Emergence of Tigecycline Resistance in Multidrug Resistant (MDR) Organisms Isolated At Tertiary Care Hospital, Ahmedabad

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Abstracts: Objective & Background: Tigecycline is the first glycylcycline antibiotic to be approved by US Food and Drug Administration (FDA) and therapeutic agent for serious infections caused by multidrug resistant organism. This study is to evaluate the emergence of Tigecycline resistance in MDR organisms. Method: This study was conducted over a period of July 2016 to 15th Nov 2016. A total of 217 Multi Drug Resistant Organisms (MDRO) isolated from various clinical specimens except urine, as per standard protocols and antibiotic sensitivity. Out of which 36 Methicillin Resistant Staphylococcus (MRSA) , 3 Vancomycin Resistant Enterococci (VRE) ,91 Multidrug resistant Acinetobacter spp and 87 MDR Enterobacteriaceae, are tested for Tigecycline (TIG) resistance by disc diffusion Method and Minimum inhibitory concentration (MIC) was determined by E-test gradient strip. Result: All the isolates for MRSA, VRE shows 0% resistant to Tigecycline but 6 (6.89%) of MDR Enterobactiaceae and 15 (16.48 %) of MDR Acinetobacter spp. were found to be resistant to Tigecycline. Conclusion: Tigecycline was found to be highly effective against gram positive bacteria and resistance is observed in Acinetobacter spp. and Enterobacteriaceae, especially in multidrug-resistant strains. So before starting the treatment, susceptibility to Tigecycline should be assessed, to prevent the development and the dissemination of resistance against this one of the last available promising and safe therapeutic options which is available to the clinicians for combating these bacteria. [Mamta K NJIRM 2017; 8(2):153-157]

Key Words: Tigecycline, MDR, E-test, MIC, Acinetobacter, MRSA, VRE, Enterobacteriaceae.

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Introduction: Tigecycline (TIG) derivative of minocycline that is modified to overcome tetracycline resistance, is the first glycylcycline antibiotic to be approved by US Food and Drug Administration (FDA) and therapeutic agent for serious infections caused by multidrug resistant organism. It is active in vitro against a broad range of gram-positive and gram-negative bacteria, anaerobes, 'atypical' bacteria as well as against many species of drug-resistant strains [e.g. Vancomycin Resistant Enterococci (VRE), methicillin-Resistant Staphylococcus aureus (MRSA), Multidrug Resistant Organisms Enterobacteriaceae and multidrug-resistant Acinetobacter baumannii]. Similar to tetracyclines, TIG inhibits protein translation by reversibly binding to the 30S subunit of the bacterial ribosome, which impedes amino acid synthesis1. Although the ribosomal binding sites of TIG are similar to those of tetracycline, TIG binds five times more effectively than tetracyclines. This allows TIG to evade the common ribosomal protection mechanisms associated with resistance to tetracyclines2. TIG has received high attention and has been regarded as the last resort to treat pan drug-resistant bacteria.

The US Food and Drug Administration (FDA) approved TIG in 2005 for the treatment of complicated intra-abdominal infections and complicated skin and skin-structure infections, in 2009 community-acquired bacterial pneumonia. However, reports of TIG resistance have increased year by year.

In our hospital study the prevalence of antimicrobial resistance is extremely high across both gram positive and gram-negative bacterial genera due to the immense antibiotic pressure[3, 4]. In view of the increased resistance in both gram-positive and gram-negative pathogens in India and across the world, this study was conducted to evaluate the emergence of TIG resistance in MDRO.

Method: This study was conducted over a period of July 2016 to 15th Nov 2016 with MDRO isolated from various clinical specimens except urine. The organisms were isolated and identified as per standard microbiological techniques. Antimicrobial Susceptibility testing was performed as per CLSI guidelines (2016) by modified Kirby-Bauer method[5]. MDR isolates were subjected to TIG susceptibility testing and interpretation was done as per US FDA criteria. A total of 10654 samples received at tertiary

care hospital, Ahmedabad, Gujarat, Study were conducted in two parts.

