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Susceptibility of Aminoglycoside Resistant *Acinetobacter baumannii* Toantibiotic Combinations

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ACINETOBACTER baumannii (*A. baumannii*) is considered one of the predominant antibiotic resistance pathogens involved in hospital acquired infections worldwide problem. The study investigated the effect of antibiotic combination of β-lactams (ceftriaxone, cefixime, carpabenem and impenim) and aminoglycosides against 5 clinical isolates of *A. baumannii* multidrug resistant. Over one year, 250 bacterial isolates were collected from 5 Egyptian hospitals from various infection sites. Two hundred out of 250 bacterial isolates were identified as *A. baumannii* based on phenotypic and genotypic techniques. The susceptibility of two-hundred *A. baumannii* strains against 21 different antibiotics was studied. The results showed that the highest resistance was to Cephalosporins, group was 99% followed by Quinolones & Fluoroquinolone was 90, 5 followed by penicillin 87.5, then Sulfa drugs was 75.5 then Carbapenem was 73 and finally Aminoglycosides was 60.5%. The minimum inhibitory concentration (MICs) values of aminoglycosides resistant *A. baumannii* strains ranged from 32 to >512mg/ml and β-lactam group ranged from 16 to >512mg/ml. Forty-five combined microtitre checkerboards were performed against the 5 totally aminoglycoside *resistant A. baumannii* strains to assess the potential for combination therapy. Combination of aminoglycoside antibiotics with β-lactams showed synergy action in thirty- eight (84%) of total forty-five combinations. Synergy was achieved with 100%, with the following combinations GN/IMP, GN/CRO, GN/CFM, AK/CRO, AK/CFM, TOB/CRO and TOB/CFM. No synergism was observed with combination between amikacin and imipenem.

Keywords: *Acinetobacter baumannii*, Aminoglycosides, β-lactams combination. Synergy.

Introduction

Acinetobacter baumannii is considered from the major causes of nosocomial outbreaks and is resistant to most available antibiotics. It can cause serious infections like (VAP), skin and soft tissue infection, wound infection, secondary meningitis, blood infection nosocomial infections such as found in bloodstream, respiratory tract and wound infection. (Almasaudi, 2018) *A. baumannii* is commonly resistant to clinically available antimicrobial agents, including β-lactams and fluoroquinolones . Aminoglycoside was treatment options for *Acinetobacter* infections but their resistance has increased in the recent years (Asif et al., 2018). Antimicrobial resistance in gram-negative bacteria is one among the 3 greatest threats to human health (Allen & Hartman, 2010; Bergogne-Berezin et al., 1987). *Acinetobacter baumannii* is one among the 3 most difficult gram-negative pathogens, particularly in medical aid units. Close to fourteen thousand critically sick patients with *A. baumannii* infections were extremely related to magnify mortality and high morbidity rates (Bouvet & Grimont, 1986). It is often causing a multiple infection like blood stream, respiratory tract, and wound infections (Bouvet et al., 1987, 1990; Anstey, 1992; Allen &

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Hartman, 1995; Peleg et al., 2008; Mortensen et al., 2014).

Multidrug-resistant *A. baumannii* strains are a critical concern, resulting in a major outbreak worldwide. Traditionally, β-lactams and aminoglycosides were successful used to treat *A. baumannii* (Chopade et al., 1985), however sadly, with susceptible *A. baumannii* increasing abuse, strains have emerged immune to nearly all antibiotics in monotherapy (Crombach et al., 1989). These days Carbapenem were until now thought of the treatment of selection against severe *A. baumannii* infections, carbapenem-resistant *A. baumannii* isolates square measure speedily increasing (Devaud et al., 1982). Aminoglycoside monotherapy was caused vital killing of *A. baumannii* however followed by speedy and intensive resistance emergence in vitro and in patients (Drusano,, 1991; Douboyas et al., 1994; Eliopoulos & Eliopoulos, 1988). B-Lactam antibiotics square measure wide used and really safe, also as clinicians' square measure well trained on the safe use of aminoglycosides (Joly-Guillou et al., 1987). Aminoglycoside and β-lactam antibiotics have completely different mechanisms of action and resistance; there's no effluence pump that affects each of those antibiotic categories in an exceedingly *A. baumannii* (Joly-Guillou et al., 1990). This implies that β-lactams could kill aminoglycoside-resistant bacterium and contrariwise (Klastersky et al., 1977; Marques et al., 1995) to boot, aminoglycoside disrupt the outer membrane of *A. baumannii* in that enhance the target website penetration of β-lactams, since the outer membrane of *A. baumannii* is around 2- to 7-fold less leaky than that of *Pseudomonas aeruginosa* and around 50-fold less leaky than that of *E. coli* (Martinez-Martinez et al., 1995; Meyers et al., 1991).

