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Original Article

Microbiology Section

Effectiveness of Minocycline in Multidrug Resistance Gram Negative Bacilli: A Cross-sectional Study

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ABSTRACT

Introduction: Antibiotic resistance is a global public health threat and remains a challenge for the physicians. Due to increased incidence of resistance to the commonly prescribed antibiotics, a newer drug or a re-emerge of an older class of antibiotic will be a choice of treatment of the Multidrug Resistant (MDR) organisms.

Aim: To determine the effectiveness of minocycline in MDR gram negative bacterial isolates by determining its Minimum Inhibitory Concentration (MIC) and to compare its effectiveness with imipenem and meropenem.

Materials and Methods: A cross-sectional periodical study was conducted during May 2016 to May 2017 using 150 non repetitive MDR gram negative bacterial isolates recovered from various clinical specimens sent to Central Laboratory, Department of Microbiology, Sri Ramachandra Medical College and Research Institute, SRIHER, Porur, Chennai, India. All the isolates were subjected for antibiotic susceptibility testing by disc diffusion method for the routine antibiotics and MIC determination by Epsilometry test (E-strip) for minocycline and meropenem and interpreted as per Clinical and Laboratory Standards Institute (CLSI) guidelines 2016. Statistical analysis was calculated with Open Epi using two by two table version 3.01.

Results: Out of the 150 study isolates minocycline was susceptible in 105 (70%) followed by imipenem and meropenem both susceptible for isolates 94 (62.7%). Minocycline was also susceptible among the 79 (84%) out of 94 of meropenem susceptible strains with a statistically significant p-value of <0.05. Similarly 26 (46.4%) out of 56 of meropenem resistant strains were susceptible for minocycline which was also statistically significant with a p-value <0.05.

Conclusion: Considering the cost of treatment with colistin which is the choice of treatment for carbapenemase producing gram negative bacteria's, minocycline can be considered as it is cheaper and less toxic. The possibility to switch from injectable to oral formulation is also possible with minocycline and so can also be considered as an alternative for colistin in such conditions.

Keywords: Colistin, E-strip, Meropenem, Minimum inhibitory concentration

INTRODUCTION

Antibiotic resistance is a global public health threat. Infections caused by Multidrug Resistant (MDR) bacteria continue to be a challenge for the physicians in treating their patients. The morbidity and mortality are on the rise of the hospitalised patients because of Hospital Acquired Infections (HAI), which are many times are due to Multi or Pan Drug Resistance (PDR) organisms which are very difficult to treat as there is no new antibiotics are available in the recent past [1]. World Health Organisation (WHO) estimates these infections to occur among hospitalised patients at a rate of 7-12% globally [2].

In India HAI is on the rise, so also the drug resistance. Many centres have reported on an average resistance to aminoglycosides ranging from 32.6-83.6%, resistance to β-Lactams (BL) and β-lactamase Inhibitors (BL-BLI) ranged from 41-80% [3]. The carbapenem resistance rate among Gram Negative Bacilli (GNB) is 36.4%, whereas resistance to quinolones is approximately 30% [4,5]. Percentage of Extended Spectrum Beta Lactamases (ESBL) producers range from 66.8-71.5% [6]. Colistin resistance rate is approximately 20% [7].

Minocycline is a second-generation tetracycline derivative, was first introduced in 1967 [8]. Minocycline acts by inhibiting the bacterial protein synthesis by binding to the 30s ribosomal subunit and specifically preventing the enzyme binding of aminoacyl-tRNA (transfer RNA) to its acceptor site, and are considered bacteriostatic agents [9]. Minocycline is a broad-spectrum antibiotic with a wide range of activity against aerobic and anaerobic gram positive cocci and gram negative organisms. In addition, they also have activity against *Mycoplasma*, Chlamydia, *Rickettsia,* Spirochetes, *Nocardia* and *Legionella* [10]. As there is increase in the incidence of resistance to all of the commonly prescribed antibiotics a newer drug or a re-emerge of an older class of antibiotic will be a choice in the treatment of these MDR organisms. In this pursue, the current study was undertaken to evaluate minocycline susceptibility in MDR GNB.

MATERIALS AND METHODS

A cross-sectional periodical study was conducted between May 2016 to May 2017 in the Department of Microbiology, South India a tertiary care centre after obtaining Institutional Ethics Committee (IEC) approval, REF: CSP-MED/16/JUN29/72.

