

DETECTION OF *tetM* GENE FOR TETRACYCLINE RESISTANCE IN *Streptococcus suis* CLINICAL ISOLATES AT RS. NGOERAH IN DECEMBER 2021 – APRIL 2022

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ABSTRACT

Background: *Streptococcus suis* is a zoonotic pathogenic bacterium that infects pigs and can be transmitted to humans, causing serious illnesses like meningitis. This infection is commonly treated with antibiotics, but improper use can lead to increased resistance. This has become a significant problem because *S. suis* has developed mechanisms to survive various types of antibiotics, especially tetracycline, which possesses the resistance gene *tetM*. This study aimed to detect the presence of the *tetM* gene for tetracycline resistance in clinical *Streptococcus suis* isolates at Prof. Ngoerah Hospital during the period of 2021-2022.

Method: This research was a cross-sectional descriptive study. The study sample consisted of 20 *S. suis* isolates from clinical specimens at Prof. Ngoerah Hospital, which were test used the PCR method to detect the presence of the *tetM* gene.

Results: The results of the PCR and electroforesis test showed that out of the 20 samples, 16 (80%) were positive for the *tetM* gene, while 4 (20%) were negative. Of the 16 isolates positive for the *tetM* gene, they came from various specimens 6 (30%) were from blood specimens and 10 (50%) were from CSF (Cerebrospinal Fluid) specimens.

Conclusion: The *tetM* gene was detected in 80% of the clinical *Streptococcus suis* isolates at Prof. Ngoerah Hospital during the period of December 2021 to April 2022. The high prevalence of the *tetM* gene in clinical *S. suis* isolates indicates that this gene plays a significant role in the bacterium's mechanism of resistance to tetracycline antibiotics.

Keywords: *Streptococcus suis*, *tetM* gene, tetracycline, PCR

INTRODUCTION

Cases of meningitis caused by *Streptococcus suis* (*S. suis*) have recently become a growing concern, due to the increasing number of reported meningitis cases. Considering the medical condition, symptoms of meningitis due to *S. suis* can rapidly progress into a life-threatening condition if not handled promptly. Furthermore, the rise in antibiotic resistance cases is also a particular concern in meningitis, as the excessive and unwise use of antibiotics can exacerbate the problem of resistance to *S. suis* bacteria.

S. suis is an alpha-hemolytic gram positive bacterium found in pigs. *S. suis* can infect both humans and animals and is naturally found in the respiratory and digestive tracts of pigs.¹ In humans, *S. suis* infection commonly occurs through direct contact with pigs or pig-derived products, such as blood or meat, that are contaminated with the bacteria. Human infections frequently occur in individuals working in the livestock industry, slaughterhouses, or pig product processing, who are at high risk of direct exposure.² Clinical symptoms in humans can vary, ranging from fever

and headache to severe complications such as meningitis, sepsis, neurological disorders, and hearing loss.³

Globally, the prevalence of human *S. suis* infection cases in 2013 was reported to be 1,600, with the highest cases being in Thailand (36%), Vietnam (30%),

and China (22%).⁴ In Indonesia, data from Prof. Dr. I.G.N.G Ngoerah Hospital indicates 44 *S. suis* meningitis cases out of a total of 71 bacterial meningitis cases between 2014 and 2017.² Although the recovery rate without complication is fairly good (70%), the global case-fatality rate for this infection reaches 12.8% per year, highlighting the importance of rapid and effective therapy.⁴

Tetracycline is a broad-spectrum antibiotic that is effective as a first-line therapy for *S. suis* infection due to its ability to inhibit bacterial protein synthesis.⁵ However, the continuous and unwise use of antibiotics drives the evolution of bacteria to develop resistance mechanisms.⁶ The highest prevalence of tetracycline resistance in 2019 was reported in China (98%), Canada (97%), India (95%), and Thailand (92%).⁶ In Indonesia, based on research

conducted at Prof. I.G.N.G. Ngoerah Hospital concerning the antibiotic susceptibility of *S. suis* in Bali, tetracycline resistance is greater than 90%.⁷ Molecularly, the *tetM* gene is a specific gene frequently detected and responsible for the tetracycline resistance mechanism in *S. suis*.⁸

Based on this background, the aim of this study was to detect the *tetM* gene and the presence of *tetM* gene for tetracycline resistance in clinical *Streptococcus suis* isolates at Prof. Ngoerah Hospital during period of December 2021 to April 2022.

