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Synergistic Combination of Carbapenems and Colistin against *P. aeruginosa* and *A. baumannii*

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ABSTRACT

Background: Intubated patients are particularly at risk of developing infections caused by these pathogens, specifically, *P. aeruginosa* and *A. baumannii*. In the past fifteen years, Carbapenems were known to be the drugs of choice for these bacteria. With the increase in the use and misuse of antibiotics, these bacteria became highly resistant, and almost all available antibiotics, including Carbapenems, became inefficient. Synergistic combination therapy may be a useful strategy in slowing as well as overcoming the emergence of resistance. The aim of this study was to evaluate the antibacterial activity on *P. aeruginosa* and *A. baumannii* of the combination of two antibiotics: Colistin and a Carbapenem (Meropenem or Imipenem). **Methods:** The antibacterial activity was assessed by determining the MIC. Then, the effect of combining the antibiotics was studied using the Checkerboard Technique described by White *et al.*, 1996. The Fractional Inhibitory Concentration (FIC) for each strain was then calculated and classified as synergy, additive, indifference or antagonism. 11 strains of *A. baumannii* and 11 strains of *P. aeruginosa* were tested in the presence of Meropenem combined with Colistin or Imipenem combined with Colistin. **Results:** For the combination of Meropenem and Colistin, 6 strains of *A. baumannii* and 3 strains of *P. aeruginosa* showed synergy while 5 strains of *A. baumannii* and 7 strains of *P. aeruginosa* showed additive effect, only 1 strain of *P. aeruginosa* showed antagonism. For Imipenem and Colistin, only 1 strain of *A. baumannii* and 3 strains of *Pseudomonas* showed synergy while 8 strains of *Acinetobacter* and 8 strains of *Pseudomonas* showed additive effect. **Conclusion:** The "*in vitro*" combination Colistin-Carbapenem is associated with an improvement in MIC. In the majority of the cases, this improvement suggests a synergistic combination or an additive effect.

Keywords: Antibiotic Combination; Antibiotic Synergy; MIC; FIC; *Acinetobacter baumannii*; *Pseudomonas aeruginosa*; Colistin; Meropenem; Imipenem

1. Introduction

Nosocomial infections are mostly encountered in patients admitted to the intensive care units [1]. Among these, catheterized or critically ill patients are considered as number one group developing hospital acquired infections [2]. Gram positive as well as many gram negative bacteria can be responsible for such infections [3,4]. *P. aeruginosa* and *A. baumannii* are opportunistic pathogens that mostly cause hospital acquired pneumonia (HAP) and ventilated associated pneumonia (VAP) [5]. They are usually more resistant than community acquired pathogens due to extensive exposure to antibiotics in hospitals.

Due to the lack or inefficiency of infection control programs in many hospitals, random/extensive use of antibiotics and many others reasons, resistance highly emerged within these pathogens and they became known as highly resistant microorganisms [6]. Carbapenem resistant *P. aeruginosa* and *A. baumannii* are nowadays widely spread. By nature, these pathogens are more resistant than other Gram negative organisms because of their outer membrane that is less permeable [7] and their ability to form biofilm [8]. Furthermore, the main mechanisms of resistance acquired by these pathogens can

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be summarized by the decrease/modification in the porin channels that is usually coupled by efflux pumps [9] and by the hydrolytic enzymes such as Metallo Beta Lactamases (MBL) and Oxacillinases. For example, a decrease in OprD causes increased level of resistance to Carbapenems since they resemble amino acids that usually gain entry via these channels [10,11]. In a recent study, carbapenems resistant *P. aeruginosa* (CRPA) isolated in Russia produced MBL [12]. Furthermore, 73% and 95% of carbapenems resistant *Acinetobacter baumanii* (CRAB) isolated in 2010 and 2011 respectively from Greece and Brazil were due to OXA-23 acquisition [13].

Similarly to the worldwide scenario, resistance in the Middle East in general and specifically in Lebanon is increasing and many strains were found to be carbapenem resistant [14-16]. In a study including hospitals from different Middle Eastern countries, it was found that CRAB isolated from a Lebanese hospital carried the oxa-58, oxa-23 and oxa-72 genes conferring the carbapenems resistance [17].

