

RESEARCH ARTICLE

## A Newer in Vitro Model for the Study of Development of in Vitro Resistance to Penicillin in a Novel Isolate of *Streptococcus tigurinus*

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### ABSTRACT

**Objectives:** *Streptococcus tigurinus* is a new member of the *Streptococcus mitis* group. It causes various invasive infections. Its isolation from the oral cavity has been reported recently for the first time in our previous study. An attempt has been made to develop a novel in vitro model for the study of development of resistance to penicillin and to find out its effect on susceptibility pattern of other antimicrobial agents in an isolate of *S. tigurinus*.

**Materials and Methods:** An in vitro model mimicking in vivo conditions was developed to induce resistance in *S. tigurinus* with MIC value of 0.12 µg/ml to penicillin by 10 serial passages by sequential exposure to increasing concentration of penicillin.

**Results:** The in vitro manipulation of original strain of *S. tigurinus* resulted in an increase in MIC value to 8 µg/ml indicating development of resistance to penicillin and a major increase in MIC value of ampicillin from 0.25 to 16 µg/ml, and increase in MIC values of cefepime (0.12 to 0.25 µg/ml), erythromycin (0.12 to 0.25 µg/ml), azithromycin (0.5 to 1 µg/ml) and clarithromycin (0.25 to 0.5 µg/ml) indicating development of cross-resistance to these antimicrobial agents.

**Conclusion:** Results indicate that exposure to increasing concentrations of penicillin can induce the development of resistance to penicillin and cross-resistance to other antimicrobial agents. As in vitro conditions are quite similar to in vivo situation, the results obtained are more appreciable than routine methods using artificial media. *J Microbiol Infect Dis* 2017; 7(4):194-197

**Keywords:** *Streptococcus tigurinus*, in vitro resistance, cross-resistance, penicillin

### INTRODUCTION

*Streptococcus tigurinus* has recently been described as a new member of the *Streptococcus mitis* group. It causes various invasive infections like infective endocarditis, spondylodiscitis, bacteremia, meningitis, prosthetic joint infections and thoracic empyema [1,2]. *S. tigurinus* has been found to have close relation with *S. mitis* phenotypically as well as genotypically. Recently, its isolation, for the first time, has been reported from the oral cavity from a case of periodontitis in our previous study [3]. Development of resistance to penicillin and cross-resistance to other antibiotics during therapy has been frequently reported in viridans

group streptococci (VGS) [4-7] but in vitro studies are mostly overlooked and rarely reported in VGS. However, in vitro studies on development of resistance are commonly reported in *Staphylococcus aureus* and *Pseudomonas aeruginosa* [8-11]. The in vitro development of resistance to penicillin has been reported in other species of VGS in our previous study [12] but the in vitro development of resistance to penicillin in *S. tigurinus* has not been studied and reported so far.

In the present study, an attempt has been made to develop an in vitro model to induce in vitro resistance to penicillin in a newer isolate of *S. tigurinus*, which has not been exposed to

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antimicrobial agents previously and to study its effect on the susceptibility pattern of *S. tigurinus* to other antimicrobial agents. To the best of our knowledge this is the first report on in vitro development of penicillin resistance in *S. tigurinus* and its effect on the susceptibility pattern of other antimicrobial agents.

## METHODS

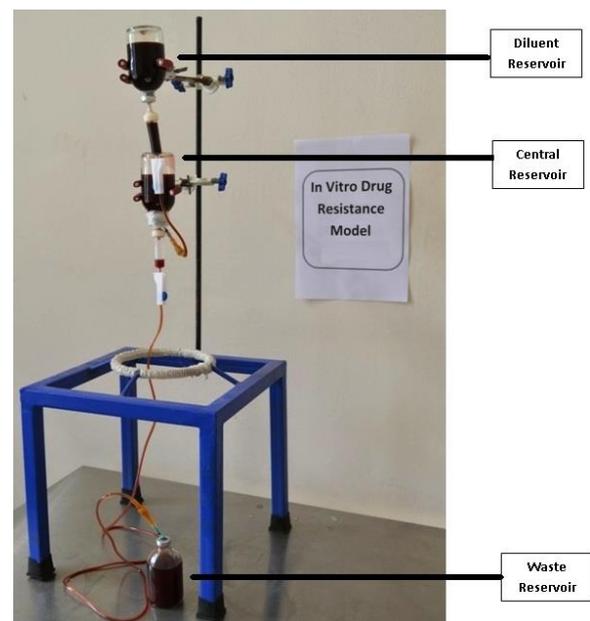
A novel isolate of *S. tigurinus*, identity of which was confirmed by performing 16S rRNA gene sequence analysis (GenBank / EMBL / DDBI accession number KJ575557) was subjected to find out MIC values against 15 different antimicrobial agents - penicillin G, ampicillin, cefepime, cefotaxime, ceftriaxone, vancomycin, erythromycin, azithromycin, clarithromycin, tetracycline, levofloxacin, ofloxacin, clindamycin, linezolid and quinupristin/dalfopristin by using automated Vitek 2 (bioMérieux) system in accordance with CLSI standards [13]. The isolate of *S. tigurinus* was manipulated in a newer in vitro model to induce resistance to penicillin. A newer in vitro model consisting a central reservoir containing *S. tigurinus*, a diluent reservoir and a waste reservoir (Figure 1). This model was used to study the development of resistance to penicillin. Increasing concentrations of penicillin were added to the central reservoir containing a complex media mimicking/simulating human in vivo like condition designed for this model (Brain – Heart infusion broth, meat particles of beef heart, proteose peptone and sterile defibrinated human blood) through the diluent reservoir. An equal volume of penicillin and test organism containing medium was removed through the waste reservoir. This model offers a continuous culture system, which mimics human in vivo conditions like blood circulation.

