GAVIN PUBLISHERS

Journal of Microbiology and Genetics

Chinnappan JAI, et al. J Microbiol Genet 05: 128.

Research Article

DOI: 10.29011/2574-7371.100028

Determination of Tigecycline and Colistin Susceptibility among Carbapenemase Producing Isolates of Gram Negative Bacteria in a Tertiary Care Hospital

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Citation: Chinnappan JAI, Lakshmipriya N, Umadevi U, Thasneem BS (2020) Determination of Tigecycline and Colistin Susceptibility among Carbapenemase Producing Isolates of Gram Negative Bacteria in a Tertiary Care Hospital. J Microbiol Genet 05: 128. DOI: 10.29011/2574-7371.100028

Received Date: 15 October, 2020; **Accepted Date:** 12 November, 2020; **Published Date:** 18 November, 2020

Abstract

Introduction: Management of infections with carbapenemase producing Gram negative bacteria has become challenging. Colistin and tigecycline are the drug considered for the treatment of these multidrug resistance bacterial infections. This study was aimed to determine the colistin and tigecycline susceptibility among carbapenemase producing Gram negative bacteria.

Materials and Methods: This study was conducted in Institute of Microbiology, Madras Medical College, Chennai with 75 carbapenemase producing Gram negative bacteria from various clinically relevant samples. Bacterial identification was done by standard microbiological protocol and the carbapenemase production was confirmed by Modified Hodge Test. The Colistin and Tigecycline MIC value were determined by E-strip (HIMEDIA) method and interpreted using EUCAST-BSAC 2017 guidelines.

Result: Among the 75 isolates included in the study, 26.6% were *Acinetobacter* spp, 22.7% *Klebsiella pneumoniae*, 18.7% *E.coli, 16% Klebsiella oxytoca* and 16% *Pseudomonas spp.* The Colistin MIC_{50} was 1 µg/ml and 0.75 µg/ml for *Enterobacteriacae* and Non-Fermentering Gram Negative Bacilli (NFGNB) respectively whereas colistin MIC_{90} was 1.5 µg/ml for both *Enterobacteriaceae* and NFGNB. Both the colistin MIC_{50} and MIC_{90} for *Enterobacteriaceae* and NFGNB were found to be susceptible. The Tigecycline MIC_{50} and MIC_{90} were 0.094 µg/ml and 0.5 µg/ml respectively for both *Enterobacteriacae* and NFGNB. Tigecycline MIC_{90} for NFGNB was found to be resistant. One among the 75 carbapenemase producing gram negative bacilli tested i.e *Acinetobacter spp* was resistant to colistin with MIC of 8µg/ml. Two *Klebsiella oxytoca* showed intermediate susceptibility and six *Acinetobacter spp* showed resistance to tigecycline. The *Acinetobacter spp.* which was resistant to colistin was also resistant to tigecycline with MIC of 0.75 µg/ml.

Conclusion: The study showed the frequency of colistin and tigecycline resistance to be 1.3% and 12.5% respectively. With these findings we conclude that colistin and tigecycline may be considered for the treatment of Carbapenemase producing Gram negative bacterial infections. However surveillance studies are recommended to organise treatment protocol and determine the dosage based on these surveillance data.

Keywords: Carbapenemase producing Gram negative bacteria; Colistin; MIC; Tigecycline

Introduction

In this era of worsening antimicrobial resistance, gram negative bacteria resistant to carbapenem poses a special clinical challenge because they have been the most active and potent agents for a longtime against multidrug resistant gram negative bacteria. This is further supported by World Health Organization priority pathogens list for research and development of new antibiotics in 2017 which enlists carbapenem-resistant Enterobacteriaceae (CRE), carbapenem-resistant *Acinetobacter baumannii*, and carbapenem-resistant *Pseudomonas aeruginosa* as critical priority [1].