Inclusion criteria: All the culture specimens sent to the laboratory during the study period growing non repetitive MDR GNB isolates were included in the study.

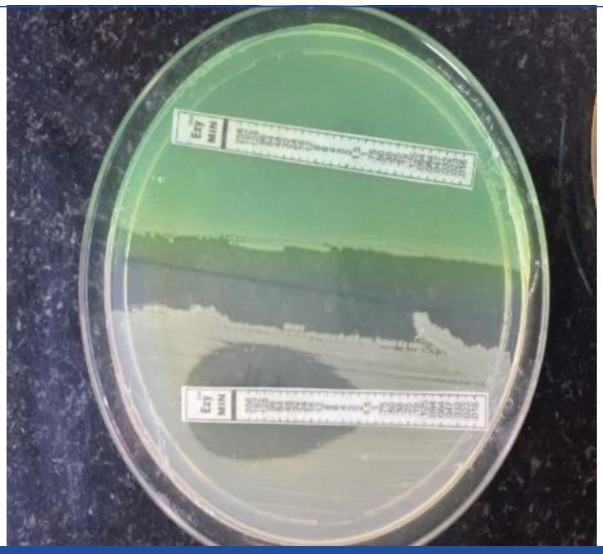
Exclusion criteria: Repetitive samples and highly susceptible (Non MDR) gram negative bacterial isolates were excluded from the study.

Study Procedure

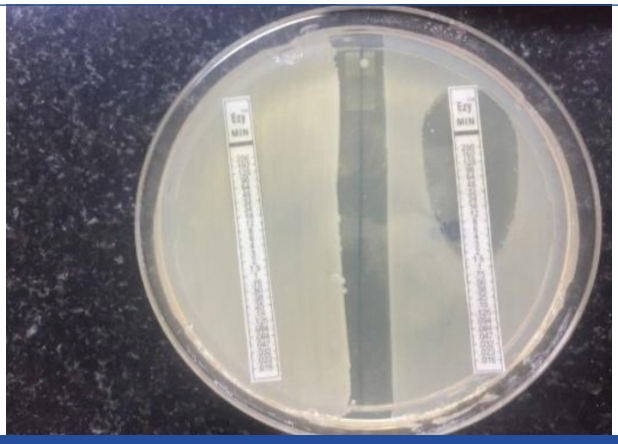
A total of 150 non repetitive MDR gram negative bacterial isolates from clinical specimens of exudates, Broncho-Alveolar Lavage (BAL), urine and blood were included [Table/Fig-1]. Study isolates were identified upto species level by conventional methods (Biochemical reactions like indole test, urease enzyme production, citrate utilisation. Triple sugar iron, manitol fermentation and motility test, phenyl alanine deaminase test, oxidase test, catalase test etc.,) and/automated methods (VITEK-2 system (Vitek2 GN-card; BioMerieux, Brussels, Belgium) [11]. Antibiotic susceptibility testing

of the isolates for ampicillin (10 µg), cephelexin (30 µg), cefotaxime (30 µg), cephalexin (30 µg), trimethoprim- sulfamethoxazole (1.25 µg and 23.75 µg), cefepime (30 µg), amikacin (30 µg), cefaperazone sulbactum (15/10 µg), piperacillin tazobactum (100 µg and 10 µg), nitrofurantoin (300 µg), ciprofloxacin (5 µg), levofloxacin (5 µg), imipenem (10 µg), meropenem (10 µg), ertapenem (10 µg) and tobramycin (10 µg) was done by disc diffusion method and interpreted as per Clinical and Laboratory Standards Institute (CLSI) guidelines 2016 [12]. MIC of the study isolates to minocycline, colistin and meropenem was done by Epsilometery strip (E-strip) obtained from HiMedia, Mumbai and interpreted as per CLSI guide lines 2016 [Table/Fig-2-4] [12]. *Escherichia coli* ATCC 25922 and *Pseudomonas aeruginosa* ATCC 27853 (Procured from HiMedia) were used as controls and the controls were satisfactory.

