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ORIGINAL ARTICLE

Evaluation of potential synergistic activity of antimicrobial combinations against Colistin resistant *Acinetobacter baumannii*

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ABSTRACT

Background: *Acinetobacter baumannii (A. baumannii) is one of the most important nosocomial pathogen. With the introduction of colistin as a last resort in treatment of A. baumannii, resistant strains. Using combined antibiotics could increase the success of treatment and reduce resistance.* ***Objectives:*** *To assess potential in-vitro synergistic activity of colistin when combined with (vancomycin, teicoplanin, rifampicin, tigecycline, meropenem, amikacin, and ceftazidime) against colistin resistant A. baumannii (CRAB). Also, to screen the prevalence of plasmid mediated colistin resistance (mcr) genes (mcr-1 to mcr-5) in colistin resistant isolates.* ***Methodology:*** *A. baumannii strains were isolated from different microbial specimens. Antibiotic susceptibility testing was done for all isolates by disk diffusion method while broth micro dilution (BMD) was performed to determine colistin minimum inhibitory concentration (MIC). Genotypic detection of mcr genes was done using multiplex PCR. Checkerboard method was done to detect potential synergistic activities between colistin and other tested antibiotics against CRAB.* ***Results:*** *A total of 94 A. baumannii strains were isolated from 373 different clinical samples. Colistin resistance was detected in 44/94 (46.8%) of isolated A. baumannii. Only 2 out of 44 CRAB (4.5%) carried mcr-1 gene and neither of other mcr genes could be detected. All isolated CRAB were resistant to 7 tested antibiotics by BMD. But when colistin was included in a checkerboard pattern, colistin-based combinations with vancomycin, meropenem, rifampicin, teicoplanin and ceftazidime showed synergy in 93.2%, 90.9%, 88.7%, 86.4% and 79.5% of isolated CRAB respectively. For both tigecyclin and amikacin more than 90% of CRAB showed indifference in combination with colistin.* ***Conclusion:*** *Further studies are needed to determine the ability of colistin based antimicrobial combinations as an alternative therapy to treat CRAB infections and confirm that synergy.*

INTRODUCTION

Acinetobacter baumannii is a Gram-negative pathogen attached to the surface of medical instruments making it one of the most common pathogens of nosocomial infection. *A. baumannii* causes a wide spectrum of infections in both hospital and community, including skin and soft tissue, urinary tract infections, meningitis, bacteremia, and pneumonia, with the latter being the most frequently reported infection[1].

Colistin recently is the last line of treatment against infections with multidrug-resistant *A. baumannii*. Colistin, a positively charged peptide, exerts its antibacterial effect through electrostatic interactions with phosphate groups of lipid A which is negatively charged and an important component of lipopolysaccharide (LPS) of Gram-negative bacilli cell wall[2].

With colistin reintroduction to treat *A. baumannii*, various mechanisms of its resistance have been documented, such as LPS total loss, LPS alteration by adding phosphoethanolamine (PEA) moieties to lipid A through either chromosomal pmrCAB operon and eptA gene-encoded enzymes or *mcr* genes encoded by plasmid and colistin efflux from the cell. Additionally, widespread hetero-resistance, which is a characteristic of *A. baumannii*, results in failure of treatment with colistin. It is noteworthy that the risk of patient mortality obviously increased because of the emergence of colistin resistant strains[3].

MCR decreases the overall negative charge on the bacterial membrane and thereby reduces colistin binding leading to development of bacterial resistance. About 10 variants of *mcr* genes (*mcr-1* through *mcr-10*) have been discovered[4].

Limited range of potentially available effective antimicrobials for treatment of *A. baumannii* resistant strains, makes it urgent to develop novel antimicrobial options[5].

Colistin combination regimens have been recommended to combat bacterial regrowth following colistin monotherapy, either by lowering resistance or by improving bacterial killing through the two antimicrobials' synergistic action. Better antimicrobial impact is achieved via sub-population or mechanistic synergy which act concomitantly. In sub-population synergy, the resistant sub-populations of one antimicrobial are eliminated by the other and vice versa. For mechanistic synergy, two antimicrobials with different mechanism of action that enhance the killing of one another[6].

Vancomycin and teicoplanin cannot act against Gram-negative bacilli. However, colistin could enhance their penetration by compromising the Gram-negative bacterial membrane structure, so it could increase these hydrophobic antibiotics' activity which would normally have no effect[7-8].

Here, we attempted to determine the potential in-vitro activity (synergy) of colistin when combined with (vancomycin, teicoplanin, rifampicin, tigecycline, meropenem, amikacin, and ceftazidime) against CRAB. Also, to screen (mcr) genes (*mcr-1* through *mcr-5*) prevalence in colistin resistant isolates.

METHODOLOGY

Study design and setting:

This cross-sectional study was conducted from April 2022 to April 2023 in Medical Microbiology and Immunology Departments, Faculty of Medicine and National Liver Institute, Menoufia University, Egypt. The study has been approved by the ethical committee, National Liver Institute, Menoufia University (NLI IRB protocol number 00470/2023, NLI IRB 00003413 FWA0000227).

