Indexed by : DOAJ Google Crossref Dimension sinta GARUDA

*J.Biomed.Transl.Res*
*ISSN: 2503-2178*

JOURNAL OF BIOMEDICINE AND TRANSLATIONAL RESEARCH

Available online at JBTR website: https://jbtr.fk.undip.ac.id



Original Research Article

# Antibiotic-Resistant Phenotype and Genotype of S. suis serotype 2 (SS2) Isolated from Humans in Bali, Indonesia

**I Putu Bayu Mayura[1*], Ni Made Adi Tarini[1], Ni Made Susilawathi[2], Putu Yuliandari[3]**

[1]*Department of Microbiology, Faculty of Medicine, Universitas Udayana, Indonesia*
[2]*Department of Neurology, Faculty of Medicine, Universitas Udayana, Indonesia*
[3]*Center for Biomedical Research, Research Organization for Health, National Research and Innovation Agency, Indonesia*

**Article Info**
History
Received: 29 Jan 2024
Accepted: 27 Aug 2024
Available: 30 Aug 2024

**Abstract**

**Background:** In recent times, meningitis, an infection primarily attributed to the zoonotic bacteria *Streptococcus suis*, has emerged as a significant public health concern in Bali, Indonesia. Their resistance to a multitude of antibiotics has emerged as a contemporary threat, as opposed to their virulence. There is a current lack of reported information regarding the genetic or phenotypic susceptibility pattern of *S. suis* to antibiotics in Bali.

**Objective:** The objective of this research endeavor was to ascertain the antibiotic susceptibility pattern of *S. suis* isolates in Bali, either through phenotypic or genetic means.

**Methods:** Glycerol stock isolates of *S. suis* from various specimen sources, including CSF, blood, and pleural fluid from April 2016 until April 2022 which had been assessed for species identification and antimicrobial susceptibility test (AST) using the VITEK 2 Compact (Biomeriuex®) were subjected to determine the serotype and antibiotic resistance genetically.

**Results:** Successful isolation of sixty-six *S. suis* isolates occurred primarily from cerebrospinal fluid. The results demonstrated that all isolates exhibited phenotypic resistance to tetracycline, with one isolate (MKPNH0071) demonstrating co-resistance to tetracycline and erythromycin. It is additionally corroborated genetically through the amplification of the *tetM* gene in every isolate, including those that exhibited concurrent resistance to erythromycin and tetracycline. The *intTn* gene, a member of the conjugate transposon Tn916 family which plays a role as horizontal media gene transfer on plasmids for carrying the resistance genes *ermB* and *tetM*, was amplified in an isolate that exhibited tetracycline co-resistance with erythromycin (MKPNH0071).

**Conclusion:** This research represents the initial investigation into the antibiotic resistance phenotype and genotype of *S. suis* serotype 2 (SS2) isolated from human subjects in Bali, Indonesia. Our findings suggest that the isolate of SS2 (MKPNH0071), which demonstrated tetracycline co-resistance with erythromycin, might be facilitated by the horizontal acquisition of the genetic element Tn916.

**Keywords:** *Streptococcus suis; antibiotic co-resistance; Bali*
**Permalink/ DOI:** https://doi.org/10.14710/jbtr.v10i2.21985

## INTRODUCTION

Pigs harbor the zoonotic pathogen *Streptococcus suis*. The upper respiratory tract of piglets, including the pharynx and tonsils, becomes colonized by this bacterium. Despite the fact that this bacterial colonization frequently results in the absence of symptoms (asymptomatic carrier status), the risk of invasive diseases such as sepsis, meningitis, endocarditis, pneumonia, and arthritis must be considered.[1,2]

* Corresponding author:
E-mail: *bayu_mayura@unud.ac.id*
(I Putu Bayu Mayura)

