

RESEARCH ARTICLE

Tigecycline Resistance among Clinical Isolates of *Staphylococcus aureus* from North-east India

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ABSTRACT

Objective: The current study investigated the resistance against cycline group of antibiotic among clinical isolates of *Staphylococcus aureus* and molecular characterization with special reference to phenotypic screening of tigecycline resistance.

Methods: 197 coagulase producing *Staphylococcus* spp. were collected from private diagnostic laboratories and Silchar Medical College and Hospital, India. Phenotypic screening was done with cycline groups of antimicrobials and genotypically characterized by PCR, targeting *coa* gene. Detection of *tet(K)*, *tet(M)*, *tet(O)* and *tet(X)* genes was performed by PCR. Antibigram profiling was performed and clonality was also determined.

Results: 127 coagulase positive *S. aureus* were phenotypically screened with cycline group of antibiotics in which prominent resistant type showed highest non-susceptibility against tigecycline 53.5% (n=68) while lowest against minocycline 20.5% (n=26). Multiplex PCR assay showed that *tet(K)* gene to be the most common cycline group of antimicrobial resistance determinant in *S. aureus*, followed by *tet(M)* gene. Multiple genes were also observed. However, resistant gene *tet(O)* was not reported. Antibiotic susceptibility testing and MIC for therapeutic purpose showed highest susceptibility rate against linezolid. DNA fingerprinting experimented with Pulsed Field Gel Electrophoresis had shown seven pulsotypes.

Conclusion: Resistance to cycline group of antibiotics with special reference to tigecycline is not frequently reported from India. Thus, this study is of clinical interest in terms of selection of proper antimicrobial chemotherapy and also using tigecycline for phenotypic screening of cycline group of antibiotic resistance in *Staphylococcus aureus*. *J Microbiol Infect Dis* 2017; 7(4):173-177

Keywords: tigecycline, *tet* genes, *Staphylococcus aureus*, North-east India

INTRODUCTION

Tigecycline, a well-tested bacteriostatic drug, acts as an inhibitor of protein synthesis, prevents entry of aminoacyl-tRNA into the A site of the ribosomal RNA [1]. This antibiotic is very active against multidrug resistant bacteria [2,3]. The increasing trends of antibiotic resistance have restricted their clinical usefulness, although the tetracyclines have been used in both human and veterinary medicine [4]. Tetracycline resistance is mediated by efflux pump function and ribosomal protection in *Staphylococcus aureus*. These resistance mechanisms are acquired from plasmid encoded *tet(K)* and *tet(L)* genes and transposon mediated *tet(M)* and

tet(O) determinants [5]. Currently modern antibiotics are becoming ineffective and difficult to treat severe bacterial infections [6]. Recently *tet(X)* gene conferring flavin-dependent monooxygenase activity is responsible for tegicycline resistance [7]. Thus observing the emergence of resistance worldwide the following objective were framed which deals with the screening of cycline group of antimicrobial resistance within clinical isolates of *Staphylococcus aureus* and molecular characterization of tigecycline resistance.

METHODS

Sample collection and identification:

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In total, 197 consecutive non-duplicate, different clinical specimens of *Staphylococcus spp.* have been collected during the period of one year from April 2014 to March 2015 from Silchar Medical College and Hospital and also from two private diagnostic microbiological laboratories of Silchar, India. The isolates were confirmed by Gram staining, growth on Mannitol Salt Agar and Chrom Agar (HiMedia, Mumbai) and also by coagulase test and latex agglutination test (HiMedia, Mumbai). For molecular characterization of *S. aureus*, PCR was performed targeting *coa* gene as described earlier [9].

Phenotypic screening of cycline groups of antimicrobial resistance in *Staphylococcus aureus*:

Screening of cycline groups of antimicrobial resistance was tested against tetracycline (30 µg), doxycycline (10 µg), minocycline (30 µg) and tigecycline (15 µg) by Kirby Bauer disk diffusion method. Later MRSA was screened by oxacillin and ceftiofloxacin disk diffusion test. The zone dimension was measured and results were interpreted as per Clinical Laboratory Standard Institute (CLSI 2014) [9] guidelines and FDA break point [10].

Molecular characterization of *tet* genes:

For genotypic characterization of cycline resistance, multiplex PCR was performed by targeting *tet(K)*, *tet(M)*, *tet(O)*, and *tet(X)*. The amplification was done by using primers (Table 1) [11,14,17] under the following reaction condition. Initial denaturation was at 95 °C for 3 min, 30 cycles of 95 °C for 20 sec, 50 °C for 30 sec, 72 °C for 1.2 min and final extension at 72 °C for 7 min.

Antibiotic susceptibility testing and minimum inhibitory concentration:

Antibiotic susceptibility against different group of antimicrobials viz: clindamycin (10 µg), erythromycin (10 µg), pristinomycin (15 µg) were tested by disk diffusion methods and Minimum Inhibitory Concentration was done for Oxacillin, vancomycin, teicoplanin and linezolid by agar dilution method and results were noted as per CLSI guidelines [9]. For the study isolates colonies were picked from an overnight culture and were transferred to sterile saline. The suspension was adjusted to 0.5 McFarland

standards and incubated on MH agar plates containing 0.5, 1, 2, 4, 8, 16, 32 and 64 mg/L of the above antibiotics.