Different clinical strains were obtained from patients admitted to Menoufia University Hospitals. Prior to sample collection**,** written informed consent was obtained from each patient or the patient's legal guardian after the study and its objectives were explained. Sample size was calculated using the Open Epi program with power of study 80% and confidence level 95%.

Patients were subjected to full history taking including name, age, gender, antimicrobial administration, date of admission, exposure to invasive procedures as central venous lines and endotracheal tubes and associated co-morbidities as DM, hypertension, cardiac disease, chronic respiratory disease or malignancy.

Specimen collection and processing:

Based on a clinical suspicion of infection in accordance with accepted definitions, several microbiological specimens have been collected under aseptic settings. Specimens included respiratory, urine, blood specimens and wound swab. The samples were then transported in suitable transport media (if required) to be processed in the Microbiology Laboratory. Then, collected samples were cultured on blood agar, MacConkey agar and CLED for urine (Merck, Darmstadt, Germany) and incubated aerobically at 37°C for 24 hours. Blood culture bottles were directly incubated in BACT/ALERT 3D (Biomeriuex, France). Positive cases were subjected to subculture on blood agar and MacConkey medium.

Identification of *A. baumannii*:

Identification of obtained colonies were performed via conventional techniques[9] then, confirmed using VITEK2 compact device system (Biomeriuex, France) using GN ID cards. For acinetobacter, the analytical profile index (API) (Biomeriuex, Craponne, France); API 20NE for non-fermentative and oxidase tests were used.

Antibiotic susceptibility testing:

For all *A. baumannii* isolates test for antimicrobial susceptibility by the modified Kirby–Bauer disk diffusion method on Muller Hinton agar was performed then, confirmed using VITEK2 compact device system (Biomeriuex, France) using Gram Negative Susceptibility card (AST-N292). The used antibiotic susceptibility disks (Oxoid, UK) were piperacillin (100µg), ampicillin-sulbactam (10/10µg), ceftazidime (30µg), cefepime (30µg), imipenem (10µg), amikacin (30µg), doxycycline (30µg), ciprofloxacin (5µg) and trimethoprim-sulfamethoxazole (1.25/23.75µg). *Escherichia coli* ATCC 25922 was utilized as quality control strain. All results of antibiotic susceptibility were interpreted in accordance with CLSI guidelines[10].

Phenotypic detection of colistin resistance:

Colistin MIC was performed by BMD method. Regarding CLSI recommendations, Colistin MIC of ≤ 2 µg⁄mL was considered intermediate, whereas MIC of ≥ 4 µg⁄mL was considered resistant[10].

Genotypic detection of plasmid-encoded *mcr* genes:

A. baumannii strain that had colistin MIC value ≥ 4 µg⁄mL was then investigated for the existence of (*mcr*) genes (*mcr-1* to *mcr-5*). Bacterial DNA extraction and purification was done with QIAamp DNA Mini Kit 50 tests ***(Qiagen, Germany, cat. no. 51304)*** following the Manufacturer`s instructions. The sequences of used primers were illustrated in table 1. The PCR amplification was performed on pre-programmed thermal cycler ***(Biometra, Germany)*** under the following conditions: The amplification cycle was: 15 min at 94°C, followed by 25 cycles of 30 s at 94°C, 90s at 58 °C, 1 min at 72°C, and a final extension time of 10 min at 72°C. Electrophoresis was done with gel 2% for 20 minutes then the products were visualized by UV and compared with DNA ladder[11]. A previously discovered *mcr*-1 gene-carrying *E. coli* isolate was used as positive control.

Table 1: Primers used for multiplex PCR for detection of *mcr* genes

Target Gene	Primer Sequence (5'-3')	Size (bp)
mcr-1	Forward AGTCCGTTTGTTCTTGTGGC	320
	Reverse AGATCCTTGGTCTCGGCTTG	
mcr-2	Forward CAAGTGTGTTGGTCGCAGTT	715
	Reverse TCTAGCCCGACAAGCATACC	
mcr-3	Forward AAATAAAAATTGTTCCGCTTATG	929
	Reverse AATGGAGATCCCCGTTTTT	
mcr-4	Forward TCACTTTCATCACTGCGTTG	1116
	Reverse TTGGTCCATGACTACCAATG	
mcr-5	Forward ATGCGGTTGTCTGCATTTATC	1644
	Reverse TCATTGTGGTTGTCCTTTTCTG	

Testing synergistic activity by checkerboard method:

