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Original Article

COLISTIN-CARBAPENEM COMBINATION THERAPY AGAINST CARBAPENEM RESISTANT GRAM NEGATIVE BACILLI INFECTIONS: CLINICAL AND AN *IN VITRO* SYNERGY STUDY

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ABSTRACT

Objective: Combination therapy is recommended for carbapenem resistant Gram negative bacilli (CR GNB) infections. However, limited data exists on the clinical effectiveness of antibiotic combinations. The purpose of this study was to evaluate the efficacy of colistin-carbapenem combination against CR GNB infection in a clinical study and an *in vitro* synergy study using Etest.

Methods: A study was conducted in a tertiary care hospital to evaluate the clinical outcome of patients with CR GNB infections who were treated with colistin-carbapenemcombination between January to April, 2013. It was comprised of 33 patients with CR GNB infection. Detection of *in vitro* synergy was performed by Etest for colistin-meropenem combination on five isolates. These isolates were also screened for the resistant genes $bla_{\text{OXA-23}}$, $bla_{\text{VIM}}$and bla_{NDM} using single target PCR.

Results: 33 CR GNB included *Acinetobacter*spp. (19), *Pseudomonas aeruginosa* (7) and *Enterobacteriaceae* spp. (7). Overall clinical success of 60.6% was observed in patients receiving colistin-carbapenem combination therapy. In respiratory infection, the clinical success rate was only 25%, whereas in soft tissue infection it was 57.1%. In bloodstream infection 100% clinical success was observed. All five isolates screened using PCR was carrying bla NDM gene, whereas isolate of *Acinetobacter baumannii* also carried $bla_{\text{OXA-23}}$ and bla_{VIM} gene. Indifferent interactions were observed between colistin and meropenem against all five isolates.

Conclusion: We observed low clinical success rate for colistin-carbapenem combination therapy, probably due to indifferent interactions between colistin and meropenem against NDM producing strain. In addition, probable pharmacokinetic concern of colistin may have a role to play.

Keywords: Carbapenem resistance, Etest, New Delhi metallo-β-lactamases, Synergy.

INTRODUCTION

Carbapenems are commonly used to treat severe infections in critically ill patients[1]. Unfortunately, Gram-negative bacilli (GNB), the most clinically relevant being *Acinetobacter baumannii*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae* and other *Enterobacteriaceae*, are becoming increasingly non-susceptible to carbapenems [2]. Carbapenem resistance is predominantly conferred by carbapenemases, such as oxacillinase (OXA)-type enzymes and metallo-β-lactamases (MBLs) of Imipenemase (IMP), Verona Italy metallo-β-lactamases (VIM) and New Delhi metallo-β-lactamases (NDM) types, and serine carbapenemases of *Klebsiella pneumoniae* carbapenemases (KPC) type [2]. Although all the resistant mechanisms are being increasingly identified worldwide, there are some clear endemic areas, such as NDM producers in India [3].

Carbapenem resistance often show concurrent carriage of additional resistance determinants to many other classes of antimicrobials such as quinolones and aminoglycosides. Therefore, therapeutic options for these infections are extremely limited and with that clinicians have returned to the use of colistin (COL) [2]. However, the clinical use of COL is hindered by side effects, mainly nephrotoxicity, in addition to unclear optimal dosing. In order to improve clinical success, various combination therapies have been used with COL. One of the antibiotic classes most commonly used in combination with COL is the carbapenem[1].

The combination of COL and carbapenem against Gram negative bacilli is supported *in vitro* by high synergy and bactericidality rates, with low antagonism and less resistance development. However, reported strain to strain variation suggests that individualized or centre based synergy testing is of value [1].

The aim of this study was to report our experience of clinical effectiveness of COL-carbapenem combination against carbapenem resistant Gram negative bacilli (CR GNB) infections including *in vitro* synergy testing of representative strains using Etest.

MATERIALS AND METHOD

The study was conducted at a 750 bedded tertiary care hospital in Mumbai, India. This study was approved by the Institutional Scientific and Ethics Board (ISEB) of the hospital. General consent was taken from all the patients for testing required for diagnosis and use of results for epidemiological or research based purposes.

