biofilm formation and Antimicrobial Resistance Pattern

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ABSTRACT

Background *Acinetobacter spp are important opportunistic pathogens responsible for nosocomial infections.* ***Objectives****: detection of EsβL and carbapenemase, the ability of biofilm formation and their relation to antimicrobial resistance.* ***Methodology****: A total of 230 clinical samples from patients admitted to Menoufia University Hospitals were obtained. Acinetobacter spp were identified by standard microbiological methods and Vitek-2 system. The antibiogram of Acinetobacter isolates was tested by the modified Kirby Bauer disk diffusion method, detection of extended-spectrum β-lactamases and carbapenemase by EsβL NDP and CANP tests. Biofilm production was detected by modified Congo red agar and PCR.* ***Results*** *Acinetobacter spp. represented (20.8%) of all the collected isolates. Vitek-2 system showed that the predominant spp. was Acinetobacter baumannii complex (80%). Acinetobacter isolates were highly resistant to cefepime and tobramycin (90% for each), ceftriaxone (88%), piperacillin, and ampicillin –sulbactam (86% for each), piperacillin- tazobactam (84%) and tetracycline (78%). About 64% and 68% of the Acinetobacter isolates were susceptible to tigecycline and colistin respectively. The sensitivity of EsβL NDP for detection of EsβL producing Acinetobacter isolates was 93.8 %. The Carba NP and carbAcineto NP tests detect carbapenemse production in 6% and 56% of Acinetobacter isolates respectively. Biofilm production was found among 56% isolates by MCRA method, while conventional PCR showed fimH and CsgA genes among 60% and 18% isolates respectively.* ***Conclusion:*** *Acinetobacter spp are serious nosocomial pathogens as they can produce ESβL and carbapenemase, and produce biofilm that is related to their antimicrobial resistance. Therefore, their adequate prevention and control is imperative.*

INTRODUCTION

Acinetobacter spp were considered as an opportunistic pathogen and recently has been emerged as an important nosocomial pathogen all over the world mostly involving patients with impaired host defense[1]. They are an important human pathogen that causes a variety of infections as ventilator-associated pneumonia, meningitis, bacteremia, wound and soft-tissue infections, peritonitis and urinary tract infections [2].

Acinetobacter baumannii was included within the group of *Enterococcus faecium, Staphylococcus aureus, Klebsiella pneumoniae, Pseudomonas aeruginosa and Enterobacter* spp (ESKAPE group) which is a group of opportunistic pathogens in healthcare settings, responsible for up to 40 % of infections in intensive care units with high rates of antibiotic resistance [3].

Acinetobacter spp. are resistant to many antibiotics and it can accumulate components of resistance mechanisms encoded on plasmids, integrons, and transposons in hospital settings associated with high antibiotic consumption[4].

Biofilm is formed of complex, sessile communities of microbes attached to a surface or buried firmly in an extracellular matrix as aggregates. The biofilm matrix makes the bacteria tolerant to harsh conditions and resistant to antibiotics[5]. Biofilms have major role in antibiotic resistance, as they play a role in antibiotic trapping and impairment and plasmid exchange. Therefore, they can lead to persistent infections of many pathogenic microbes. Moreover, they are important in indwelling medical device, dental plaque, and upper respiratory and urogenital tract infections [6].

The aim of this study was to detect ESβL and carbapenemase producing *Acinetobacter* isolates, to detect biofilm-producing *Acinetobacter* strains phenotypically and genotypically (*FimH gene*, and csgA gene), and also to assess the relation between the ability of biofilm production and antimicrobial drug resistance.

METHODOLOGY

This study was performed in Medical Microbiology and Immunology Department, Faculty of Medicine, Menoufia University. All patients were subjected to full history taking and thorough clinical examination.

Collection of samples:

A total of 230 clinical samples including (100 respiratory secretions, 60 urine, 30 burn swabs, 20 blood samples and 20 pus swabs) were obtained from infected patients.