Detection of potential synergistic activities using checkerboard method was done according to *Schwalbe et al*. method[12]. Antibiotic powders that were used have been purchased (***Sigma-Aldrich, USA***). Antibiotic concentrations prepared before dilution were four times over the maximum concentration to be examined. For instance, we started with a concentration of 256 µg/ml for antibiotic A and 64 µg/ml for antibiotic B if the greatest concentration for A is 64 µg/ml and for antibiotic B is 16 µg/ml. Checkerboard was done by using 96-well plates that contained colistin with one of the other seven antimicrobials. The checkboard pattern was prepared by serial doubling dilutions of one of the 7 tested antimicrobials in the horizontal wells and colistin in the vertical wells. Then, each well of a 96-well microtiter plate was inoculated with approximately 50µl of bacterial suspension from an overnight culture with initial inoculum concentration of approximately $5x10^5$CFU/ml (a half McFarland bacterial suspension diluted to 1:100), then incubated aerobically at 37˚C for 18-24 hours. After that, wells with turbidity (identified against a dark background) were considered as positive for bacterial growth. MICs (lowest concentration with bacterial growth inhibition) for each individual antibiotic in the checkerboard were defined. Data from the checkerboard were analyzed using the lowest fractional inhibitory concentration index (FICI), which was used to define synergy. The FICI was calculated as follows: FICI = FIC A + FIC B, where FIC A is the MIC of drug A in the combination/MIC of drug A alone, and FIC B is the MIC of drug B in the combination/MIC of drug B alone. 'Synergy', 'additivity', 'indifference' and 'antagonism' were interpreted when the FICI was ≤0.5, >0.5 to ≤1, >1 to ≤4 and >4, respectively. Synergy is considered when the two antimicrobials could increase each other's effect; additivity means the additional effect of two antimicrobial actions without synergism; antagonism is considered when the combined effect of the two antimicrobials is lower than the most effective one used individually; and indifference indicates none of the before mentioned phenomena[6,13,14].

No susceptibility breakpoints are available for *A. baumannii* for tigecycline, vancomycin, teicoplanin and rifampicin in the CLSI guidelines. Consequently, *staphylococci* CLSI criteria were used and vancomycin (MIC ≥32 µg/ml), teicoplanin (MIC ≥32 µg/ml) and rifampin (MIC ≥4 µg/ml) were considered resistance[15]. For tigecycline, the European Committee on Antimicrobial Susceptibility Testing (EUCAST) criteria for *Enterobacteriaceae* were applied (MIC >2 µg/ml as resistance)[16].

Statistical analysis:

Data were tabulated & analyzed by SPSS version 20 ***(SPSS Inc., Chicago, IL, USA)***. Continuous variables are expressed as mean and SD. Categorical variables are expressed as frequencies and percent. Chi-square and Mann-Whitney tests were used. A significance level of $P<0.05$ was used in all tests.

RESULTS

Of 373 different clinical samples collected from hospitalized patients who had nosocomial infections (evident 48 hours or more after hospital admission), ninety-four *A. baumannii* were isolated. Duplicate isolates were excluded.

Demographic and Clinical characteristics of patients infected with *A. baumannii* are listed in table 2. *A. baumannii* nosocomial infection was higher among males (73.4%), patients who had undergone invasive procedures (79.8%) and patients with other comorbidities (75.5%). The types of collected specimens included respiratory tract sputum and tracheal aspirates (47/94; 50%), blood (22/94; 23.4%), urine (13/94; 13.8%) and wound (12/94;12.8%). CRAB isolates were significantly (P<0.05) higher among patients aged >60 years old (25%), hospitalized >10 days (31.8%), and those in intensive care unit (ICU) (56.8%) and among respiratory (38.6%) and urine samples (22.7%).

Table 2: Demographic and Clinical characteristics of patients infected with *A. baumannii*

Demographic and clinical characteristics	Total	Studied groups				χ2	P value
		Colistin susceptible (n=50)		Colistin resistant (n=44)			
		NO.	%	NO.	%		
Age (years):							
Mean±SD	40.84± 17.38	36.24 ± 16.88		46.06± 16.62		U=	0.006
Range	10.0-70.0	10.0 – 65.0		10.0– 70.0		2.73	S
Age groups							
10-30	35 (37.2)	24	48.0	11	25.0	6.74	0.03
31-60	43 (45.7)	21	42.0	22	50.0		S
>60	16 (17.0)	5	10.0	11	25.0		
Gender:							
Male	69 (73.4)	36	72.0	33	75.0	0.10	0.74
Female	25 (26.6)	14	28.0	11	25.0		NS
Duration of hospitalization (days):							
3-6	22 (23.4)	18	36.0	4	9.1	12.86	0.002
7-10	53 (56.4)	27	54.0	26	59.1		S
>10	19 (20.2)	5	10.0	14	31.8		
Antimicrobial administration							
Yes	94 (100.0)	50	100.0	44	100.0	NA	NA
No	0 (0.0)	0	0.0	0	0.0		
Invasive procedures							
Yes	75 (79.8)	42	84.0	33	75.0	1.17	0.27
No	19 (20.2)	8	16.0	11	25.0		NS
Associated comorbidities:							
Yes	71 (75.5)	35	70.0	36	81.8	1.76	0.18
No	23 (24.5)	15	30.0	8	18.2		NS
Specimen type:							
Respiratory samples	47 (50.0)	30	60.0	17	38.6	9.07	0.02
Blood	22 (23.4)	13	26.0	9	20.5		S
Urine	13 (13.8)	3	6.0	10	22.7		
Wound	12 (12.8)	4	8.0	8	18.2		
Department							
ICU	50 (53.2)	30	49.2	25	56.8	13.80	0.007
Pediatric oncology	5 (5.3)	2	3.3	5	11.4		S
Burn unit	15 (16.0)	9	14.8	10	22.7		
Chest department	14 (14.9)	14	22.9	0	0.0		
Surgery	10 (10.6)	6	9.8	4	9.1		

