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Original article

Effect of meropenem-colistin and meropenem-amikacin combinations against carbapenem-resistant *Pseudomonas aeruginosa* isolates in Suez Canal University Hospitals

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ABSTRACT

Background: Healthcare associated infections (HAIs) caused by carbapenem-resistant *Pseudomonas aeruginosa* are considered as an overwhelming problem in hospitals due to its resistance to most effective antibiotic classes. Consequently, various antimicrobial combinations have been suggested as an alternative in clinical practice**.** So, our aim was to improve the antibiotic policy in Suez Canal University Hospitals (SCUHs) in the treatment of carbapenem-resistant *Pseudomonas aeruginosa* infections and to reduce morbidity and mortality rates due to these infections**. Method:** A cross-sectional descriptive study was carried out on 36 carbapenem-resistant *Pseudomonas aeruginosa* strains collected from different wards in SCUHs. A checkerboard assay was carried on these strains to assess the effect of meropenem colistin and meropenem-amikacin combination. **Results:** The synergy testing of the meropenem-amikacin combination on carbapenem-resistant *Pseudomonas aeruginosa* showed 50% synergy, 8.3% addition, 36% indifference and 5.7% antagonism. For the meropenem-colistin combination, it showed 39% synergism, 30.5% addition and 30.5% indifference with no antagonism was observed. Although, the mean fractional inhibitory concentration (FIC) value of meropenem was higher in the meropenem-amikacin combination than in the meropenem-colistin combination, the difference was statistically insignificant. **Conclusions:** Both combinations (meropenem colistin and meropenem-amikacin) showed high rates of synergy against carbapenem-resistant *Pseudomonas aeruginosa* isolates and can offer good alternatives in the clinical practice for treatment of the carbapenem-resistant *Pseudomonas aeruginosa* strains.

Introduction

Healthcare-associated infections (HAIs) caused by *Pseudomonas aeruginosa* (*P. aeruginosa*) is considered a serious problem due to the pathogen's inherent and acquired resistance to almost all available antibiotic classes and the paucity of novel effective antibiotics [1]. The improper use of carbapenems, the drugs of last choice, has led to the spread of carbapenem resistance among *P. aeruginosa* strains with the serious rising rates of morbidity and mortality due to infections by this pathogen [2].

The use of antibiotic combinations has emerged as an alternative option for dealing with this situation seeking the synergism with suppressing the bacterial resistance and achieving less toxicity of the used antibiotics [3]. In the combination, colistin disrupts the bacterial outer membrane facilitating the entry of meropenem that acts on inhibition of bacterial

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wall synthesis [4]. The addition of amikacin to meropenem is effective in vitro. The destruction of cell wall peptidoglycan polymers by meropenem enhances the entry of the amikacin that inhibits the protein synthesis [5].

The aim of this study was to improve the antibiotic policy in Suez Canal University Hospitals (SCUHs) in the treatment of carbapenem-resistant *Pseudomonas aeruginosa* infections through evaluation of the effect of meropenem-colistin and meropenem-amikacin combinations against carbapenem-resistant *Pseudomonas aeruginosa*.

Methodology

Study population

This cross-sectional descriptive study was conducted in SCUHs in Ismailia, Egypt, during the period from April 2019 to December 2019. Thirty-six carbapenem-resistant *P. aeruginosa* clinical isolates were recovered from 490 patients admitted to different wards in SCUHs. Both males and females were represented from all age groups. Ethics committee of Faculty of Medicine, Suez Canal University had approved the study.

Specimen collection and processing

Urine, sputum, endotracheal aspirate, pus, and blood specimens were properly collected under complete aseptic conditions and processed in Microbiology and Immunology Lab, Faculty of Medicine, Suez Canal University, for isolation and identification of carbapenem-resistant *P. aeruginosa* strains.

Cultures were done on blood agar, MacConkey's agar and Pseudomonas agar. The cultured plates were incubated aerobically at 35± 2˚C for 24 hours. Colonies suspected to be *P. aeruginosa* (being non-lactose fermenter on MacConkey's agar and Gram-negative bacilli by Gram stain) were confirmed by oxidase test and by production of the blue pyocyanin pigment on Pseudomonas agar [6].

Antibiotic susceptibility testing

Antibiotic susceptibility testing for the isolated *P. aeruginosa* strains was performed according to the Clinical and Laboratory Institute guidelines, 2019 [7] using the Kirby-Bauer disc diffusion method. The following antibiotic discs (Oxoid, UK) were included: Pipracillin (100 µg), pipracillin-tazobactam (100/10 µg), ceftazidime (30 µg), cefepime (30 µg), aztreonam (30 µg), gentamycin (10 µg), tobramycin (10 µg), amikacin (30 µg), ciprofloxacin (5 µg), levofloxacin (5 µg), Imipenem (10 µg) and meropenem (10 µg). *Pseudomonas aeruginosa* strain was considered carbapenem-resistant when the diameter of the zone of inhibition is ≤ 15mm around meropenem and/or imipenem discs [7].