The high rates of resistance in an exceedingly. *A. baumannii* highlight the required want for another treatment choices, like rationally optimized combination therapies. Therefore, we have a tendency to conduct during this study to examine the susceptibleness pattern of resistant *Acinetobacter baumannii* against usually out their antibiotics in our discovered and establish synergistic microorganism killing and overcome of resistance for mixtures of a β-lactam with aminoglycoside against *A. baumannii* as substantial treatment choices.

Materials and Methods

Collection and identification of bacterial isolates

Two hundred and fifty of Gram-negative bacterial isolates (250) were collected from clinical samples from different infection site (blood, urine, stool, sputum, wound and endotracheal tube) from microbiological laboratories belonging to five hospitals in Cairo, Egypt (*Nasser Institute*, El-Kasr Al-Aini Hospital, Abu El-Reesh, El-harem Hospital and Hussein Hospital) through November 2016 to December 2017. All bacterial isolates were identified using conventional methods depending on cultural and biochemical characteristics on blood and MacConkey agar medium and as oxidase negative and catalase positive isolates. The positive 200 *Acinetobacter* isolates were confirmed using PCR detection of *bla-oxa-51* gene with amplicon size 353bp that is characteristic for *Acinetobacter baumannii* and is intrinsic to the species, using the primers sequences as following:

5"-TAATGCTTTGAT CGGCCTTG-3"

3"-TGGATTGCACTTCATCTTGG-5"

Antimicrobial susceptibility testing

Antimicrobial susceptibility testing of identified *Acinetobacter* strains was carried out by disk diffusion method using the Kirby–Bauer technique1966 (Meyers et al., 1991) and as the recommendations of Clinical and Laboratory Standards Institute (CLSI) document M2-A41 (CLSI, 2018). Antibiotics to be tested; were selected referring to CLSI document M100-S28 (CLSI, 2018), and they included the first- and second-line antibiotics commonly used for treatment of *Acinetobacter* infections. The tested antibiotics included; gentamicin, tobramycin, amikacin (10µg), meropenem (10µg), imipenem (10µg), Amoxycillin Clavulanate (30µg), cefixime (30µg), Ampicillin (5µg), Cefoperazone (10µg), Cefoperazone-Sulbactam (30µg), Cefotaxime (thirty) Cefoxitin (30µg), Ceftazidime (thirtyµg), Ceftriaxone (thirty µg), Cefuroxime (thirty µg), Ciprofloxacin (5µg), Co-trimoxazole (10 µg), Levofloxacin (5µg), Piperacillin (10µg), Ofloxacin (5µg), Norfloxacin (10µg).

Determination of minimum inhibitory concentrations (MICs) of antibiotics against Acinetobacter baumannii

Minimum inhibitory concentrations (MICs)

of different antibiotics against 5 clinical *A. baumannii* strains selected according to phenotypic pattern (1.5×10^8 CFU/ml) were determined by broth microdilution method in Mueller-Hinton broth MHB (Oxoid, USA) according to Clinical and Laboratory Standards Institute methods (CLSI, 2014). The different antibiotic standards include: cefixime, ceftriaxone, imipenem, Gentamicin, Tobramycin and Amikacin which used in this study .

Combinations of antibiotics using Checkerboard method

Combination of antibiotics was done by using checkerboard method (Eliopoulos & Moellering, 1996) for five the most resistant *A. baumannii* strains namely, ASN1, ASN4, ASN12, ASN15 and ASN18. The checkerboard dilution test is widely used *in vitro* for the evaluation of combination potential synergetic effect of both individual and combined antibiotics as represent by FIC index. The concentration range of each used antibiotic combination tested in range from 1/4 XMIC up to 2X MIC dilution. Each test was performed in triplicate with starting inoculum at concentration of 5×10^5 CFU/ml. The fractional inhibitory concentration (FIC) index is an efficient key for interpretation of the interaction of antibiotics, and was calculated for every antibiotic in each combination according to the subsequent formula.

