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Susceptibility of Minocycline against Carbapenem Resistant Gram-Negative Bacilli

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ABSTRACT

Objective: Pathogens resistant to ≥3 antimicrobial agents are referred to multi-drug resistant (MDR) pathogens. Although several antibiotics are available, antibiotics with broader spectrum of activity is required. The present study aimed to evaluate the in vitro susceptibility of minocycline against Carbapenem resistant gram-negative bacilli and its concordance sensitivity as compared to Tigecycline.

Methods: Non-repetitive, consecutive isolates carbapenem resistant gram-negative bacilli including MDR *Klebsiella pneumoniae* (*K. pneumoniae*) and *Acinetobacter spp*. were used for evaluation using Epsilometer test (E-test). The minimum inhibitory concentration (MIC) cut-off range for minocycline was based on Clinical and Laboratory Standards Institute (CLSI, 2016) and the interpretation (sensitive<4, intermediate=8, resistant ≥16) was considered valid for both, *Enterobacteriaceae* and *Acinetobacter*. Concordance susceptibility of minocycline was compared with that of tigecycline.

Results: Overall, 18 isolates from MDR *Acinetobacter spp*., 20 isolates from MDR Enterspp.obacter spp. (18 *E. coli* isolates and 2 Enterobacter, undefined), and 63 isolates of MDR Klebsiella spp. (58 *K. pneumoniae* isolates and 5 Klebsiella isolates) were evaluated. In vitro, sensitivity of minocycline was 50.0% (9/18) in Acinobacter species; 45.0% (9/20) in *Enterobacter spp*. and 36.5% (23/63) in Klebsiella spp., respectively. Of the 101 isolates used, concordance between tigecycline and minocycline was observed in 42 isolates (41.6%) and sensitivity to both minocycline and tigecycline was observed in 12 isolates (28.6%). The Cohen Kappa values showed that the overall concordance between tigecycline and minocycline was 0.11 (non-slight agreement).

Conclusion: Minocycline is effective against all the three carbapenem resistant gram-negative bacteria included in the study. *J Microbiol Infect Dis 2018; 8(4):140-146.*

Keywords: *minocycline, Titgecycline, sensitivity, carbapenem resistant gram-negative bacilli, in vitro susceptibility*

INTRODUCTION

Treatment of infections caused by bacteria resistant to antibiotics has remained a challenge over the years for the medical community [1,2]. These antibiotic-resistant micro-organisms were termed as "ESKAPE" pathogens (*Enterococcus faecium*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Acinetobacter baumanii*, *Pseudomonas aeruginosa*, and *Enterobacter spp*.) as per the update published by the Infectious Diseases Society of America. This terminology represented those pathogens which easily escape the effects of antibiotics and cause persistent infection [3]. More pathogens are becoming resistant to antibiotics and those which are resistant to several antibiotics have been termed as multi-drug resistant (MDR) pathogens. Although these include both gram-positive and gram-negative bacteria, the infections caused by gram-negative MDR bacteria are more serious. However; MDR and extensive drug resistant (XDR) pathogens are not the same. While there is no internationally accepted definition which may differentiate these two terms, MDR may be described as resistance of at least one pathogen to three or more antimicrobial agents and XDR may be described as resistance of least one pathogen to all but two or fewer antimicrobial agents [4,5]. Infections due to gram-negative bacteria may

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result in more severe infections and increased duration of hospital stay [6, 7].

Evaluation of MDR bacterial isolates in a prospective study from South India showed that 11% (23 of 210 isolates) were carbapenem-resistant. These included *E. coli* (six isolates) *K. pneumoniae* (three isolates), P. aeruginosa (five isolates) and A. baumannii (nine isolates) [1]. According to the Center for Disease Dynamics, Economics and Policy (CDDEP), Methicillin-resistant *Staphylococcus aureus* (MRSA) resistance was observed in 47% Indian population in 2014 while 13% of the Indian population was *E. coli* resistant to carbapenems in 2013. [8] Of the many gram-negative bacteria, Acinetobacter is a complex genus which causes nosocomial infections. [9] Acinetobacter has an extreme drug resistance (XDR) phenotype which demands early initiation of therapy. A case series conducted by Wong et al reported up to 70% mortality rate in individuals with infections caused by XDR strains [9].