The enrolled patients were hospitalized patients with CR GNB infections who were treated with COL- carbapenem combination between January 2013 and April 2013.160 to 240 mg (2 to 3 MIU) of colistimethate sodium (CMS) per 8 or 12 h was administered and doses were adjusted according to the renal functions along with 1g dosing of carbapenem every 8 h. Exclusion criteria were as follows: Administration of COL-carbapenem combination as empirical treatment or for infections involving organisms other than CR GNB and patients for whom the duration of combination treatment was < 48 h.

GNB isolates obtained from clinical specimen received for cultural studies during the study period were identified as carbapenem resistant by imipenem and/or meropenem resistant results from routine laboratory test system (Vitek 2; bio Merieux). Antibiotic susceptibility was interpreted as per criteria published by CLSI [4]. Susceptibility of tigecyclin was determined by the use of minimum inhibitory concentration (MIC) breakpoints approved by the US Food and Drug Administration (US-FDA) [5]. For COL, break points proposed by a European Committee on Antimicrobial Susceptibility Testing (EUCAST) were used because relevant break points were not available from CLSI [6].

Carbapenemase production was detected using modified Hodge's test (MHT) and MBLs production using Ethylene-diamine-tetra-acetic acid (EDTA) disk synergy test, as described [7].

One representative isolate from each genera comprising *Acinetobacter baumanii*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, *Escherichia coli* and *Enterobacter cloacae* was selected based on susceptibility profile. DNA was extracted from these strains by heat boil method and were screened for the resistant genes $bla_{\text{OXA-23}}$, bla_{VIM} and bla_{NDM} by single target PCR using previously published primers, OXA-23, Oxa-23-F 5'-GATCGGATTGGAGAACCAGA-3'/ Oxa-23-R 5'-ATTTCTGACCG CATTTCCAT-3'; VIM, Vim-F 5'-GATGGTG TTTGGTCGCATA-3'/ Vim-R 5'-CGAATGCGCAGCACCAG-3'; NDM-F 5'-GGTTTGGCGATCT GGTTTTC-3'/ NDM-R 5'-CGGAATGGCTCATCACGATC-3'[8-10].

Further, *in vitro* synergy between COL and meropenem (MER) against these strains were determined using Etest. Mueller-Hinton agar plates (HiMedia, Mumbai, India) were used for Etest MIC and synergy testing. The MICs of COL and MER were determined using Etest strips (bioMerieux). Synergy screening was performed using a method employing two Etests applied at right angles to each other, as described [11]. The following formulas were used to calculate the fractional inhibitory concentration (FIC) index: FIC of MER = MIC of MER in combination/MIC of MER alone; FIC of COL = FIC of COL in combination/MIC of COL alone; FIC index = FIC MER + FIC of COL. Synergy is defined as an FIC index of ≤0.5. Indifference is defined as an FIC index of >0.5 but of ≤4. Antagonism is defined as an FIC index of >4[11]. Data recorded for each patient included the following: demographic details, associated co-morbidities, previous antimicrobial regimens, site of infection, causative pathogen and length of stay. Only single clinically significant isolate from the patient was included. Outcome at the end of treatment was defined as successful (partial or complete improvement of signs/symptoms of infection or positive microbial response in terms of sterile culture results post or during the treatment), or failure (no improvement or deterioration of signs/symptoms of infection or negative microbial response in terms of persistent positive culture results with the same organism 3 days after initiation of antibiotic therapy). Final disposition was defined as death, discharged during illness or transferred to ward or discharged.

RESULTS

A total of 33 patients received COL-carbapenem combination therapy for CR GNB infections during the study period. Patient and pathogen characteristics are recorded in Table 1. All patients had received other antimicrobial agents prior to acquiring infection by CR GNB. The total number is more than 100%, since most of the patients received more than one antimicrobial agent.