FIC index= FICA + FICB

FICA= MIC of drug A in combination/ MIC of drug A alone

FICB= MIC of drug B in combination/ MIC of drug B alone

The FIC indices were taken as: Synergy (was outlined when)= FIC≤0.5.

Additive or indifferent (was outlined when)= FIC > 0.5 ≤4.0.

Antagonism (was outlined when)= FIC >4.0.

The checkerboard method (Microtitre method) was performed in 96 well microtiter plates containing Cephalosporins plus aminoglycosides and Carbapenem plus aminoglycosides antibiotics.

Results

Isolation and identification of bacterial cultures

Two hundred and fifty bacterial isolates were collected from Egyptian hospitals from different infection sites. However, the most common clinical specimen were endotracheal infections 47.2, followed by blood, 20.4 then urine 13,6 sputum, 12.8 and finally wounds 6%, respectively (data not shown). Upon phenotypic identification using morphological, cultural and biochemical properties, 220 bacterial isolates were identified as *Acinetobacter* spp. Out of 220, two-hundred *Acinetobacter* spp. were further confirmed as *Acinetobacter baumannii* using molecular identification by detection of *blaoxa-51*gene that is characteristic for *A. baumannii* at 353pb.

Antimicrobial suscipcibility

The phenotypic resistance patterns represented in Table 1, The results showed that the highest resistance was to Cephalosporins, group was 99% with cefixime (CFM) followed by Quinolones & Fluoroquinolone was 90.5 with Levofloxacin (LEV) followed by penicillin 87.5 with Amoxycillin/clavulanate (AMC), then Sulfa drugs was 75.5 then Carbapenem was 73 with Meropenem (MEM) and finnaly Aminoglycosides was 60.5% with Gentamicin (GN).

According to the results of susceptibility profile twelve (12) *Acinetobacter baumannii* strains that showed the widest spectrum resistance to aminoglycosides were detected for determination of MIC values namely ACN1, ACN1N, ACN2, ACN3, ACN4, ACN5, ACN7, ACN10, ACN12, ACN13, ACN15, and ACN18. Table 2 showed the MIC values of antibiotics belonging to aminoglycoside and β-lactam groups. All *Acinetobacter baumannii* strains showed high MICs concentration for all antibiotics tested in a range from 16 to ≥ 512mg/L for tested aminoglycoside groups while β-lactam groups showed MIC values in a range from 32 to ≥ 512 mg/L. For gentamicin, tobramycin and amikacin MIC values varied between 256 to ≥ 512, 128 to ≥ 512mg/L and 16 to ≥ 512mg/L, respectively. β-lactam antibiotics, MIC vales of imipenem, ceftriaxone and cefixime were varied between (32 to ≥ 512mg/L), (256 to ≥ 512mg/L) and (128 to ≥ 512mg/L) respectively.

TABLE 1. Percentage of resistance patterns of *A. baumannii* to amginolycosides resistant.

Antibiotic groups		Antibiotics	Sensitive (S)		Resistance (R)	
			%	No.	%	No
Aminoglycosides		Gentamicin (GN)	39.5	79	60.5	**121**
		Tobramycin (TOB)	45	90	55	110
		Amikacin (AK)	47	**94**	53	106
β-lactam	Penicillin	Amoxycillin/clavulanate (AMC)	12.5	25	87.5	175
		Ampicillin (AMP)	17.5	35	82.5	165
		Piperacillin (PRL)	43	86	57	114
	Cephalosporins	Cefepime (FEP)	17	34	83	166
		Cefoperazone (CEP)	39	78	61	122
		Cefoperazone-Sulbactam	8.5	12	91.5	183
		Cefotaxime (CTX)	12	24	88	176
		Cefoxitin (FOX)	4	8	96	192
		Ceftriaxone (CRO)	2	4	98	196
		Cefuroxime (CXM)	4	8	96	192
		Cefixime (CFM)	1	2	99	**198**
	Carbapenem	Imipenem (IMP)	35	70	65	130
		Meropenem (MEM)	27	54	73	146
Quinolones & Fluoroquinolone		Ciprofloxacin (CIP)	23.5	47	76.5	153
		Levofloxacin (LEV)	9.5	19	90.5	181
		Ofaxacin (OFX)	53.5	107	46.5	93
		Norfloxacin (NOR)	60.5	121	39.5	79
Sulfa drugs		Co-trimoxazole (STX)	24.5	49	75.5	151

TABLE 2. MICs concentration determination of aminoglycosides and β-lactam against selected 12 clinical *A. baumannii* strains.