Although several antibiotics are available for the treatment of bacterial infections, an antibiotic with a broader spectrum of activity is the need of the hour. Minocycline, a tetracycline is one such broad spectrum antibiotic which inhibits the protein synthesis in bacteria by binding to the 30S ribosomal subunit [10]. Minocycline is effective against resistant clinically important pathogens, has excellent pharmacokinetic properties, and limited toxicity when used in serious infections [11]. Due to its clinically relevant therapeutic effect against MDR gram–negative infections, most studies have evaluated its effect against *Acinetobacter baumannii* where it has demonstrated good in vitro activity [12]. Additionally, minocycline is effective for the treatment of minocycline-susceptible MDR *Acinetobacter baumannii* [13]. Tigecycline is another tetracycline which is a glycylcycline and an analogue of minocycline. Tigecycline exhibits a unique mechanism of action against gram-negative bacteria viz. MDR A. baumannii, P. aeruginosa, *Escherichia coli* (*E. coli*), *K. pneumoniae*, *K. oxytoca*, and *Enterobacter aerogenes* (*E. aerogenes*) when evaluated for its in vitro activity. [14,15]

In this scenario, the present study aimed to evaluate the in vitro susceptibility of minocycline against Carbapenem resistant gram-negative bacilli. The study further evaluated the concordance of Minocycline sensitivity in carbapenem resistant strains with Tigecycline.

METHODS

The present study evaluated the in vitro susceptibility of minocycline in non-repetitive, consecutive isolates carbapenem resistant gram-negative bacilli including MDR *Klebsiella pneumoniae* (*K. pneumoniae*) and *Acinetobacter spp*. using Epsilometer test (E-test).

The study was conducted in the Department of Microbiology, Aster Medcity, Kochi, Kerala between March 2016 and October 2016.

Study Variables

Minimum Inhibitory Concentration (MIC): The MIC cut-off range for minocycline was based on Clinical and Laboratory Standards Institute (CLSI, 2016) and the interpretation (sensitive <4, intermediate =8, resistant ≥16) was considered valid for both, *Enterobacteriaceae* and *Acinetobacter* [16].

Tigecycline's MIC was interpreted as per the European committee on antimicrobial susceptibility testing (EUCAST, 2016) guidelines. The MIC (sensitive=1; resistant=4) was determined as per the susceptibility pattern provided by the automated system BD Phoenix/Vitek [17].

The MIC values of the bacteria were interpreted as S (Sensitive), I (Intermediate) or R (Resistant) by comparing the breakpoint values of minocycline with the criteria recommended by CLSI. MIC was marked at the point where ellipse intersects the scale and the MIC value at complete inhibition of all growth was also marked. In the presence of variation in the intersect on either side of the strip, the greater value MIC was marked. Any growth at the edge of the strip was ignored.

Concordance: In addition to evaluation of MIC values, concordance susceptibility of minocycline was compared with that of tigecycline. Concordance was defined as the probability that if tigecycline is sensitive, minocycline is also sensitive versus if tigecycline is resistant; minocycline is also resistant for samples of Carbapenem resistant gram-negative bacilli. The MIC of tigecycline was evaluated only for the evaluation of concordance

and not for the comparison of antibacterial effect of minocycline and tigecycline. Further, concordance was reported using Cohen Kappa values.

Preparation of Inoculum and Application of E-Strips

The inoculum was prepared using three to four individual test strain colonies by emulsifying and transferring to a tube of saline. The turbidity was compared to that in the 0.5 McFarland standards and turbidity was adjusted accordingly. Inoculation was then done in the Mueller Hinton Agar media by using a sterile cotton swab dipped into the inoculum. The swab was streaked over the entire surface and the inoculation completed. E-strips were applied to the agar surface facing upwards with the scale visible and 'E' end at the edge of the plate. The entire procedure was repeated for Quality Control Strain (*E. coli* ATCC 25922). Both the plates were incubated at 37°C for 18-24 hrs. Uniform depth of agar plates was 4 mm and the materials used to carry out all evaluations included forceps, sterile cotton swabs, and sterile normal saline. The E- test (supplied by BioMerieux, New Delhi, India) detects MIC ranging from 0.016-256 µg/mL (Figure 1) [18].

Statistical Analysis

Descriptive statistics were used to represent all variables. The proportion of isolates which were sensitive or resistant was expressed as numbers and percentages. Cohen Kappa values were used to report concordance. Kappa values of ≤0 indicated no agreement and 0.0-0.20 indicated none to slight agreement, 0.21-0.40 indicated fair, 0.41-0.60 moderate, 0.61-0.80 substantial, and 0.81 1.00 as almost perfect agreement.

RESULTS

Isolate Distribution

Overall, 18 isolates from MDR *Acinetobacter spp*., 20 isolates from MDR *Enterobacter spp*. (18 *E. coli* isolates and two Enterobacter isolates), and 63 isolates of MDR Klebsiella spp. (58 *K. pneumoniae* isolates and five Klebsiella isolates: all five were Klebsiella oxytoca) were evaluated for in vitro activity of minocycline. The isolate-wise and sample-wise distribution is presented in Table 1.