1) MDR organisms isolate.

a) A Total of 360 Staphylococcus aureus isolated and were screened for MRSA by Cefoxitin for presence of MecA mediated Oxacillin resistance. If screening test was positive, it was subjected to Oxacillin MIC and results were interpreted as per CLSI guidelines[6], 36 (MRSA) were identified.

b) Among 47 isolated Enterococci, only 3 Vancomycin resistance enterococci were confirmed by determining the minimum inhibitory concentration (MIC) of Vancomycin by E-test with a concentration range 0.016-256 mcg/ml, (HiMedia, India) [7]. The isolated VRE MIC >256 ug/ml was found.

c) Among 1591 isolated Enterobacteriaceae (Escherichia coli and klebsiella spp.), 87 MDR Enterobacteriaceae isolated by Carbapenamase production was phenotypically confirmed by Modified Hodge test and double disc synergy test[5b].

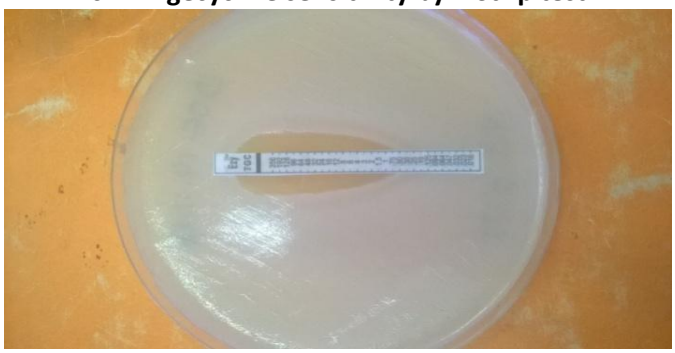
d) Among 480 clinical isolates of Acinetobacter spp. 91 MDR Acinetobacter spp. were identified by Imipenem-EDTA combined disc method as described by Yong et al[8].

Quality control for antimicrobial susceptibility testing is done as per CLSI guidelines recommended by ATCC strains.[5b]

2) Tigecycline susceptibility Testing: TIG susceptibility in these isolates was done by the disc diffusion method, using TIG disc (15 µg/disc, HiMedia, Mumbai, India). The interpretation of zone diameters for all gram negative bacteria (including Acinetobacter spp.) was done using the US FDA. TIG susceptible breakpoints listed for Enterobacteriaceae (MIC ≤ 2 µg/ml and ≥ 19 mm zone size) [9]. Interpretation of zone diameters of all gram-positive bacteria was done using the US FDA criteria. TIG susceptible breakpoints listed for S. aureus (MIC ≤ 0.5 µg/ml and ≥ 19 mm zone size) and E. faecalis (Vancomycin susceptible only) (MIC ≤ 0.25 µg/ml and ≥ 19 mm zone size) [10]

TIG resistance with Minimum inhibitory concentration (MIC) was determined using E-test gradient strips with a concentration range of 0.016 to 256 µg/ml (HiMedia, Mumbai, India) on Mueller-Hinton agar plates. Resistance was defined as MIC ≥ 8µg/ml and zone size ≤ 14 mm[9]. Interpretation of zone Diameters of all gram positive bacteria was done using the US FDA criteria.

Pic: 1 Tigecycline sensitivity by E-Strip test



Pic : 2 Tigecycline resistant by E-test

Quality control by the disc diffusion technique using the 15 mcg Tigecycline disk on ATCC 25923 Staphylococcus aureus (zone diameter 20-25 mm), Escherichia coli ATCC 25922 disc diffusion zone diameter 20-27 mm and MIC 0.03-0.25 ug/ml, the criteria provided in US FDA should be achieved

Result: A total of 217 MDR clinical isolates were evaluated in this study. It included 36 confirmed isolates of MRSA, 3 VRE, 87 isolates MDR Enterobacteriaceae and 91 MDR Acinetobacter spp. Since Tigecycline has no or limited activity against Pseudomonas and Proteus spp., these were not included in our study[11, 12, 13]. All the isolates for MRSA, VRE shows 0% resistant to Tigecycline by disc diffusion method, all isolates seemed sensitive as per US FDA criteria (Tigecycline susceptible breakpoints ≥ 19 mm zone diameter) however, 6 (6.89%) isolated MDR Enterobactiaceae were resistant to Tigecycline by disc diffusion technique as ≤14 mm zone size) as per US FDA criteria and Tigecycline resistant isolates by disc diffusion method were also tested by E –strip for MIC testing. The MIC of all the isolates tested was more than > 8 ug/ml and 15 (16.48 %) MDR Acinetobacter spp. were found to be resistant to Tigecycline by disc diffusion method. Tigecycline

