ORIGINAL ARTICLE

In Vitro Activity of Tigecycline Against Multi Drug Resistant Bacteria

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Author`s Contribution

[1]sample collection, lab work, analysis of results, [2]Proof reading [3]Supervision, study design. [4, 5,6]Helped in lab work, data analysis and discussion

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ABSTRACT

Objective: To determine *in vitro* efficacy of tigecycline against multi-drug resistant bacteria.

Study design: Descriptive cross-sectional study.

Place and duration of study: Department of Microbiology, Army Medical College/Armed Forces Institute of Pathology, Rawalpindi over a period of two years.

Materials and Methods: Methicillin resistant *Staphylococcus aureus* were identified by using cefoxitin 30µg disc. Extended spectrum β lactamase producers were detected employing double disc synergy test. Whereas detection of vancomycin resistant enterococci was done using E-strips of vancomycin. Susceptibility of the isolates to tigecycline was done by employing modified Kirby Bauer disk diffusion technique. Minimum inhibitory concentrations of the isolates were determined by using E-strips (bioMerieux) of tigecycline. Results were interpreted according to recommendations.

Results: All methicillin resistant *Staphylococcus aureus* isolates were sensitive to tigecycline. All extended spectrum β-lactamase producers (100%) were susceptible to tigecycline by modified Kirby-Bauer disk diffusion technique but (97.5%) by E-strip method. All vancomycin resistant enterococci (100%) were susceptible to tigecycline.

Conclusion: The results demonstrated that tigecycline, has excellent *in vitro* activity against MDR bacteria. This drug is thus a promising therapeutic option in an era of rapidly growing antibiotic resistance.

Key words: Antibiotic susceptibility, Methicillin resistant Staphylococcus aureus, Tigecycline Vancomycin resistant enterococci.

Introduction

Antimicrobial resistance is a growing concern all over the world. Among infections caused by gram-positive pathogens, methicillin-resistant *Staphylococcus aureus* MRSA) has taken central stage, now accounting for well over 50% of all documented staphylococcal infections in the US.[1] The emergence of extended spectrum β-lactamase (ESBL) producers has posed a problem in the use of many classes of antibiotics.[2] Likewise the infections caused by vancomycin-resistant enterococci (VRE) have been implicated in increasing mortality, and cost of hospitalization.[3] According to a recent multi-centre study, the frequency of MRSA in Pakistan is estimated to vary between 2-61%, with highest frequency seen in major cities of the country.[4] Data on incidence of VRE in Pakistan is lacking, however a regional study has revealed it to be 9.5 % in Iran.[5] The prevalence of ESBL producers is 35% among nosocomial isolates in one study carried out in a tertiary care hospital, Lahore and

incidence of ESBL in major hospitals of India has been reported to be as high as 6%-87%.[6,7]

Due to emergence of these antibiotic resistant organisms the focus is on a new class of antimicrobial agents, glycylcyclines, represented by tigecycline. It is 9-*t*-butylglycylamido derivative of minocycline. It specifically inhibits bacterial protein synthesis. It has a novel, often bactericidal mode of action of binding to the 30S ribosomal subunit, blocking aminoacyl-tRNA entry into the acceptor site. It has a broad spectrum of activity against many Gram positive, Gram negative and anaerobic organisms.[8]

The rapid emergence of multidrug resistant organisms (MDRs) necessitates the study of new drugs. This study is aimed at detecting the efficacy of tigecycline against MDR bacteria including MRSA, VRE and ESBL producing enterobacteriacae. This study will help in defining the role of tigecycline in treating infections caused by such resistant organisms and to aid our clinicians in finding a suitable alternative against such resistant bacteria.

Materials and Methods

This was a descriptive study and performed in Microbiology department of Army Medical College, Rawalpindi, Pakistan from 1st January 2010 to 31st December 2011. Study was approved from ethical review board. None probability consecutive sampling technique was used for sample collection. Clinical specimens including urine, blood, pus, sputum, high vaginal swab(HVS), aspirates, central venous lines, chest tubes, nasobronchial lavage (NBL) and catheter tips sent for culture and sensitivity to the department of Microbiology Army Medical College, Rawalpindi were processed for Gram staining and cultured on blood and Mac Conkey agar (Oxoid, Basingstoke, UK) and incubated for 24-48 hours at 37°C to get bacterial growth. *Staphylococcus aureus* organisms were tested for methicillin resistance by modified Kirby-Bauer disk diffusion method with 30µg cefoxitin disk (Oxoid, UK) on Mueller Hinton agar plates as recommended by Clinical and Laboratory Institutes (CLSI) guidelines. Culture plates were placed in incubator at 33-35°C for 24 hours. Cefoxitin zones were noted as per CLSI criteria. A zone diameter of ≥22 was taken as sensitive and a zone diameter of ≤ 21 as MRSA.[9]