Through history, these pathogens were treated using different anti-pseudomonal and anti-*Acinetobacter* agents such as aztreonam, aminoglycosides and fluoroquinolones [18]. Carbapenems were considered as the drugs of extreme cases of resistance. With the increase in resistance against carbapenems, most of the available antimicrobial agents are becoming useless [17]. Physicians have limited solution for the treatment of such infections. The old antibiotic Colistin, in spite of its toxicity and side effects, is considered nowadays as the last resource when these multi-drug organisms are observed [18,19]. The idea of combining Colistin with other antibiotics seeking a synergistic activity, and probably a less toxicity, looks promising. Combination therapy limits and suppresses bacterial resistance, decreases antibiotic toxicity, covers a broad range of pathogens with greater efficacy and most importantly leads to synergy [20,21].

The aim of this study was to evaluate the antibacterial activity on *P. aeruginosa* and *A. baumannii* of the combination of two antibiotics: Colistin and a Carbapenem (Meropenem or Imipenem) using Checkerboard technique.

2. Materials and Methods

Bacterial strains: 11 strains of *A. baumannii* and 11 strains of *P. aeruginosa* of different susceptibility profiles were isolated from patients of Centre Hospitalier du Nord (CHN), North Lebanon. Most of the strains were resistant or intermediate to Carbapenems and susceptible to Colistin.

Inoculum preparation: Strains were incubated overnight at 37˚C. Two to 3 colonies were added to Muller Hinton Broth and then the turbidity was standardized to 0.5 McFarland (10^8 CFU/mL). Dilutions were done in order to match a final concentration of 5×10^5 CFU/mL that was used for MIC determination and Checkerboard technique.

Antibiotic preparation: Imipenem (Merck Sharp & Dohme B/V, Netherlands), Meropenem (AstraZeneca UK Limited, United Kingdom) and Colistin (Forest laboratories, United Kingdom) were obtained as powder. Aliquots were prepared and stored at −20˚C.

MIC determination: The MICs of Imipenem, Meropenem and Colistin for the 22 strains were determined by broth macrodilution method as described by Clinical and Laboratory Standard Institute-CLSI [23]. For each strain, duplicate sets of 16 tubes for serial dilutions each were prepared. The MIC was defined as the lowest concentration of antibiotic that inhibits the visual growth of bacteria after 18 hours of incubation. The final inoculum was 5×10^5 CFU/mL. The tubes were incubated for 16 h - 20 h at 37˚C and then interpreted.

Synergy testing by Checkerboard technique: The combinations tested for each strain of each microorganism were Meropenem plus Colistin and Imipenem plus Colistin. The concentration of Colistin ranged from 1/32 × MIC to 32 × MIC while that of the carbapenem (Imipenem or Merpenem) ranged from 1/8 × MIC to 8 × MIC. The checkerboard technique consists of the following steps: In panel B, MHB was added in all wells except row A and H. 100 µL of Imipenem or Meropenem was then added into the raw G (G1 → G12) and raw H (H1 → H11; except H12) to give a final concentration of 8MIC. Serial dilution from G to B was performed. In panel A: 50 µL of MHB was added in all the wells except column 1 and 12. Then, 50 µL of Colistin was added to the column 11 (A11 → H11) and column 12 (A12 → G12; except H12) to give a final concentration of 32 MIC. Serial dilution from column 11 to 2 was performed. After serially diluting panel A and Panel B, 50 µL was taken from each well of panel B and dispensed into the corresponding well of panel A. Then the bacterial inoculum was prepared and added into the wells. The plates were incubated for 18 - 24 h at 37˚C and then interpreted.

FIC calculation: To evaluate the antibacterial effect of each combination, the Σ FIC was calculated.

$$\Sigma \text{FIC} = \text{FIC of drug A (Colistin) and FIC of drug B (Meropenem or Imipenem)} = \frac{\text{MIC of drug A in combination}}{\text{MIC of drug A alone}} + \frac{\text{MIC of drug B in combination}}{\text{MIC of drug B alone}}.$$

The results were then classified as: synergy for Σ FIC ≤ 0.5; additive for Σ FIC between 0.5 and 1.5; and indifference for values of Σ FIC between 1.5 and 2; Antagonism was linked to values above 2 [22,24,25].

