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IN VITRO ACTIVITY OF COLISTIN AGAINST MULTIDRUG RESISTANT GRAM NEGATIVE BACILLI (MDR GNB) ISOLATED FROM VARIOUS CLINICAL SPECIMENS

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ABSTRACT

Introduction: 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] Colistin resistant organisms are reported in various parts of world.

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

Results: Isolates consisted of 64 *Escherichia coli*,45 *Pseudomonas aeruginosa*, 44 *Klebsiella pneumoniae*,32 *Citrobacter koseri*,13 *Acinetobacter baumanii* & 2 *Proteus mirabilis*. Of these, 69 (34.5%) were resistant to Colistin. Colistin resistance in highest number was detected in *Escherichia coli* isolates obtained from various samples.

Conclusion: As colistin resistance is emerging rapidly, measures are necessary to restrict indiscriminate use of antimicrobials & to adhere strictly to the hospital antibiotic policy. It is also the need of an hour that newer antimicrobials get discovered soon to tackle such MDR microorganisms.

KEYWORDS : Gram negative, Multi-drug resistant, Colistin

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. Morbidity and mortality because of gram negative MDR nosocomial pathogens is high. 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. 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]

Multi-resistance in other 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-β-lactamases are being seen with increasing frequency worldwide [4]. Some species such as*Acinetobacter 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 β-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.

In this study, we report spectrum of multidrug resistant gram negative bacilli (MDR GNB) isolated from various clinical specimens & evaluate in vitro activity of colistin against these 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. Various 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 which were further tested for their susceptibility to colistin after incubating them for 18-24 hours at 37^0c on Muller Hinton Agar by Kirby Bauer Disc Diffusion Test. Interpretation of results was done according to CLSI guidelines.[15]

Disk diffusion

Disk diffusion testing was done using 10 µg colistin disk (Himedia Mumbai) and the disk zone diameters were interpreted according to the CLSI guidelines for colistin (resistant ≤10 mm and susceptible ≥11 mm) [15] The bacterial inoculum was adjusted for disk diffusion, method strictly according to CLSI guidelines using 0.5 Mac Farland turbidity standard. Standard ATCC strains were used as controls for disk diffusion testing.

RESULTS

Spectrum of MDR GNB isolated

A total of 200 MDR GNB were included in the the study. Identification of GNB isolates was done by set of standard biochemical reactions for GNB. These isolates consisted of 64 *Escherichia coli*,45 *Pseudomonas aeruginosa*,44 *Klebsiella pneumoniae*,32 *Citrobacter koseri*,13 *Acinetobacter baumanii* & 2 *Proteus mirabilis*.

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)

Of the 200 isolates, 69 (34.5%) were resistant to Colistin and 131 (65.5%) were sensitive to it. 91(45.5%) isolates were resistant to all tested antimicrobials. Amongst total 64 MDR *E.coli* isolates,24 (34.78%) were resistant to Colistin & 40 were sensitive to it.Amongst total 45 MDR *Pseudomonas* isolates, 13 (18.84%) were resistant to Colistin & 32 were sensitive to it.Out of 44 *Klebsiella* isolates,15 (21.73%) were resistant to Colistin & 29 were sensitive. 12 (17.39%) out of 32 *Citrobacter* isolates were resistant to Colistin & 20 were sensitive. 5 (7.24%) out of 13 *Acinetobacter* isolates were resistant and 8 were sensitive to colistin.Both *Proteus mirabilis* isolates included in this study were sensitive to colistin.

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) However increasing development of resistance is a cause of concern.Colistin is used mainly against infections caused by gram negative bacteria including *Pseudomonas aeruginosa, Acinetobacter baumanii & Klebsiella pneumoniae*. Susceptibility & resistance breakpoints & dosage are another problems because of their geographic differences.

Reports on Colistin resistant bacteria from various parts of the world suggest that there is developing resistance towards Colistin among gram negative bacteria, although the mechanism of resistance is not clear. 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].

In present study, we have included different MDR gram negative bacilli isolated in our laboratory from various samples from different patients. We found colistin resistance in highest number from *Escherichia coli* isolates obtained from various samples.More over 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 against common infections & adherence to the hospital antibiotic policy, which will further limit dissemination of MDR bacteria.It is also the need of an hour that newer antimicrobials get discovered soon to tackle such MDR microorganisms.

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