The mechanisms responsible for carbapenem resistance including porin mutations, efflux pump upregulation and

carbapenemase production are explained in detail by Nordmann and Poirel [2]. Of significance are chromosomally encoded porin gene mutation (such as OprD), overexpression of genes encoding for efflux pumps (such as MexAB-OprM, MexXY-OprM, or MexCD-OprJ) and the carbapenemases like KPC, VIM, IMP, NDM and OXA-48 types [2-4]. Resistance due to carbapenemase production is clinically most important as they not only hydrolyze almost all beta-lactams and confer high Minimum Inhibitory Concentration (MICs) but are also encoded by genes transferable horizontally by plasmids or transposons which also have genes encoding for other resistance determinants [5].

Reports of carbapenemase-mediated resistance continue to increase globally [6,7], leading clinicians to resort to other antibiotics with therapeutic success against carbapenemase-producing Gram-negative bacteria. Currently, colistin (polymyxin E) and tigecycline have become the antibiotics of last resort for carbapenemase- producing Gram-negative bacteria [8,9]. Increasing colistin and tigecycline consumption has been concurrent with increasing reports of tigecycline and colistin resistance, especially during therapy. Already, clinical outbreaks involving colistin resistant KPC-producing *Klebsiella pneumoniae* have been reported in the United States and Italy with worrying recurrence leaving very limited treatment options [5,6]. Increasing nonsusceptibility to colistin and tigecycline during colistin and tigecycline monotherapy has engendered the use of colistin and/or tigecycline in double and triple combinations with a carbapenem, an aminoglycoside (amikacin, gentamycin and tobramycin), rifampicin, fosfomycin or fluoroquinolone [8,9].

In view of the above, this study was conducted to isolate and identify the carbapenemase producing Gram negative bacteria from patients admitted in various wards and determine their colistin and tigecycline susceptibility pattern.

Materials and Methods

Study Design

This cross-sectional study was carried out in the Institute of Microbiology Madras Medical College and Rajiv Gandhi Government General Hospital, Chennai. The study was conducted for a period of 6 months from (April 2017 to September 2017)

Sample Size: 75 carbapenemase producing Gram negative bacteria

Inclusion Criteria

- Non-repetitive isolates of Carbapenemase producing Gram negative bacterial isolates.
- Only one isolate per sample in polymicrobial infection were included.

Bacterial Identification and Phenotypic Analysis of Carbapenems

Non-repetitive clinically relevant isolates of Gram negative bacteria from various clinical samples including blood, sputum, pus, wound swabs, bronchial wash, tracheal aspirates and urine were collected. The isolates were identified as per Standard Microbiological protocol. Antimicrobial susceptibility testing of the isolates were done by Kirby Bauer's disc diffusion technique on Mueller-Hinton agar, as per Clinical Laboratory Standard Institute (CLSI) guidelines 2017. Isolates with intermediate susceptibility or resistance to meropenem and imipenem by disc diffusion method were screened for the production of carbapenemase.

Phenotypic Detection of Carbapenemases

A phenotypic detection of carbapenemases was done by Modified Hodge test.

Modified Hodge Test (MHT) [10]: 0.5 McFarland standard suspension of *E.coli* ATCC 25922 was prepared in saline and diluted 1:10 in saline. A lawn culture of 1:10 dilution *E.coli* ATCC 25922 was done on to a Mueller Hinton Agar plate and allowed to dry for 3-5 minutes. A 10µg meropenem disc is placed in the center of the test area. In a straight line, the test organism was streaked from the edge of the disc to the edge of the plate and incubated at 37˚C for 16-20 hours. Positive and negative controls were included.

Interpretation

Enhanced growth (Clover-leaf indentation) = Positive

No enhanced growth = Negative

Colistin Suceptibility Test

Colistin MIC was determined by Epsilometer Test (E-test) using colistin E-strips of concentration gradient 0.016 to 256µg/ml (HIMEDIA) as per the EUCAST guidelines 2017 [11]. *E.coli* ATCC 25922 was used for Quality control (QC range, 0.25-2µg/ml).