Susceptibility of Minocycline

The in vitro susceptibility of minocycline against *Klebsiella* showed sensitivity in 36.5% (23/63) of the isolates while 41.2% (26/63) of the isolates were resistant. Additionally, minocycline demonstrated sensitivity in 50% (9/18) of *Acinetobacter spp.* while 33.3% (6/18) were resistant. Among the 20 *Enterobacter spp*., sensitivity was observed in 9 isolates (45%) and resistance was observed in 5 isolates (25%). The detailed susceptibility is presented in Table 2.

Degree of Concordance

Of the total 101 isolates used in the present study, concordance between tigecycline and minocycline was observed in 42 isolates (41.5%). Of these, 69.0% of isolates were resistant towards both tigecycline and minocycline (29/42), 28.6% isolates were sensitive (12/42), and 2.4% isolates (1/42) had intermediate susceptibility towards both tigecycline and minocycline. The Cohen Kappa values showed that the overall concordance between tigecycline and minocycline was 0.11 (non-slight agreement). The detailed degree of concordance and Cohen Kappa values are presented in Table 3.

In addition, it is important to note that a few isolates which showed intermediate/resistant susceptibility towards tigecycline were sensitive to minocycline. Of these, highest proportion of such susceptibility was observed with Klebsiella spp. wherein 20 of the 63 isolates (31.7%) which were sensitive towards minocycline, showed either intermediate or resistant susceptibility towards tigecycline. The detailed susceptibility is presented in Table 4.

Distribution of Isolates	Number (N)
Isolate-wise Distribution	
Klebsiella	63
K. pneumoniae	58
Klebsiella	5
Enterobacteriaceae	20
E. coli	18
Enterobacter, undefined	2
Acinetobacter spp.	18
Sample-wise Distribution	
Respiratory	15
Urine	40
Pus/Tissue	14
Blood	8
Other Body Fluids	24

Table 2. In vitro Susceptibility of Minocycline towards Carbapenem resistant Gram-negative bacteria.

Isolate	Sensitive n (%)	Intermediate n (%)	Resistant n (%)
Klebsiella	23 (36.5)	14 (22.2)	26 (41.2)
K. pneumoniae	22 (37.9)	12 (20.6)	24 (41.3)
Klebsiella spp.	1 (20)	2 (40)	2 (40)
Enterobacteriaceae	9 (45)	6 (30)	5 (25)
E. coli	9 (50)	5 (27.7)	4 (22.2)
Enterobacter, undefined	-	1 (50)	1 (50)
Acinetobacter spp.	9 (50)	3 (16.6)	6 (33.3)

Table 3. Degree of Concordance in the Susceptibility to Minocycline and Tigecycline and against *K. pneumoniae* and *Acinetobacter spp*..

Isolates	Concordance n, (%)	Sensitive Concordance* n, (%)	Cohen Kappa Values^	Concordance
Klebsiella	25 (39.6)	3 (12)	0.052	None-Slight
K. pneumoniae	23 (39.6)	3 (13)		
Klebsiella	2 (40)	-		
Enterobacteriaceae	8 (40)	6 (75)	-0.004	No agreement
E. coli	6 (33.3)	6 (100)		
Other Enterobacter spp.	2 (100)	-		
Acinetobacter spp.	9 (50)	3 (33.3)	0.25	Fair

*The sensitive concordance column represents the number of isolates that were sensitive to both minocycline and tigecycline of the total isolates which demonstrated concordance

^The overall Cohen Kappa value for concordance was 0.11 (none-slight agreement)

Table 4. Isolates sensitive to Minocycline and with intermediate/resistant susceptibility towards Tigecycline.

Isolates	Sensitive to Minocycline + Resistant/Intermediate Susceptibility to Tigecycline, n (%)
Klebsiella	20 (31.7)
K. pneumoniae	19 (32.7)
Klebsiella	1 (20)
Enterobacteriaceae	3 (15)
E. coli	3 (16.6)
Enterobacter, undefined	Nil
Acinetobacter spp.	6 (33.3)

DISCUSSION

The prevalence of infections caused by MDR pathogens is showing an upward graph. More bacteria are now becoming resistant to several antibiotics which have made treatment of such infections challenging. This has created the need to proactively initiate therapy to avoid adverse consequences [19]. Treatment of infections caused by MDR pathogens requires careful evaluation of several factors so that the right therapeutic agent is used for therapy. These factors include evaluation of pharmacokinetics/pharmacodynamics of the therapeutic agent, well-designed clinical trials to evaluate the efficacy, and implementation of infection control practices to prevent horizontal spread of antibacterial resistance [20].