- **Respiratory secretions** were collected from morning sputum samples, endotracheal aspirates, and bronchial aspirates.
- **Blood samples** were collected under strict aseptic conditions and inoculated into culture bottles
- **Urine samples**: 10–20 ml of midstream urine was collected.
- **Pus** by a sterile cotton swab from infected wounds**.**

Identification of bacterial isolates:

All the specimens were cultured on different media (Oxoid, UK). The growing *Acinetobacter* isolates were examined by the standard microbiological methods and VITEK 2 System to identify the growing bacteria. The confirmed *Acinetobacter* isolates were preserved in nutrient broth supplemented with 16% glycerol and stored frozen at -80°C [7]**.**

Antimicrobial susceptibility testing by disk diffusion method

Different antibiotic susceptibility disks (Oxoid) were used including: piperacillin (PRL) (100 µg), ampicillin/sulbactam (SAM) (10/10 µg), piperacillin/ tazobactam (TZP)(100/10 µg), ceftazidime (CAZ) (30 µg), cefotaxime (CTX) (30 µg), ceftriaxone (CRO) (30 µg), cefepime (CPM) (30 µg), meropenem (MEM) (10 µg), imipenem (IPM) (10 µg), amikacin (AK) (30 µg), gentamicin (GM) (10µg), tobramycin (TOB)(10 µg), tetracycline (TET)(30 µg), doxycycline (DOX) (30µg),ciprofloxacin (CIP)(5 µg), levofloxacin (LEV) (5 µg), trimethoprim-sulfamethoxazole (SXT) (1.25/23.75 µg), colistin (10µg) and tigecycline (TGC) (30µg).

Phenotypic detection of ESBL in *Acinetobacter* isolates:

Screening of ESBL production

According to [8]***CLSI, 2019***, ceftazidime (30µg), cefotaxime (30µg), and ceftriaxone (30µg) were used. A zone diameter of ≤ 18 mm for ceftazidime, ≤ 23 mm for cefotaxime, and ≤ 21 mm for ceftriaxone were considered potential ESBL-producers.

Phenotypic confirmation of ESBLs production by cephalosporin/clavulanate combination disks:

Confirmation of ESBL-production was performed using ceftazidime (30 µg) and cefotaxime (30 µg) disks alone and in combination with clavulanic acid. An increase of at least 5 mm in zone diameter for antimicrobial combination with clavulanic acid versus its zone when tested alone confirms ESBL production [9].

The ESBL NDP (Nordmann-Dortet-Poirel) test:

This is a phenotypic detection of ESBL enzymes by colorimetric method. The test identifies the hydrolysis of the lactam ring of cephalosporin (cefotaxime), which generates a carboxyl group, by acidifying the culture media. The change in pH resulting from this hydrolysis is identified by the color change (from red to yellow/orange) generated using a pH indicator (phenol red). Inhibition of ESBL activity is evidenced by adding tazobactam[10].

Detection of carbapenemase production by *Acinetobacter* spp:

a. Screening for the resistance to carbapenems was done and interpreted according to [8]***CLSI 2019*** by using imipenem disk diffusion method. Results were categorized as Resistant if zone diameter ≤ 18

b. ***Carba NP Test:*** This is a phenotypic method developed for carbapenemase detection. It is based on *invitro* hydrolysis of imipenem by a bacterial lysate, which is detected by changes in pH values using the indicator phenol red (red to yellow/orange)[11].

c. ***CarbAcineto NP Test****:* This is the modified test of Carba NP used for *Acinetobacter* spp. in which the lysis buffer was replaced by 100 µl 5M NaCl solution, avoiding any buffer effect, the bacterial inoculum was doubled from one-third to one-half of a calibrated loop to a full calibrated loop in order to increase the enzyme quantity then proceed as Carba NP test [12].