U: Mann-whitney test, χ2: chi square test, NA: not applicable, S: significant (P value < 0.05), NS: not significant (P value > 0.05).

The pattern of antibiotic susceptibility of tested isolates was highly resistance to: piperacillin (89.3%), ampicillin-sulbactam (85.1%), trimethoprim-sulfamethoxazole (84%), ceftazidime (81.9%), amikacin (77.7%), doxycycline (76.6%), cefepime (74.5%), ciprofloxacin (70.2%) and imipenem (69.1%). Colistin resistance was found in 44/94 (46.8%) of isolated *A. baumannii* as seen in figure 1 (A). Regarding distribution of colistin MIC in isolated *A. baumannii*, (25/94; 26.6%), (17/94; 18.1%), (8/94; 8.5%), (10/94; 10.6%), (25/94; 26.6%) and (9/94; 9.6%) had MIC ≤0.05µg/ml, 1µg/ml, 2µg/ml, 4µg/ml, 8µg/ml and ≥16µg/ml respectively as shown in figure 1 (B).

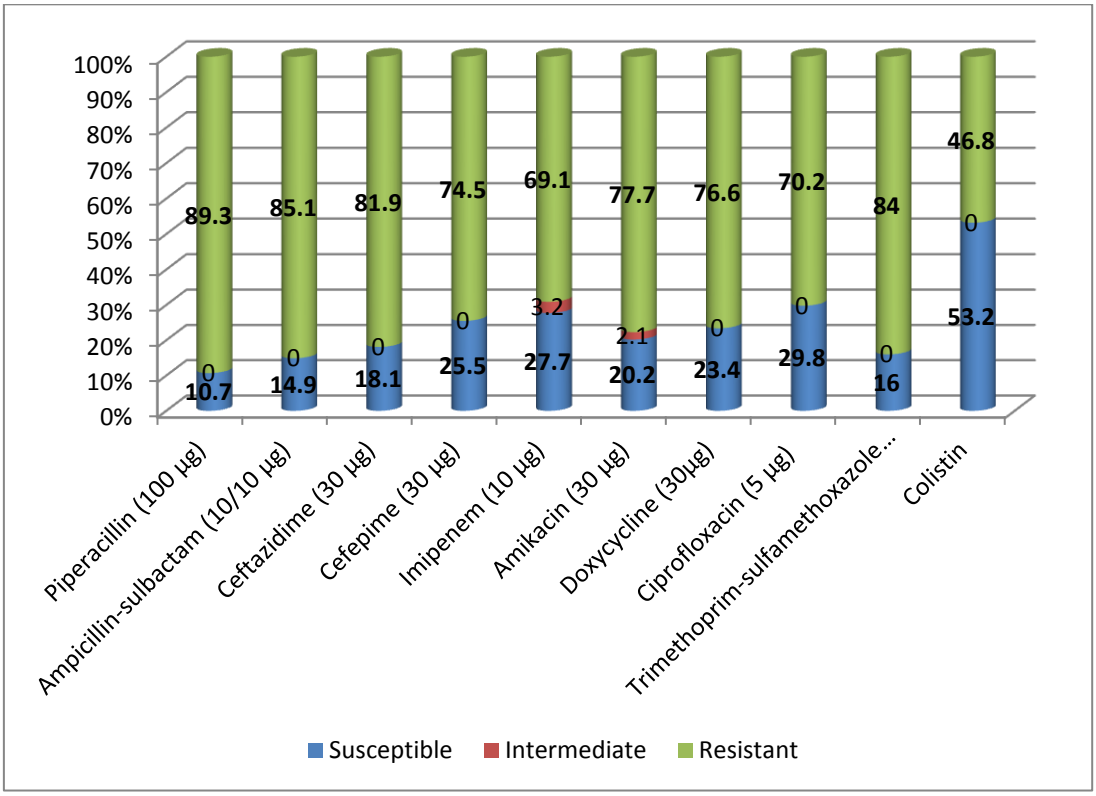

Fig. 1 (A): Antibiotic susceptibility pattern of isolated *A. baumannii*

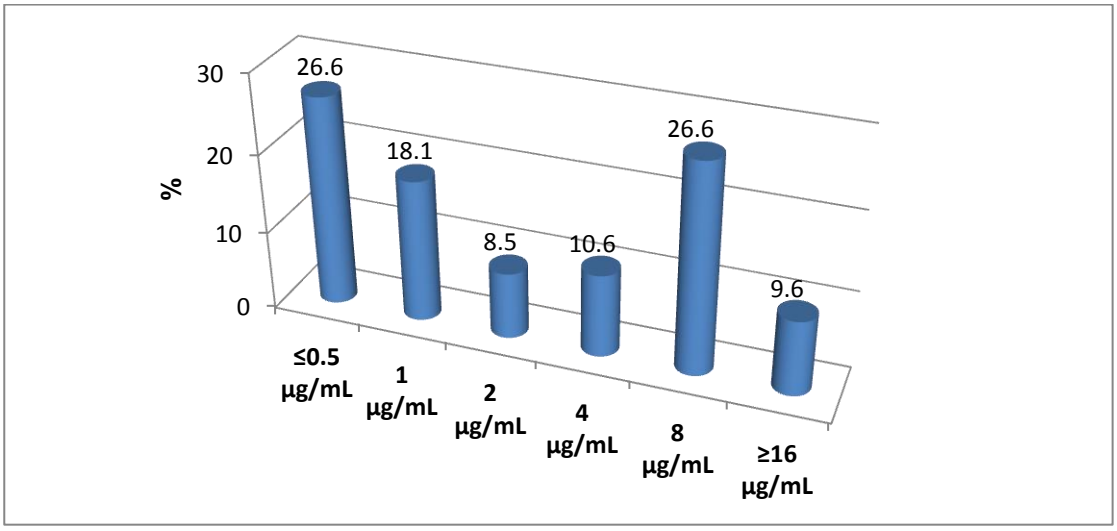

Fig. 1 (B): Colistin susceptibility pattern (MIC) of isolated *A. baumannii*

By multiplex PCR, only mcr-1 gene was found in 2 out of 44 representing 4.5% of CRAB isolates, while *mcr*-2, -3, -4, and -5 were not detected in any of them as shown in figure 2.

Fig. 2: Gel electrophoresis showing the amplified product of the *mcr-1* gene (320 bp). Lane 1: 100 bp DNA ladder (Cleaver Scientific, UK). Lane 2-3: *mcr-1* positive isolate; Lanes 4–6: negative isolates; Lane 7: positive control for *mcr-1* gene; Lane 8: negative control.

For each isolated CRAB, the checkerboard synergy test has been done against 7 colistin based antimicrobial combinations. All strains showed resistance to teicoplanin, vancomycin, rifampicin, tigecycline, imipenem, amikacin, and ceftazidime by BMD. Nevertheless, by addition of colistin in checkerboard pattern with vancomycin, meropenem, rifampicin, teicoplanin and ceftazidime then calculating the FICI to analyze the results, synergy was detected in 93.2%, 90.9%, 88.7%, 86.4% and 79.5% of CRAB respectively. Regarding tigecycline and amikacin more than 90% of CRAB displayed indifference in combination with colistin as shown in table 3.