3. Results

Minimum Inhibitory Concentrations: The MICs obtained for each antibiotic are shown in **Table 1** and **2**. All *P. aeruginosa* strains used were sensitive to Colistin. 4 strains were resistant to Meropenem and Imipenem. All the other strains were intermediate for both carbapenems. P21 had the lowest MIC to Colistin while P24 had the highest MIC to Colistin. For Meropenem, P27 had the lowest MIC while P28 had the highest MIC. For Imipenem, P11, P21 and P25 had the lowest MIC while P30 had the highest MIC. For *A. baumannii*, 10 of the 11 strains used were sensitive to Colistin. 5 strains were resistant to Meropenem and Imipenem. A2, A4, A5, A8, A9, A10, A12 and A13 had the lowest MIC to Colistin while A100 had the highest MIC to Colistin. For Meropenem, A8 had the lowest MIC while A2, A5 and A6 had the highest MIC. For Imipenem, A9 had the lowest MIC while A5, A6, A12 and A100 had the highest MIC.

3.1. Checkerboard Results

Table 3 shows the FICs calculated for all the *Acinetobacter* strains using the 2 combinations of antibiotics; while table 4 shows the FICs calculated for all the *Pseudomonas* strains using the 2 combinations of antibiotics.

For the combination of Meropenem and Colistin on *A. baumannii*, 6 of the 11 strains showed synergy while 5 strains showed additive results. The average of the "Mean of Σ FIC" for the 11 strains of *A. baumannii* is 0.5393 ± 0.180 with Meropenem combined to Colistin. For the combination of Imipenem and Colistin on *A. baumannii*, 1 of the 11 strains showed synergy while 8 strains showed additive results and 2 strains showed antagonism. The average of the "Mean of Σ FIC" was 1.2019 ± 0.942 with Imipenem combined to Colistin.

For the combination of Meropenem and Colistin on *P.aeruginosa*, 3 of the 11 strains showed synergy while 7 strains of 11 showed additive results. Antagonistic result

Table 1. MICs results by broth macrodilution of Colistin (COL), Meropenem (MERO), and Imipenem (IMP) for 11 *A. baumannii* strains.

Strain number	MIC of COL (IU/ml)	MIC of MERO (mg/ml)	MIC of IMP (mg/ml)
A2	0.9765	31.25	15.625
A3	1.9950	7.8125	7.8125
A4	0.9765	7.8125	15.625
A5	0.9765	31.25	31.25
A6	1.955	31.25	31.25
A8	0.9765	0.061	0.25
A9	0.9765	1.955	0.123
A10	0.9765	7.8125	7.8125
A12	0.9765	15.625	31.25
A13	0.9765	7.8125	3.9063
A100	62.5	15.625	31.25

Table 2. MICs of Colisitn (COL), Meropenem (MERO), and Imipenem (IMP) against 11 strains of *P. aeruginosa*.

Strain number	MIC of COL (IU/ml)	MIC of MERO (mg/ml)	MIC of IMP (mg/ml)
P11	0.9765	1.955	0.9765
P19	0.9765	3.9063	3.9063
P20	0.488	15.625	7.8125
P21	0.061	3.9063	0.9765
P22	0.25	7.8125	15.625
P24	1.9765	3.9063	1.955
P25	0.9765	1.955	0.9765
P27	0.488	0.9765	1.955
P28	1.95	31.25	15.625
P29	0.9765	15.625	15.625
P30	0.488	15.625	31.25

Table 3. FICs calculated for *Acinetobacter baumannii* in Meropenem + Colistin and Imipenem + Colistin combinations.

