In vitro study of meropenem and colistin combination against multidrug resistant clinical isolates from patients with burns

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Abstract

Background: Nosocomial infections are known in patients treated at tertiary care hospitals. However this acquires significance as some of these are resistant to most of the major categories of antibiotics. Treatment of such infections become more challenging while treating patients in burn wards. There is a pressing requirement to evaluate combinations of some of the available antibiotics to treat these nosocomial infections in burns patients. No study evaluating combination of meropenem and colistin on clinical isolates of burns patients in a tertiary care hospital is available.

Objective: To evaluate synergistic effect of meropenem and colistin antibiotic combination on common multidrug resistant bacteria isolated from the burn ward patients.

Methods: A total of 46 strains of multidrug resistant bacteria which included 23 strains each of P. aeruginosa and Acinetobacter baumannii were tested forin-vitro synergistic effect of meropenem and colistin combination. MIC and FIC index was calculated for all the bacterial isolates.

Result: 18 out of 23 strains of P. aeruginosa and 19 out of 23 strains of Acinetobacter baumannii showed synergistic activity.

Conclusion: This study suggests that the combination of meropenem and colistin could be a good alternative for the treatment of Acinetobacter and Pseudomonas infections in burns patients, until a newer antibiotic agent is available.

Keywords: Combination antibiotics, Chequer board titration, Fractional Inhibitory Concentration Indices (FICI), Meropenem-colistin combination, Multidrug-resistance

Introduction

As the skin barriers are destroyed and immune system is suppressed in patients with burns, they are at high risk of developing nosocomial infection, which is further compounded by prolonged hospitalization and invasive therapeutic and diagnostic procedures. This risk of infection increases proportionately with the size of the burn[1].

Bacteria and fungi are the most common pathogens of burn wounds. These microbes form multi-species biofilms on burn wounds within 48–72 hours of injury[1]. These organisms either originate from the patient's own skin, gut and respiratory flora, or through contact with contaminated health care environments and workers[1,2-10]. Gram-positive bacteria are some of the first to colonize burns, followed quickly by Gram-negative. Fungal infection tends to occur in the later stages after the majority of bacteria have been eliminated by topical antibiotics[1].

The most common causes of burn wound infections are bacteria, with *Pseudomonas aeruginosa* being the most important species[9–11]. Clinicians are increasingly opting for two or more antibiotics as empiric choice to ensure clinical cure. Antibiotic combinations are sought to provide synergistic killing. Synergistic interactions are usually thought of as advantageous, since, for a given amount of drug, they more effectively inhibit the growth of drug-sensitive pathogens[12].

One of the parameters which has been used to show interactions during combination therapy are the FIC (Fractional Inhibitory Concentration) indices, derived from chequer board titrations[13]. To the best of our information, there are only few studies in tertiary care hospitals to find in-vitro effect of meropenemand colistin combination on multidrug resistant clinical isolates from burns patients, using chequer board titrations.

Materials and Methods

This study was conducted at Department of Microbiology, of a tertiary care hospital. Study period was from Sep 2014 to Sep 2015. Clinical isolates were characterized using conventional methods and identified using a Vitek™ Automated Microbiology System (BioMerieux, USA).

Forty six multidrug resistant bacterial strains were selected for the study. These included 23 strains each of *Pseudomonas aeruginosa* and *Acinetobacter baumannii*. All of these bacterial strains were resistant to meropenem and susceptible to colistin.

Antimicrobial agents: The following antimicrobial agents were used: colistinsulfome thatesodium and meropenem. The MIC (Minimum inhibitory Concentration) values of colistin and meropenem were determined for all 46 bacterial isolates.

MIC determination: The MIC values of meropenem and colistin were determined for all 46 bacterial isolates by broth microdilution method as described by Clinical and Laboratory Standard Institute-CLSI[14]. Meropenem and colistin were tested at concentrations up to six times above and below the MICs of the drugs[14].

Growth Control and sterility controls were also tested. The bacterial inoculum used was 5×10^5 CFU/mL (Colony forming units per ml).

Synergy testing by Chequer board technique: Synergy testing of meropenem with colistin was performed by chequerboard method. The combinations of antibiotics tested for each strain of bacteria was meropenem plus colistin.

Interaction was determined according to calculated FIC (Fractional Inhibitory Concentration) index.

FIC index calculation

FIC index: FIC of drug A (meropenem) and FIC of drug B(colistin)

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

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

Interpretative definitions: Results of calculations were interpreted as previously described[15,16]. A calculated FIC index value of ≤0.5 represented a synergistic effect (i.e. total effect greater than the sum of the individual antibiotic effects), a value between >0.5 and <2 represented an additive effect (i.e. no additional contribution from including the second antibiotic, compared with use of the first antibiotic alone), and a value of ≥2 represented an antagonistic effect (i.e. total effect less than the sum of the individual effects)[15,16].

Results

The MICs obtained for each antibiotic are shown in Table 1 and 2. All *Pseudomonas aeruginosa* Paeruginosa strains used were sensitive to colistin and resistant to meropenem. For *Pseudomonas aeruginosa*, isolate numbers P 8 and P 12 had the lowest MIC to colistin, while P 6, P9 and P11 had the highest MIC to colistin. For meropenem, isolate number P21 had the lowest MIC, while P2 and P8 had the highest MIC.

For *A.baumannii*, all 23 strains used were sensitive to colistinand resistant to meropenem. Isolate number A8 had the lowest MIC to colistin, while A6, A9 and A11 had the highest MIC to colistin. For meropenem, A15 and A21 had the lowest MIC while A6 had the highest MIC.