The production of ESBL enzyme was detected employing double disc synergy (DDS) test using 30 µg discs of aztreonam, ceftazidime and 10µg cefpodoxime (Oxoid, Basingstoke, UK) placed 15 mm (edge to edge) from amoxycillin-clavulanate (20/10µg) disc. Inoculated plates were incubated overnight at 35 ± 2°C. Zone of enhancement between amoxycillin-clavulanate and any one of the above (aztreonam, ceftazidime and cefpodoxime) for an organism was considered as ESBL producer.[9]

Enterococcus isolates were tested by modified Kirby-Bauer disk diffusion technique using 30µg vancomycin disk (Oxoid, Basingstoke, UK) on Mueller-Hinton agar (Oxoid, Basingstoke, UK).The plates were incubated at 35 ± 2°C for 24 hours. Susceptibility to vancomycin was interpreted as per CLSI criteria. A zone diameter of ≥ 17 mm was taken as susceptible; 15-16 mm was intermediate and ≤ 14 mm as resistant. The confirmation of VRE was done by determining MICs using E-strips (bioMerieux).[9]

Susceptibility of the isolates to tigecycline was done, employing Kirby Bauer disk diffusion technique, according to the guidelines provided by the FDA. The MICs of all three mutidrug resistant organisms were determined by E-strips (bioMerieux) of tigecycline. According to FDA guidelines, inocculum (0.5 McFarland) of bacterial suspensions of MRSA, ESBL producers and VRE isolates were placed on Mueller-Hinton agar, followed by placing 15µg disk of tigecycline (Oxoid, England) onto the medium. The plates were incubated aerobically at 35 ± 2°C for 16 – 18 hours.

The results of MIC and disk diffusion for tigecycline were interpreted according to FDA approved criteria as given below.(Table I)

Table 1: FDA approved criteria for tigecycline 10

	MIC (µg/mL)			Zone diameters (mm)		
Pathogens	S	I	R	S	I	R
MRSA	≤0.5	--	--	≥19	15-18	≤14
VRE	≤0.25	--	--	≥19	15-18	≤14
ESBL producing enterobacteriacae	≤2	4	≥8	≥ 19	15-18	≤14

MSSA ATCC 25923, MRSA 33591 were used as control strains for MRSA. *Klebsiella pneumoniae* ATCC 700603 and *E. coli* ATCC 25922 were used as controls for ESBL producers. *Enterococcus faecalis* ATCC 29212 – susceptible and *Enterococcus faecalis* ATCC 51299 – resistant were used as controls for VRE. Descriptive

analysis of all the data was done, as categorical variables were summarized in proportions and percentages while quantitative informations in mean, SD and range accordingly. To compare differences 95% LOS was used, chi square for categorical variables and t-test for quantitative information were used accordingly.

Results

From A total of 100 multidrug resistant organisms including 50(50%) MRSA, 40(40%) ESBL producers and 10(10%) VRE were studied. Pus was the most common specimens followed by blood from which MRSA was isolated also presented in Table II. The ESBL producers were isolated mostly from urine followed by pus samples also presented in Table III. The most common ESBL producer among GNRs was *Escherichia coli* 24(60%) followed by *Klebsiella pneumoniae* 9(22.5%), *Enterobacter cloacae* 4(10%), *Acinetobacter baumannii* 2(5%) and *Morganella morganii* 1(2.5%). The distribution of specimens from which VRE were isolated is presented in Table IV.

Table II: Source of MRSA isolates (n=50)

Sample source	Frequency(%age)
Pus/pus swab	38(76%)[a]
Blood	4(8%)[b]
Catheter tips	3(6%)[b]
Body fluids	2(4%)[b]
NBL	1(2%)[b]
Urine	1(2%)[b]
Throat swab	1(2%)[b]

LSD (0.05)= 32.05

Values sharing a letter in common are not significantly different from each other at P ≥ 0.05

Table III: Source of ESBL producing isolates (n=40)

Sample source	Frequency(%age)
Urine	22(55%)[a]
Pus/pus swab	06(15%)[b]
Catheter tips	05(12.5%)[b]
Blood	03(7.5%)[b]
HVS	02(5%)[b]
NBL	01(2.5%)[b]
Sputum	01(2.5%)[b]

LSD (0.05)= 21.25

Values sharing a letter in common are not significantly different from each other at P ≥ 0.05

The range of zone diameters with 30µg disk of tigecycline for MRSA isolates was 21 to 31 mm. Mean zone diameter was 25.5±1.9 mm (mean±SD). The range of MICs of tigecycline for MRSA was 0.047 to 0.32µg/mL. Mean MIC was 0.099±0.059µg/mL. Thus all fifty isolates (100%) of MRSA were susceptible to tigecycline both by modified Kirby-Bauer disk diffusion technique as well as E-strip method.

