

DETECTION AND PREVALANCE OF THE MACROLIDE RESISTANCE GENE *ermB* IN CLINICAL *Streptococcus suis* ISOLATES IN BALI, INDONESIA

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ABSTRACT

Background: *Streptococcus suis* is a significant zoonotic pathogen causing high morbidity and mortality in humans, particularly in Southeast Asia. Resistance to macrolide antibiotics, often mediated by the *ermB* gene, has become a serious global threat, with a very high prevalence in many countries. However, molecular data on this resistance from Indonesia, especially Bali, remain scarce. This study aimed to detect the presence and determine the prevalence of the *ermB* gene in clinical *S. suis* isolates from a tertiary hospital in Bali. This study employed a descriptive cross-sectional design on 20 archived clinical *S. suis* isolates from the period of December 2021 to April 2022.

Methods: Detection of the *ermB* gene was performed using the Polymerase Chain Reaction (PCR) method with specific primers.

Results: The results showed that the *ermB* gene was detected in 1 out of 20 isolates analyzed, yielding an overall prevalence of 5%. The single positive isolation originated from a cerebrospinal fluid specimen. In conclusion, the *ermB* gene is present in the clinical *S. suis* population in Bali, but its prevalence is significantly lower compared to reports from other Asian countries.

Conclusion: This likely indicates lower selective pressure from macrolide use in the local environment. These data provide a crucial baseline for antimicrobial resistance surveillance and antibiotic stewardship guidelines in this region.

Keywords : *Streptococcus suis*, Macrolide Resistance, *ermB*

INTRODUCTION

Streptococcus suis is a significant emerging zoonotic pathogen, posing a global public health threat as a major cause of morbidity and mortality in both the swine industry and humans. Its relevance is particularly pronounced in Southeast Asia, where *S. suis* is a leading cause of bacterial meningitis in adults, especially in Thailand and Vietnam¹. Globally, over 1,500 human cases were reported by the end of 2012, with major outbreaks occurring in Thailand, Vietnam, and China.¹ High incidence in this region is often linked to cultural practices such as the consumption of raw or undercooked pork products and close contact with infected pigs². In Bali, Indonesia, where pork consumption is common, the first confirmed human case was reported in 2014. By 2017, 44 cases of *S. suis*-associated meningitis were recorded, with transmission strongly linked to the local tradition of preparing and consuming raw pork meat and blood³.

As a Gram-positive, non-motile, and catalase-negative coccus⁴, *S. suis* is classified into 34 serotypes based on its capsular polysaccharide (CPS), a primary virulence factor that helps the bacterium evade phagocytosis. While many serotypes can infect pigs, serotype 2 is the most virulent and frequently isolated from human clinical cases⁵.

As a commensal organism, *S. suis* colonizes the upper respiratory tract of up to 80% of healthy pigs^{2,6}. Zoonotic transmission to humans occurs primarily through occupational exposure or consumption of raw pork products⁷. The pathogenesis involves colonization, invasion into the bloodstream, and systemic dissemination, which can lead to severe conditions such as septicemia, arthritis, endocarditis, and meningitis⁸. The management of *S. suis* infections is complicated by the escalating crisis of antimicrobial resistance (AMR)⁹. Extensive antibiotic use in both human medicine and livestock farming has driven the selection and spread of resistant strains⁹. Reports from across Asia paint a concerning picture, with macrolide resistance rates in *S. suis* frequently exceeding 90%^{10,11}. For

instance, a study in China found that by 2017, 97% of isolates had become resistant to macrolides¹¹. This widespread resistance severely limits treatment options and elevates the risk of therapeutic failure.

The predominant mechanism of macrolide resistance in *S. suis* is target-site modification, mediated by erythromycin-resistance-methyltransferase enzymes encoded by *erm* genes. These enzymes methylate the 23S rRNA of the 50S ribosomal subunit, which prevents macrolide antibiotics from binding effectively and thus renders them inactive¹².

