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Original article

In vitro comparison of colistin-versus tigecycline-based combinations against carbapenem resistant *Acinetobacter baumannii* intensive care unit clinical isolates

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ABSTRACT

Background: *Acinetobacter baumannii (A. baumannii)* has emerged as a nosocomial pathogen especially in the intensive care units (ICUs). It's enlisted at the top of urgent threat level organisms in centers for disease control and prevention (CDC's) antibiotic resistance threats report. **Objectives:** To assess prevalence, risk factors of health care associated infection by *A. baumannii*, and to compare the in-vitro efficacy of colistin sulfate- tigecycline combinations versus their individual combination with levofloxacin and meropenem against carbapenem resistant *A. baumannii* clinical isolates from an Egyptian tertiary care hospital ICUs. **Methods:** The study included 250 ICU patients, samples were collected according to the site of infection. *Acinetobacter baumannii* was isolated, identified and tested for antibiotic susceptibility by disc diffusion. Broth microdilution method was used for assessment of colistin, tigecycline, levofloxacin, and meropenem. Thirty isolates resistant to all carbapenems were tested by the checkerboard method to assess effect of antibiotic combinations. **Results:** forty-six *A. baumannii* were isolated, with highest prevalence in respiratory secretions. Prior antibiotic administration and failure of empirical antibiotic therapy were found to be a major risk factors of infections by *A. baumannii*. Colistin combination with meropenem showed the highest synergy (50%). Tigecycline-meropenem combination had the highest antagonistic effect (66.7%). **Conclusion:** No antagonistic effect of colistin combination with meropenem was confirmed in this study. Only colistin-based combinations, particularly those with meropenem may confer therapeutic benefits against carbapenem-resistant *A. baumannii*.

Introduction

Multidrug-resistant *Acinetobacter baumannii (A. baumannii)* is a considerable pathogen causing health care associated infection (HAIs), especially in critically ill patients admitted to intensive care units (ICUs) [1]. *Acinetobacter baumannii* is characterized by its great intrinsic and acquired resistance to many antibiotics leading to the frightening reality of its treatment failure [2].

Infectious Diseases Society of America (IDSA) classified *A. baumannii* among the six most problematic multidrug-resistant (MDR) pathogens in hospitals [3]. Lack of new antibacterial drugs for clinical use in current time against resistant microbes intensifies the global health crisis of

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antimicrobial resistance [4]. Even the promising agents such as cefiderocol with activity towards carbapenem resistant microorganisms including carbapenem-resistant *A. baumannii* are quiet in early clinical development stages and will be eventually available only in the coming years [5]. Besides, the new βlactam-β-lactamase inhibitor combinations are not active against carbapenem-resistant *A. baumannii* [6]. All of these reasons have led to the reliance on polymyxins as salvage therapy.

Colistin use has many constraints including toxicity, hetero-resistant isolates development, and finally the clinical and laboratory and standard inistitute (CLSI) warning of the limited clinical efficacy even if intermediate *in vitro* susceptibility results are obtained that drives clinicians to try other treatment strategies including antimicrobial combinations [7].

Tigecycline, a semi-synthetic tetracycline product, proved to have *in vitro* antimicrobial activity against carbapenem-resistant *A. baumannii* isolates. Unfortunately increasing resistance has been reported in different localities, in addition to reports of tigecycline monotherapy failure suggest a need for tigecycline combination with other antimicrobials [8,9].

Synergistic combination therapy using available antibiotics offers a promising and tangible option to treat infections by MDR bacteria. For carbapenem-resistant *A. baumannii*, there is no consensus on optimal antimicrobial treatments for such strains [10].

This study aimed to assess prevalence, risk factors of HAI by *A. baumannii*, and to compare the *in vitro* efficacy of colistin sulfate- tigecycline combinations versus their individual combination with levofloxacin and meropenem against carbapenem resistant *A. baumannii* clinical isolates from an Egyptian tertiary care hospital ICUs.