Four or five identical colonies were inoculated into 5ml of saline and incubated for 2 hours to match 0.5 McFarlands standards. Lawn culture of the suspension was made onto Mueller Hinton Agar plate. The E strip was placed on the Mueller Hinton Agar plate using a sterile applicator and incubated at 37˚C for 20-24 hrs. After incubation whereby bacterial growth becomes visible, an elliptical zone of inhibition correlating with the gradient concentration of antibiotic. The MIC value is read from the scale in terms of µg/ml where the point of ellipse intersects the strip (Table 1).

Organism	Minimum Inhibitory Concentration (MIC) - µg/ml		
	Sensitive	Intermediate	Resistant
Enterobacteriaceae	≤2	-	>2
Pseudomonas spp.	≤2	-	>2
Acinetobacter spp.	≤2	-	>2

Table 1: MIC interpretive criteria for Colistin [11].

Tigecycline Susceptibility Test

Pseudomonas species is intrinsically resistant to Tigecycline, hence *Enterobacteriaceae* and *Acinetobacter spp* were tested for Tigecycline MIC by using tigecycline E-strips of concentration gradient 0.016 to 256µg/ml (HIMEDIA) as per the EUCAST guidelines 2017. *E.coli* ATCC 25922 was used for Quality control (QC range, 0.03-0.25µg/ml) (Table 2).

Organism	Minimum Inhibitory Concentration (MIC) - µg/ml		
	Sensitive	Intermediate	Resistant
Enterobacteriaceae	≤1	>1 to ≤2	>2
Acinetobacter spp.	≤0.25	-	≥0.5

Table 2: MIC interpretive criteria for Tigecycline [11,12].

Statistical Analysis: Data were entered in Microsoft Excel 2013. MIC_{50} and MIC_{90} were calculated for colistin and Tigecycline among various organisms. MIC_{50} and MIC_{90} were defined as lowest concentration of antibiotics(lowest MIC) at which 50% and 90% of the tested strains were inhibited.

Results

A total of 112 Carbapenem resistant gram negative isolates by disc diffusion were screened for Carbapenemase production by Modified Hodge Test, out of which 75 isolates tested positive. Clinical samples for the isolates included in the study and distribution of isolated bacteria are provided in (Table 3).

Clinical samples	*E. coli*	*K.pneumoniae*	*K.oxytoca*	*Pseudomonas aeruginosa* & other *Pseudomonas spp.*	*Acinetobacter spp.*	Total
Urine	11	8	7	5	7	38
Pus	2	4	3	5	6	20
Tracheal aspirate	1	3	2	2	1	9
Sputum	0	2	0	0	2	4
Body fluids	0	0	0	0	2	2
Bronchial wash	0	0	0	0	1	1
Blood	0	0	0	0	1	1
Total	14	17	12	12*	20	75
*Out of 12 *Pseudomonas spp.* 7 were *Pseudomona aeruginosa*						

Table 3: The source and distribution of isolates in clinical samples (n=75).

Among the 75 Modified Hodge Test positive(Carbapenemase producers) isolates, 50.6% isolates were from urine sample and the most frequently isolated organism was *Acinetobacter spp* (26.6%) followed by *Klebsiella pneumoniae*, *E.coli, Klebsiella oxytoca* and *Pseudomonas spp.*

Susceptibility pattern of the isolates to colistin and tigecycline is provided in (Table 4).

Organism	Colistin(n=75)		Tigecycline(n=63)		
	S	R	S	I	R
E.coli	14	0	14	0	0
K.pneumoniae	17	0	17	0	0
K.oxytoca	12	0	10	2	0
Pseudomonas aeruginosa & other *Pseudomonas* species	12	0	-*	-*	-*
Acinetobacter spp.	19	1	14	-	6
Total	74	1	55	2	6
**Pseudomonas spp.* is intrinsically resistant to Tigecycline hence Tigecycline MIC was not performed for them.					