Human infections are also caused by emerging *S. suis* in Southeast Asia (Hong Kong, Thailand, Vietnam, and China).[1,2,3] This zoonotic pathogen is the most common cause of bacterial meningitis in adults in Vietnam and Hongkong.[3] Asia is documented as the region with the highest incidence of infections caused by this pathogen, at 0.8 cases per 100,000 individuals.[2,3,4] There have been reports of this infection also emerging in Western European countries, albeit with an incidence rate that is ten times lower.[4] In 2007, Wertheim documented 409 human cases of *S. suis* infection, the majority of which originated in Southeast Asia.[5] In 2008, instances of *S. suis* infection were identified in pig joint fluid samples collected in the Timika region of Papua, Indonesia.[6] Susilawathi *et al*. recently reported that 44 of the 71 cases of bacterial meningitis collected at Sanglah Central General Hospital between 2014 and 2017 were confirmed to be meningitis caused by *S. suis*.[7]

Among the 29 serotypes of *S. suis*, serotype 2 (SS2) is the most prevalent in both pigs and humans, causing infections.[8,9,10] Serotype 2 continues to be the most prevalent in Europe, North America, South America, Asia, and Australia. Infection cases caused by *S. suis* exhibit a diverse distribution of serotypes across all geographical regions. North America (Canada) exhibits the highest prevalence of serotypes 1, 1/2, and 2. This is in contrast to South America, where serotypes 1/2, 2, and 3 predominate. It has been reported that serotypes 2, 4, 7, and 9 are more prevalent in Europe.[4,11] Susilawathi *et al*. reported that serotypes 2 and 1/2 are the most frequently found in Indonesia.[7]

Given the lack of a viable vaccine against *S. suis*, there has been a growing reliance on antibiotics to manage and control infections caused by SS2. Among these, macrolides, β-lactams, tetracyclines, and sulfonamides are the most commonly prescribed.[12,13] With the escalating utilization of antibiotics to treat infections induced by SS2, a novel peril emerges: antibiotic resistance to this pathogen on a global scale. The most frequently reported antibiotic resistance among *S. suis* isolates was to macrolides (>70%) and tetracyclines (>90%).[14] A research investigation was conducted at Prof. I.G.N.G. Ngoerah Hospital regarding antibiotic susceptibility of *S. suis* in Bali. The findings revealed that throughout the period from 2016 to 2021, all 55 clinical isolates of *S. suis* exhibited resistance to tetracycline.[15] Human infections with *S. suis* that are co-resistant to tetracycline and macrolide/lincosamide have been documented in multiple studies.[4,16,17] The co-resistance mechanism between tetracycline and macrolide/lincosamide is purportedly facilitated through the action of transposons Tn916, which belong to the transposon conjugate family and serve as a conduit for horizontal gene transfer on the SS2 plasmid.[16]

As the prevalence of antibiotic resistance in *S. suis* infections rises globally, preventative measures and suitable treatment are required to contain instances of this pathogen-caused antibiotic resistance. Consequently, this research was conducted to further the sample study initiated by Dwijayanti *et al*.[15] Additionally, the genetic analysis will be explored more deeply including the finding of co-resistance to tetracycline and macrolide/lincosamide isolate in this study.

## MATERIALS AND METHODS

### Ethics approval

This study was approved by The Research Ethics Committee of the Faculty of Medicine, Universitas Udayana (Denpasar, Bali, Indonesia) No:1387/UN14.2.2.VII.14/LT/2023 dated May 25, 2023

### Isolate information data

The isolate data, including species identification and antibiotic susceptibility, were obtained using the VITEK 2 Compact (bioMérieux®) with the VITEK® 2 GP card and VITEK® 2 AST-ST03 card. These results were adjusted according to the 2021 Clinical Laboratory Standards Institute (CLSI) guidelines.[18] The antibiotics Benzylpenicillin, Ampicillin, Cefotaxime, Ceftriaxone, Levofloxacin, Erythromycin, Clindamycin, Linezolid, Vancomycin, and Tetracycline were evaluated for susceptibility. Subsequently, the antibiotic susceptibility patterns of the *S. suis* serotype 2 clinical isolates in Bali were ascertained utilizing Microsoft Excel 2019.