Study design and participants

This cross sectional study was executed in Anesthesia and surgical ICU, emergency ICU, Tropical Medicine ICU, and Medical Microbiology and Immunology Department, Faculty of Medicine, Zagazig University throughout 12 months from April 2019 to April 2020.

This comprehensive study included all patients admitted to ICUs during study period if they developed fever, leukocytosis and other evidences of infection after 48 hours of hospital admission. Patients were excluded if there was evidence of infection prior to hospital admission.

Ethical approval

The study was approved by Zagazig University Institution Review Board (ZU-IRB) (Approval code 6353). This study was carried out in accordance with The Code of Ethics of the World Medical Association (Declaration of Helsinki). Informed consent was obtained from patients or their relatives.

Microbiological work up

Clinical samples collection and *A. baumannii* identification

Clinical samples were aseptically collected according to the site of infection from ICU admitted patients with evidence of HAI. Samples included endotracheal aspirates, urine sample, pus, and blood. Detailed history of patients included age, sex, length of hospital stay, use of invasive medical devices, problem obliging ICU admission and previous administration of empirical antibiotics were stated. Samples were transported and processed for isolation and identification of the causative organism. *Acinetobacter baumannii* initial identification was done via conventional biochemical methods [11] and confirmed to the species level by API 20NE (Bio-Mérieux, France). Only one *A. baumannii* isolate per patient was included.

Antimicrobial susceptibility of isolates

Antimicrobial susceptibility test was performed by disk diffusion method according to CLSI guidelines for the 9 members of group A antimicrobials suggested by Food and Drug Administration (FDA) including ampicillin-sulbactam (10/10 µg), ceftazidime (30 µg), ciprofloxacin (5 µg), levofloxacin (5 µg), Gentamicin (10 µg), tobramycin (10 µg), imipenem (10 µg), meropenem (10 µg), and doripenem (10 µg**) (Oxoid, UK)**. *Pseudomonas aeruginosa* ATCC®27853 was used as a quality control strain **(American Type Culture Collection [ATCC], Manassas, VA, USA)** [7].

Carbapenem resistance was defined as *A. baumannii* that test resistant to imipenem, meropenem or doripenem based on current CLSI M100 standards [12]. Isolates that were resistant to all tested carbapenems (n=30) were used for subsequent steps in the study.

Determination of minimal inhibitory concentration (MIC), MIC_{50}, MIC_{90} of antibiotics to be used in combination study

The antimicrobial agents used in combination assessment were selected based on its empirical combination use for treatment of carbapenem-resistant *A. baumannii* and the previous *in vivo* and *in vitro* studies suggesting their efficacy in combination [13-15].

The broth microdilution method was used for the estimation of minimum inhibitory concentration (MIC) for colistin sulfate (Hebei Shengxue Dacheng Pharmaceutical, china), Tigecycline (Suzhou greenway Biotech / china), levofloxacin and meropenem (Sigma- Aldrich, USA). CLSI standards regarding solvents for stock solution preparation, dilutions, timing of preparation, and broth media were followed. *Pseudomonas aeruginosa* ATCC®27853 was used as a quality control strain (American Type Culture Collection [ATCC], Manassas, VA, USA) [7].

Interpretation of results were done according to CLSI standards except for tigecycline as no breakpoints were available regarding *A. baumannii*. MIC_{50} and MIC_{90} were estimated and reported for each individual antibiotic.

Synergism testing by checkerboard method

The selected antimicrobials were tested non-combined and in combination by microdilution checkerboard method. The checkerboard method was performed according to the method described [16,17]. Briefly, in a sterile 96 well microtiter plates doubling dilutions of one antibiotic were done in the horizontal wells and the other antibiotic dilutions in the vertical wells. Bacterial suspension (1/100 dilution of 0.5 McFarland bacterial suspension) was added to each well. The final antibiotic concentration ranged from 1/8 up to 4 folds of predetermined MIC for the tested isolate. Serially diluted antibiotics without combination, growth control and sterility control wells were included. The plates were covered and incubated at 35˚C for 18-24 h. The wells with no visible growth were identified visually against a dark background, the MICs for antibiotics in combination were recorded. Fractional inhibitory concentration index (FICI) was used to describe different combination effects [16].