Detection of biofilm formation:

Phenotypic detection by modified Congo red agar method (MCRA):

Congo red medium was incubated for 48 h at 37°C and subsequently 2-4 days at room temperature. A black color interpreted as positive biofilm producing strains in contrast with red colonies which was interpreted as negative biofilm producers[13]**.**

Detection of biofilm-associated genes (fimH and CsgA) by conventional PCR:

- DNA extraction: Bacterial DNA was extracted and purified using the gene JET™ genomic DNA purification kit (ThermoFisher Scientific, UK).

Table I: Primers used in the study[14]:

Primername	Sequence	Product size (bp)
FimH	F:5`TGCAGAACGGATAAGCCGTGG 3` R: 5`GCAGTCACCTGCCCTCCGGTA 3`	508
CsgA	F: 5`ACTCTGACTTGACTATTACC 3` R: 5`AGATGCAGTCTGGTCAAC 3`	200

PCR conditions for detection of *fimH* and *CsgA* genes in *Acinetobacter* spp[14]

PCR was performed in atotal volume of 25µL for each gene containing 12.5µl DreamTaq green PCR Master Mix (2x),1 µl of each primers F & R, 10µl DNA Extract, 0.5 µl Water nuclease –free.

PCR amplification were performed with the following amplification scheme, an initial denaturation at (95°C for 4 min) for both genes, followed by 34 cycles and 30 cycles of [DNA denaturation (94°C for 1 min and 95°C for 50 sec), primer annealing (56°C for 45sec and 58°C for 1 min), primer extension (72°C for 1 min and72°C for 45 sec)], and final extension at 72°C for 10min and 72°C for 8min for *FimH* and CsgA respectively. The amplified DNA was electrophoresed using 2% agarose gel (Fermentas, Lithuania) stained with ethidium bromide (Sigma, USA), and the bands at (508 for *fimH* and 200 bp for *csgA*) were visualized and photographed (Samsung, WB30F, Korea).

Statistical analysis:

Computer SPSS program version 20 was used. The results were expressed as number and percentage. Chi-square test was performed ($P < 0.05$ and <0.001 were considered significant and highly significant, respectively), and accuracy was represented using the terms sensitivity, specificity, positive predictive value, negative predictive value, and overall accuracy.

RESULTS

Fifty *Acinetobacter* isolates were obtained from 230 hospitalized patients admitted to different departments and ICUs. *Acinetobacter* isolates were derived mostly from ICUs (54%). about 48%, 26%, 4%, 12% and 10% of clinical *Acinetobacter* isolates were derived from respiratory secertions, blood, urine, pus and burn unit respectively as shown in [table 1] **.**Vitek2 system results found that *A.baumannii Complex* was the most predominant isolated species (80%)

Table 1: Distribution of *Acinetobacter* isolates (n=50) according to different samples among hospital departments.

Acinetobacter Samples	Departments												Total	
	ICU		Surgery		Pediatric		Chest		Burn unit		Internal medicine			
	N	%	N	%	N	%	N	%	N	%	N	%	N	%
1.Respiratory samples														
- Sputum	11	68.75	0	0	0	0	3	18.75	0	0	2	12.5	16	32
- Tracheal aspirate	5	100	0	0	0	0	0	0	0	0	0	0	5	10
- Bronchial aspirate	0	0	0	0	0	0	3	100	0	0	0	0	3	6
- Total	16	66.7	0	0	0	0	6	25	0	0	2	8.3	24	48
2-Blood	9	69.2	0	0	4	30.8	0	0	0	0	0	0	16	26
3-Urine	2	100	0	0	0	0	0	0	0	0	0	0	2	4
4-Pus	0	0	6	100	0	0	0	0	0	0	0	0	6	12
5-Burn	0	0	0	0	0	0	0	0	5	100	0	0	5	10
Total	27	54	6	12	4	8	6	12	5	10	2	4	50	100

- Regarding antimicrobial susceptibility pattern by disk diffusion method. *Acinetobacter* isolates were highly resistant to cefepime and tobramycin (90% for each), ceftriaxone (88%), piperacillin, and ampicillin –sulbactam (86% for each), piperacillin- tazobactam (84%) and tetracycline (78%). On the other hand, 64%, 68% and 50 % of the *Acinetobacter* isolates were susceptible to tigecycline, colistin and doxycycline respectively as shown in [table 2].