Table 4: Determination of the MIC values of the antibiotics tested for the isolates.

Among the 75 carbapenemase producing gram negative bacilli tested, one isolate of *Acinetobacter spp.* (*Acinetobacter baumannii*) from the pus sample of pyothorax was resistant to colistin with MIC of 8µg/ml. Two *Klebsiella oxytoca* showed intermediate susceptibility and six *Acinetobacter spp.* showed resistance to tigecycline. The *Acinetobacter spp.* which was resistant to colistin was also resistant to tigecycline with MIC of 0.75 µg/ml.

The distribution of colistin MIC among the carbapenemase producing gram negative bacteria is shown in (Table 5).

Organism	Number of isolates with colistin MIC (µg/ml)					Total
	0.75	1	1.5	2	8	
E.coli	4	8	2	0	0	14
K.pneumoniae	9	6	1	1	0	17
K.oxytoca	5	6	1	0	0	12
Pseudomonas aeruginosa & other *Pseudomonas* species	8	2	2	0	0	12
Acinetobacter spp.	10	7	2	0	1	20
Total	36	29	8	1	1	75

Table 5: Distribution of colistin minimum inhibitory concentrations in carbapenemase producing Gram negative bacteria (n=75).

Maximum isolates (36/75) showed colistin MIC of 0.75 µg/ml, followed by 1 µg/ml(29/75).

The distribution of tigecycline MIC among the carbapenemase producing gram negative bacteria is shown in (Table 6).

Organism	Number of isolates with tigecycline MIC (µg/ml)											Total
	0.047	0.064	0.094	0.125	0.25	0.32	0.5	0.75	1	1.5	2	
E.coli	1	2	3	2	2	1	3	0	0	0	0	14

K.pneumoniae	6	4	2	1	1	0	3	0	0	0	0	17
K.oxytoca		3	2	0	1	0	3		1	1	1	12
Acinetobacter spp.	3	5	3	1	2	0	4	2	0	0	0	20
Total	10	14	10	4	6	1	13	2	1	1	1	63

Table 6: Distribution of tigecycline minimum inhibitory concentrations in carbapenemase producing Gram negative bacteria (n=63).

Maximum isolates (14/63) showed tigecycline MIC of 0.064 µg/ml, followed by 0.5 µg/ml(13/63).

The colistin and tigecycline MIC_{50} and MIC_{90} was calculated for *Enterobacteriaceae* and non-fermenters separately are listed in (Table 7).

Antibiotic	Organism	MIC Range	MIC_{50} (µg/ml)	Interpretation*	MIC_{90} (µg/ml)	Interpretation*
Colistin	*Enterobacteriaceae*	0.75-2	1	Susceptible	1.5	Susceptible
	NFGNB	0.75-8	0.75	Susceptible	1.5	Susceptible
Tigecycline	*Enterobacteriaceae*	0.047-2	0.094	Susceptible	0.5	Susceptible
	NFGNB	0.047-0.75	0.094	Susceptible	0.5	Resistant
*The interpretation of the MIC_{50} and MIC_{90} of all antibiotics tested for all pathogens was performed using the EUCAST-BSAC 2017 guidelines. NFGNB- Non-fermentering Gram negative bacilli						

Table 7: Determination and interpretation of the MIC values of the antibiotics tested (n=75).

The Colistin MIC_{50} was determined to be 1 µg/ml and 0.75 µg/ml for *Enterobacteriacae* and NFGNB respectively whereas colistin MIC_{90} was determined to be 1.5 µg/ml for both *Enterobacteriaceae* and NFGNB. Both the colistin MIC_{50} and MIC_{90} for *Enterobacteriaceae* and NFGNB was found to be in the susceptible.

The Tigecycline MIC_{50} and MIC_{90} were determined to be 0.094 µg/ml and 0.5 µg/ml respectively for both *Enterobacteriacae* and NFGNB.