Chequerboard Results

Table 1 shows the FICs calculated for all the *A. baumannii* strains using the 2 combinations of antibiotics; while Table 2 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*, 19 of the 23 strains showed synergy, while 4 strains showed additive results. The average of the "Mean of FIC index" for the 23 strains of *A. baumannii* is 0.413±0.188 with meropenem combined with colistin. For the combination of meropenem and colistin on *P.aeruginosa*, 18 of the 23 strains showed synergy while 5 strains of 23 showed additive results. The average of the "Mean of FIC index" for the 23 strains of *P.aeruginosa* is 0.443±0.222 with meropenem combined with colistin.

Table 1: MIC index and FIC index of 23 Strains of *Acinetobacter baumannii*

Acinetobacter baumannii						
Strains	**MIC Meropenem(µg/ml)**		**MIC Colistin(µg/ml)**		**FIC Index**	**Outcome**
	Alone	**With Colistin**	**Alone**	**With Meropenem**		
A1	32	4	0.5	0.125	0.375	Synergy
A2	32	2	0.5	0.125	0.25	Synergy
A3	16	1	0.25	0.0625	0.313	Synergy
A4	32	8	0.5	0.125	0.5	Synergy
A5	32	4	0.5	0.125	0.375	Synergy
A6	>128	32	1	0.125	0.375	Synergy
A7	64	16	0.25	0.125	0.75	Additive
A8	16	8	0.125	0.0625	1	Additive
A9	32	4	1	0.25	0.375	Synergy
A10	32	4	0.5	0.125	0.375	Synergy
A11	32	4	1	0.125	0.25	Synergy
A12	16	1	0.25	0.0625	0.313	Synergy
A13	32	16	0.25	0.25	0.25	Synergy
A14	16	8	0.5	0.0625	0.625	Additive
A15	8	1	0.5	0.0625	0.25	Synergy
A16	32	8	0.5	0.125	0.5	Synergy
A17	16	8	0.5	0.0625	0.625	Additive
A18	16	1	0.25	0.0625	0.313	Synergy
A19	32	4	0.5	0.125	0.375	Synergy
A20	32	16	0.25	0.25	0.25	Synergy
A21	8	1	0.5	0.0625	0.25	Synergy

A22	16	1	0.25	0.0625	0.313	Synergy
A23	32	8	0.5	0.125	0.5	Synergy

Table 2: MIC index and FIC index of 23 Strains of *Pseudomonas aeruginosa*

Pseudomonas aeruginosa						
Strains	**MIC Meropenem(µg/ml)**		**MIC Colistin(µg/ml)**		**FIC Index**	**Outcome**
	Alone	**With Colistin**	**Alone**	**With Carbepenem**		
P1	64	16	0.5	0.125	0.375	Synergy
P2	128	2	0.5	0.125	0.25	Synergy
P3	32	1	0.25	0.0625	0.313	Synergy
P4	64	8	0.5	0.125	0.5	Synergy
P5	32	4	0.5	0.125	0.375	Synergy
P6	64	32	1	0.125	0.375	Synergy
P7	64	16	0.25	0.125	0.75	Additive
P8	128	8	0.125	0.0625	1	Additive
P9	32	4	1	0.25	0.375	Synergy
P10	32	4	0.5	0.125	0.375	Synergy
P11	32	4	1	0.125	0.25	Synergy
P12	16	8	0.125	0.0625	1	Additive
P13	32	16	0.25	0.25	0.25	Synergy
P14	16	8	0.5	0.0625	0.625	Additive
P15	8	1	0.5	0.0625	0.25	Synergy
P16	32	8	0.5	0.125	0.5	Synergy
P17	16	8	0.5	0.0625	0.625	Additive
P18	16	1	0.25	0.0625	0.313	Synergy
P19	32	4	0.5	0.125	0.375	Synergy
P20	32	16	0.25	0.25	0.25	Synergy
P21	8	1	0.5	0.0625	0.25	Synergy
P22	16	1	0.25	0.0625	0.313	Synergy
P23	32	8	0.5	0.125	0.5	Synergy

Discussion

Most *P. aeruginosa* and *A. baumannii* infections are treated using different anti-pseudomonal and anti-acinetobacter agents such as aztreonam, aminoglycosides and fluoroquinolones. Carbapenems are considered to be drugs used for extreme resistant cases. However, with the increase in resistance against carbapenems, most of the available antimicrobial agents are proving to be virtually useless[17].

In our study all strains of *P. aeruginosa* and *A. baumannii* were resistant to meropenem but susceptible to colistin (**Tables 1** and **2**). On the other hand, the combination of meropenem and colistin was synergistic in majority of strains isolated. This suggests that the combination of meropenem with colistin could be a good alternative for the treatment of *Acinetobacter* and *Pseudomonas* infections from burns patients, until an alternative antibiotic agent is successfully developed.

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[18].

Due to the labor-intensiveness of chequerboard titrations, difficult nature of test that needs expertise and interpretation as well as difficulty in providing quick reports to the treating Physicians, newer methods for an optimized approach is being looked into. One of these methods is the Xact™ test that has been developed by AB Biodisk, Solna, Sweden that endeavors to measure the different gradients of antibiotic combinations in perpendicular fashion, employing a 50 X 50 mm plastic carrier that has a combination of two antibiotics immobilized over it. This carrier is used over a lawn culture of the organism that is being studied. The FICI are read using a software that indicates different outcome (Synergy, Additiveness and Antagonism) at the touch of a button. This method has been found to have good correlation with chequerboard titration. The method can be adapted for use in multi-resistant fungal isolates as well[19].

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