Among the various *erm* genes, *erm(B)* is the most frequently detected in macrolide-resistant *S. suis* isolates globally and is considered a primary driver of this resistance phenotype^{13,14}. The high prevalence of macrolide resistance is strongly correlated with the presence of the *ermB* gene. In a study on isolates from pigs in China, *ermB* was identified in up to 97.2% of macrolide-resistant strains¹⁴. Furthermore, a study in Japan showed that of 64 strains resistant to macrolide antibiotics, 100% were positive for the *ermB* gene¹⁵.

The *erm(B)* gene is often located on mobile genetic elements (MGEs), such as transposons and Integrative and Conjugative Elements (ICEs), which also frequently carry tetracycline resistance genes like *tet(O)*¹⁶. This genetic linkage facilitates the horizontal transfer and co-selection of resistance to both antibiotic classes, accelerating the spread of multidrug resistance.

Despite extensive AMR data from mainland Southeast Asia, there is a significant lack of molecular data from Indonesia, particularly Bali. This knowledge gap hinders a comprehensive understanding of the regional AMR landscape and limits the development of evidence-based local antibiotic stewardship strategies. Therefore, this study aimed to provide the first molecular evidence on this issue by detecting the presence and determining the prevalence of the *ermB* gene in clinical *S. suis* isolates from Prof. dr. I. G. N. G. Ngoerah General Hospital in Denpasar, Bali.

MATERIALS AND METHODS

Study Design

This was a descriptive observational study with a cross-sectional design. The presence of the *ermB* gene was observed in archived clinical *S. suis* isolates without any intervention on the subjects.

Study Sample

The sample consisted of 20 archived clinical *S. suis* isolates collected via consecutive sampling from Prof. dr. I. G. N. G. Ngoerah General Hospital from December 2021 to April 2022. The research was conducted at the Integrated Biomedical Laboratory, Microbiology Laboratory Division, Faculty of Medicine, Udayana University. The inclusion criterion was all identified *S. suis* isolates from the specified period; the exclusion criterion was any isolate that failed to regrow upon subculture. The archived isolates had been previously identified and tested for antimicrobialsusceptibility using the VITEK 2 Compact system (bioMérieux®) with VITEK® 2 GP and AST-ST03 cards, results for erythromycin were used for phenotypic analysis.

Research Procedure

Bacterial Culture

S. suis isolates stored at -80°C were subculture on Nutrient Agar media and incubated at 37°C for 24 hours. All procedures were performed aseptically inside a biosafety cabinet to ensure culture purity.

DNA Extraction

Plasmid DNA was extracted from pure bacterial colonies using the *QIAprep® Spin Miniprep Kit* (Qiagen, Hilden, Germany) according to the manufacturer's protocol. The procedure involved bacterial cell lysis, protein precipitation, and elution of pure DNA. *ermB* Gene Amplification by Polymerase Chain Reaction (PCR)

The *ermB* gene was amplified using specific primers¹⁷ with the following sequences:

Forward Primer: 5'-GAAAAGGTACTCAACCAAATA-3'
Reverse Primer: 5'-AGTAACGGTACTTAAATTGTTTAC-3'

The expected amplicon size was 639 base pairs (bp). The PCR reaction was performed in a total volume of 10 µL, containing 5 µL *GoTaq® Green Master Mix* (Promega, USA), 0.5 µL of each primer (10 µM), 3 µL of nuclease-free water, and 1 µL of DNA template. Thermal cycling conditions were initial pre-denaturation at 95°C for 5 minutes; 35 cycles of denaturation at 95°C for 1 minute, annealing at 48°C for 1 minute, and extension at 72°C for 1 minute; followed by a final extension at 72°C for 5 minutes. The positive control was sourced from a clinical *Streptococcus suis* isolate that is co-resistant to tetracycline and erythromycin, and a negative control (nuclease-free water) was included in each run for validation.