Table 3. The checkerboard test of colistin-resistant *A. baumannii* isolates

Antimicrobial combinations	**Studied isolates N=44**			
	Synergy (FIC index is ≤0.5) N (%)	**Additive (FIC index >0.5 to ≤1) N (%)**	**Indifference (FIC index >1 to ≤4) N (%)**	**Antagonism (FICI is >4) N (%)**
Colistin- vancomycin	41 (93.2)	0 (0.0)	3 (6.8)	0 (0.0)
Colistin- teicoplanin	38 (86.4%)	2 (4.5)	4 (9.1)	0 (0.0)
Colistin- rifampicin	39 (88.7%)	3 (.6.8)	2 (4.5)	0 (0.0)
Colistin- tigecycline	0 (0.0)	4 (9.1)	40 (90.9)	0 (0.0)
Colistin- meropenem	40 (90.9)	3 (6.8)	1 (2.3)	0 (0.0)
Colistin- amikacin	0 (0.0)	3 (6.8)	41 (93.2)	0 (0.0)
Colistin- ceftazidime	35 (79.5)	0 (0.0)	9 (20.5)	0 (0.0)

DISCUSSION

The continuous rising infection with antimicrobial-resistant *A. baumannii* have forced using colistin as a final option for its treatment, leading to evolution of colistin resistance[17].

In our study, Nosocomial infection with *A. baumannii* was higher among male patients exposed to invasive procedures and with other comorbidities and mostly from respiratory specimens. These findings are similar to studies of *Okasha and Meheissen* [7], in Egypt, *Al Bshabshe et al.*[18], in KSA and *Novović and Jovčić* [3].

Old age and longer duration of hospitalization, ICU admission and specimen type were significant risk factors to acquire CRAB. In agreement, the study of *Elham and Fawzia* [19] confirmed that most of patients having CRAB strains were on mechanical ventilation (50%) in ICU (64%). ***Jiang*** et al.,[20] explained increased incidence of being infected with multi-drug resistant organisms in hospitals, especially in ICUs which

provide life support for critically sick patients with reduced host immunity, and because of use of invasive device.