Table 2: Antimicrobial susceptibility pattern of *Acinetobacter* isolates by disk diffusion method

Antimicrobial group	Antimicrobial Agent	Abbreviation	Disk content (µg)	Total n=50					
				Sensitive		Intermediate		Resistant	
				N	%	N	%	N	%
Penicillins	**Piperacillin**	**PRL**	100	0	0	7	14	43	86
β- Lactam/ β-lactamase inhibitor combination	**Ampicillin. Sulbactam**	**SAM**	10/10	5	10	2	4	43	86
	Piperacillin. Tazobactam	**TZP**	100/10	6	12	2	4	42	84
Cephalosporins	**Cefotaxime**	**CAZ**	30	14	28	0	0	36	72
	Ceftazidime	**FEP**	30	15	30	0	0	35	70
	Cefepime	**CTX**	30	3	6	2	4	45	90
	Ceftriaxone	**CRO**	30	2	4	4	8	44	88
Carbapenems	**Imipenem**	**IMP**	10	21	42	5	10	24	48
	Meropenem	**MEM**	10	26	52	0	0	24	48
	Doripenem	**DOR**	10	21	42	0	0	29	58
Aminoglycosides	**Gentamycin**	**CN**	10	14	28	3	6	33	66
	Tobramycin	**TOB**	10	4	8	1	2	45	90
	Amikacin	**AK**	30	21	42	3	6	26	52
Fluoroquinolones	**Ciprofloxacin**	**CIP**	5	19	38	0	0	31	62
	Levofloxacin	**LEV**	5	21	42	0	0	29	58
	Gatifloxacin	**GAT**	5	11	22	4	8	35	70
Tetracyclines	**Tetracycline**	**TE**	30	10	20	1	2	39	78
	Doxycyclin	**DO**	30	25	50	0	0	25	50
Folate pathway Inhibitors	**Trimethoprim sulfamethoxazole**	**SXT**	1.25/ 23.75	17	34	0	0	33	66
Lipopeptides	**Colistin**	**CT**	10	34	68	0	0	16	32
Glycylcyclines	**Tigecycline**	**TGC**	30	32	64	6	12	12	24

- ESBL production was detected among 16/50 (32%) of *Acinetobacter* isolates by double disc diffusion (ceftazidime + ceftazidime-clavulanate) and 15/50 (30%) of isolates were detected as ESβL producer By ESBL NDP with a statistically no significant difference between the two methods as in [table 3] and [figure 1]. Considering double disc diffusion test as a gold standard the sensitivity, specificity, PPV, NPV, and accuracy of ESBL NDP test were as follows 93.8%, 100%, 100%, 97% and 98% [table 4].
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Table 3: Comparison between ESBL.NDP test and ESBL by combined disk diffusion method used for detection of ESβL- producing *Acinetobacter*

	ESBL by confirmatoty combined disk		ESBL. NDP		Z test	P value
	No	%	No	%		
Positive	16	32	15	30	0	1
Negative	34	68	35	70	0	1
Total	50	100	50	100		