Strains No.	MIC					
	Aminoglycosides			β-lactam		
	Gentamicin	Tobramycin	Amikacin	Imipenem	Ceftriaxone	Cefixime
ACN1	≥512	≥512	≥512	32	256	128
ACN1N	≥512	≥512	≥512	64	≥512	≥512
ACN2	≥512	256	≥512	>512	≥512	≥512
ACN3	≥512	128	≥512	≥512	≥512	≥512
ACN4	≥512	128	128	≥512	>512	>512
ACN5	256	≥512	16	128	≥512	≥512
ACN7	≥512	≥512	32	64	≥512	≥512
ACN10	≥512	256	128	≥512	≥512	256
ACN12	256	≥512	>512	128	≥512	≥512
ACN13	≥512	≥512	>512	256	>512	>512
ACN15	≥512	128	128	256	>512	256
ACN18	≥512	256	256	128	≥512	256

Results showed that out of the 5 selected strains 4 were 100% resistance to antibiotics and only strain ASN12 was sensitive to cefixime and ofloxacin antibiotics (Fig. 1, Table 3).

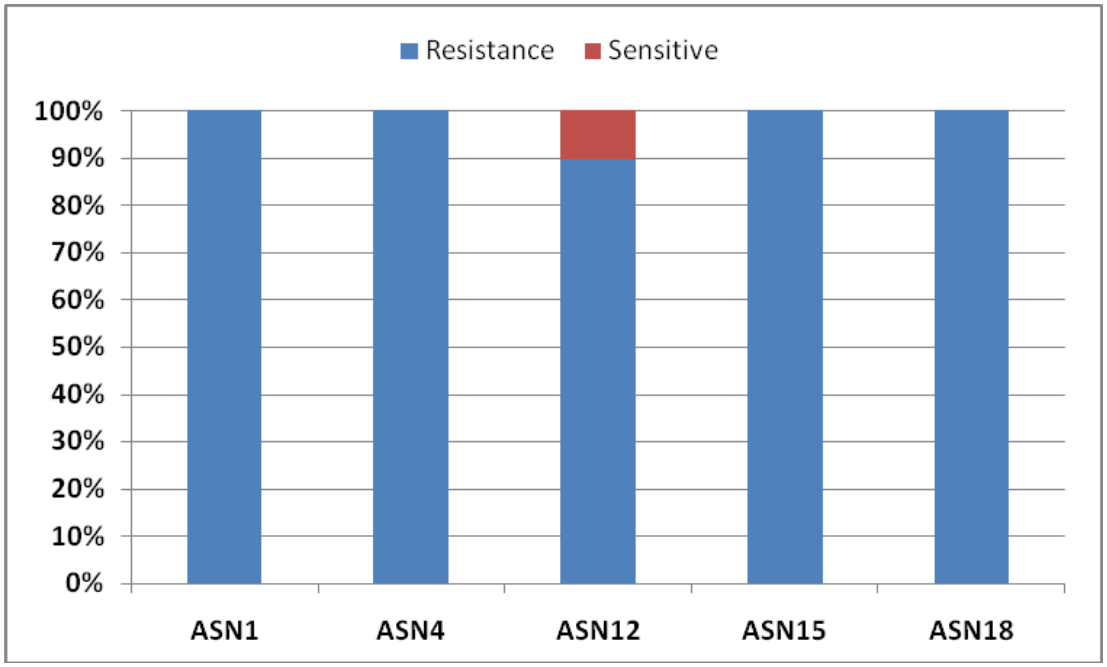

Fig. 1. Phenotypic resistance patterns of five selected *A. baumannii* strains against Aminoglycosides antibiotic.

TABLE 3. MICs of tested antibiotics against five selected *A. baumannii* strains.