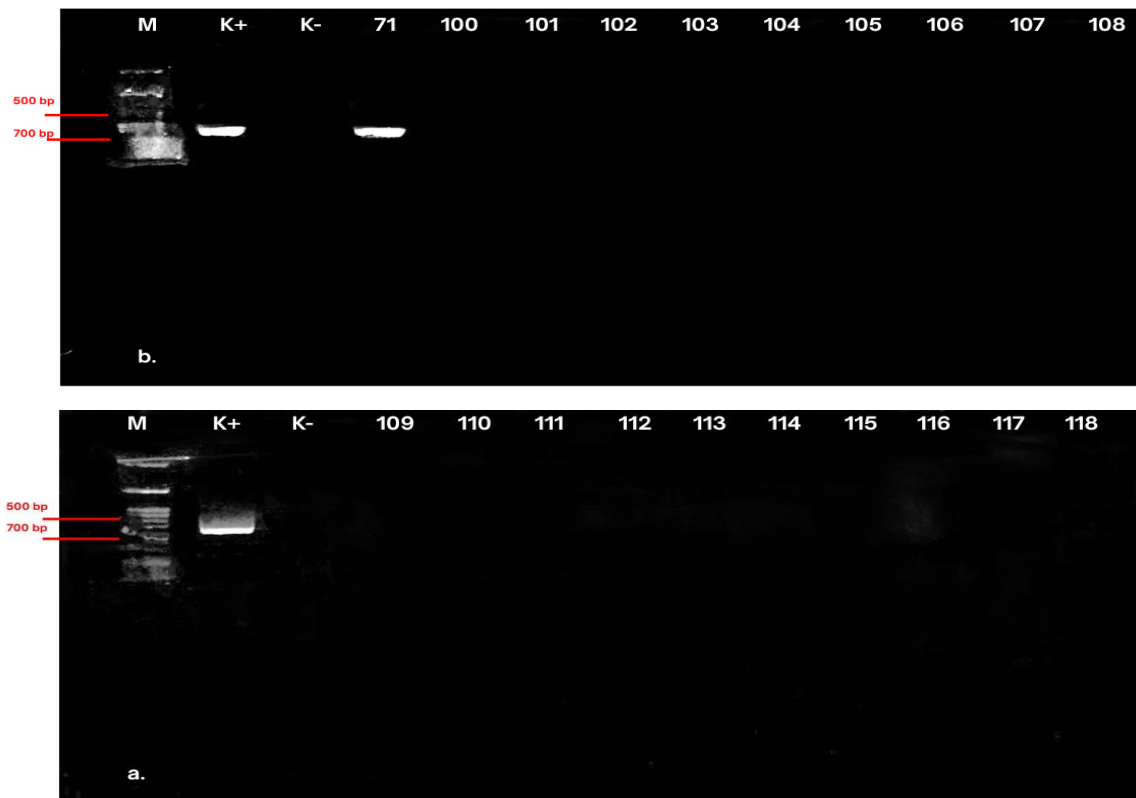


Figure 1. Agarose gel electrophoresis result of the *ermB* gene. The figure shows the 1% agarose gel electrophoresis of PCR amplification products for the *ermB* gene from 20 clinical *S. suis* isolates. M, indicated as a marker, 100 bp DNA ladder; K+, indicated as positive control; K-, indicated as negative control; 71 through 118, indicated as the clinical isolate ID numbers. Among the 20 isolates tested, only isolate 71 successfully amplified the *ermB* gene, showing a distinct DNA band with the expected PCR product size of 639 bp, which is aligned with the positive control.

Data Analysis

The presence of the *ermB* gene was determined by the observation of a DNA band of the appropriate size (approx. 639 bp). Data were analyzed descriptively. The prevalence of the *ermB* gene was calculated as the number of positive isolates divided by the total number of isolates, expressed as a percentage.

RESULTS

Isolate Characteristics

A total of 20 archived clinical *S. suis* isolates were successfully subcultured. nutrient agar, all isolates displayed typical colony morphology, small and grayish colonies. Of the 20 isolates, 13 (65%) were from cerebrospinal fluid (CSF) specimens, and 7 (35%) were from blood cultures.