Table IV: Sample distribution of VRE isolates

Sample source	Frequency(%age)
Catheter tip	05(50%)a
Blood	02(20%)a
Urine	02(20%)a
Pus swab	01(10%)a

LSD (0.05)= 4.22

Values sharing a letter in common are not significantly different from each other at P ≥ 0.05

The range of zone diameters with 15µg disk of tigecycline for ESBL producing isolates was 19 to 29 mm. Mean zone diameter was 21.8±2.2 mm (mean±SD). The range of MICs of tigecycline for ESBL producing isolates was 0.094 to 3.0µg/mL. Mean MIC was 1.03±0.73µg/mL. Thus all forty isolates (100%) of ESBL producers were susceptible to tigecycline by modified Kirby-Bauer disk diffusion technique but thirty nine (97.5%) ESBL producers were susceptible to tigecycline by E-strip method and one (2.5%) isolate was not susceptible.

The range of zone diameters with 15µg disk of tigecycline for VRE isolates was 25 to 39 mm. Mean zone diameter was 31.6±4.9 mm (mean±SD). The range of MICs of tigecycline for VRE isolates was 0.023 to 0.50µg/mL. Mean MIC was 0.077±0.143µg/mL. Thus all ten isolates (100%) of VRE were susceptible to tigecycline by modified Kirby-Bauer disk diffusion technique but nine (90%) VRE isolates were susceptible to tigecycline by E-strip method and one (10%) isolate was not susceptible, having MIC more than 0.25µg/mL.

Discussion

Bacterial resistance is rising which is real threat both community as well as hospital acquired infections by multi drug resistant organisms. This is basically a result of irrational use of antibiotics worldwide. Problem is more aggravated in developing countries where infection control practices and antibiotic policies are nonexistent. Multidrug resistant bacteria like MRSA, ESBL producers and VRE are increasingly reported all over the world. Mortality, morbidity and treatment cost have increased due to infections caused by these pathogens.[12]

Although treatment options are available for these pathogens, however there are reports of developing

resistance against these options. Carbapenems are considered the last resort for the treatment of ESBL producing enterobactericiae. However, carbapenem resistance in ESBL producers has been reported from several parts of the world, including Greece, Korea, United States, Israel, and China.[13] Vacomycin is considered as treatment of choice for MRSA but Tiwari et al reported two MRSA isolates which were vancomycin resistant from a tertiary care hospital, nothern India in 2006.[14]

Linezolid is among the first-line therapeutics against all VRE infections. However, Sedaat et al has reported that if patients infected with VRE were treated with linezolid for four weeks or longer, there are chances of development of linezolid resistance.[15] These findings reveal that treatment options for treating the superbugs like MRSA, ESBL producers and VRE are reducing. Hence, we have to find new alternatives to treat these superbugs. In this study we have tested a new antibiotic tigecycline which is effective against these multidrug resistant isolates.

All MRSA isolates were sensitive to tigecycline by disk diffusion method and MICs were also in the sensitive range in our study. Same results were shown by Reinert et al in a study conducted on the isolates collected from Asia/Pacific Rim, Europe, Latin and North America. He showed 100% sensitivity of MRSA isolates to tigecycline by microdilution method.[16] Kasbekar et al conducted a study on 265 MRSA isolates and found MIC_{90} of 0.25 µg/mL.[17] Gales et al conducted a study in Latin America and showed that tigecycline (MIC_{50}, 0.25 µg/mL) was eight-fold more potent than minocycline (MIC_{50}, 2 µg/mL) against oxacillin-resistant S. aureus.[18] Sauli et al showed that 99% of MRSA isolates were inhibited at a concentration of ≤0.5 µg/mL.[11] All these results are in concordance with our results.

Activity of tigecycline against ESBL producers is 97.5% in our study. Similar results have been shown by other studies. Reinert et al reported >93% sensitivity of isolates collected from Asia/Pacific Rim, Europe and Latin and North America.[16] Sauli et al revealed 97% sensitivity against ESBL producers in a Greek hospital.[11] Waites et al also reported that more than 90% sensitivity of tigecycline against ESBL producers among hospitalized patients of USA. Among ten VRE isolates only one isolate was resistant (MIC 0.50 µg/mL) in our study. Waites et al showed hundred percent sensitivity of VRE to tigecycline with MIC range (0.06-0.12 µg/mL).[19] Due to limited resources we selected less number of isolates which was the limitation of our study. Further studies should be continued to interpret the results properly.

Conclusion

The results demonstrated that tigecyline has good *in vitro* activity against MRSA, ESBL producers and VRE isolates. This drug is a promising therapeutic option in an era of rapidly growing antibiotic resistance, especially when we have to treat co-infections caused by gram-positive and gram-negative super bugs.

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