### Bacterial isolate

From April 2016 to April 2022, we gathered 66 bacterial isolates from various specimen sources e.g., cerebrospinal fluid, blood, and pleural fluid (Supplementary data). The inclusion criteria for this study were as follows: all *S. suis* isolates identified as *S. suis* by the VITEK® 2 GP card, with antimicrobial susceptibility testing (AST) performed using the VITEK® 2 AST-ST03 card; a complete microbiology request form; and a medical record.

### Bacterial culture conditions

A collection of 66 isolates of *S. suis* was maintained at a temperature of -80 °C in tryptic soy broth (TSB) media containing 50% glycerol. For further study, 66 glycerol stock isolates of *S. suis* were cultivated on 5% defibrinated sheep blood agar plate (DSBAP) and incubated in 5% $CO_2$ at 37 °C for 18 to 24 hours. All 66 isolates were grown as colonies on DSBAP, reconfirmed as *S. suis* using VITEK 2 Compact (bioMérieux®), and then subjected to further investigation.

### Bacterial DNA isolation

*S. suis* chromosomal DNA was isolated utilizing a Roche High Pure PCR Isolation Kit Template (Roche Life Science, Indianapolis, U.S.A.). However, for antibiotic resistance genes that predominantly carried by plasmid DNA were extracted using the QIAprep® Spin Miniprep Kit (Qiagen, Hilden, Germany). *S. suis* colonies were suspended in 200 µl of phosphate-buffered saline (PBS) with a pH of 7.3. Isolation of DNA from the bacterial suspensions was carried out in accordance with the guidelines provided by the manufacturer.

### Polymerase Chain Reaction (PCR) condition

Primers designated for identification, genotyping, and antibiotic resistance genes were utilized in this investigation. The PCR was performed with Go Taq® Green Master Mix (Promega, Madison, USA) with primer concentrations of 0.3 M were utilized. Then, the amplicons were electrophoresed for 35 min on a 1.5% agarose gel in TBE buffer at 100 volts. The DNA was

**Table 1.** Target gene and PCR primers used in this study

| Target genes | Primer sequence 5' – 3' | Amplicon size (bp) | Temperature | References |
|---|---|---|---|---|
| ***S. suis* genes target** | | | | |
| *recN* | CTACAAACAGCTCTCTTCTAGTC<br>ACAACAGCCAATTCATGGCGTGATT | 336 | 60$^0$C | Ishida *et al.* |
| **Major *S. suis* serotype capsular genes** | | | | |
| *cps2J* | GTTGAGTCCTTATACACCTGTT<br>CAGAAAATTCATATTGTCCACC | 459 | 60$^0$C | Nutravong *et al.* |
| ***Macrolide Resistance Genes*** | | | | |
| *ermB* | GAAAAGGTACTCAACCAAATA<br>AGTAACGGTACTTAAATTGTTTAC | 639 | 48$^0$C | Gygax *et al.* |
| ***Lincosamide Resistance Genes*** | | | | |
| *lnuB* | CCTACCTATTGTTTGTGGAA<br>ATAACGTTACTCTCCTATTC | 944 | 47$^0$C | Gygax *et al.* |
| ***Tetracycline Resistance Genes*** | | | | |
| *tetM* | GTTAAATAGTGTTCTTGGAG<br>CTAAGATATGGCTCTAACAA | 657 | 45$^0$C | Agerso *et al.* |
| ***Tn916-Like Transposon Family*** | | | | |
| *intTn* | GGTCTTCGTATTTCAGAGTTTGG<br>GTTGCATGTGCGTAATAGTTCAG | 473 | 53$^0$C | Agerso *et al.* |

visualized using GelRedTM Nucleic Acid Gel Stain (Biotium, Hayward, CA 94545) and then documented using Gel Doc (Bio-Rad). The PCR conditions corresponding to each primer pair are detailed in Table 1.