Acinetobacter baumannii strains	Concentration (mg/L)					
	Aminoglycosides group			β-lactam group		
	Gentamicin	Tobramycin	Amikacin	Imipenem	Ceftriaxone	Cefixime
ASN1	≥512	≥512	≥512	64	512≥	512≥
ASN4	≥512	128	128	512≥	>512	>512
ASN12	256	≥512	>512	128	512≥	512≥
ASN15	≥512	128	128	256	>512	256
ASN18	≥512	256	256	128	>512	256

Therapy combination

In order to study the overcome of resistance problem, we decided to focus on evaluate the MICs of selected antibiotic alone and in combination. Data in Table 4 showed the *Acinetobacter baumannii* susceptibility to combination of aminoglycosides and β-lactams antibiotics by employing checkerboard method. Results showed that Synergism was achieved with (100%) in all gentamycin combination with the five tested strains. However, 93.33% and 66.66% was found for all tobramycin and amikacin combination, respectively. In addition, according to FIC index, cephalosporins antibiotics were found to have synergistic effect when used with aminoglycosides other than carbapenem (imipenem). Antagonism and additive were detected in 40 and 60% of selected strains in combination between amikacin and imipenem (AK & IMP) respectively according to FIC index. While combination between tobramycin with imipenem (TOB/IMP), showed synergy and addative with 60 and 40% respectively. This means that synergism was most observed in all antibiotic combination against tested strains whereas the least effective combination was in amikacin with imipenem.

TABLE 4. Combination of aminoglycosides and β-lactams against five *A. baumannii* strains by Chekerboard.

Antibiotic combination	Concentration (mg/L)											
	Conc.	FIC	Activity	Conc.	FIC	Activity	Conc.	FIC	Activity	Conc.	FIC	Activity	Conc.	FIC	Activity
GN/IMP	32/8	0.158	S	16/4	0.03	S	16/4	0.09	S	16/4	0.04	S	16/4	0.06	S
GN/CRO	4/4	0.015	S	4/4	0.011	S	32/128	0.37	S	32/128	0.188	S	4/4	0.01	S
GN/CFM	8/64	0.14	S	4/4	0.013	S	8/128	0.28	S	8/128	0.5	S	4/4	0.10	S
AK/IMP	512/256	5	Ang	128/64	0.625	Adv	128/64	0.628	Adv	512/128	4.5	Ang	64/64	0.75	Adv
AK/CRO	16/4	0.038	S	8/128	0.158	S	8/128	0.25	S	32/32	0.282	S	8/128	0.28	S
AK/CFM	16/128	0.28	S	8/128	0.04	S	16/32	0.078	S	16/32	0.25	S	4/16	0.03	S
TOB/IMP	16/128	2.03	Adv	32/64	0.375	S	32/64	0.56	Adv	32/64	0.5	S	8/32	0.28	S
TOB/CRO	4/4	0.01	S	4/4	0.034	S	4/32	0.07	S	4/32	0.06	S	8/128	0.28	S
TOB/CFM	4/32	0.07	S	4/4	0.035	S	16/4	0.037	S	16/4	0.14	S	8/128	0.2	S

FIC: Fractional inhibitory concentration, S: Synergy, Ad: Additive, Ag: Antagonism.

Discussion

Bacterial isolates in our study were identified as *A.baumannii* by phenotypic and genotypic methods and by analysis of PCR products on agarose gel which revealed a DNA fragment at 357bp. Traditional biochemical identification for *A. baumannii* to species level is inapplicable, so PCR was performed to determine the presence of bla OXA-51-like gene which this enzyme is intrinsic to *A. baumannii* that naturally found on the chromosome of this species and is an indication that chromosomally encoded enzyme has been under considerable selective pressure from antibiotic use, and this enzyme is not benign and plays a role in resistance (Woodford et al., 2006; Fazeli et al., 2014). This approach is in agreement with other studies which provided evidence that detection of bla OXA-51-like gene can be used as a simple and reliable way of identifying *A. baumannii* as ubiquitous nature in *A. baumannii* (Turton et al. 2006; Goli et al. 2017; Tchuinte et al., 2019).