Synergy testing by checkerboard technique [8]

Synergy testing for meropenem-colistin and meropenem-amikacin combinations was performed against carbapenem-resistant *P. aeruginosa* strains using the checkerboard technique as follows:

- Meropenem, colistin sulfate, and amikacin were obtained as pure powders (Sigma Aldrich, Germany). Antibiotics were stored at 4°C until use. The antibiotic powders were dissolved in water to obtain the stock antibiotic solutions. According to the [7], the stock solutions of the antibiotic powders were diluted in appropriate volume of cation-adjusted Muller Hinton broth (CAMHB) to get the final working concentrations (µg/ml).
- Three to five isolated colonies from fresh blood agar culture were transferred to sterile Trypticase soy broth (TSB) and incubated then it was adjusted to achieve a turbidity equivalent to 0.5 McFarland standards. Fifty microliters (50 µl) from the bacterial suspension were transferred to 5 ml TSB in order to achieve a final inoculum size of 5 x105 CFU/ml.
- Plain untreated 96-well (12 columns and 8 rows) microtiter plates were used. Two plates were used for each isolate; one for the meropenem-colistin combination and the other for the meropenem-amikacin combination **(Figure 1)**.
- Fifty microliters of each antibiotic dilution were transferred to the microtiter plate wells. Meropenem was transferred from column 1 to column 9 to obtain range of concentrations from 1 to 256 µg/ml while amikacin and colistin were put from row A to row G with concentration range 4 to 256 µg/ml for amikacin and 0.5 to 32 µg/ml for colistin.
- In column 10, the wells were filled by 100 µl of the serial dilutions of colistin or amikacin only while row H wells were filled by 100 µl of meropenem dilutions only. Therefore, the minimum inhibitory concentration (MIC) of colistin or amikacin was determined from column 10 and that of meropenem was determined from row H.
- Ten microliters of the standardized bacterial suspension were transferred to all wells of the plate except for column 12 that contained only 100 µl of CAMHB as a sterile negative control while column 11 contained only the bacterial

suspension to be used as a positive control. Blood agar plates were used as check plates.

- The plates were incubated at 35 -37°C for 24 hr. The results were obtained by reading MICs of each antibiotic alone and in the combination defined as the lowest concentration of the antibiotic alone and in combination that inhibited the growth of the organism and showed no turbidity as judged by naked eye.
- Synergy was determined according to the fractional inhibitory concentration index (FICI) that was calculated for each combination. FICI was calculated as following:

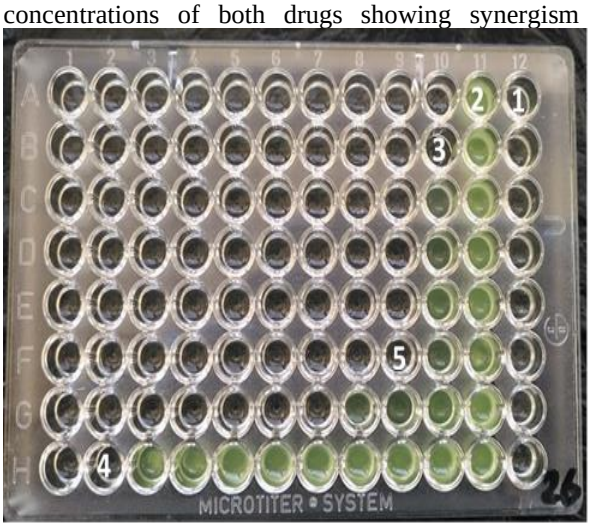
For colistin-meropenem combination

- FIC of colistin = MIC of colistin in combination with meropenem / MIC of colistin alone.
- FIC of meropenem = MIC of meropenem in combination with colistin / MIC of meropenem alone.
- FIC index = FIC of colistin + FIC of meropenem.

Interpretation of the results:

- FIC index ≤ 0.5 → Synergistic.
- FIC index > 0.5 and ≤ 1.0 → Additive.
- FIC index >1.0 and ≤ 4.0 → Indifferent.
- FIC index > 4.0 → Antagonistic.