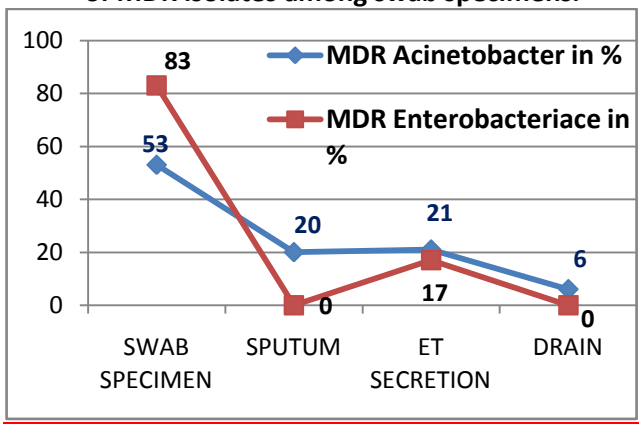
resistant isolates by disc diffusion method were also tested by E –strip for MIC testing. The MIC of all the isolates tested was more than > 32 ug/ml

Table: 1 show Tigecycline resistant isolated from MDRO

Organisms	Total Isolates	n-217	Tigecycline resistant	%
Staphylococcus aureus	360	36	0	0
Enterococci	47	3	0	0
Enterobacteriaceae	1591	87	6	6.89
Acinetobacter	480	91	15	16.48

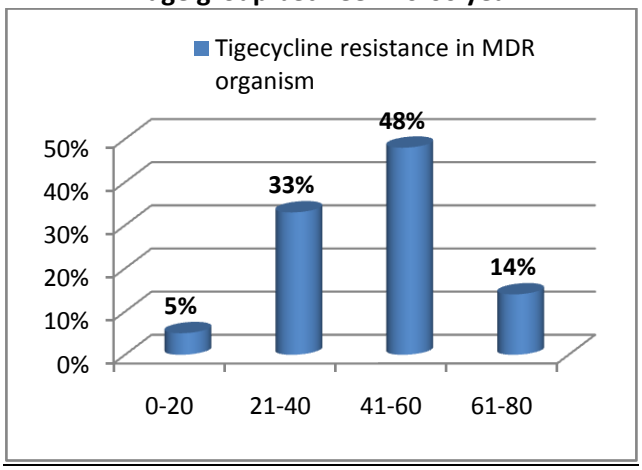
Table: 1 n- MDRO (217) include MRSA,VRE, MDR organisms.

Graph: 1 Represent highest Tigecycline resistance of MDR isolates among swab specimens.



Graph: 1 Tigecycline resistance in MDR Acinetobacter spp. and MDR Enterobacteriaceae isolated from various specimen.

Graph: 2 shows highest Tigecycline resistance among age group between 40-60 year



Graph:2 Age wise distribution of Tigecycline resistance in MDR isolates (n-21), n-total Tigecycline resistance

Discussion: This study has a number of limitations including those related to the considerable technical difficulties associated with TIG susceptibility testing. There are no MIC break point exist for TIG to Acinetobacter spp. at the time of study we used same FDA break point for Acinetobacter spp. that was set for Enterobactiaceae in US FDA criteria.

Table : 2 Worldwide reports of Tigecycline resistance rates in different pathogens.

Study	Pathogen	Susceptibility breakpoints (mg/L)	TIG Resistant(%)
Our study, Ahmedabad ,India	MRSA,VRE, Enterobacteriaceae (MDR) Acinetobacter (MDR)	As per US FDA criteria	0/0/6.89/ 16.48
Bijayini Behera et.al 2009,AIIMS, Delhi [14]	MRSA,VRE, Enterobacteriaceae bacteria (ESBL producing), Acinetobacter(MDR)	As per US FDA criteria	0/0/0/7.6
Dipender Kaur Najotra et.al[15], 2012	Acinetobacter baumannii (MDR)	>8 ug/ml as per US FDA criteria	3
Araj and Ibrahim,2008 Lebanon[16]	E.coli(MDR), Klebsiella pneumonia (MDR), Acinetobacter spp. (MDR)	Inhibition zones (mm): K.pneumoniae,≥19; A cinetobacter spp,≥16	0/3/ 2
Naesens etal, 2009 Belgium [17]	Klebsiella (ESBL producing),E.coli (ESBL producing), Enterobacter spp. (MDR)	≤1, EUCAST	100; 35; 96
Livermore et al, 2011, uk [18]	Enterobacteriaceae (carbapenem-resistant)	≤1, EUCAST	53.1

(US Food and Drug Administration; MDR, multidrug-resistant; ESBL, extended-spectrum β-lactamase; EUCAST, European Committee on Antimicrobial Susceptibility Testing ;MRSA, meticillin resistant Staphylococcus aureus.VRE;Vancomycin resistant enterococci

a) Susceptibility breakpoints for Enterobacteriaceae were used.
b) Resistance breakpoints were provided.)