Colistin resistance was discovered in this study in (46.8%) of isolated *A. baumannii*. The same results were reported by *Seleim et al* [17], in Egypt (49%), *Gerson et al.* [21] in Germany (48%) and *Papathanakos et al* [22] in Greece (41%). In the same line, *Nowak et al.*[23], reported that colistin resistanc was 47.7% among isolated *A. baumannii* from patients with ventilator-associated pneumonia in Greece, Italy and Spain, whereas *Al-Kadmy et al.* [24] in Iraq, reported higher rate (76%). In contrary, *Hameed et al.*[25] noted lower rates in Pakistan (9.6%) and *Kandee* [26], in Egypt (2.8%).

Only *mcr*-1 gene was detected in 2 isolates (4.5%) of our CRAB isolates, while *mcr*-2, -3, -4, and -5 were not detected at all. This is nearly matched with *Seleim et al* [17], in Egypt and *Hameed et al.* [25], in Pakistan who documented that 1% and 1.6% respectively of CRAB had *mcr*-1 gene. Also, in *Ajlan et al.*[27], study, in Egypt, they stated that only 3 carbapenem resistant Gram-negative bacilli isolates (6.98%) carried *mcr*-1 while other *mcr* genes were not detected. However, *Rahman and Ahmed* [28], in India and *Al-Kadmy et al* [24], found that 20% and 73.5% respectively of isolated *A. baumannii* were *mcr-1* gene positive. *Khoshnood et al.*[29], in Iran, did not detect *mcr-1* gene in any of the seventy isolated *A. baumannii*. Such differences in results could be explained by variance in sample form and number, differences in patient characters, or geographic discrepancy between countries.

Colistin resistance *A. baumanni* strains because of monotherapy increased the need to find effective antimicrobial combinations [6]. The mechanism by which monotherapy causes resistance is by selection of colistin-resistant subpopulations in heteroresistant strains or chromosomal mutations, also by the transmission of plasmid-mediated resistance[30-31].

In the current study, about 93.2% of CRAB displayed synergy with colistin vancomycin combinations. In accordance, *Okasha HA and Meheissen*[7], study, in Egypt and *Gordon et al.*[32] revealed that there was a synergy in all isolates of *A. baumanni* resistant strains with colistin-vancomycin combination. They explained synergy mechanism via disruption of bacterial membranes by colistin that was detected through electron microscope. Moreover, Colistin and vancomycin combination demonstrated synergy in earlier *in vitro* and *in vivo* investigations[33-34].

This study revealed synergy in 90.9% and 88.7% of CRAB in colistin combination either with meropenem or rifambicin respectively. This is consistent with previous studies that demonstrated *in vitro* synergistic effect for polymyxin combinations with carbapenem and rifampicin against both colistin-sensitive and -resistant MDR or XDR *A. baumannii* isolates [6, 35, 36]. Another Systematic review and *A. baumannii* meta-analysis documented synergy rates of 17.5–98.3% for polymyxin-carbapenem combinations[37-38]. The fluctuation in results may be due to different applied methods for testing synergy, number of tested isolates, their antibiotic susceptibility profile and their clonal diversity.

Our results showed that synergy was demonstrated in about 86.4% of our resistant strains with colistin/teicoplanin combination. This coincides with *Rady et al.*, study in which *in vitro* synergistic and bactericidal activity at 6 hour was found against all isolated *A baumannii* that did not show bacterial regrowth at 24 h on combination of 1 mg/l colistin with 10 mg/l teicoplanin[8].

Previous studies testing colistin with tigecycline did not detect synergy *in vitro* or *in vivo*[39-40]. Moreover, *Almutairi* [14], stated that adding colistin with tigecycline or amikacin mostly displayed indifference and colistin-ceftazidime showed synergism against examined strains. These previous findings matched our results.

CONCLUSION

It is recommended that further studies *in vivo* and *in vitro* are encouraged to define the therapeutic benefits of these tested antimicrobial combinations. Also, other techniques as time-kill assay could be performed to validate such synergy.

Consent for publication: Not applicable

Availability of data and material: Data are available upon request

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REFERENCES

1. Hu Y, Lyu Y, Jia X, Yue C, Deng S, Zhang X. Non-antibiotic prevention and treatment against *Acinetobacter baumannii* infection: Are vaccines and adjuvants effective strategies?. Frontiers in Microbiology. 2023 Jan 25;14:120
2. Andrade FF, Silva D, Rodrigues A. Colistin update on its mechanism of action and resistance, present and future challenges. Microorganisms 8: 1716.
3. Novović K, Jovčić B. Colistin Resistance in *Acinetobacter baumannii:* Molecular Mechanisms and Epidemiology. Antibiotics. 2023 Mar 4;12(3):516.
4. Hanpaibool C, Ngamwongsatit N, Ounjai P, Yotphan S, Wolschann P, Mulholland AJ, Spencer J, Rungrotmongkol T. Pyrazolones Potentiate