MATERIALS AND METHODS

Ethics Approval

This study was approved by the Research Ethics Committee of the Faculty of Medicine, Universitas Udayana.
No: 0552/UN14.2.2.VII.14/LT/2025

Study Design and Ethics

The type of study used was observational with intervention performed on *Streptococcus suis* clinical isolates. The samples were taken within the period of December 2021 to April 2022 from *Streptococcus suis* clinical isolates at Prof. dr. I. G. N. G. Ngoerah Central General Hospital, and tested for the *tetM* gene using molecular detection, with a cross-sectional study design.

Bacterial Culture

This study utilized 20 stored clinical isolate samples originating from clinical specimens, namely blood and Cerebrospinal Fluid (CSF). The isolates were previously stored at -80°C in the Clinical Microbiology Laboratory of Prof. I.G.N.G. Ngoerah Central General Hospital, Denpasar. First, the bacterial culture medium (nutrient agar), a disposable loop (or inoculation loop), and the stored *S. suis* clinical isolates were prepared. A small amount of the *S. suis* clinical isolate sample was retrieved using the loop. The tip of the loop was gently streaked onto the agar medium, filling four quadrants. Subsequently, the agar medium was placed in an incubator at 37°C.

Bacterial DNA Isolation

DNA isolation was performed using the DNA High Pure Purification KIT (Roche Life Science, Indianapolis, U.S.A.). The procedure began by resuspending 5–10 bacterial colonies in 200 µL of TE buffer and 200 µL of PBS buffer, followed by incubation at -20°C. The lysis step involved adding lyse blue and RNase A to buffer P1. The bacterial pellet was resuspended in 250 µL of buffer P1, followed by the addition of 250 µL of buffer P2 and 350 µL of buffer N3, then centrifuged at 13,000 rpm for 10 minutes. The supernatant was transferred to a spin column and sequentially washed with 500 µL of buffer PB and 750 µL of buffer PE, with centrifugation for 1 minute at each step. Finally, DNA was eluted from the column using 30 µL of buffer EB and stored at -20°C.

Polymerase Chain Reaction (PCR) condition

In the Polymerase Chain Reaction (PCR) step, *tetM* gene uses the forward primer F: GTTAAATAGTGTCTTGGAG and the reverse primer R: CTAAGATATGGCTCTAACAA.²³ Used 5 µL of master mix, 0.5 µL each of forward and reverse *tetM* primers, 3 µL of dH₂O, and 1 µL of sample DNA were added into each tube. The mixture was homogenized using a micropipette for a total of 20 samples. Positive and negative controls were prepared in separate tubes. The positive control was derived from a clinical *S. suis* isolate that was confirmed to amplify the *tetM* gene through sequencing.¹⁵ The PCR conditions were set as follows: pre-denaturation at 95°C for 5 minutes, denaturation at 95°C for 1 minute, annealing at 45°C for 1 minute, extension at 72°C for 1 minute, and final extension at 72°C for 5 minutes, repeated for 35 cycles. After completion, the PCR products were stored at 4°C.

Electrophoresis Method

Agarose gel preparation began by dissolving 0.7 grams of agarose powder in 70 mL of 1× TBE buffer to achieve a 1% concentration. The solution was homogenized and heated in a microwave for 2 minutes. After heating, 2 µL of Red Nucleic Acid 10,000× stain was added, and the solution was poured onto a glass plate with a comb in place until solidified. The solidified gel was then transferred to an electrophoresis chamber filled with 0.5× TBE buffer. Subsequently, 2 µL of DNA sample, 2 µL of positive control (obtained from a clinical isolate of *S. suis* confirmed to amplify the *tetM* gene through sequencing), 2 µL of negative control, and 1 µL of marker were loaded into the wells. Electrophoresis was performed for 35 minutes at 50 volts. After completion, the gel was removed, and the resulting DNA bands were visualized under UV light (transilluminator) and documented.