DNA fingerprinting by Pulsed Field Gel Electrophoresis:

Typing of isolates, harboring resistance genes was done by Pulsed Field Gel Electrophoresis (PFGE), using *Sma*I restriction enzyme for digestion of bacterial agarose plugs.

RESULTS

Identification of *Staphylococcus aureus*:

A total of 127 isolates were identified as *S. aureus* and further considered for the study.

Phenotypic screening of cycline group of antimicrobials resistance:

When phenotypically screened for resistance against selected cycline group of antimicrobials, 11 different combinations of antibiogram types were observed. Among them most prominent resistant type showed highest non-susceptibility towards tigecycline 53.5% (n=68), followed by tetracycline 41.7% (n=53), then doxycycline 40.2% (n=51) and minocycline 20.5% (n=26) and the as illustrated in Table 2.

Genotypic characterization of resistance genes:

When all the combinations of 74 cycline resistant isolates were screened by multiplex PCR assay, thirty three showed different combination of *tet* genes. Among them *tet(K)* (15.0 %) was found to be the most prevalent resistance determinant followed by *tet(M)* (7.9%). The combination of *tet(K)/tet(M)* (3.1%) and *tet(K)/tet(X)* (0.8%) were also detected and illustrated in Table 3.

Antibiotic susceptibility testing and minimum inhibitory concentration:

Disc diffusion result showed that out of one hundred twenty seven isolates, 49 (38.6%) were susceptible to clindamycin, followed by 16 (12.6%) to pristinamycin and 8 isolates (6.29%) to erythromycin. While examining minimum inhibitory concentration, linezolid (81.1%) and teicoplanin (72.4%) came up with good activity followed by vancomycin (66.1%) and oxacillin (22.8) shown in Table 4.

DNA Fingerprinting of coagulase positive *Staphylococcus aureus* by PFGE:

PFGE was performed for clonal dissemination/diversity of coagulase harboring

isolates, where seven types of clonal relatedness was observed among the isolates.

Table 1. Primer used for PCR amplification of *tet* genes.

Primer pairs	Target	Sequence (5'-3')	Amplified product size (bp)	References
<i>TetK</i> -F:-	<i>Tet(K)</i>	TTATGGTGGTTGTAGCTAGAAA	361	[12]
<i>TetK</i> -R:-		AAAGGGTTAGAACTCTTGAAA		
<i>TetM</i> -F:-	<i>Tet(M)</i>	AGT GGA GCG ATT ACA GAA	159	[12]
<i>TetM</i> -R:-		CAT ATG TCC TGG CGT GTC TA		
<i>TetO</i> -F:-	<i>Tet(O)</i>	AATGAAGATTCGACAATTT	801	[15]
<i>TetO</i> -R:-		CTCATGCGTTGTAGTATTCCA		
<i>TetX</i> -F:-	<i>Tet(X)</i>	CAA TAA TTG GTG GTG GAC CC	468	[18]
<i>TetX</i> -R:-		TTC TTA CCT TGG ACA TCC CG		

Table 2. Phenotypic screening of Cycline resistance.

Resistant Antibiotic/s	Number of Isolates
TGC	14
TET	1
TET, DOX	3
TET, TGC	3
DOX, TGC	1
MIN, TGC	2
TET, DOX, MIN	2
TET, DOX, TGC	26
TET, MIN, TGC	4
DOX, MIN, TGC	4
TET, DOX, MIN, TGC	14
OX, CEF	98

Table 3: PCR results of amplified *tet* genes.

Resistant antibiotic	Number of resistant gene/ <i>Staphylococcus aureus</i>
TGC	<i>tetK</i> (n=3), <i>tetM</i> (n=3), <i>tetK/tetX</i> (n=1)
TET, DOX	<i>tetK</i> (n=2)
TET, TGC	<i>tetK</i> (n=1), <i>tetM</i> (n=2)
DOX, TGC	<i>tetM</i> (n=1)
TET, DOX, MIN	<i>tetK/tekM</i> (n=1)
TET, DOX, TGC	<i>tetK</i> (n=12), <i>tetM</i> (n=1)
DOX, MIN, TGC	<i>tetM</i> (n=1)
TET, DOX, MIN, TGC	<i>tetK</i> (n=1), <i>tetM</i> (n=2), <i>tetK/tetM</i> (n=2)

TET=Tetracycline; DOX=Doxycycline; MIN=Minocycline; TGC=Tigecycline

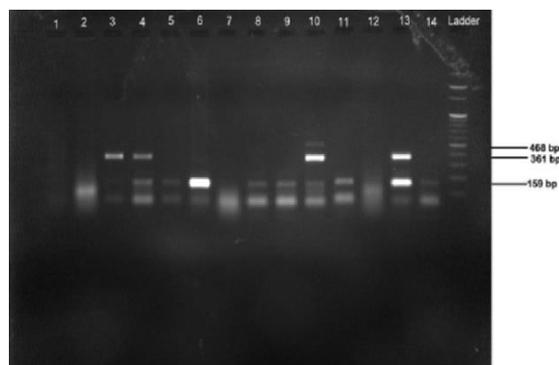


Figure 1. Showing 159 bp, 361 bp and 468 bp of DNA bands of *tet* genes in which lane 3,4,5,6,8,9,10,11 & 13 showing positive result for *tet(M)* gene while lane 3,4,10 and 13 showing positive result for *tet(K)* and lane no. 10 showing positive bands for *tet(X)* gene respectively, lane 1 showing negative result, lane 14 showing positive control and lane 15 as DNA ladder.