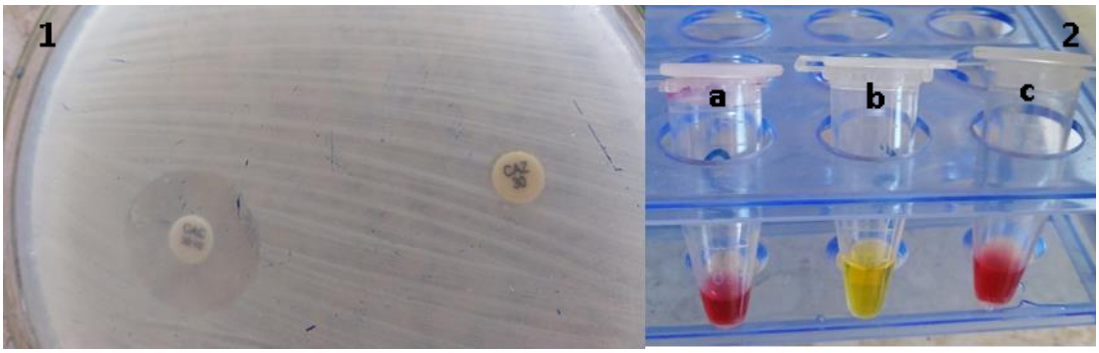

Fig. 1: Confirmatory test for ESβL production in *Acinetobacter* isolates by combined disk method(1) where there is an increase of inhibitory zone diameter ≥5 mm around ceftazidime/clavulanate (CAC) and by **EsβL NDP test (2)** (color changed from red to yellow) in tube b and remains red in tube c by adding tazobactam (B-lactam inhibitor)

Table 4: ESBL.NDP test in relation to ESBL by combined disk as agold standard test

ESBL NDP	ESBL by combined disk (gold standard)				Total (50)		Sensitivity	Specificity	PPV	NPV	Accuracy
	Positive (16)		Negative (34)								
	No	%	No	%	No	%					
Positive	15	93.8	0	0	15	30	**93.8%**	**100 %**	**100 %**	**97 %**	**98 %**
Negative	1	6.3	34	100	35	70					
Total	16	100	34	100	50	100					

PPV =positive predictive value NPV =negative predictive value

- Regarding Carbapenemase detection, 29/50 (58%) of *Acinetobacter* isolates were carbapenemase producer by imipenem disk diffusion method, 3/50 (6%) of isolates were producers by carba NP test and 28/50 (56%) by CarbAcineto Np test, with a statistically significant difference (p**<0.001**) as shown in [table 5] and [figure 2].

Table 5: Comparison between different methods of carbapenemase detection in *Acinetobacter* isolates

	Imipenem screening		Carba NP		CarbAcineto Np		χ2	P value
	No	%	No	%	No	%		
Positive	29	58	3	6	28	56	36.17	**0.001<**
Negative	21	42	47	94	22	44		
Total	50	100	50	100	50	100		