Amongst the 19 isolates of *Acinetobacter* spp., 18 were *Acinetobacter baumannii* and 1 was identified as *Acinetobacter junii. Enterobacteriaceae* spp. (7) included *Klebsiella pneumoniae* (4), *Escherichia coli* (2), and *Enterobacter cloacae* (1). Susceptibility profile of these isolates is given in Table 2, 3 and 4. With MHT carbapenemase production was detected in 31/33 (93.9%) isolates, whereas EDTA-disk synergy test detected MBL production in 30/33 (90.9%) isolates.

Table 1: Characteristics of patients treated with COL-carbapenem combination

Variable	*n* (%)
Demographics	
Age [median (range)]	58 yrs (1 month - 86 yrs)
Sex (male)	20 (60.6)
Comorbidity	
Heart dysfunction	8 (24.2)
Malignancy	7 (21.2)
Diabetes Mellitus	9 (27.3)
Hyper tension	12 (36.4)
Chronic renal failure	7 (21.2)
Admission to ICU	31 (93.9)
Prior surgery	3 (9.1)
Prior antibiotic use	33 (100)
BL/BLI	26 (78.8)
Carbapenem	18 (54.5)
Aminoglycosides	5 (15.2)
Colistin	4 (12.1)
Fluoroquinolones	3 (9.1)
Tigecyclin	1 (3.0)
Prior hospitalization	9 (27.3)
Type of infection	
Respiratory infection	12 (36.4)
Bloodstream infection	12 (36.4)
Soft tissue infection	7 (21.2)
Urinary tract infection	2 (6.0)
Pathogens	
Acinetobacter spp.	19 (57.6)
Pseudomonas aeruginosa	7 (21.2)
Enterobacteriaceae spp.	7 (21.2)
Time to develop infection with CRGNB (days) [mean ± SD (range)]	16.0 ± 22.57 (0 to 127)
Duration of hospitalization (days) [mean ± SD (range)]	42.6 ± 31.49 (12 to 143)

Note: BL/BLI - β-lactam/β-lactamase inhibitor, ICU - intensive care unit, SD - Standard deviation.

All five representatives isolate screened using PCR analysis were found to be carrying bla_{NDM} gene, whereas isolate of *Acinetobacter baumannii* also carried $bla_{\text{OXA-23}}$ and bla_{VIM} genes. Results of *in vitro* synergy testing using Etest for COL and MER combination against these five representative isolates are shown in Table 5. All isolates showed indifferent results.

Table 2: Susceptibility profile of *Acinetobacter* spp isolates

	Acinetobacter spp.			
Antimicrobial agent	**MIC range mg/l**	**MIC_{50} mg/l**	**MIC_{90} mg/l**	**% Sensitivity**
Imipenem*	≥16	≥16	≥16	0
Meropenem*	ND	-	-	-
Amikacin*	ND	-	-	-
Tobramycin*	≤1 to ≥16	8	≥16	42
Gentamycin*	≤1 to ≥16	8	≥16	5
Colistin**	≤0.5	≤0.5	≤0.5	100
Tigecyclin***	≤0.5 to 4	2	4	47
Ciprofloxacine*	≥4	≥4	≥4	0

Overall in-hospital mortality of 42.4% (14/33) was observed in this study. Three patients died because of the reasons unrelated to infection and attributed mortality of the infection was found to be 33.3% (11/33). Successful clinical outcome of the infection was observed in 20 out of 33 patients (60.6%). A total of 11 out of 13 patients whose infections were unresponsive to therapy died andtwo were discharged during illness. Table 6 presents the clinical response associated with the site of infection and the causative pathogen for patients treated with COL-carbapenem combination. COL-MER combination (16/24 successful cases) was more commonly used compared to COL-Imipenem combination (4/9 successful cases).

DISCUSSION

Infections caused by CR GNB constitute a major challenge for current medical practice due to limited therapeutic options for these infections and there are no established guidelines for their management.

Many CR GNB isolates are only susceptible to colistin; however, colistinpresents some drawbacks, which have discouraged its use in monotherapy. In this context, along with strong pre-clinical evidence of benefit in combining antimicrobials against CR GNB, the clinical use of combination therapy has been raised as an interesting strategy to overcome these potential limitations of a single agent[12]. The commonly used combination of COL, especially COL and MER against GNB is supported *in vitro* by high synergy and bactericidality rates[1]. Nevertheless, clinical studies validating *in vitro* findings are limited.