**DNA sequencing**

The positive control in this study was not included; however, the sample that successfully amplified the *tetM*, *ermB*, and *lnuB* genes target, respectively was then subjected to perform DNA sequencing commercially in 1stBase (Selangor, Malaysia). The DNA sequencing results from each target gene were analyzed using SnapGene version 7.2.1. Then, the nucleotide sequence was aligned with sequence database using the BLAST® tool, which is available at the National Centre for Biotechnology Information (NCBI) platforms (https://blast.ncbi.nlm.nih.gov/Blast.cgi).

## RESULTS

**The majority of specimen sources come from cerebrospinal fluid (CSF)**

Based on the register data, 66 samples that satisfied the inclusion criteria were identified as *S. su*is. Based on the analysis of the 66 samples data, it was determined that cerebrospinal fluid (CSF) provided the greatest number of specimens, 51 (77.27%), followed by blood with 14 (21.21%), and one specimen source derived from pleural fluid (1.51%).

**Almost every isolate of *S. suis* was identified as SS2.**

Based on the results of bacterial identification tests conducted using VITEK 2 Compact Automatic System (BioMérieux®) and the VITEK® 2 GP card, *S. suis* was identified in 66 of the samples analyzed. All samples underwent a second confirmation through the detection of *recN*, a gene that is conserved in *S. suis*. The findings indicated that the gene *recN* was amplified in every sample (data not shown). The entire sample was subsequently subjected to an additional serotype test, and the results determined that it belonged to *S. suis* serotype 2 (SS2) (data not shown).

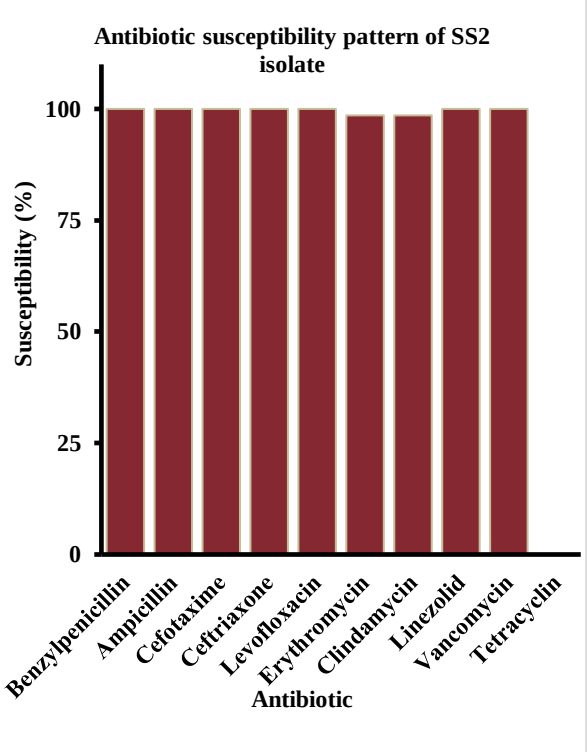


**Figure 1.** Antibiotic susceptibility pattern of SS2

Antibiotic susceptibility data was obtained from VITEK 2 compact. This result showed that all isolates (100%) were susceptible toward Benzylpenicillin (BZP), Ampicillin (AMP), Cefotaxime (CTX), Ceftriaxone (CRO), Levofloxacin (LVX), Linezolid (LNZ), Vancomycin (VAN). However, all isolates (100%) resistance toward Tetracycline (TCY). Isolate MKPNH0071 which exhibited resistant to Tetracycline (TCY), also show resistance toward Erythromycin (ERY), and Clindamycin (CLI).