Figure 1. Checkerboard plate to evaluate the effect of meropenem-colistin combination on the carbapenem-resistant *P. aeruginosa* (CRPA) strains. 1: negative control. 2: positive control.3: MIC of colistin alone. 4: MIC of meropenem alone .5: the lowest concentrations of both drugs showing synergism



Statistical analysis

Statistical analysis was performed using SPSS-17 software (SPSS Inc., Chicago, Illinois, USA). Quantitative data were described in terms of range and mean (±SD), while nonnumeric data were described as frequencies and percentages.

Results

The study included 110 *P. aeruginosa* isolates with the prevalence of 22.4% among the different isolated organisms. Enterobacteriaceae were isolated at the rate of (34.7%) followed by Gram positive organisms (28.4%) , other non-fermenters (9%) and candida (4%) while no growth occurred in (1.5%) of specimens. Out of the 110 isolated *P. aeruginosa* strains, 36 (32.7%) strains were carbapenem-resistant, and 74 (67.3%) strains were carbapenem-sensitive.

Most (41.7%) of the CRPA strains were isolated from the intensive care unit (ICU). Neonatal ICU (NICU) was the least (2.8%) ward from which CRPA strains were isolated **(Table 1)**.

Antibiotic susceptibility testing of the CRPA isolates showed that all the isolates were resistant to ceftazidime, cefepime, ciprofloxacin, gentamicin, levofloxacin, tobramycin, imipenem, and meropenem. Resistance to amikacin, aztreonam, piperacillin and piperacillin- tazobactam was 86.1%, 83.3%, 94.5% and 91.7% respectively. By the broth microdilution method, 13 (36.1%) of the CRPA isolates were colistin-resistant and 23 (63.9%) were colistin-sensitive.

Synergy testing of the meropenem-amikacin (MEM-AMK) combination on CRPA showed 50% synergy, 8.3% addition, 36% indifference and 5.7% antagonism. For the meropenem-colistin (MEM-colistin) combination, it showed 39% synergism, 30.5% addition, 30.5% indifference however no antagonism was observed for this combination. Synergy was observed in the meropenem-amikacin combination more than the meropenem-colistin combination (50% vs. 39%) **(Table 2)**.

The mean MIC of meropenem, colistin and amikacin decreased significantly in the combination compared to their mean MICs alone. **Table 3** shows the mean MICs of meropenem, amikacin and colistin when used alone and in combinations.

The mean FIC value of meropenem was higher in the meropenem-amikacin combination than in the meropenem-colistin combination (0.660 ± 1.4 vs. 0.243 ± 0.4) but the difference was statistically insignificant ($p > 0.05$). **Table 4** shows the range and mean value of FIC of the two combinations.

Meropenem-colistin combination showed synergistic effect on 69.2% of the colistin-resistant strains and 21.7% of the sensitive strains. It also

showed additive effect on 15.4% and indifferent effect on 15.4% of the colistin-resistant strains. This combination has no antagonistic effect neither on colistin-sensitive nor colistin-resistant strains **(Table 5)**.

Table 1. Frequency distribution of the isolated CRPA according to the hospital wards.

Hospital ward	No. of isolates	Percentage (%)
ICU	15	41.7%
Surgical wards	8	22.2%
Internal medicine wards	6	16.7%
Burn unit	4	11.1%
Obstetrics/gynecology	2	5.7%
NICU	1	2.8%
Total	**36**	**100%**

ICU: intensive care unit, NICU: neonatal intensive care unit

Table 2. Synergy testing results of the meropenem-amikacin and meropenem-colistin combinations on CRPA isolates (n = 36).

Combination	Synergistic	Additive	Indifferent	Antagonistic
MEM-AMK*	18 (50%)	3 (8.3%)	13 (36%)	2 (5.7%)
MEM-colistin	14 (39%)	11 (30.5%)	11 (30.5%)	0 (0%)

*MEM-AMK: meropenem-amikacin, Synergism: FICI ≤ 0.5, additive: FICI >0.5 and ≤ 1.0, indifference: FICI>1.0 and ≤ 4.0, antagonism: FICI >4.0.MEM: meropenem, AMK: amikacin.

Table 3. Reduction in the mean MIC of meropenem, amikacin and colistin in the MEM-AMK and MEM-colistin combinations.

	MEM-AMK combination			
	Mean MIC alone	Mean MIC combined	Mean difference	***p* value**
Meropenem	287	147.4	139.6	*0.0034**
Amikacin	194.5	168	26.5	*0.0008**
	MEM-colistin combination			
	Mean MIC alone	Mean MIC combined	Mean difference	***p* value**
Meropenem	287	82	205	*0.00006**
Colistin	7.9	2.9	5	*0.017**

*Significant (*p value < 0.05*)

Table 4. FIC of the two combinations for the 36 CRPA isolates.