RESULTS

Subculture Result of *Streptococcus suis*

The culture results again showed the growth of *Streptococcus suis* colonies on nutrient agar medium. The colonies appeared round with smooth, shiny, yellowish surfaces. After 24 hours of incubation and achieving optimal growth, the culture was subsequently processed for DNA isolation for the next stage of analysis.

Detection of the *tetM* Gene in Clinical *Streptococcus suis* Isolates using PCR and Electrophoresis

From the recultured *Streptococcus suis* bacteria, DNA isolation was carried out using the DNA High Pure Purification KIT. The obtained plasmid DNA was then subjected to polymerase chain reaction (PCR) and electrophoresis. The amplification results were visualized through electrophoresis, where the presence of a DNA band at 657 bp indicated a positive result for the *tetM* gene, as

shown in Figures 5.4 and 5.5, which display the marker, test samples, positive control, and negative control. Based on the electrophoresis results in Figure 5.4, samples 71 and 100–108, along with the positive control, showed bands with a 657 bp amplicon, while the negative control showed no band formation. The presence of a band indicates that these samples successfully amplified the tetracycline resistance

gene *tetM*. In Table 5.5, samples 109, 110, 116, 118, and the negative control showed no band formation, whereas samples 111, 112, 113, 114, and 115, along with the positive control, showed bands with a 657 bp amplicon. The absence of a band indicates that the samples did not amplify the tetracycline resistance gene *tetM*.

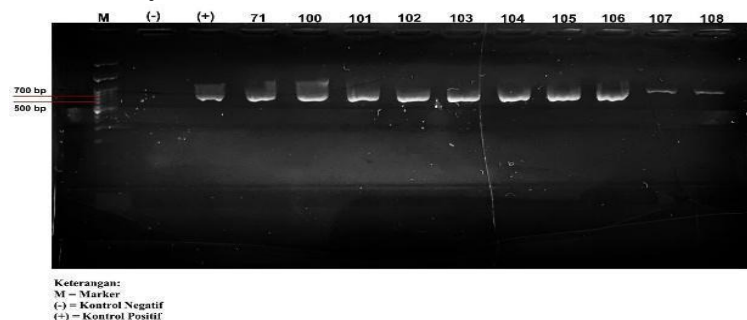


Figure 1. Electrophoresis results of samples 71 and 100–108 showing band formation with an amplicon length of 657 bp.

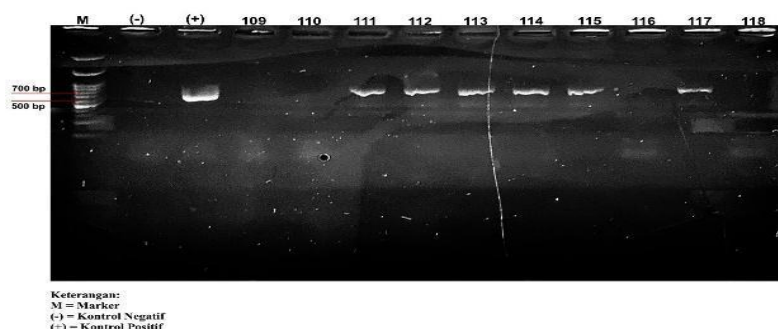


Figure 2. Electrophoresis results of samples 109, 110, 116, 118, and the negative control showing no band formation, while samples 111, 112, 113, 114, 115, 117, and the positive control showed band formation with an amplicon length of 657 bp.

Table 1 presents the percentage of PCR results from a total of 20 samples, showing that 16 samples (80%) contained the *tetM* gene, while 4 samples (20%) showed no gene

detection. The high detection rate of this gene indicates a widespread resistance to tetracycline-class antibiotics among the isolates.

