ORIGINAL RESEARCH PAPER

Microbiology

STUDY OF DUAL IN VITRO ACTIVITY OF COLISTIN & MEROPENEM AGAINST MULTIDRUG RESISTANT GNB

KEY WORDS: Gram negative, Multi-drug resistant, Colistin, Meropenem

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ABSTRACT

Background: Bacterial resistance towards antibiotics is a clinical threat because it increases the problem of infectious disease. Currently colistin is increasingly being used against multidrug resistant gram negative bacteria which include *Pseudomonas aeruginosa,,Acinetobacter baumanii, Escherichia coli, Klebsiella pneumoniae & Salmonella enterica*. [1]. Combination antibiotic therapy is frequently used to treat severe gram negative infections though controversial & debatable.

Methods: Total 200 MDR GNB isolates were included in the study which were further tested for their susceptibility to Colistin & Meropenem by Kirby Bauer Disc Diffusion Test.

Results: Of 200 MDR GNB isolates, 69(34.5%) isolates showed resistance to Colistin.Out of 69 Colistin resistant isolates, 55(79.71 %) were resistant to Meropenem as well.

Conclusion: As multidrug resistance is emerging rapidly,measures are necessary to restrict indiscriminate use of antimicrobials against common infections & adherence to the hospital antibiotic policy, which will further limit dissemination of bacteria that accumulate resistance to both colistin & carbapenems &/or to multiple antibiotics.

INTRODUCTION

Bacterial resistance towards antibiotics is a clinical threat because it increases the problem of infectious disease.Concern regarding multidrug resistant bacteria (MDR),especially nosocomial pathogens is attracting more interest because new drugs to overcome resistant bacteria in the drug development pipeline are not readily available.Because of irrational use of antibiotics pathogens can develop and share resistance to common antimicrobials and the development of new drugs appears distant.This growing resistance has rekindled interest in Colistin,one of the oldest antibiotics.The use of colistin against panresistant nosocomial infections caused especially by *Pseudomonas and Acinetobacter spp.* has been reported recently.

Multi-resistance in Gram-negative bacteria, including strains resistant to carbapenems, is also emerging as a global health issue [2], [3]. Now clinical isolates with mutational fluoroquinolone resistance and metallo- -lactamases are being seen with increasing frequency worldwide [4]. Some species such asAcinetobacter baumannii strains only susceptible to polymyxins, have become a common problem especially in intensive care units [5].

Colistin, an old antibiotic also known as polymyxin E, has attracted more interest recently because of its significant activity against multi-resistant *P. aeruginosa*, *Acinetobacter baumannii* and *Klebsiella pneumoniae*, and the low resistance rates to it.When the use of a -lactam, aminoglycoside, or quinolone is ineffective, the polymyxins, particularly colistin, remain drugs of last resort [6]. Recent studies suggest that colistin administered as monotherapy or combination therapy is an effective and safe antimicrobial agent for multidrug-resistant Gram-negative bacteria infections. [7].Colistin resistant organisms are reported in various parts of world,including resistance of *Pseudomonas aeruginosa* in cystic fibrosis from UK[8], carbapenemase producing *Klebsiella pneumoniae* resistant to colistin [9,10,11], *Acinetobacter baumanii* [12,13], and polymyxin resistant *Escheichia coli* [11,14]. Increasing numbers of reports regarding colistin resistant bacteria indicates a developing threat to future treatment options for diseases caused by gram negative bacteria.

Combination antibiotic therapy is frequently used to treat severe gram negative infections though controversial & debatable.Definitive combination therapy is recommended for carbapenemase producing enterobactericeae & should also be considered for severe infections with *Pseudomonas* & *Acinetobacter* spp. when B-lactams cannot be used.

In this study, we report spectrum of multidrug resistant gram negative bacilli (MDR GNB) isolated from various clinical specimens.We also evaluate in vitro activity of colistin against these MDR GNB & further study in vitro activity of Meropenem against Colistin resistant MDR GNB.

MATERIALS AND METHODS

A prospective study was conducted at MIMER medical college, Talegaon –Dabhade, Pune during the period of June 2016 to October 2016 after obtaining approval from institutional ethical committee. Samples received in Microbiology laboratory were cultured on Blood agar & MacConkey agar. GNB isolates obtained were identified as MDR by using CDC Criteria (Isolate nonsusceptible to atleast 1 agent in > 3 antimicrobial categories) by Kirby Bauer Disk Diffusion Susceptibility Test. GNB isolates susceptible to antimicrobials in > 3 antimicrobial categories were excluded by the study.