Colistin Activity against MCR-1-Producing Resistant Bacteria: Computational and Microbiological Study. ACS omega. 2023 Feb 20

5. Green KD, Chandrika NT, Vu LY, Pang AH, Tsodikov OV, Garneau-Tsodikova S. Aromatic hydrazides: A potential solution for *Acinetobacter baumannii* infections. European Journal of Medicinal Chemistry. 2023 Mar 5;249:115165.
6. Mantzana P, Protonotariou E, Kassomenaki A, Meletis G, Tychala A, Keskilidou E, Arhonti M, Katsanou C, Daviti A, Vasilaki O, Kagkalou G. In Vitro Synergistic Activity of Antimicrobial Combinations against Carbapenem-and Colistin-Resistant *Acinetobacter baumannii* and *Klebsiella pneumoniae*. Antibiotics. 2023 Jan;12(1):93.
7. Okasha HA, Meheissen MA. In Vitro Activity of Colistin and Vancomycin or Azithromycin Combinations on Extensively Drug Resistant *Acinetobacter baumannii* Clinical Isolates. Advances in Microbiology. 2017;7(01):71.
8. Rady OM, El-Attar L, Amine A. In vitro synergistic activity of colistin and teicoplanin combination against multidrug-resistant Acinetobacter spp. The Journal of Antibiotics. 2022 Mar;75(3):181-4.
9. Tille PM. Bailey and Scott 's Diagnostic Microbiology 2020; 18th edition.
10. Clinical and Laboratory Standards Institute (CLSI). Performance Standards for Antimicrobial Susceptibility Testing; 33rd edition. CLSI supplement M100. Clinical and Laboratory Standards Institute, USA; 2023
11. Rebelo AR, Bortolaia V, Kjeldgaard JS, Pedersen SK, Leekitcharoenphon P, Hansen IM, et al. Multiplex PCR for detection of plasmid-mediated colistin resistance determinants, mcr-1, mcr-2, mcr-3, mcr-4 and mcr-5 for surveillance purposes. Euro Surveill. 2018 Feb;23(6):17-00672.
12. Schwalbe, R., Steele-Moore, L. and Goodwin, A.C. (Eds.). Antimicrobial Susceptibility Testing Protocols. CRC Press, New York. 2007
13. Bae S, Kim MC, Park SJ, Kim HS, Sung H, Kim MN, et al. *In Vitro* Synergistic Activity of Antimicrobial Agents in Combination against Clinical Isolates of Colistin-Resistant *Acinetobacter baumannii*. Antimicrob Agents Chemother. 2016 Oct 21;60(11):6774-6779.
14. Almutairi MM. Synergistic activities of colistin combined with other antimicrobial agents against colistin-resistant *Acinetobacter baumannii* clinical isolates. PLoS One. 2022 Jul 13;17(7):e0270908.
15. Clinical and Laboratory Standards Institute (CLSI). Performance Standards for Antimicrobial Susceptibility Testing; 33rd edition. CLSI supplement M100. Clinical and Laboratory Standards Institute, USA. Zone diameter and MIC breakpoints for staphylococcus spp in CLSI Performance Standards for Antimicrobial Susceptibility Testing 2023: 94-105.
16. European Committee on Antimicrobial Susceptibility Testing Steering C. EUCAST technical note on tigecycline. Clin Microbiol Infect. 2006; 12(11):1147–9. https://doi.org/10.1111/j.1469-0691.2006.
17. Seleim SM, Mostafa MS, Ouda NH, Shash RY. The role of pmrCAB genes in colistin-resistant *Acinetobacter baumannii*. Scientific Reports. 2022 Dec 5;12(1):20951.
18. Al Bshabshe A, Joseph MRP, Al Hussein A, Haimour W and Hami M. Multidrug Resistance Acinetobacter Species at the Intensive Care Unit, Aseer Central Hospital, Saudi Arabia: A One Year Analysis. Asian Pacific Journal of Tropical Medicine 2016; 9: 903-908.
19. Elham B, Fawzia A. Colistin resistance in *Acinetobacter baumannii* isolated from critically ill patients: clinical characteristics, antimicrobial susceptibility and outcome. African Health Sciences. 2019 Nov 5;19(3):2400-6
20. Jiang Y, Ding Y, Wei Y, Jian C, Liu J, Zeng Z. Carbapenem-resistant *Acinetobacter baumannii:* A challenge in the intensive care unit. Frontiers in Microbiology. 2022;13.
21. Gerson S, Lucassen K, Wille J, Nodari CS, Stefanik D, Nowak J, et al. Diversity of amino acid substitutions in PmrCAB associated with colistin resistance in clinical isolates of *Acinetobacter baumannii*. International journal of antimicrobial agents. 2020 Mar 1;55(3):105862.
22. Papathanakos G, Andrianopoulos I, Papathanasiou A, Priavali E, Koulenti D, Koulouras V. Colistin-resistant *Acinetobacter baumannii* bacteremia: a serious threat for critically ill patients. Microorganisms. 2020 Feb 20;8(2):287.
23. Nowak J, Zander E, Stefanik D, Higgins PG, Roca I, et al. High incidence of pandrug-resistant *Acinetobacter baumannii* isolates collected from patients with ventilator associated pneumonia in Greece, Italy and Spain as part of the MagicBullet clinical trial. J. Antimicrob. Chemother. 2017; 72: 3277–3282.
24. Al-Kadmy IM, Ibrahim SA, Al-Saryi N, Aziz SN, Besinis A, Hetta HF. Prevalence of genes involved in colistin resistance in *Acinetobacter baumannii*: first report from Iraq. Microbial Drug Resistance. 2020 Jun 1;26(6):616-22.
25. Hameed F, Khan MA, Muhammad H, Sarwar T, Bilal H, Rehman TU. Plasmid-mediated mcr-1 gene in *Acinetobacter baumannii and Pseudomonas*