	Drug	**FIC Range**	**Mean ± SD**	***p* value**
MEM-AMK	Meropenem	0.01 - 8.00	0.660 ± 1.4	*0.09**
	Amikacin	0.03 - 4.00	0.700 ± 0.9	
MEM-Colistin	Meropenem	0.002 - 1.035	0.243 ± 0.4	
	Colistin	0.02 - 1.00	0.512 0.3	

*Insignificant (*p value >0.05*).

Table 5. Synergy results for the meropenem-colistin combination in colistin-resistant strains and colistin-sensitive strains.

	Colistin-resistant strains (*n* = 13)	**Colistin-sensitive strains** (*n* = 23)
Synergistic	9 (69.2%)	5 (21.7%)
Additive	2 (15.4%)	9 (39.1%)
Indifferent	2 (15.4%)	9 (39.1%)
Antagonistic	0 (0%)	0 (0%)

Discussion

Carbapenem-resistant *P. aeruginosa* infections are a significant public health challenge worldwide due to the difficulty in treating these infections [9]. The usage of monotherapy may lead to extensive resistance and results in clinical treatment failure. Therefore, combination therapies with antibiotics that have different antimicrobial mechanisms have been proposed as good options for treating these infections [10].

This study included 110 *P. aeruginosa* isolates collected from 490 patients admitted in different wards in SCUHs in Ismailia. Of all the *P. aeruginosa* strains isolated in this study, 32.7% were carbapenem-resistant. This relatively agrees with the previous studies that were done by **Hashem et al.** [11] in Egypt in SCUHs and **Ismail and Mahmoud** [12] who reported the rate of isolation of CRPA in their study was 32% and 27.3% respectively. On the other hand, the studies of **McCann et al.** [13] and **Terahara and Nishiura [**14] reported a carbapenem-resistance rate 14.6 % and 11.9% in *P. aeruginosa* isolates, respectively. Application of strict antibiotic policies in the management of carbapenem-resistant *Pseudomonas* infections is responsible for such variation in the prevalence rates in different studies.

The highest isolation rate of carbapenem-resistant *P. aeruginosa* was from the ICU (41.7%), while neonatal ICU (NICU) was the least (2.8%) ward from which CRPA strains were isolated. This agrees with **Tsao et al.** [15] and **McCann et al.** [13] who reported that the highest prevalence of CRPA was in the ICU. The high prevalence of carbapenem resistance in the ICU is attributed to many factors such as prolonged hospitalization, immune suppression, prior prolonged antibiotic therapy particularly

carbapenems, presence of indwelling devices, and use of mechanical ventilators.

Antibiotic susceptibility testing of our carbapenem-resistant *P. aeruginosa* isolates showed that all the isolates were resistant to ceftazidime, cefepime, ciprofloxacin, gentamicin, levofloxacin, tobramycin. Resistance to amikacin, aztreonam, piperacillin and piperacillin-tazobactam was 86.1%, 83.3%, 94.5% and 91.7% respectively. In the study of **El-Mahdy and El-Kannishy** [16] the resistance rate in CRPA reached up to 58%, 63%, 61% and 82% to levofloxacin, ciprofloxacin, piperacillin-tazobactam and aztreonam, respectively. Ceftazidime and cefepime were the least effective antibiotics, with a resistance rate 97%.

By the broth microdilution method, 36.1% of the carbapenem-resistant *P. aeruginosa* isolates were colistin-resistant and 63.9% were colistin-sensitive. This was in disagreement with the studies of **Tsao et al.** [15] and **Meradji et al.** [17] who reported that 12.7% and 25% of the isolated carbapenem-resistant *P. aeruginosa* strains were colistin-resistant, respectively. This discrepancy in colistin-resistance pattern among different studies can be due to the misuse of drugs, dissimilar policies of hospitals for controlling the infection and topographical distribution.

Before 2016, all of the detected colistin-resistance mechanisms were attributed to chromosomal genes. In 2016, plasmid-borne colistin-resistance genes, *mcr-1* and *mcr-2*, were reported to perform horizontal transfer in bacteria [18]. In addition, *P. aeruginosa* can produce biofilm on the hospital surfaces and catheters which can lead to cross-resistance based on the low penetration of antibiotics into the bacterial community after biofilm formation [19].

Synergy testing for the two combinations was done by the checkerboard technique. It is one of the most commonly used synergy testing methods because it is easy to perform and has the advantage of testing multiple concentrations of the antibiotics at one time. However, more than two antibiotics cannot be tested at a time, different methods of interpreting results are present, and only antimicrobials with a fixed incubation time can be tested [3].