Table 3: Susceptibility profile of *Pseudomonas aeruginosa* isolates

Pseudomonas aeruginosa				
Antimicrobial agent	**MIC range mg/l**	**MIC_{50} mg/l**	**MIC_{90} mg/l**	**% Sensitivity**
Imipenem*	4 to ≥16	8	≥16	0
Meropenem*	8 to ≥16	≥16	≥16	0
Amikacin*	16 to ≥64	≥64	≥64	14
Tobramycin*	≥16	≥16	≥16	0
Gentamycin*	≥16	≥16	≥16	0
Colistin**	≤0.5 to 3	≤0.5	≤0.5	100
Tigecyclin***	NA	-	-	-
Ciprofloxacine*	≥4	≥4	≥4	0

Table 4: Susceptibility profile of *Enterobacteriaceae* spp isolates

Enterobacteriaceae spp.				
Antimicrobial agent	**MIC range mg/l**	**MIC_{50} mg/l**	**MIC_{90} mg/l**	**% Sensitivity**
Imipenem*	2 to ≥16	4	≥16	0
Meropenem*	4 to ≥16	≥16	≥16	0
Amikacin*	16 to ≥64	32	≥64	42
Tobramycin*	≥16	≥16	≥16	0
Gentamycin*	≤1 to ≥16	≥16	≥16	28
Colistin**	≤0.5	≤0.5	≤0.5	100
Tigecyclin***	≤0.5 to ≥8	2	≥8	57
Ciprofloxacine*	≥4	≥4	≥4	0

Note: MIC, Minimum inhibitory concentration; MIC_{50}, MIC (mg/l) required to inhibit the growth of 50% of organism, MIC_{90}, MIC (mg/l) required to inhibit 90% of organism, MICs (mg/l) were determined by broth dilution method, * MICs were interpreted in accordance with the CLSI, ** MICs were interpreted in accordance with EUCAST, *** MICs were interpreted in accordance with US-FDA, ND – Not Done, NA – Not Applicable

Table 5: MER and COL Etest MICs and synergy testing by Etest

Clinical Isolate	**MIC of MER**	**MIC of MER in combination**	**FICI of MER**	**MIC of COL**	**MIC of COL in combination**	**FICI of COL**	**FIC of combination**
A. baumannii	≥32	24	0.75	0.125	0.094	0.75	1.5 ind
P. aeruginosa	≥32	≥32	1	3	3	1	2ind
K. pneumoniae	8	8	1	0.25	0.25	1	2ind
E. coli	4	4	1	0.125	0.125	1	2 ind
Enterobacter cloacae	4	3	0.75	0.25	0.19	0.76	1.51 ind

Note: FICI – Fractional inhibitory concentration index, ind – indifference

Table 6: Outcome of patients treated with COL-carbapenem combination associated with type of infection and causative pathogen

Carbapenem resistant clinical isolates	**Total (*n*)**	**Success (%)**	**Successful outcome (%)**			
			Respiratory Infection	**Blood stream Infection**	**Soft Tissue Infection**	**Urinary Tract Infection**
Acinetobacter spp.	19	11(57.9)	3/9 (33.3)	4/4 (100)	4/6 (66.7)	-
Pseudomonas aeruginosa	7	3 (42.9)	0/3 (0)	2/2 (100)	0/1 (0)	1/1 (100)
Enterobacteriaceae spp.	7	6 (85.7)	-	6/6 (100)	-	0/1 (0)
Total	33	20 (60.6)	3/12 (25)	12/12 (100)	4/7 (57.1)	1/2 (50)

In this study, we examined the clinical effectiveness of COL-carbapenem combination against CR GNB infection in 33 hospitalized patients. On evaluating the patient characteristics it was observed that all the patients were exposed to multiple antibiotics, 93.9% were admitted to ICU, and overall long hospital stay. These are some of the risk factors making them more vulnerable to acquiring CR GNB infection [13, 14]. These risk factors along with underlying comorbidities indicated the patients group in the present study to be critically ill. Looking at the susceptibility profile of the clinical isolate. It could be ascertained that there were very few treatment options for serious infection caused by CR GNB. 90.9% positivity of EDTA-disk synergy test suggested that carbapenem resistance was largely conferred by MBLs in the present study cohort.