Detection of the *ermB* Gene and Phenotypic Resistance

Ethical Clearance

This study received ethical approval from the Research Ethics Committee of the Faculty of Medicine, Udayana University (Protocol number: 0255/UN14.2.2.VII.14/LT/2025)

A perfect correlation between the genotypic and phenotypic results was observed. Genotypic analysis via PCR and gel electrophoresis showed that 1 out of 20 isolates (sample No. 71) was positive for the *ermB* gene (Figure 1). Concurrently, phenotypic analysis from the VITEK 2 system showed that 1 out of 20 isolates was resistant to erythromycin. Crucially, the isolate that was phenotypically resistant was the same isolate (No. 71) that tested positive for the *ermB* gene. The remaining 19 isolates were negative for the *ermB* gene and sensitive to erythromycin (Table 1)

Tabel 1. Correlation of genotypic (*ermB* detection) and phenotypic (erythromycin resistance) results for 20 clinical *S. suis* isolate

	Isolate Code	Isolate Source	<i>ermB</i> Detection (PCR)	Erythromycin (VITEK) Resistance
	71	CSF	Positive (+)	Resistance
	100	Blood	Negative (-)	Sensitive
	101	CSF	Negative (-)	Sensitive
	102	CSF	Negative (-)	Sensitive
	103	Blood	Negative (-)	Sensitive
	104	CSF	Negative (-)	Sensitive
	105	Blood	Negative (-)	Sensitive
	106	CSF	Negative (-)	Sensitive
	107	Blood	Negative (-)	Sensitive
	108	CSF	Negative (-)	Sensitive
	109	Blood	Negative (-)	Sensitive
	110	CSF	Negative (-)	Sensitive
	111	CSF	Negative (-)	Sensitive
	112	Blood	Negative (-)	Sensitive
	113	Blood	Negative (-)	Sensitive
	114	CSF	Negative (-)	Sensitive
	115	CSF	Negative (-)	Sensitive
	116	CSF	Negative (-)	Sensitive
	117	CSF	Negative (-)	Sensitive
	118	CSF	Negative (-)	Sensitive
Total	20	CSF: 13, Blood: 7	Positive: 1, Negative: 19	Resistance: 1, Sensitive : 19

Prevalence of the *ermB* Gene

Based on the molecular detection, the overall prevalence of the *ermB* gene in this cohort of clinical *S. suis* isolates was

5% (1 out of 20). The single positive isolate was derived from a CSF specimen. Consequently, the prevalence of the *ermB* gene was 7.7% (1 out of 13) among CSF isolates and 0% (0 out of 7) among blood isolates (Table 1 and Table 2).

Tabel 2. Distribution of positive results by clinical specimen source

Specimen Type	<i>ermB</i> Detection Results	
	Positive, n (%)	Negative, n (%)
CSF	1 (7.7%)	12 (92.3%)
Blood	0 (0%)	7 (100%)
Total	1 (5%)	19 (95%)

DISCUSSION

This study provides the first molecular evidence of the macrolide resistance gene *ermB* in clinical *Streptococcus suis* isolates in Bali, Indonesia, revealing a prevalence of 5%. The most significant finding is the perfect 1:1 correlation observed between the genotypic result (presence of the *ermB* gene) and the phenotypic result (resistance to erythromycin) within the same cohort of isolates. This provides powerful internal validation for our findings and strongly suggests that the *ermB* gene is the primary mechanism of macrolide resistance in this population.

The 5% prevalence of the *ermB* gene stands in stark contrast to reports from other Asian countries. For example, a study in China detected the *ermB* gene in 97.2% of all isolates examined¹⁴, while research in Japan found that 100% of macrolide-resistant strains carried the *ermB* gene¹⁵. Although these findings appear to contradict the majority of similar studies, they are consistent with phenotypic data from another study on clinical *S. suis* isolates in Bali, which found resistance to macrolides (specifically erythromycin) to be below 10%¹⁸. This indicates that the results of this study align with the literature, which establishes that the *ermB* gene plays a crucial role in macrolide resistance;

because macrolide resistance in Bali is quite low, the prevalence of the *ermB* gene is consequently also low.