The FIC and FICI was calculated using the following equations:

The FIC drug A = MIC of drug A in combination / MIC of drug A alone

The FIC drug B = MIC of drug B in combination / MIC of drug B alone

FICI = FIC of drug A + FIC of drug B

The results of the FICI were interpreted as shown in **table (I)**.

Table I. interpretation of FICI of antibiotic combination.

FICI ≤ 0.5	Synergism
0.5 < FICI ≤ 1	Addition
1 < FICI ≤ 4	Indifference
FICI > 4	Antagonism

Patients' outcomes assessment

The ICU consultants were informed about the results of synergism testing. The outcomes of surviving patients who received combination therapy assessed clinically (fever regression- signs of infection resolution - laboratory investigations) and microbiologically by recollection and testing of samples from previously proved infected sites 3-5 days after onset of antibiotic combination administration.

Statistical analysis

All data were analyzed using SPSS 22.0 for windows (SPSS Inc., Chicago, IL, USA) & MedCalc 13 for windows (MedCalc Software bvba, Ostend, Belgium).Categorical variables were expressed as a number (percentage). Chi square test was used to compare percentage. All tests were two sided. $p<0.05$ was considered statistically significant (S), $p<0.001$ was considered highly statistically significant (HS) and $p\geq0.05$ was considered non-significant (NS).

Results

A total of 250 clinical samples were included in the study from ICU admitted patients (144 males and 106 females) with clinical and laboratory evidence of HAI. Their age ranged from 18 to 80 years (mean ± SD = 56 ± 19). Of the tested 250 clinical samples, 46 *A. baumannii* isolates were recovered. *Acinetobacter baumannii* accounted for 18.4 % of HAI in ICUs. *Acinetobacter baumannii* showed higher prevalence in endotracheal aspirate samples (22.1 %) with higher percent among patients with late onset ventillator associated pneumonia than early onset ones (27.5% versus 11.8%) respectively with no statistically significance difference between early and late onset VAP. Lower prevalence 14% and 8.3 % in pus and

blood culture were detected respectively with no statistical significant difference (*P >0.05*) **(Table 1)**.

Admission to other hospital wards before ICU and failure of empirical antibiotic were statistically highly significance risk factors for increased prevalence (%) of *A. baumannii* isolates [(*p-value=0.001*** for both, Odds (95% CI=3.9(2.03-7.7) & 3.1(1.6-6.21)] respectively. Intensive care unit admission for more than a week showed higher prevalence of *A. baumannii* isolation but no statistical significant difference from ICU stay for less than a week (*p-value=0.08*) . In addition the reason for ICU admission wasn't statistically significance risk factor for increased prevalence (%) of *A. baumannii* isolates (*p-value=0.7*) **(Table 2)**.

Assessment of antibiotic susceptibility of 9 FDA suggested group A antimicrobials against *A. baumannii* isolates showed that all isolates were resistant to ampicillin-sulbactam, ceftazidime, and ciprofloxacin. The least detected resistance was to meropenem and doripenem (65.2%) **(Table 3)**.

Estimated minimal inhibitory concentration of different antibiotics showed MIC_{50} / MIC_{90} of the tested antibiotics were (0.5 / 1 µg/mL) for colistin and (0.5 / 1 µg/mL) for tigecycline. Three isolates out of 30 tested carbapenem resistant *A. baumannii* (10%) had tigecycline MIC > 2 µg/mL **(Table 4)**.