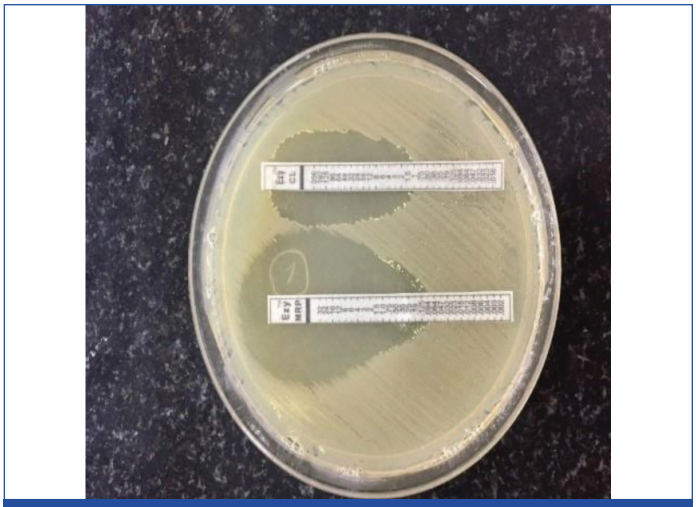
[Table/Fig-1]: MDR isolate.



[Table/Fig-2]: Minocycline E-strips with ATCC *Escherchia coli* and ATCC *Pseudomonas aeruginosa* controls.



[Table/Fig-3]: Minocycline E-strip- Showing resistant (left) and suceptible (right) to the test isolates.



[Table/Fig-4]: E-strip- isolates showing suceptiblity to meropenem and colisitin.

STATISTICAL ANALYSIS

Statistical analysis was calculated with Open Epi using two by two table version 3.01. The p-value was calculated by Chi-square test and p-value <0.05 was considered to be statistically significant.

RESULTS

A total of 150 gram negative bacterial isolates screened by disc diffusion as MDR from various clinical specimens, urine 84 (56%), pus 45 (30%), blood 13 (8.7%) and BAL 8 (5.3%), were included in this study. Out of these 150 samples, 87 were isolated from males and 63 from females (M:F ratio=1.4:1). The age of the study population ranged from one day to 94 years. (Mean age: 46.4 years). Bacterial isolates break up from the various clinical samples is shown in the [Table/Fig-5] and antibiotic susceptibility percentage of the study isolates for the routine antibiotics by disc diffusion method is shown in the [Table/Fig-6].

Organisms	Urine n (%)	Pus n (%)	Blood n (%)	BAL n (%)	Total n (%)
Escherichia coli	37 (44.1)	14 (31.1)	6 (46.1)	2 (25)	59 (39.3)
Klebsiella pneumoniae	21 (25)	11 (24.5)	3 (23.1)	3 (37.5)	38 (25.3)
Enterobacter spp.	2 (2.4)	2 (4.4)	0	1 (12.5)	5 (3.4)
Acinetobacter spp.	6 (7.1)	8 (17.8)	1 (7.7)	1 (12.5)	16 (10.7)
Proteus spp.	4 (4.8)	1 (2.2)	0	0	5 (3.4)
Providencia spp.	5 (6)	0	0	0	5 (3.4)
Pseudomonas spp.	8 (9.5)	6 (13.3)	1 (7.7)	1 (12.5)	16 (10.7)
Citrobacter spp.	0	0	1 (7.7)	0	1 (0.7)
Burkholderia cepacia	0	0	1 (7.7)	0	1 (0.7)
Morganella spp.	1 (1.1)	3 (6.7)	0	0	4 (2.7)
Total	84	45	13	8	150

[Table/Fig-5]: Specimen wise distribution of the bacterial isolates.

Antibiotics	Sensitive (%)	Resistant (%)	Total
Ampicillin	-	100	150
Cephalexin	-	100	150
Cefuroxime	-	100	58
Cephotaxim	-	100	150
Ceftazidime	-	100	92
Cefepime	13 (8.7)	137 (91.3)	150
Cefaperazone sulbactum	60 (40)	90 (60)	150
Piperacillin tazobactum	60 (40)	90 (60)	150
Amikacin	108 (72)	42 (28)	150
Tobramycin	108 (72)	42 (28)	150
Ciprofloxacin	13 (19.7)	53 (80.3)	66
Ofloxacin	29 (19.3)	131 (87.3)	150

Imepenem	100 (66.6)	50 (33.4)	150
Meropenem	100 (66.6)	50 (33.4)	150
Polymyxin B	129 (95.5)	6 (4.5)	135

[Table/Fig-6]: Antibiotic susceptibility results by disc diffusion.