Discussion

The knowledge of antibiotics susceptibility pattern of the bacteria is necessary to overcome the problem of treating infections with resistant isolates. Available treatment options for infection caused by carbapenemase producing GNB are limited currently with colistin and tigecycline being the primary treatment. Increasing use of colistin and tigecycline for carbapenemase producing GNB infections has led to the emergence of colistin and tigecycline resistance in several countries worldwide. The prevalence of this emerging resistance varies between regions based on the availability and use of these antibiotics [13]. Hence this study was aimed to determine the colistin and tigecycline susceptibility pattern in Carbapenemase producing Gram negative bacteria isolated from clinical samples in a tertiary care hospital, Tamil Nadu.

A recent Global surveillance program study involving 39 countries, revealed 88% and 91.1% of carabapenemase producing *Enterobacteriaceae* were susceptible to colistin and tigecycline respectively [14] and a study conducted in Bangalore by Chandran, et al revealed 100% and 94.05% of carabapenemase producing *Enterobacteriaceae* were susceptible to colistin and tigecycline respectively [15]. While in the present study, the colistin and tigecycline susceptibility among *Enterobacteriaceae* were 100% and 94.43% respectively, which indicates lower prevalence of resistance comparatively.

The MIC_{50} and MIC_{90} for colistin and Tigecycline among *Enterobacteriaceae* in the present study were 1µg/ml & 1.5µg/ml and 0.094 µg/ml & 0.5 µg/ml respectively while in the Global Surveillance Program were 0.12 µg/ml& 4 µg/ml and 1 µg/ml & 2 µg/ml; and in the study conducted in Bangalore by Chandran et al 0.094 µg/ml & 0.125 µg/ml and 1 µg/ml & 2 µg/ml respectively [14,15]. This breakdown helps us identify, the MIC_{50} of colistin among *Enterobacteriaceae* is high in the present study compared to the other studies indicating a impeding rise of resistance in the near future, though the prevalence of colistin resistance is low currently. Thus, prompting implementation of appropriate approach in using these antibiotics.

In the present study, 100% and 95% of *Pseudomonas spp* and *Acinetobacter spp.* were susceptible to colistin with MIC_{50} and MIC_{90} being 0.75 µg/ml and 1.5 µg/ml respectively for both organism. While 70% of the *Acinetobacter spp.* were susceptible to Tigecycline with MIC_{50} and MIC_{90} being 0.094 µg/ml and 0.5µg/

ml. In a study conducted in Egypt 2015 by Wesam Hatem Amer, *Acinetobacter spp.* revealed 100% and 43.3% susceptibility to Colistin and Tigecycline respectively with MIC_{50} and MIC_{90} 0.5 µg/ml & 1 µg/ml and 2 µg/ml & 4 µg/ml respectively [16]. In the study conducted by Sangeetha et al in 2012 at Vellore revealed Colistin and Tigecycline MIC_{50} and MIC_{90} of *Pseudomonas spp.* and *Acinetobacter spp* as 1 µg/ml & 2 µg/ml and 0.5 µg/ml & 64 µg/ml respectively [17]. These values strengthen the finding that the prevalence of resistance among NFGNB against colistin is much lower in the present study, though the same cannot be said for tigecycline but with proper guidelines the development of resistance can be delayed significantly.

There are few limitations in the study. There are chances for inaccuracies due to lack in use of genotypic methods. Polymyxins diffuses poorly in the media, hence the use of E-test tends to underestimate the resistance pattern.

Conclusion

With the findings in the current study, it is concluded that colistin and Tigecycline can be considered as an option in treating infections with carbapenemase producing Gram negative bacteria, however national and global surveillances studies are recommended to regulate the treatment protocol and dose adjustment. Emerging resistance against these drugs are also to be kept in mind, as it leaves us with very minimal options to treat them. Hence their judicious use and regular monitoring of their susceptibility pattern are also recommended.

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