	Meropenem + Colistin			Imipenem + Colistin		
	FIC min	**FIC max**	**Mean of SFIC**	**FIC min**	**FIC max**	**Mean of SFIC**
A2	0.1875	0.5625	0.3438	0.25	1.0313	0.5871
A3	0.375	1.0313	0.6763	0.1875	1.0625	0.4732
A4	0.25	0.625	0.4531	0.625	1.25	0.993
A5	0.1875	0.625	0.356	0.375	1.125	0.811
A6	0.1875	1.0625	0.4236	0.5	1.125	0.8125
A8	0.5625	1.125	0.9107	0.75	2.25	1.525
A9	0.25	0.625	0.4188	0.53125	0.75	0.640625
A10	0.375	1.0625	0.6625	0.516	0.75	0.6224
A12	0.375	0.75	0.5391	1.25	2.25	2.094
A13	0.1875	0.625	0.38	0.5	2.03125	1.005
A100	0.5	1.0625	0.7688	1.0078	17	3.6566
Mean	**0.3125**	**0.8324**	**0.5393**	**0.5902**	**2.7841**	**1.2019**
STD	**0.128**	**0.221**	**0.180**	**0.299**	**4.748**	**0.942**

was seen with the strain P25. The average of the "Mean of Σ FIC" for the 11 strains of *P. aeruginosa* is 0.9342 ± 0.572 with the combination of Meropenem and Colistin.

For the combination of Imipenem and Colistin on *P. aeruginosa*, 3 stains showed synergy while 7 strains of 11 showed additive results and 1 showed indifference. The best synergy was detected with the strain P30. The average of the "Mean of Σ FIC" for the 11 strains of *P. aeruginosa* is 0.9619 ± 0.431 with the combination of Imipenem and Colistin.

The antibacterial effect of both combinations on both *A. baumannii* and *P. aeruginosa* showed mostly synergistic or additive results. To a significant extent Meropenem and Colistin showed a better synergy when compared to Imipenem and Colistin.

4. Discussion

Most *P. aeruginosa* and *A. baumannii* are becoming multidrug resistant, the major issue being resistance to Carbapenems [26]. All the strains used in this study were resistant or intermediate to Carbapenems however susceptible to Colistin except the strain A100 that was highly resistant to Colistin (**Tables 1** and **2**). The only differences in susceptibility to Carbapenems were seen with the following strains P22, P20 and A4. Strain P22 showed intermediate resistance to Meropenem but full resistance to Imipenem. It has been described that Meropenem has greater *in vitro* efficacy than Imipenem against *P. aeruginosa* [23]. P20 strain is the only intermediate strain for Imipenem but resistant for Meropenem. Such profile may be mostly related to an upregulation in the efflux system MexAB/OprM resulting in increased resistance to Meropenem as compared to Imipenem [25]. Strain A4 showed intermediate resistance for Meropenem but fully resistance for Imipenem and this might be explained by a down-regulation in the OprD porin channel which will cause an increase in the resistance to Imipenem as compared to Meropenem [26].

On the other hand, the combination of Meropenem and Colistin can be considered as a better combination for the treatment of *A. baumannii* and *P. aeruginosa* when compared to the combination Imipenem and Colistin because the means of FICs for Meropenem/Colistin were 0.5393 ± 0.180 and 0.9342 ± 0.572 respectively while those of Imipenem/Colistin were 1.2019 ± 0.942 and 0.9619 ± 0.431 respectively.

The best synergy rate and subsequent highest antibacterial activity were noticed for the combination of Meropenem and Colistin. This suggests that the combination could be a good alternative for the treatment of *Acinetobacter* and *Pseudomonas* infections until the successful development of a better antibiotic agent. The reason for the increased synergy with Meropenem than Imipenem might be that most OXA and MBLs carbapenemases target with greater affinity Imipenem as compared to Meropenem [28,29]. In addition, the synergistic or additive effect might be influenced by the ability of Colistin to disrupt the bacterial outer membrane and increase its permeability for Carbapenems [30-32] and therefore stop the cross linking of the new synthesized polymers.

Another advantage for combination therapy is the delay in emergence of bacterial resistance and specifically the rapidly developing resistance and heteroresistance toward Colistin [6]. It must be mentioned that not only synergy is considered as an advantage for the therapy but also additive result is by itself beneficial, because even a miniature raise in the antibacterial activity using the combination therapy may help clinical success and re-

covery.

Our study offers data in support for the combination therapy to treat infections caused by multi-drug-resistant *Acinetobacter* and *Pseudomonas*. However, in order to be successfully implemented, such a decision requires more pharmacokinetic and pharmacodynamic studies mainly to specify the doses that need to be administered in order to maintain an acceptable profile of toxicity of these combinations.

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