When effects of antibiotic combinations were assessed on *A. baumannii* isolates resistant to all tested carbapenems, the highest synergy (50%) was found for combinations of colistin with meropenem, followed by combination of colistin with levofloxacin (16.7%). Tigecycline-meropenem combination had the highest antagonistic effect (66.7%). Tigecycline exerted synergistic activity only with colistin in only 3 isolates (10%) (Isolates with tigecycline MIC>2 µg/mL) while this combination showed no antagonistic effect in any of tested isolates. Neither levofloxacin nor meropenem exerted synergistic activity when combined with tigecycline in any of the tested isolates **(Table 5)**.

Colistin-meropenem combination was prescribed for 15 patients. Tigecycline was not available for use during study period. Among patients who received colistin-meropenem combination (n=15) the infection was cured in 10 patients (the surviving patients).

Table 1. Distribution of *Acinetobacter baumannii* isolates (n=46) among different samples (n=250).

Samples	**Number of samples**	**N (%) of carbapenem resistant Acinetobacter isolates**	**X^2**	***P***
Endotracheal aspirate				
< 5 days (early onset VAP)	17	2 / 17 (11.8%)		
≥ 5 days (late onset VAP)	51	14 /51 (27.5%)		
Total	68	16/ 68 (22.1 %)		
urine	120	22 (18.3%)	**2.1**	***0.5***
Pus	50	7 (14 %)		
blood	12	1 (8.3 %)		
Total	250	46 (18.4 %)		

$^{*}\boldsymbol{p} < 0.05$ is significant

Table 2. Risk factors for *A. baumannii* infections in the ICU.

	Total number of samples (n=250)	**Prevalence (%) of *A. baumanii* isolates (n= 46)**	***p-value#***	**Odds (95% CI)**
Reason for ICU admission				
Trauma	87	19 /87 (21.8%)	*0.7*	-------
Surgical emergency	60	11 /60 (18.3%)		
Hepatic failure	91	14 /91 (15.4%)		
others	12	2 /12 (16.7%)		
Admission to other hospital wards before ICU				
Admitted	68	24 / 68 (35.3%)	*0.001***	3.9
Not admitted	182	22 / 182 (12.1%)		(2.03-7.7)
Period of ICU admission			*0.08*	
Less than 1 week	87	11/ 87 (12.6%)		1.9
More than 1 week	163	35 / 163 (21.5%)		(0.9-3.9)
Prior antibiotic administration				
No prior antibiotic	150	17/ 150 (11.3%)	*0.001***	3.1
Failure of emperical antibiotic	100	29 / 100 (29%)		(1.6-6.21)
Total	250	46/250 (18.4%)		

#P-value for Chi Square test,** $p < 0.001$ =highly statistically significant (HS)

Table 3. *In-vitro* susceptability of FDA suggested group A antimicrobials against *A. baumannii* isolates (n=46).

Antibiotic	**susceptible**	**intermediate**	**resistant**
Ampicillin-sulbactam (10/10 µg)	0 (0%)	0 (0%)	46/46 (100%)
Ceftazidime (30 µg)	0 (0%)	0 (0%)	46/46 (100%)
Ciprofloxacin (5 µg)	0 (0%)	0 (0%)	46/46 (100%)
Levofloxacin (5 µg)	1 (2.1%)	3 (6.5%)	44/46 (91.3%)
Gentamicin (10 µg),	2 (4.3%)	7 (15.2%)	37/46 (80.4%)
Tobramycin (10 µg)	1 (2.2%)	6 (13%)	39/46 (84.8%)
Imipenem (10 µg)	1 (2.2%)	12 (26.1%)	33/46 (71.7%)
Meropenem (10 µg)	5 (10.9%)	11 (23.9%)	30/46 (65.2 %)
Doripenem (10 µg)	7 (15.2%)	9 (19.7%)	30/46 (65.2%)

Table 4. Minimal inhibitory concentration (MIC), MIC50, MIC90 of different antibiotics used for combination against carbapenem resistant *A. baumannii* isolates (n=30).

antibiotic	**MIC range (mg/l)**	**MIC_{50}**	**MIC_{90}**
Levofloxacin	4 - 64	16	32
Meropenem	4 -32	8	16
Tigecycline	0.125-8	0.5	1
Colistin	0.125-1	0.5	1

Table 5. Drug combination effect determined by the checkerboard method on *A. baumannii* isolates resistant to all tested carbapenems (n=30).