Total 200 MDR GNB isolates were included in the study & were tested for their susceptibility to Colistin (10 g) (Himedia Mumbai) by Kirby bauer disk diffusion test.Colistin resistant isolates were further tested for Meropenem susceptibility. Zone diameters were interpreted according to CLSI guidelines [15].

RESULTS

Spectrum of MDR GNB isolated

A total of 200 MDR GNB were included in the the study.A set of standard biochemical reactions was used for identification of GNBs.

Colistin susceptibility testing

Table 1- Organism wise Colistin susceptibility testing results

MDR ORGANISM	Total(n=200)	Colistin resistant (n=69) (%)
Escherichia coli	64	24 (34.78)
Pesudomonas aeruginosa	45	13 (18.84)
Klebsiella pneumoniae	44	15 (21.73)
Citrobacter koseri	32	12 (17.39)
Acinetobacter baumannii	13	5 (7.24)
Proteus mirabilis	2	0 (0)

Meropenem resistance among colistin resistant isolates (Fig.1)

Out of 69 Colistin resistant isolates, 55(79.71 %) were resistant to Meropenem as well. A total of 27.5% isolates were found to be resistant to both meropenem and colistin. Isolates resistant to both Colistin and Meropenem constituted *Escherichia coli* (22/55=40%),
Klebsiella pneumoniae (13/55=23.63%),*Citrobacter koseri* (7/55=12.72%), *Pseudomonas aeruginosa* (10/55=18.18%) & *Acinetobacter baumannii* (3/55=5.45%).

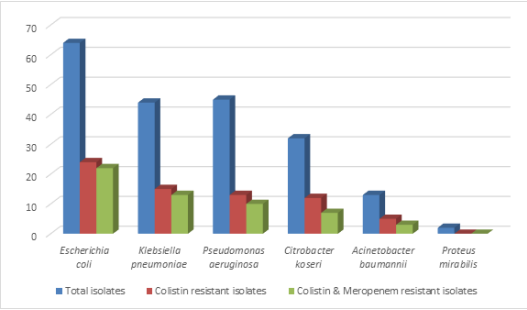

Fig.1 Comparison of the total number of isolates of each bacterial species with their Colistin resistance and Meropenem resistance.

DISCUSSION

Knowledge of antimicrobials to which bacteria are susceptible is essential to overcome the problem of evolving bacterial resistance to commonly used antimicrobials. Interest in Colistin has reemerged because of its antibacterial activity that finds use against many carbapenem resistant bacteria (16-19).

Reports on Colistin resistant bacteria from various parts of the world suggest that there is developing resistance towards Colistin among gram negative bacteria.A study of *Acinetobacter baumanii* from Spain suggest that among 115 isolates, 19 % were resistant [20], in Korea, 27.9% of 214 isolates were resistant [21] and in Australia 93.8% of 16 isolates were heteroresistant to colistin [22]. In present study,we detected 7.24% of MDR *Acinetobacter* isolates showing resistance to Colistin which is lesser than results of previous studies [20-22]. *Klebsiella pneumoniae* studies indicate that 18 isolates obtained from patients in Greece [23], 55 [6.8%] of 221 from South Korea [24] were resistant to colistin.In our study,we detected 21.73% of Colistin resistant MDR *Klebsiella pneumoniae* .This is in concordance with the study from Australia which showed 6 [27%] of 22 Colistin resistant *Klebsiella pneumoniae* [25]. *Pseudomonas aeruginosa* from patients with cystic fibrosis may have resistance to colistin [26].

We found colistin resistance in highest number from *Escherichia coli* isolates.Also combined Colistin & Meropenem resistance was found in significant number from *E.coli* isolates followed by *Klebsiella* & *Pseudomonas* isolates.

27.5 % MDR GNB isolates in our study showed resistance to both colistin & Meropenem , narrowing the treatment option for the patients infected with these organisms. This percentage of both Colistin & Meropenem resistant isolates is somewhat higher than similar study from Tamilnadu [1].Moreover some isolates were resistant to all tested antimicrobials, which is a serious concern, further restricting the treatment options for infections caused by such strains.

Hence, measures are necessary to restrict indiscriminate use of antimicrobials & adherence to hospital antibiotic policy, to limit dissemination of bacteria resistant to both colistin & carbapenems &/or to multiple antibiotics.

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