As per our study among confirmed multidrug resistant organisms, Acinetobacter spp. was most resistant to TIG (16.48%), Enterobacteriaceae showing resistant (6.89%) but Tigecycline is most effective against MRSA and VRE Isolates. A similar study was done in 2009 at AIIMS Delhi with US FDA break point, according to this study, all the isolates of MRSA, VRE, Vancomycin resistant Streptococcus spp. and ESBL producing Enterobacteriaceae bacteria were sensitive to Tigecycline by the E-test and disc diffusion methods. However out of total 26 MDR Acinetobacter baumannii, 2 (7.6 %) had an MIC of 8 µg/ml (resistant) and 13 (50%) had MICs ranging between 3-6 µg/ml (intermediate) [14.] Another study at Acharya Shri Chander College of Medical Sciences and Hospital, Jammu, India, had been done from 2010 to 2012,result were 81.5% of the MDR isolates were sensitive to TIG and the MICs of TIG for these MDR isolates ranged from 0.25 to 32 µg/ml and 3% resistant to Tigecycline[15]. One international study by George F Araj et.al[16] at American University of Beirut Medical Centre, according to them, TIG resistant MDR E.coli were 0%, MDR klebsiella pneumonie 3% and MDR Acinetobacter were 2% .

TIG is used as a last line of defense against MDR strains and increasing rates of resistance are a growing concern clinically. Resistance has been reported in many types of pathogens including Acinetobacter spp., Enterobacteriaceae, E. faecalis, S. aureus, Furthermore, the prevalence of these resistances varies worldwide and is mention in above (table 2.) 1, 2, 3 are Indian study and 4,5,6 are international study. More studies are needed to investigate the inter-method agreement of Tigecycline in vitro susceptibility testing so that breakpoints and disc diffusion guidelines can be formulated for Acinetobacter spp.. This will minimize interpretative difference amongst various studies and provide the true magnitude of resistance in this genus. Since Tigecycline has a long terminal half-life and a large volume of distribution, it can be used as a life saving antimicrobial in polymicrobial infections due to gram-positive and gram-negative bacteria[14, 21].

Acinetobacter baumannii is an emerging cause of nosocomial outbreaks worldwide and is considered one of the top six deadliest micro-organisms by the Infectious Diseases Society of America (IDSA) of particular concern is the multidrug resistance of A. baumannii, which is defined as resistance to almost all available antibiotics TIG commonly, remain the only active antibiotic.

Conclusion: Tigecycline was found to be highly effective against gram positive bacteria and resistance is observed in Acinetobacter spp. and Enterobacteriaceae, especially in multidrug-resistant strains. The treatment options for the infections which are caused by multidrug resistant organisms are very limited, and Tigecycline is rapidly finding a role in the treatment of severe infections, as this antimicrobial has a favorable activity against a wide variety of organisms, which include the MDR Acinetobacter spp.. But the Tigecycline resistance among the MDR isolates that had not previously been exposed is worrisome[19]. So before starting the treatment, susceptibility to TIG should be assessed, to prevent the development and the dissemination of resistance against this one of the last available promising and safe therapeutic options which is available to the clinicians for combating these bacteria.

Acknowledgment: We extend our gratitude to Medical Superintendent, Civil Hospital Ahmedabad, Dean of B.J. Medical College, Ahmedabad and Director, Post Graduate Studies and Research, B.J. Medical College, Ahmedabad for their kind support in this study.

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Conflict of interest: None

Funding: None

Cite this Article as: Mamta K, Soni S, Vasdmiya M, Vegad M, Rathod A, Goswami A. Emergence of Tigecycline Resistance in Multidrug Resistant (MDR) Organisms Isolated . Natl J Integr Res Med 2017; 8(2):153-157