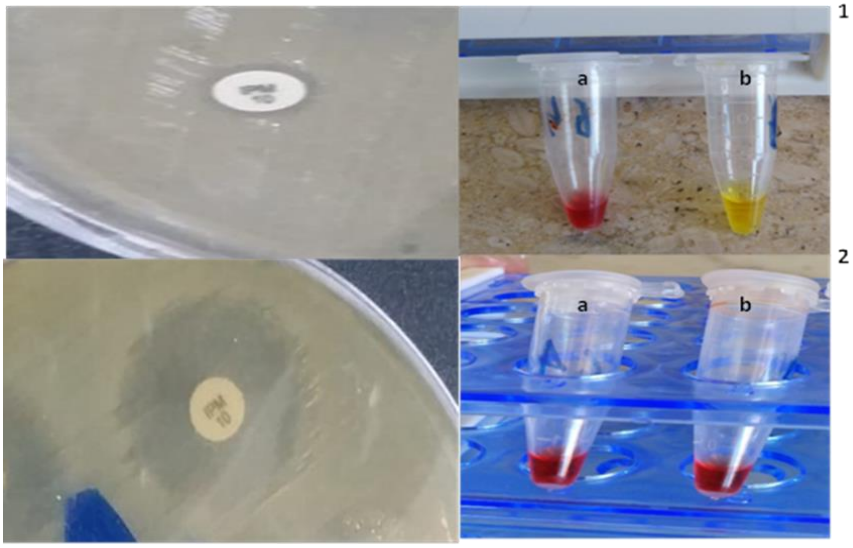

Fig. 2: Representative results obtained by both Carba NP and CarbAcineto NP tests; 1 represents results obtained by imipenem- resistant strains where a tube (red colour/control tube) is a negative and tube b (yellow colour) is a positive result i.e., carbapenemase-producing isolate. **2** represents results obtained by imipenem susceptible strain where both tubes (a,b) are negative results i.e., carbapenemase-non producing isolates .

- Modified Congo red agar detected 28/50 (56%) of *Acinetobacter* isolates as biofilm positive while 22/50 (44%) as biofilm negative. On the other hand, 60% and 18% of *Acinetobacter* isolates were positive for the *fimH* gene and CsgA gene by conventional PCR [figure 3 and 4].

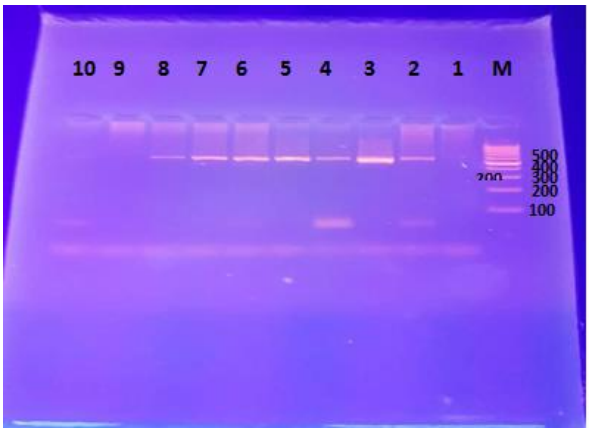

Fig. 3: Agarose gel electrophoresis for the PCR amplified products of *Acinetobacter FimH* gene
Lane M: DNA molecular size marker (1000 bp).
Lanes 2, 3, 4, 5, 6, 7 and 8 were positive for *FimH* gene (508bp).
Lanes 1,9 and 10 were negative for *FimH* gene (508bp).

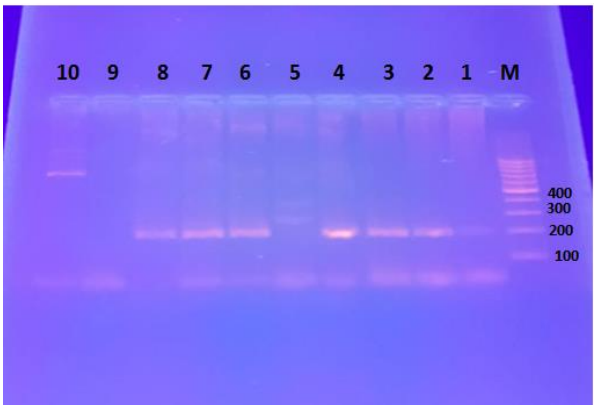

Fig. 4: Agarose gel electrophoresis for the PCR amplified products of *Acinetobacter CsgA* gene.
Lane M: DNA molecular size marker (1000 bp).
Lanes 1, 2, 3, 4, 6, 7 and 8 were positive for ***CsgA*** gene (200 bp).
Lanes 5, 9 and 10 were negative for ***CsgA*** gene (200 bp)

- About 23/28 (82.1%) of the biofilm-producing *Acinetobacter* isolates were positive for the *FimH* gene, while 7/22 (31.8%) of non-biofilm producing isolates had *FimH* gene with statistically significant difference between them and 7/28 (25%) of the biofilm-producing isolates were positive for the CsgA gene while, 2/22 (9.1%) of the non-biofilm producing isolates were positive for the CsgA gene with no statistically significant difference between them [table 6]