Combinations	**Synergy**	**Addition**	**Indifference**	**Antagonism**
Colistin+ levofloxacin	16.7%	0%	63.3%	20%
Colistin+ meropenem	50%	40%	10%	0%
Colistin +tigecycline	10 %	23.3%	66.7%	0%
Tigecycline + meropenem	0%	0%	33.3%	66.7%
Tigectcline + levofloxacine	0%	3.3%	56.7%	40%

Discussion

Acinetobacter baumannii has emerged as one of the important nosocomial pathogens. Moreover, its remarkable ability to upregulate and acquire resistance determinants, further limits available therapeutic choices [1].

Acinetobacter baumannii accounted for 18.4% of HAIs in ICU admitted patients. These results more or less similar to that reported in a national surveillance of health care–associated infections in 91 ICUs in 28 Egyptian hospitals in which *A. baumannii* accounted for 13.7% of ICU-onset infections [18]. Other Egyptian studies reported that *A. baumannii* was isolated from 10.7% and 28.3% of HAI [19,20]. *Acinetobacter baumannii* was detected in 22.1 % of ventilator associated pneumonia. Slightly lower prevalence was reported by **Meawad et al.** where A. *baumannii* accounted for 15.9% of ventilator associated pneumonia [19].

Failure of empirical antibiotic therapy was a considerable risk factor for acquiring A. *baumannii* infection (*p<0.05*), *A. baumannii* isolation rate increased to reach 29% of total isolates versus only 11.3% of total isolates from patients with no previous antibiotic therapy. **Zilberberg and coworkers** in their study reported MDR *A. baumannii* isolation as the single strongest predictor of receiving inappropriate empirical therapy [21]. Prior admission to other wards also increased risk of A. *baumannii* infection similar to the report of Blanco et al., 2018 that hospital stay prior to ICU admission was associated with increased isolation rate of MDR *A. baumannii* from ICU acquired infections [22].

Concerning the antibiotic susceptibility of *A. baumannii* isolates from ICUs, all isolates were resistant to ampicillin-sulbactam these results were concordant with other studies which reported resistance to penicillin- beta-lactamase inhibitors in all *A. baumannii* isolates [23, 24].

Regarding carbapenem resistance, 71.7 % of isolates were carbapenem resistant. These results were comparable to another Egyptian study as 70% of isolates were imipenem resistant [25]. In a previous study performed in ICUs of our institute during years 2013-2014, imipenem resistance was only 20% in *A. baumannii* isolates [19]. The resistance rate raised during 2016-2017 to reach 37% and 33.3% for imipenem and meropenem respectively [23]. In a recent study resistance rate reached 93.3% for imipenem [20]. This worrisome progressive reduction in *A. baumannii* susceptibility to carbapenems makes implementation of antimicrobial stewardship (AS) is an indispensable choice [26]. A study performed in Turkey showed similar high imipenem resistance rate (70%) [27].

On the other hand in USA according to the fact sheet published as a part of CDCs 2019 antibiotic resistant threat reports, there was reduction in carbapenem- resistance in Acinetobacter isolates from hospitalized patients over time thanks to continued infection control practices and appropriate antibiotic use [28].

All isolates in our study were of intermediate susceptibility for colistin according to CLSI breakpoints declared in 2020. In other Egyptian study colistin resistance was reported to be 6.25% among *A. baumannii* isolates [29]. MIC_{90} for both colistin and tigecycline were 1µg/ml. This suggests that both colistin and tigecycline are available therapeutic choices for carbapenem-resistant *A. baumannii* infections based on *in vitro* susceptibility testing.