Aminoglycosides resistance in *Acinetobacter* spp. has emerged as a tremendous health problem due to that therapeutic option was very limited. Here, our investigated isolates showed resistance or reduced in phenotypic susceptibility normally to all the tested antimicrobial. Aminoglycoside resistance is not unusual in *Acinetobacter* spp. These results were in agreement with Lambert et al. (1997). Specially consequences from inactivation of the antibiotic by way of specific modifying enzymes which include acetyltransferases, phosphotransferases (Khoshnood et al., 2017; Magallon et al., 2019) and many reports documented the excessive charges of antibiotic insusceptible discovered in *Acinetobacter* spp. These organisms are regularly proof against multiple antimicrobial dealers; lately, there are numerous reviews on lines proof against most clinically applicable tablets (Lu et al., 2008). Variations in antibiotic susceptibility had been found between countries, probably because of environmental factors and specific patterns of antimicrobial utilization (Giamarellou et al., 2008). It was noticed that greater than 80% of isolates to be resistance to cephalosporin, aminoglycosides and quinolones especially 2nd and 0.33-generation (Gaur et al., 2008). Findings of our have a look at confirmed the resistance charge to imipenem, ampicillin/tobramycin, ceftazidime, cefixime, gentamicin, amikacin

and ciprofloxacin have been greater than 90% in decided on multidrug-resistant *Acinetobacter baumannii* this observation is constant with Livermore (2002).

In the gift observe, endotracheal infections were the foremost clinical specimen of *Acinetobacter* spp. The frequency of isolation and kind of bacterium found in clinical specimens in several countries wide varies (Shiri et al., 2005; Van Looveren & Goossens, 2004). Ability hazard factors for constitution or infection of hospitalized patients with multidrug-resistant *Acinetobacter* strains embrace length of ICU keep, underlying diseases, or conditions as exposure to carbapenems or third-generation antibiotic drug, hospitalization and victimisation urinary catheterization (Prashanth & Badrinath, 2006; Cisneros et al. 2005). The findings showed that clinical isolates of *Acinetobacter* strains in our hospital carrying varied styles of aminoglycoside resistance. One amongst the common approaches to overcome antibiotic resistance was combination of gentamycin, amikacin and tobramycin with imipenem, ceftriaxone and cefixime. Our results virtually extend to the results of previous studies on aminoglycosides in combination with beta lactam against *Acinetobacter baumnnii*, the checkerboard methodology was done to assess the synergy between antimicrobials against *Acinetobacter* strains. in several of those studies antibiotic combos have confirmed the synergistic or bactericidal effects towards bacteria that have been proof against the individual drugs by way of using checkerboard methodology As an example, synergistic outcomes had been confirmed for double and triple antibiotic mixtures including an aminoglycoside, an anti-pseudomonal beta-lactam, colistin, a fluoroquinolone, a macrolide, or rifampin against multidrug-resistant *Pseudomonas* spp. (Aoki et al.,2009; Fish et al., 2002; Saiman et al., 2002).

The aminoglycoside/ β -lactam combinations were developed in the early and mid-1980s from animal studies data and become very popular to apply on human management infections and subsequently, the following studied depend on this concept. 1-4 to prevent or delay the antimicrobial resistance emergence in pathogens (Gerber et al., 1982; Johnson & Thompson, 1986; Paul et al., 2004). Double and triple antibiotic mixtures including an aminoglycoside, ampicillin/sulbactam, a carbapenem, colistin, rifampin, tigecycline, or vancomycin had been powerful in opposition to multidrug-resistant *Acinetobacter* spp. (Urban et al., 2010; Kiffer et al., 2005; Hornsey & Wareham, 2011), each drug combination was evaluated in duplicat. This study revealed that various antimicrobial combinations could be synergistically *in vitro* against multidrug-resistant most *Acinetobacter* spp. the checkerboard technique is hired for this reason. The results obtained in our examine showed the rate of synergy were became found in most antibiotic mixtures.

The result of mixtures of imipenem, ceftriaxone and cefixime with a second group (gentamycin, amikacin and tobramycin) exhibited mostly synergism. Combos of those antibiotics with gentamycin exhibited synergy in 100% of the performed assessments with the five *Acinetobacter* spp. in combination between amikacin and β-lactams (AK plus IMP, CRO and CFM) was 100% and also in case combination between tobramycin with β-lactams (TOB plus IMP, CRO and CFM) was 100%. While in 40% of selected strains antagonism was seen. This observation is consistent with the experience of others (Lim et al., 2008; Prashanth & Badrinath, 2006).