Table 6: Relation between *FimH* and *CsgA* genes by conventional PCR and biofilm formation

***FimH* gene**	**Biofilm formation**				**Total**		**Test of significance (X^2)**	**P Value**
	Positive (28)		**Negative (22)**					
	N	**%**	**N**	**%**	**N**	**%**		
Positive	23	82.1	7	31.8	30	60	13	**< 0.001**
Negative	5	17.9	15	68.2	20	40		
***CsgA* gene**								
Positive	7	25	2	9.1	9	18	2.113	0.266
Negative	21	75	20	90.9	41	82		

- About 13/16 (81.3%) of ESβL-producing *Acinetobacter* isolates were biofilm-positive while 3/16 were biofilm negative [table 7]

Table 7: Number and percent of Biofilm formation among ESβL-producing *Acinetobacter* isolates (No=50)

Combined ESBL	**Biofilm formation**				**Total**		**Test of significance (X^2)**	**P value**
	Positive		**Negative**					
	N	**%**	**N**	**%**	**N**	**%**		
Positive	13	81.3	3	18.7	16	32	6.088	**0.014***
Negative	15	44.1	19	55.9	34	68		
Total	28	56	22	44	50	100		

DISCUSSION

Acinetobacter species are considered challenging pathogens responsible for serious opportunistic infections[15]**.** In the present study, 50 *Acinetobacter* strains were isolated from 230 hospitalized patients, Similar rates were found in Iran[16] (20.8%) and in Nepal[17] (20%). the highest isolation rate of *Acinetobacter* isolates was from ICUs (54%) and from respiratory samples (48%). In agreement with this, the study in Egypt[18] reported that ICU was the main source of *Acinetobacter* samples (38.5%) isolated from respiratory secretions (42.3%). On the other hand, Rebic et al.[1] found that Most of the positive *Acinetobacter* isolates were from the general surgery (48.65%) while, intensive care unit (ICU) were (20.27%)

Antimicrobial resistance of *A. baumannii* to antibiotics has become a problem worldwide. This resistance causes difficulty in treating infections caused by such organisms[19]. In this study, *Acinetobacter* isolates were highly resistant to cefepime and tobramycin (90% for each), ceftriaxone (88%), piperacillin, and ampicillin –sulbactam (86% for each), piperacillin- tazobactam (84%) and tetracycline (78%). this results agreed to much extent with *Yang et al.*[20]that recorded the resistance to cefepime to be the most common (96.2%) then, resistance to carbenicillin (88.39%), sulfamethoxazole-trimethoprim (75.6%), ticarcillin (74.23%), piperacillin (69.75%), ceftazidime (69.7%), ciprofloxacin (65.8%), gentamicin (60.8%), tigecycline (57.6%) and amikacin (56.17%). In this study the resistance of *Acinetobacter* Isolates to carbapenems (imipenem, meropenem and doripenem) was 48%, 48% and 58% respectively, similar results (48.1%) were recorded by the study in Egypt[18]. Other studies reported higher resistance rates reaching up to 100% [21]**.**

This study revealed that 64% and 68% of the *Acinetobacter* isolates were susceptible to tigecycline and colistin. Higher susceptibility to tigecyclines (91.3%) was observed in India[22] while Lower susceptibility (25.8) was reported (25.8) in Greece[23].

This study showed that the most efficient antibiotics for *Acinetobacter* infection were tigecycline and colistin with resistance rate (36 and 32%) which are the last line of treatment of XDR *Acinetobacter* isolates.

In his study 16/50 (32%) of *Acinetobacter* isolates were confirmed as ESBL-producers by double disc diffusion (ceftazidime + ceftazidime-clavulanate). Similar results (32.9%) and (27.5%) were reported by Goel et al. [24] and Kaur and Singh[25]. Higher results (71.4%) and (59%) were obtained by *Punia et al.* [26] and Abdar et al. [27]. Regarding ESBL NDP test, 15/50 (30%) were detected as ESβL producers and the sensitivity and specificity were 93.8% and 100% respectively. similar results were obtained by Nordmann et al.[10] who found that the sensitivity and specificity of ESBL NDP test 92.6% and 100% respectively. Also, the study *in Egypt* found that, the ESBL NDP test was able to diagnose all cases of *Acinetobacter*[28].