Synergy testing of the meropenem-colistin combination showed 39% synergism, 30.5% addition and 30.5% indifference. No antagonism was observed in this combination. Two studies conducted by **Lee et al.** [4] and **Aoki et al.** [20] tested the effect of imipenem-colistin combination by the checkerboard technique on Carbapenem-resistant *P. aeruginosa* and showed additive and indifferent effects with no synergy or antagonism. They emphasized that not only synergy is considered as an advantage for the therapy, but also additive effect is by itself beneficial, because even a minimal rise in the antibacterial activity using the combination therapy may help clinical success and recovery.

Ramadan et al. [21] tested meropenem-colistin combination on carbapenem-resistant *P. aeruginosa* by the E-test and their results showed 64% synergy and 36% addition with no indifference or antagonism. Different methods of synergy testing can produce different results of the same combination due to different interpretation methods and synergy definitions.

Carbapenem-aminoglycoside combination is one of the most frequently used combinations for the treatment of *P. aeruginosa* infections. In our study, the meropenem-amikacin combination showed 50% synergy, 36% indifference, 8.3% addition and 6.7% antagonism. Our results differed from those of **He et al.** [22] who tested doripenem-amikacin combination against CRPA and reported 20% synergy, 47% addition and 33% indifference. Also, **Nazli et al.** [23] used the E-test to evaluate the effect of combining amikacin and imipenem on carbapenem-resistant *P. aeruginosa* and reported 100% indifferent effect. **Wilhelm et al.** [24] studied the effect of imipenem-amikacin combination on carbapenem-resistant *P. aeruginosa* using time-kill assay and reported synergistic effect on 33% of the isolates. Variations of results from our study are due to the differences in the used methods and the tested antibiotics from each group.

Significant reduction of the mean MICs of meropenem, amikacin and colistin in the combinations was observed as compared to their MICs when tested alone. Similarly, **Loho et al.** [25] also reported significant reduction of the mean MIC of doripenem and amikacin when combined to each other compared to their MICs when tested alone.

The mean FIC value of meropenem was higher in the meropenem-amikacin combination than in the meropenem-colistin combination (0.660 ± 1.4 vs. 0.243 ± 0.4) but the difference was statistically insignificant ($p > 0.05$). **Dauod et al.** [8] also found that the average of the mean FIC for their *P. aeruginosa* strains is 0.934 ± 0.57 with the combination of meropenem and colistin.

In the current study, meropenem-colistin combination showed synergistic effect on 69.2% of

the colistin-resistant strains and 21.7% of colistin-sensitive strains. It also showed additive effect on 15.4% and indifferent effect on 15.4% of the colistin-resistant strains. This combination showed no antagonistic effect on neither colistin-sensitive nor colistin-resistant strains. These results were not in accordance to those of **Ramadan et al.** [21] which showed synergistic effect on all colistin-resistant strains and 54% of the sensitive strains and an additive effect on 36% of the sensitive strains. The variability of the results may be due to differences in methodology, definitions of synergy and choice of the strains.

In their clinical study, **Rigatto et al.** [26] evaluated the effect of colistin alone and in combination with a β-lactam on extensively drug-resistant *P. aeruginosa* infections. The 30-day mortality was 42.4% and 67.6% in combination and monotherapy groups, respectively. They reported that combination therapy was independently associated with lower 30-day mortality.

On the other hand, the clinical trial of **Falagas et al.** [27] compared the effect of using colistin alone versus colistin and meropenem combination and concluded that there was no difference in response and nephrotoxicity rates. Similarly, **Paul et al.** [28] evaluated the effect of colistin therapy alone versus colistin-meropenem combination and reported that there was no significant difference between colistin monotherapy and combination therapy after 14-days after therapy.

Conclusion

The current study reported that in vitro antibiotic interaction tests in the checkerboard technique are useful method to know whether combination of two different antibiotics will be effective to kill carbapenem-resistant *P. aeruginosa*. Our data showed synergistic effects of the meropenem-amikacin and meropenem-colistin combinations on carbapenem-resistant *P. aeruginosa*.

Because in many cases only colistin remains as an effective antibiotic for treating carbapenem-resistant *P. aeruginosa* infections, using the meropenem-colistin combination will offer the advantage of using lesser concentrations of colistin and hence minimizing its nephrotoxic effect and the development of resistance to it. A better advantage will be offered by using the meropenem-amikacin combination which will avoid using colistin at all and preserve its usage for treating more resistant strains. Additional in vivo studies are needed to assess clinical efficacy of the antimicrobial combinations.

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.

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