Amongst COL-carbapenem combination, MER was more preferred over imipenemin combination therapy. Hence, COL-MER combination was used in Etest, to evaluate it's in *vitro* effectiveness against CR GNB. All isolates selected for *in vitro* sensitivity testing were found to be NDM producers, whereas clinical isolate of *Acinetobacter baumannii* carried multiple carbapenem resistance genes along with NDM. *In vitro* synergy testing showed indifferent interactions between COL and MER against these isolates. Findings of our study are in accordance with recent observations of time-kill experiment demonstrating no bactericidal effect of COL-MER against VIM- and NDM- producing *Klebsiella pneumonia* [15].

They are also in partial agreement with the previous report showing no marked synergy or borderline synergy of colistin in combination

with carbapenem against CR *Acinetobacter baumannii* isolates producing OXA-23[16].

Although no synergistic activity was demonstrated against our isolates by COL-MER combination, all isolates were sensitive to colistin with MIC of COL as low as ≤0.5mg/l (except one *Pseudmonas aeruginosa* isolate from respiratory infection, Colistin MIC-3 mg/l). Despite this fact clinical success of 60.6% was observed in patient receiving COL-carbapenem combination therapy against CR GNB infections. Probable pharmacokinetic (PK) concerns of colistin might be related to the failure to improve infections caused by sensitive microorganisms. In 2010,Imberti*et al*. reported plasma colistinC_{max} at a steady state of 1.15–5.14 mg/l (Dosage of CMS – 3 MIU per 8 or 12 h) and 0.68–4.65 mg/l (Dosage of CMS – 2MIU per 8 h), respectively, in critically ill patients with moderate-to-good renal function[17]. However, colistin has limited tissue penetration when administered intravenously and may not achieve adequate concentrations in an important foci of infection such as respiratory tract [18]. In addition, Imberti *et al*. reported undetectable colistin concentration (limit of detection, 50 ng/ml) in bronchoalveolar lavage (BAL) at 2 h after the start of the CMS infusion[17]. Hence, with knowledge PK concerns of colistin and MIC of colistin observed in our isolates, we can only postulate why clinical effectiveness of COL-carbapenem was low for respiratory and soft tissue infection, whereas high clinical effectiveness was observed for BSI by CR GNB. However, colistin is reported to attain higher concentration in lung with alternate route of administration (nebulization), which may be used to improve clinical effectiveness of the therapy[19].

Amongst the carbapenemases conferring resistance to carbapenem in GNB, NDMs are the latest carbapenemases to be recognized and since 2008 have been reported worldwide not just in *Enterobacteriaceae* spp. But also in non-fermenters such as *Acinetobacter baumannii* with multiple other carbapenem resistance genes[20, 21]. This rapid spread of NDM attains significant global attention due to their extensive drug resistant phenotypes and with the Indian subcontinent being clearly a main reservoir of NDM producers more studies addressing NDM producing CR GNB are needed[22]. There are several limitations of our study. First, our study employed small number of patients ata single tertiary care hospital; therefore generalization to other clinical setting is limited. Second, only representative isolates were tested for *in vitro* synergy of COL-MER combination. Third, there is lack of pharmacokinetic and pharmacodynamic data.

CONCLUSION

Overall 60.6% clinical success was observed in patients receiving COL-carbapenem combination against CR GNB infection. Against NDM producing CR GNB, interaction between COL and MER was found to be indifferent despite reports of enhanced activity of colistin in combination with meropenem. Clinical success rate of 100% in BSI, 57.1% in soft tissue infection and lowest clinical success rate of 25% in respiratory infection was probably attributed to the pharmacokinetic concerns of colistin. Considering the dearth of data on effective antimicrobial combination against NDM producing isolates, more *in vitro* and *in vivo* studies are needed in order to determine the optimal antimicrobial therapy for patients with NDM producing CR GNB infections.

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