Regarding carbapenemase production, similar to this study, Dortet et al.[12] found that the CarbAcineto NP test efficiently detected OXA-type carbapenemase producers, leading to a significant improvement of the sensitivity from 11.9% for carba NP to 94.7 % for CarbAcineto NP test**.**

In this study the detection of biofilm was performed by MCRA. About (56%) of *Acinetobacter* isolates were biofilm producers by modified Congo red agar method.

Similar results (53.97%) were obtained by [17]*Dumaru et al.* in contrast to this study Ghasemi et al.[29] found that (7.1%) of the isolates were biofilm producer while Zeigham et al.[2] found that All A. *baumannii* isolates were able to produce biofilm.

Regarding the relation of EsβL producing *Acinetobacter* isolates and biofilm formation, similar to this study, Punia et al.[25] found that (76%) of ESβL producers form strong biofilm. On the other hand, Emami and Eftekhar [30] did not find an association between ESβL production and the potential to form biofilm among the burn isolates.

In the current study, the prevalence of *fimH* gene and CsgA gene were (60%) and (18%) respectively. similarily Mohajeri et al. [31]. found *fimH* in (60%), higher results (74%) were reported by Momtaz et al. [14] who found also, CsgA in 13% isolates. Other studies showed higher results for csgA (54%, 70% and 66.7%) were obtained by[31, 32, 33].

There was a positive correlation between biofilm formation capacity and antibiotic resistance. High degree of resistance among biofilm-producing isolates were seen with cefotaxime, Ceftazidime, Gentamycin, Ciprofloxacin, Levofloxacin, Tetracycline and Trimethoprim sulfamethoxazole (100%, 96.4%, 89.3%, 89.93%, 85.2%, 92.9% and 89.3%) with a statistically significant difference ($p<0.05$) between them and nonbiofilm producing isolates. Quite similar results were obtaind by Dumaru et al.[17] who found that the association between biofilm and antibiotic resistance was statistically significant for aminoglycosides, fluoroquinolones, cephalosporins, imipenem, and piperacillin. On the other hand, Qi et al.[34] found a negative correlation between biofilm formation capacity and resistance as, susceptible isolates could form stronger biofilms than non-susceptible ones ($P < 0.001$).

Regarding relation of *(fimH* and CsgA) gene and biofilm formation, Strains that form biofilm but don't have the gene might be due to the possibility that there were another genes responsible for biofilm formation as bap, ompA, csuE, epsA, blaPER-1, bfmS, ptk, pgaB, and kpsMII [2].while strains that had the genes but don't form biofilm might be due to lack of gene expression. although type 1 fimbriae (*FimH*) is important adhesion factor for bacterial initial attachment to the biological surfaces, presence of this gene is not the only determinant for biofilm development and several environmental and genetic factors may be involved with the expression of this gene [35].

Conclusion and recommendations:

Acinetobacter infection is an important nosocomial pathogen particularly in ICUs. EsBLs and carbapenemase production among *Acinetobacter* spp are increasing. There was a relationship between biofilm production and antimicrobial resistance forming a serious threat to empiric therapy of *Acinetbacter* isolates. Colistin and tigecycline can be the last treatment options left for the management of *Acinetobacter* infections

Conflicts of interest:

- The authors declare that they have no financial or non financial conflicts of interest related to the work done in the manuscript.
- Each author listed in the manuscript had seen and approved the submission of this version of the manuscript and takes full responsibility for it.
- This article had not been published anywhere and is not currently under consideration by another journal or a publisher.

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