Table 1. PCR Results of the *tetM* Gene from 20 *S. suis* Isolates

Results of <i>S. suis</i> Samples	Result	Percentage
<i>tetM</i> gene positive	16 isolate	80%
<i>tetM</i> gene negative	4 isolate	20%
Total	20 isolate	100%

The analysis of PCR and electrophoresis results was also categorized based on the type of specimen source. The distribution of the *tetM* gene in clinical specimens is presented in Table 2. These data provide a comprehensive overview of the prevalence of the *tetM* gene in

Streptococcus suis isolates from their infection sources. Most *S. suis* isolates (80%) amplified the *tetM* gene. Among blood samples, 6 (30%) were positive and 1 (5%) was negative, while among CSF samples, 10 (50%) were positive and 3 (15%) were negative.

Table 2. Distribution of Positive and Negative *tetM* Gene Results Based on Specimen Source

Specimen	<i>tetM</i> Gene Positive	<i>tetM</i> Gene Negative	Total Samples
Blood	6 (30%)	1 (5%)	7 (35%)
CSF	10 (50%)	3 (15%)	13 (65%)
Total	16 (80%)	4 (20%)	20 (100%)

DISCUSSION

The study showed that *Streptococcus suis* isolates obtained from the Clinical Microbiology Laboratory of Prof. dr. I.G.N.G. Ngoerah General Hospital during the period of December 2021 – April 2022 demonstrated amplification of the tetracycline resistance gene *tetM* at a 657 bp amplicon in samples 71, 100–108, and 111–115, while samples 109, 110, 116, and 118 did not amplify the *tetM* gene. Detection of the gene using the PCR method revealed a prevalence of the *tetM* gene in 16 samples (80%), while 4 samples (20%) showed no detection out of a total of 20 samples. This finding is consistent with previous research, which also reported a high prevalence of the *tetM* gene in clinical isolates of *S. suis*, where 100% of the analyzed samples were found to amplify the *tetM* gene in Bali, Indonesia.¹⁴

Thus, the high prevalence of the *tetM* gene in clinical isolates of *Streptococcus suis* indicates that, according to the One Health concept, this resistance gene can be transmitted to humans through direct contact with livestock workers or farmers, as well as through the consumption of animal-derived products.²¹ The empirical use of tetracycline in pig farming practices often integrated into feed or drinking water to maintain gut health and promote growth poses a high risk of contributing to antimicrobial resistance (AMR).²² Unsupervised administration leads to the development of bacterial defense mechanisms in livestock, such as the *tetM* gene, which is commonly located on plasmids. As a result, bacterial infections transmitted from animals become difficult to treat because tetracyclines are no longer effective.²³

The detection of the *tetM* gene in *Streptococcus suis* clinical isolates demonstrates that this gene plays a crucial role in resistance to tetracycline antibiotics. Its presence may limit the effectiveness of standard therapies, making it necessary to develop more appropriate infection management strategies, including the use of alternative antibiotics. Therefore, further research is needed to identify other genes or molecular pathways underlying antibiotic resistance in *S. suis*.

CONCLUSIONS AND SUGGESTIONS

Based on the results of this study, it can be concluded that the *tetM* gene, which encodes tetracycline resistance,

was detected in clinical isolates of *Streptococcus suis* at Prof. Ngoerah Hospital during the period of December 2021 – April 2022. The prevalence of the *tetM* gene as a tetracycline resistance determinant in *S. suis* clinical isolates from Prof. Ngoerah Hospital during this period was 80%.

Based on these findings, it is recommended that future studies employ an analytical design to examine the factors influencing *tetM* gene expression in *Streptococcus suis*. Further research should also be conducted to identify the structural characteristics of the *tetM* gene in clinical isolates from Prof. dr. I G.N.G. Ngoerah General Hospital, Denpasar, in order to compare them with structures previously reported in different populations.

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