DISCUSSION

New generation cycline group of antibiotics have been introduced into clinical practice for treatment of *Staphylococcus aureus*, which is

considered to be the most predominant organism among gram positive infections. Continuous exposure of cycline groups of antimicrobials has led to increase in resistant trait. As reported earlier, the treatment option for

staphylococcal infection becomes complex due to phenotypic switching, mutational capability and heterogenic resistance [8]. Beside this, patients in critical conditions subjected to broad-spectrum antibiotics have life threatening risk by this microorganism as it often exhibit a multidrug resistant phenotype [7]. Thus in order to slow down infection rate the tetracycline and its derivatives doxycycline and minocycline are regarded for promising anti-staphylococcal activity. To add the latest armour in the class, a new glycylicycline antibiotic, tigecycline has been introduced into clinical practice in 2005 with greater efficacy [14].

The present study reveals that the prevalence of tigecycline resistance is highest followed by tetracycline, doxycycline and minocycline, which is in contrast with the observation in UK in 2010 [3].

However, several other studies have reported various resistance determinants of tetracycline. It was reported by Duran et al., 2012 that 41% and 30.8% isolates were phenotypically resistant to tetracycline in *S. aureus* and CoNS, in which 42.4% *S. aureus* isolates harboured both *tet(M)* and *tet(K)* genes and 39% CoNS were also found to carry these genes [11]. A study conducted in Poland in 2000 by Trzcinski et al. observed 66% *tet(K)* gene in tetracycline-resistant MRSA [12]. From 1997-1999, 400 MRSA and 200 MSSA isolates were collected from 25 university hospitals in Europe by Schmitz et al. found that 73% MRSA and 96 % MSSA isolates carry *tet(K)* gene [13]. In 2005 a study from South Africa reported 58% (22/38) *tet(K)* in *S. aureus* from poultry processing plants [14]. Based on their studies it can be assumed that *tet(K)* gene is more prevalent worldwide and present investigation is also in agreement with the previous reports.

Our study depicts that the isolates carrying *tet(K)* were resistant to tigecycline, tetracycline and doxycycline but not to minocycline and those carrying *tet(M)* were resistant to all the four cycline group of drugs. Whereas the isolates carrying the combination of *tet(K)/tet(M)* genes, showed resistance to all the four antibiotics but combination of *tet(K)/tet(X)* has been reported in only one isolate which showed phenotypic invitro resistance to tigecycline while *tet(O)* gene has not been amplified in this study.

Therefore, findings of this study are in agreement with an earlier report [15].

Although our study focused on the tigecycline resistant MRSA, presence of *tet(K)*, *tet(M)* and combination of *tet(K)/tet(M)* and *tet(K)/tet(X)* were also observed within MSSA isolates. Trzcinski et al., in 2001 reported that *tet(K)* is responsible for tetracycline resistance in MSSA whereas *tet(M)* was predominantly present within MRSA strains [12]. In another study *tet(M)* was contributor for tetracycline and minocycline, while isolates with both *tet(K)* and *tet(M)* had a elevated MIC then those with single gene. [14]. Similar finding was too recorded in a previous study of 1990 [15]. Therefore this study suggests that *tet(K)* confers resistance towards tigecycline, is most prevalent in this area.

In India, tigecycline resistance is less frequent among hospital pathogens. Behera et al., 2008 studied the in vitro susceptibility of tigecycline against hospital isolates [5], the finding of which was in agreement with a study, conducted in Nigeria by Shittu et al., 2011, where all the *S. aureus* isolates obtained from clinical and non-clinical sources was found to be 100% susceptible to tigecycline by disk diffusion method [16]. However there is a conflicting fact in the present study in which most of the isolates showed non-susceptibility towards tigecycline. In the current study absence of any visible amplification with study primers might be due to heterogeneity of resistance determinants. Linezolid may be considered as therapeutic option as it came up with better efficacy.

In conclusion, with the advent of time the increase in the occurrence of infections caused by *Staphylococcus aureus* is partially a consequence of inadequate infection control policy and also due to the pathogen's ability to adapt to a changing environment. Thus, this study pointed out that the mechanism of cycline resistance is mainly due to the efflux pumps and ribosomal protection. Presence of *tet(X)* gene indicates that the bacteria are slowly adapting a newer mechanism of resistance i.e. enzymatic inactivation. During phenotypic screening of cycline group of antimicrobials most of the antibiograms types showed non-susceptibility to tigecycline and hence this can be used as a screening tool for detection of resistance to this particular group of antimicrobials.

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Conflicts of Interest:

None exist.

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