All the test isolates were subjected for MIC determination by Epsilometry test (E-strip) for minocycline, colistin and meropenem [Table/Fig-7-9]. Susceptible percentage of test isolates were as follows: A total of 129 (95.5%; n=135, *Protea* group, *Burkholderia* spp., were excluded) were susceptible for colistin followed by 105 (70%) isolates for minocycline and 94 (62.7%) of the test isolates had shown susceptibility to meropenem.

Isolates	Susceptible			Resistant	Total (%)
	Sensitive (≥4 µg)	Intermediate (8 µg)	Total (%)	≤16 µg	
Escherichia coli	43	9	52 (88.1)	7 (11.9)	59 (39.3)
Klebsiella pneumoniae	22	8	30 (78.9)	8 (21.1)	38 (25.3)
Enterobacter spp.	4	0	4 (80)	1 (20)	5 (3.3)
Acinetobacter spp.	12	0	12 (75)	4 (25)	16 (10.7)
Proteus spp.	1	-	1 (20)	4 (80)	5 (3.3)
Providencia spp.	-	-	-	5 (100)	5 (3.3)
Pseudomonas spp.	2	1	3 (18.8)	13 (81.2)	16 (10.7)
Citrobacter spp.	1	-	1 (100)	-	1 (0.7)
Burkholderia cepacia	-	1	1 (100)	-	1 (0.7)
Morganella spp.	1	-	1 (25)	3 (75)	4 (2.7)
Total	86 (57.3%)	19 (12.7%)	105 (70%)	45 (30%)	150 (100)

[Table/Fig-7]: Minocycline susceptibility for MDR isolates by E-strip.

Organisms	Sensitive	Resistant	Total
Pseudomonas spp.	14 (87.5%)	2 (12.5%)	16
Acinetobacter spp.	14 (87.5%)	2 (12.5%)	16
Citrobacter spp.	-	1 (100%)	1
Enterobacter spp.	5 (100%)	-	5
Escherchia coli	58 (98.3%)	1 (1.7%)	59
Klebsiella pneumoniae	38 (100%)	-	38
Total	129 (95.5%)	6 (4.5%)	135

[Table/Fig-8]: Colistin susceptibility by E-strip.

Organisms	Sensitive	Resistant	Total
Pseudomonas spp.	4 (25%)	12 (75%)	16
Acinetobacter spp.	6 (37.5%)	10 (62.5%)	16
Burkholderia cepacia	-	1 (100%)	1
Citrobacter spp.	-	1 (100%)	1
Enterobacter spp.	3 (60%)	2 (40%)	5
Escherchia coli	53 (89.8%)	6 (11.1%)	59
Klebsiella pneumoniae	21 (55.3%)	17 (44.7%)	38
Morganella spp.	3 (75%)	1 (25%)	4
Providencia spp.	1 (20%)	4 (80%)	5
Proteus spp.	5 (100%)	-	5
Total	96 (64%)	54 (36%)	150

[Table/Fig-9]: Meropenem susceptibility by E-strip.

Comparison of meropenem susceptibility by disc diffusion and E-strip method: A total of 100 (66.7%) isolates were susceptible by disc diffusion method. Whereas by E-strip method 94 (62.7%) of them were susceptible. All the test isolates (50) which were resistant by disc diffusion was also found to be resistant by E-strip method. Among the six which were susceptible by disc diffusion, the MIC ranged between 4-12 µg.

Minocycline susceptibility and its statistical significance: The comparison of susceptibility of minocycline and meropenem is shown in [Table/Fig-10]. Among the 94 meropenem susceptible strains, 79 (84%) showed susceptible to minocycline with statistical significant p-value <0.05. A total of 26 (46.4%) out of 56 the meropenem resistant strains susceptible to minocycline with statistical significant p-value of <0.05.