*S. tigurinus* was inoculated into tryptone soya broth and incubated overnight. After achieving the final bacterial concentration of  $1 \times 10^5$  CFU/ml approximately (turbidity matched with McFarland tube No.1), one ml of broth culture was inoculated into the central reservoir containing 50 ml of the complex media and incubated at 37 °C for 72 hours in a continuous culture with inflow of media from the diluents reservoir to the central reservoir, the excess medium from central reservoir to waste reservoir with a steady flow rate. *S. tigurinus* was serially exposed to

increasing concentrations of penicillin (starting from ½ of the predetermined MIC of 0.12 µg/ml). The concentration of the penicillin was increased after every 72 hours, serially in the following order starting from 0.05 µg/ml, 0.1 µg/ml, 0.2 µg/ml, 0.4 µg/ml, 0.8 µg/ml, 1 µg/ml, 1.2 µg/ml, 1.4 µg/ml, 1.6 µg/ml, 1.8 µg/ml, 2 µg/ml. Altogether a serial exposure of increasing concentration of the penicillin was done for 11 times using the in vitro model. MIC values of *S. tigurinus* were re-determined after serial passage by using Vitek 2 system to find out the changes induced by serial exposure to increasing concentrations of penicillin.

## RESULTS

An MIC value of penicillin of original strain of *S. tigurinus* was increased from 0.12 µg/ml to 8 µg/ml. A major increase in MIC value of *S. tigurinus* from 0.25 to 16 µg/ml was observed against ampicillin. The slight increase in MIC values of *S. tigurinus* (twice the MIC of original isolate) was seen with cefepime (from 0.12 to 0.25 µg/ml), erythromycin (from 0.12 to 0.25 µg/ml), azithromycin (from 0.5 to 1 µg/ml) and clarithromycin (from 0.25 to 0.5 µg/ml). However, no changes in MIC values were observed with other structurally related and unrelated antimicrobial agents after the exposure of *S. tigurinus* to penicillin (Table 1).



**Figure 1.** In vitro drug resistance model.

Table 1. MIC of *S. tigurinus* before and after exposure to increasing concentration of penicillin in vitro.

Antimicrobial agent	MIC of original strain of <i>S. tigurinus</i> ( $\mu\text{g/ml}$ )	MIC of <i>S. tigurinus</i> after exposure to increasing concentration of penicillin ( $\mu\text{g/ml}$ )
Penicillin	0.12	8
Ampicillin	0.25	16
Cefepime	0.12	0.25
Cefotaxime	0.12	0.12
Ceftriaxone	0.12	0.12
Vancomycin	0.5	0.5
Erythromycin	0.12	0.25
Azithromycin	0.5	1
Clarithromycin	0.25	0.5
Tetracycline	0.5	0.5
Levofloxacin	0.5	0.5
Ofloxacin	2	2
Clindamycin	0.25	0.25
Quinupristin/Dalfopristin	2	2
Linezolid	0.25	0.25

## DISCUSSION

Repeated exposure of susceptible strains of bacteria to antimicrobial agents is known to make an environment suitable for the development and maintenance of drug resistance in them. The development of resistance to penicillin in VGS during therapy has been reported [4-7], but the reports on in vitro development of resistance to penicillin in VGS are very rare. Although commonly studied and reported in *S. aureus* and *P. aeruginosa* to a variety of antimicrobial agents [8-11], it is very rarely studied and reported in VGS. No previous reports are available on in vitro development of resistance in VGS except for one reported by us [12]. The in vitro development of resistance to penicillin in *S. tigurinus* has not been studied and reported so far. To the best of our knowledge this is the first report on in vitro development of resistance to penicillin in *S. tigurinus*. In the present study, resistance to penicillin was induced in *S. tigurinus* by sequential exposure to increasing concentration of penicillin by using a newer in vitro model, as evidenced by increasing MIC values of *S. tigurinus* after sequential exposure to penicillin. A major change in an MIC value of *S. tigurinus* to penicillin indicates development of resistance to penicillin and a major change in MIC value to ampicillin indicates development of cross-resistance to ampicillin following exposure to penicillin (structurally similar antibiotic with similar mode of action). The slight changes in MIC of cefepime, erythromycin, azithromycin

and clarithromycin indicate low level of cross-resistance to these agents following exposure to penicillin and no major changes in the MIC of rest of the antimicrobial agents indicates no cross-resistance was developed/induced to these agents.

In vitro development of resistance by repeated exposure of susceptible strain of any bacteria using artificial media is very common but always there is variation in in vitro and in vivo susceptibility/resistance pattern because of differences in in vivo and in vitro environmental conditions. To avoid the disparities in the results, an attempt has been made to provide the environmental conditions which are very near to in vivo situation. Although environmental conditions are not exactly similar to in vivo situation, the results of present study are near to reality as they are obtained using a newer in vitro model in which conditions are quite similar to in vivo situation.

The limitations of this study are lack of experimental control group and only one isolate in a study group. It would have been more interesting to study the more number of isolates to obtain more useful and concrete conclusions but unfortunately we could not study more number of strains because of its rarity (In spite of processing 200 specimens we could get only one isolate of *S. tigurinus*).

These results indicate that exposure of susceptible strain of *S. tigurinus* to sublethal concentrations of penicillin not only results into

development of resistance to penicillin but also leads to development of cross-resistance to other antimicrobial agents. These results show that the resistance could also be developed easily in novel isolates of bacteria which are not exposed to antimicrobial agents ever before.

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