aeruginosa: first report from Pakistan. Revista da Sociedade Brasileira de Medicina Tropical. 2019 Sep 5;52.

26. Kandee A. Detection of colistin susceptibility in multi- drug resistant *pseudomonas aeruginosa* and *acinetobacter baumannii* by four different methods, Egypt. J. Med. Microbiol 2016; 25 (2): 17-23
27. Ajlan SE, Elmahdy EE, Sleem AS. Assessment of Colistin Susceptibility among Carbapenem-Resistant Clinical Isolates. Egyptian Journal of Medical Microbiology. 2022 Jul 1;31(3):109-16.
28. Rahman M & Ahmed S. Prevalence of colistin resistance gene mcr-1 in clinical Isolates *Acinetobacter Baumannii* from India. IJID 2020; 101(S1): 81
29. Khoshnood S, Savari M, Montazeri EA & Sheikh AF. Survey on genetic diversity, biofilm formation, and detection of colistin resistance genes in clinical isolates of *Acinetobacter baumannii.* Infect. Drug Resist. 2020; 13: 1547–1558 (2020).
30. Meletis G, Tzampaz E, Sianou E, Tzavaras I, Sofianou D. Colistin heteroresistance in carbapenemase-producing *Klebsiella pneumoniae.* J. Antimicrob. Chemotherap. 2011; 66: 946–947.
31. El-Sayed Ahmed MAE, Zhong LL, Shen C, Yang Y, Doi Y, Tian GB. Colistin and its role in the Era of antibiotic resistance: An extended review (2000–2019). Emerg. Microbes Infect. 2020; 9: 868–885.
32. Gordon NC, Png K and Wareham DW. Potent Synergy and Sustained Bactericidal Activity of a Vancomycin-Colistin Combination versus Multidrug-Resistant Strains of *Acinetobacter baumannii*. Antimicrobial Agents and Chemotherapy 2010; 54: 5316-5322.
33. O'Hara JA, Ambe LA, Casella LG, Townsend BM, Pelletier MR, Ernst RK, et al. Activities of Vancomycin- Containing Regimens against Colistin-Resistant *Acinetobacter baumannii* Clinical Strains. Antimicrob Agents Ch. 2013; 57(5):2103–8.
34. Hornsey M, Wareham DW. *In vivo* efficacy of glycopeptide-colistin combination therapies in a Galleria mellonella model of *Acinetobacter baumannii* infection. Antimicrob Agents Chemother. 2011; 55 (7):3534–7.
35. Lenhard JR, Nation RL, Tsuji BT. Synergistic combinations of polymyxins. Int. J. Antimicrob. Agents 2016, 48, 607–613
36. Scudeller L, Righi E, Chiamenti M, Bragantini D, Menchinelli G, et al. Systematic review and meta-analysis of in vitro efficacy of antibiotic combination therapy against carbapenem-resistant Gram-negative bacilli. Int. J. Antimicrob. Agents 2021; 57: 106344
37. Ni W, Shao X, Di X, Cui J, Wang R, Liu Y. In vitro synergy of polymyxins with other antibiotics for *Acinetobacter baumannii*: A systematic review and meta-analysis. Int. J. Antimicrob. Agents 2015, 45, 8–18.
38. Jiang Z, He X, Li J. Synergy effect of meropenem-based combinations against *Acinetobacter baumannii:* A systematic review and meta-analysis. Infect. Drug Resist. 2018, 11, 1083–1095.
39. Cheng A, Chuang YC, Sun HY, Sheng WH, Yang CJ, Liao CH, et al. Excess mortality , combination therapy for extensively drug-resistant *Acinetobacter baumannii* bacteremia: A multicenter prospective observational study. Crit. Care Med. 2015; 43: 1194–1204**.**
40. Kengkla K, Kongpakwattana K, Saokaew S, Apisarnthanarak A, Chaiyakunapruk N. Comparative efficacy and safety of treatment options for MDR and XDR *Acinetobacter baumannii* infections: A systematic review and network meta-analysis. J. Antimicrob. Chemother. 2018; 73: 22–32.