Three out of 30 isolates (10%) in our study had tigecycline MIC > 2 µg/ml (The US FDA breakpoints of tigecycline approved for *Enterobacteriaceae*). Similar results were reported by another Egyptian study that reported 9.37% of *A. baumannii* isolates as tigecycline non susceptible [29]. On the other hand 100% of *A. baumannii* isolates from ventilator associated

pneumonia in emergency ICU of Zagazig University hospitals were tigecycline susceptible [24].

On speaking about checkerboard method, our results showed that colistin with meropenem is the most active *in vitro* combination on carbapenem resistant *A. baumannii* with 50% synergy and no antagonism was observed. These results matched with results reported by **Meliani and coworkers** who reported synergy between colistin and imipenem in 57.14% of tested carbapenem resistant isolates [30] and higher synergism (80%) was reported in other studies [27].

Slightly lower synergism between colistin and meropenem (30%) was reported by Bae and co-workers [13]. This difference may be caused by difference in colistin resistance between the two studies as they tested colistin resistant isolates while all isolates in our study were of intermediate susceptibility to colistin.

Our results are the reverse of that reported by **Kheshti et al.** who found that colistin–imipenem combination exhibited the highest (40%) antagonistic effect [31]. **Sertcelik et al.** reported the synergy of colistin with meropenem was only 4.3% and additive effect "partial synergism" in 95.7% of isolates [32]. This marked discrepancy between studies' findings could be due to using different *in vitro* methods for synergism assessment [33]. Also, involved mechanism of carbapenem resistance or even MIC values of carbapenem in different isolates may explain this marked discrepancy between different studies that was confirmed by the study done by **Zhu and coworkers** who studied colistin imipenem combination and reported synergy in 93.3% of isolates with imipenem MICs of 16 μg/ml versus 16.7% of isolates with imipenem MIC of 64 μg/ml and suggested paying more attention to the MICs of single drugs for combination therapy choice [34].

Tigecycline- colistin combination showed no antagonistic effect in any of tested isolates in our study. Very limited synergistic effect (10%) of tigecycline combination with colistin was detected only in the 3 strains with MIC > 2 μg/ml. This synergism may be explained by the permeating action of colistin on bacterial outer membrane leading to better access of tigecycline into bacterial cell's target site. Lack of tested bacterial resistance to colistin may explain this synergism.

Tigecycline- colistin combination showed addition and indifference in 23.3% and 66.7% of isolates respectively. These results were comparable with a study done by **Bae and coworkers** who observed indifference of tigecycline combination with colistin in 77.8% of isolates [13].

Tigecycline-imipenem combination had the highest antagonistic effect in 66.7% of isolates, this finding greatly matched with **Güçkan et al.** [27]. This can be explained by the interference between the bacteriostatic effect of tigecycline with the bactericidal action of meropenem on bacterial cells.

Although tigecycline didn't have a good *in vitro* evidence for use in a combination to treat infections by carbapenem resistant *A. baumannii* in our study, Previous clinical study done by **Kofteridis and coworkers** have evaluated the efficacy of empirical tigecycline-carbapenem combination therapy in the treatment of pan-drug resistant bacteria to be clinically effective in 37.5% of *A. baumannii* infections[35]. Such discrepancy between *in vitro* and *in vivo* studies may be due to tigecycline pharmacodynamics properties, as tigecycline is rapidly concentrated into tissues ensuing higher tissue concentrations up to 78-fold its plasma concentration. Another explanation of such discrepancy is the labile nature of tigecycline solution due to oxidative degradation *in vitro* [36]. A rationalization of tigecycline recommendation in treatment is thus essential, it should be limited to treatment of extensive drug resistant organisms.

Conclusion

No undesirable antagonistic effects of colistin combination with meropenem were confirmed in this study. These results are fortunate as colistin / meropenem combination therapy is often given in our ICUs for treatment of carbapenem resistant Gram-negative bacteria. Only colistin-based combinations, particularly those with meropenem may confer therapeutic benefits against carbapenem-resistant *A. baumannii*.

Recommendation

Application of antibiotic stewardship is mandatory as well as strict infection control policies for prevention of development of pan-drug resistant bacteria.

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