Drug	Susceptible n (%)	Resistant n (%)	Total
Meropenem	94 (62.7%)	56 (37.3%)	150
Minocycline	105 (70%)	45 (30%)	150
	Minocycline		
Drug	Susceptible n (%)	Resistant n (%)	Total
Meropenem susceptible	79 (84%)	15 (16%)	94
Meropenem resistant	26 (46.4%)	30 (63.6%)	56
Total	**105**	**45**	**150**

[Table/Fig-10]: The comparison of susceptibility of minocycline and meropenem for MDR gram negative bacilli.

DISCUSSION

In this era of MDR infections, especially in the hospitalised patients and also with limited antimicrobials available for managing such patients, minocycline is thought to be an effective alternate at present. Akers KS et al., in his study at military centre against MDR *Acinetobacter* spp showed a favourable clinical outcome with minocycline [13]. A study done by Shankar C et al., against with *Klebsiella pneumoniae* reported 71.4% susceptibility to minocycline [14].

Colton B et al., reported 50% of colistin resistant strains were susceptible to minocycline but in this study, colistin was found to be highly efficacious against MDR strains rather than minocycline in-vitro [15]. A comparative study done by Flamm RK et al., with tetracycline, doxycycline and minocycline reported that minocycline was found to be increasingly effective against MDR organisms [16]. In current study, 70% of MDR organisms were susceptible to minocycline [16]. Similarly study done by Vento TJ et al., with MDR *E. coli* reported as minocycline was effective against 85% of study isolates [17]. In this study, 88.1% isolates showed susceptibility to minocycline. Ritchie DJ and Garavaglia-Wilson A, in their study with MDR *Acinetobacter* spp in 2014 has reported 80% susceptibility to minocycline, whereas, in this study, 75% of MDR *Acinetobacter* spp. were susceptible to minocycline [18]. Castanheira M et al., again with MDR *Acinetobacter* has given susceptibility to colistin as 98.8% and minocycline 79.1% [19]. In present study also, similar findings were observed. They also compared the efficacy of minocycline for various MDR GNB had shown the following: 81.4% susceptibility for *Citrobacter* followed by 81.4% for *Enterobacter*, 79.1% for *Acinetobacter*, 78.8% for *Escherichia coli* and 75.7% for *Klebsiella* spp. [19]. In this study, 80% of the MDR *Enterobacter* was susceptible to minocycline followed by *Acinetobacter* spp. (75%), *E. coli* (88.1%) and *Klebsiella* spp. 57.9%. Adibhesami H et al., had reported only 56% of MDR *Acinetobacter* susceptible to minocycline [20]. In contrast, this study had about 75% susceptibility. Vento TJ et al., had found 85% of MDR *E. coli* were susceptible to minocycline [17], whereas present study had only 88.1% susceptibility. Yang Y et al., has stated in their study that 82.4% of their study isolates i.e., *Acinetobacter* spp. were susceptible to minocycline [21]. In another study by Parveen A and Bhat P, reported 40.5% of their *Acinetobacter* spp. isolates susceptible to minocycline [22]; whereas in this study, 75% of *Acinetobacter* was susceptible to minocycline. Most of the findings done by various authors in different studies periods in different parts of the world have reported the efficacy of

minocycline almost equal to the present study done in 2016 [22]. A systematic review done by Fragkou PC et al., for MDR *Acinetobacter baumannii* showed a significant clinical and microbiological success rates following minocycline treatment as 72.6% and 60.2%, respectively [23]. As the treatment of choice in MDR GNB usage of carbapenem like meropenem, imipenem and ertapenem are often practiced. In present study, among the 94 meropenem susceptible bacterial isolates, 84% were also susceptible for minocycline also and so it is very important to also consider minocycline as an alternative for meropenem where minocycline is cost-effective and least toxic comparatively. Similarly 26 (46.4%) of meropenem resistant strains were susceptible to minocycline, hence can also be used in conditions where organisms are producing carbapenemase and can replace colistin in these conditions.

Limitation(s)

Being a periodic study, only 150 MDR gram negative organisms were tested and colistin susceptibility would have been compared by performing microbroth dilution method.

CONCLUSION(S)

Selection of appropriate and ideal antibiotics remains a main key for the treatment of MDR organisms. As minocycline is showing efficacy in carbapenemase producing GNB, minocycline can be used as an alternative for colistin in these conditions especially in patient admitted in Intensive Care Units as minocycline is cost-effective and less toxic. Minocycline can also replace the carbapenems in the treatment except for its bacteriostatic action.

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