In another study, Tod et al. (2000) by assessing ceftazidime plus tobramycin and piperacillin/ tazobactamplus tobramycin mixtures against multidrug-resistant *P. aeruginosa* have been evaluated and synergy ratios of sixty seven % and five hundredth, respectively were found. With relation to Fosfomycin which mentioned by Obara & Nakae (1991) and Landersdorfer et al. (2013). Synergistic interactions with alternative antibiotics were verify in 57% of the tests, rate almost like that reported formerly for multidrug-resistant *P. aeruginosa*. Fosfomycin enhances the active transport of tobramycin in *P. aeruginosa*; *in vitro* synergic actions have been additionally confirmed for polymyxin E, imipenem, ceftazidime and ciprofloxacin as discovered in other studies by Shiri et al. (2005). The speed of synergy of antibacterial combos varies in line with isolate and is not strictly related to susceptibility or resistance to imipenem. Comparison of the 2 multidrug-resistant *P. aeruginosa* (46 Rand72R) revealed a lot of frequent and great drug MIC reductions for the 46R isolate than for the 72R isolate. For this reason, it is really useful to check every multidrug-resistant isolate with the distinctive capsules in mixture.

Some of the synergy outcomes, only a few antibacterial mixtures have led to enough MIC discounts (Chastre & Trouillet, 2000). Other authors also noted synergism between third and fourth generation cephalosporin and aminoglycosides (often gentamicin, amikacin and tobramycin) against 30% to 90% of *Enterobacteriaceae* (Eliopoulos & Eliopoulos 1988; Cha, 2008).

Conclusion

Antimicrobial synergy was observed against clinical isolates of MDR *Acinetobacter* spp. A few drug combos resulted in sufficient discounts, which propose that these combos can be of medical use for infections of MDR *Acinetobacter* spp. as an alternative to antibiotic therapy, suggesting its potential as an among alternative tested aminoglycosides. Therefore, consequently, *in vitro* facts need to be tested through assessing the clinical performance of mixtures of antimicrobial agents before precise guidelines to alter current remedy guidelines for *Acinetobacter* infections are viable.

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حساسية بكتريا الاسينيتوباكتر بومنييای المقاومة للامينوجليكوسيد لمزيج المضادات الحيوية

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تم عزل 250 عزلة بكترية من اماكن مختلفة من المستشفيات المصرية وتم تعريف 200 عزلة الاسينيتوباكتر بومنييای وبدراسة حساسية هذه العزلات للمضادات الحيويه وجد منهم حوالي 120 عزلة مقاومة للامينوجليكوسيد بنسبة 60%، وحوالي 130 عزلة بنسبة 65% مقاومة للمضاد الحيوي ايمبينم وحوالي 180 عزلة بنسبة 90% مقاومة للسيفالوسبورين.

وبقياس الحد الأدنى للمضادات الحيويه وجد أن التركيزات المثبطة للعزلات تتروح ما بين 512 إلى 32 ملي جرام. وباختبار امتزاج مجموعات المضادات الحيوية في العدوي الخاصة بالمستشفيات التي تسببها بكتريا الاسينتوباكتر بومنييا باستخدام طريقه الشطرنج checkerboard وهي البيتالكتام (سيفترياكسون، سيفيكسيم، كاربابينيم، وايمينيم) مع الأمينوجليكوسيد ضد عزلات الأسينتوباكتر بومنييا المقاومة لمعظم المضادات الحيوية التي تسبب العدوي بالمستشفيات. وبهذة الطريقة لوحظ أن 60% كانت تأثير متعاون بين ايمبينيم وجينتاميسين و100% مع سيفترياكسون وسيفيكسيم وحوالي اثنين من أربعه وأربعين لوحظوا انه له تأثير سلبي. اي لايوجد سمة تعاون بين المضادين الحيوين ايمينيم وايماكسين بينما حدث العكس. وكذلك أيضا أظهرت النتائج أعلى تأثير تعاون بين اماكيسين مع سيفيرياكسون وسيفكيم بنسبة 100% ولكن في حالة التوبراميسين مع ايمينيم بنسبة 50% وبنسبة 100% في حالة التوبراميسين مع سيفترياكسون، سيفيكسيم. وحوالي اثنين من اربعه واربعين لوحظوا أنه له تأثير سلبي. أي لايوجد سمة تعاون بين المضادين الحيوين ايمينيم وايماكسين بينما كان العكس. وكذلك أيضا اظهرت النتائج أعلى تأثير تعاون بين اماكيسين مع سيفيرياكسون وسيفكيم بنسبة 100% ولكن في حالة التوبراميسين مع ايمينيم بنسبة 50% وبنسبة 100% في حالة التوبراميسين مع سيفترياكسون،سيفيكسيم.