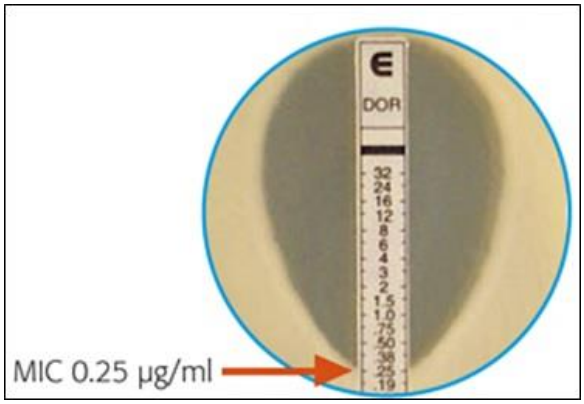

Figure 1. Graphical Representation of Epsilometer test (E- test)

Minocycline, an old antibiotic which was not widely used is now being evaluated for its effectiveness against the MDR strains [21]. In addition to evaluating the antibacterial effect of minocycline, the concordance was compared with tigecycline. Further, carbapenem resistant gram-negative bacterial strains were taken for this in vitro evaluation because these are a group of micro-organisms which are resistant to a class of antibiotics called as carbapenems which are known to have a very broad spectrum of activity and are often considered as the last resort for the treatment of an infection [14].

Overall, the sensitivity against Klebsiella was 36.5% and 22.2% of the isolates demonstrated intermediate susceptibility. A total of 26.2% *Klebsiella pneumoniae* carbapenemase (KPC)-producing Enterobacteriaceae were susceptible to minocycline in another study conducted to evaluate and compare the in vitro antibacterial activity of minocycline with other agents such as tigecycline, and doxycycline [22]. The study further concluded that minocycline has a better potential against KPC producing Enterobacteriaceae as compared to other tetracyclines such as tigecycline and doxycycline [22].

While the susceptibility to Klebsiella was 36.5%, half of the Acinobacter isolates (50%) were sensitive to minocycline. A study conducted to evaluate the in vitro effect of minocycline against *Acinetobacter baumannii*, a carbapenem resistant gram-negative bacteria using E- test demonstrated that more than half of the isolates (63.2%, 55/87) were susceptible to minocycline [23]. Arroyo et al., in a study, demonstrated that the susceptibility of minocycline (MIC50/90) against strains of *Acinetobacter baumannii* was 18% and 49.4% respectively [24]. The effect of minocycline against MDR-*Acinetobacter baumannii* was further supported by a review which concluded that minocycline is effective for the treatment of infections such as nosocomial pneumonia caused by *Acinetobacter baumannii* [25].

Among the *Enterobacter spp*., sensitivity was observed in 45% of the isolates while 30% had intermediate susceptibility. A study evaluating the overall susceptibility of minocycline against carbapenem resistant *E. coli* showed that 52% of the isolates were susceptible to minocycline. These results suggest that minocycline is effective against *E. coli* which belongs to *Enterobacter spp*. [26].

Further, the concordance between both, minocycline and tigecycline, was observed in 41.5% of the isolates used in the study. In addition, 31.7% of the Klebsiella isolates were resistant against tigecycline however; exhibited sensitive to intermediate susceptibility towards minocycline suggesting minocycline to be more effective as an antibacterial agent. Although a few studies suggest that tigecycline may have a better antibacterial activity as compared with minocycline against carbapenem resistant gram-negative bacteria, a study conducted at the Detroit Medical Center using the antimicrobial Stewardship approach concluded that in addition to a better antibacterial activity (as compared to doxycycline), minocycline demonstrates better pharmacokinetic properties (as compared to tigecycline). The study further showed that the in vitro susceptibility of minocycline against carbapenem resistant *K. pneumoniae* was 12% and against Acetobacter baumannii was 74% [27].

In conclusion, the results of the present study have demonstrated that minocycline is effective against all the three carbapenem resistant gram-negative bacteria included in the study. The study further highlighted that minocycline has also been effective against few isolates which were resistant to tigecycline. These results suggest that minocycline may be considered as a therapeutic agent for the treatment of MDR pathogens which are otherwise difficult to inhibit using other antibiotics. Inclusion of limited number of isolates may be considered as one of the drawbacks of the study. Additionally, comparison with more therapeutic agents would have provided a broader perspective of the overall effect of minocycline for the treatment of infections caused by carbapenem resistant gram-negative bacteria. However; more studies demonstrating the pharmacokinetic and pharmacodynamic properties of minocycline in addition to its drug-interactions are required to implement this therapeutic agent in real world clinical practice.

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