The low prevalence of the *ermB* gene is likely attributable to lower selective pressure from macrolide antibiotic use in the local Bali environment. The amplification of resistance genes in a bacterial population is heavily influenced by the frequency of corresponding antibiotic use. Data on antibiotic consumption in Bali indicate that macrolides are not first-line therapy for most infections, and their overall use is lower compared to beta-lactams and fluoroquinolones¹⁹. Furthermore, while antibiotics are accessible for livestock use in Indonesia, studies indicate that the use of macrolides in the livestock sector in Bali is not significant^{20,21}. This combination of relatively low clinical and agricultural use creates an environment of minimal selective pressure, which could explain why the *ermB* gene has not become widespread in the *S. suis* population in Bali.

From a clinical perspective, this low *ermB* prevalence implies that macrolides may still be an effective therapeutic option for *S. suis* infections in Bali, particularly for patients with contraindications to beta-lactam antibiotics. However, this interpretation requires caution, as other resistance mechanisms, such as the presence of other *erm* genes or efflux pumps encoded by *mef(A/E)* genes¹², could be present. Therefore, these genotypic findings do not replace the need for routine Antimicrobial Susceptibility Testing (AST) to guide patient therapy. This situation presents a "therapeutic window of opportunity" for antimicrobial stewardship. By implementing prudent antibiotic use policies now in both human and animal health sectors, the spread of resistance can be slowed, preserving the clinical utility of these important drugs.

Although small in scale, this study provides invaluable baseline data for public health. It offers the first molecular snapshot of macrolide resistance in *S. suis* in this region, which can serve as a benchmark for future surveillance programs. Continuous monitoring will enable health authorities to detect any increase in *ermB* prevalence early, before it becomes a widespread clinical problem. The threat of mobile genetic elements (MGEs) co-carrying multiple resistance genes remains a concern, as the use of other common antibiotics like tetracycline could co-select for *ermB*¹⁶. This reinforces the urgency of implementing a "One Health" approach to AMR surveillance, integrating data from human, animal, and environmental sources.

This study has several limitations. First, the small sample size (n=20) limits the generalizability of the findings. Larger-scale studies are needed for a more accurate prevalence estimate. Second, samples were sourced from a single tertiary referral hospital and may not represent resistance patterns in other hospitals or rural areas. Future studies should expand on this foundational work with larger, multi-center samples to confirm these findings on a wider scale.

CONCLUSION AND SUGGESTIONS

Conclusion:Based on the research findings, the following conclusions can be drawn:The *ermB* gene, which encodes for resistance to macrolide antibiotics, was detected in clinical *Streptococcus suis* isolates from Prof. dr. I. G. N. G. Ngoerah General Hospital during the 2021-2022 period.The prevalence of the *ermB* gene in the clinical *Streptococcus suis* isolates in this study was 5%.

Suggestions:Based on these findings, the following are recommended:For Healthcare Professionals and Hospitals: It is recommended to continue routine Antimicrobial Susceptibility Testing (AST) for all invasive *S. suis* isolates to guide appropriate therapy. Strengthening antimicrobial stewardship programs is crucial to preserve the efficacy of macrolides and other antibiotics.For Public Health Authorities: It is advisable to establish systematic and continuous molecular AMR surveillance programs for priority genes like *ermB* in *S. suis*. This surveillance should adopt a "One Health" approach,including isolates from both human and swine sources, to monitor trends and detect potential spread early.

For Future Research: It is recommended to conduct larger-scale studies involving multiple healthcare centers across Bali to obtain a more robust prevalence estimate. Future research should integrate genotypic data with phenotypic data and whole-genome sequencing to gain a more comprehensive understanding of resistance mechanisms and their genetic context.

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