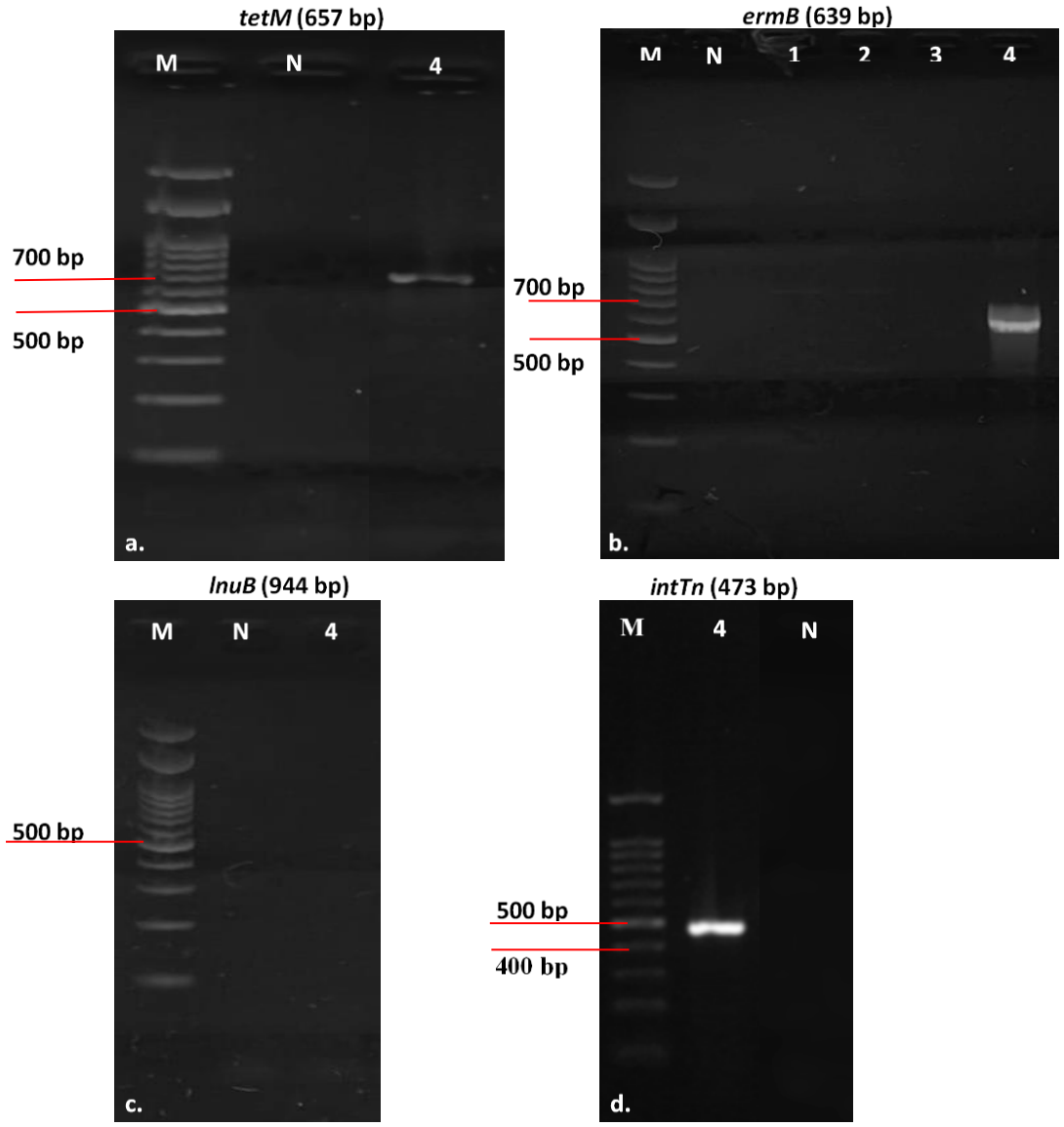


**Figure 2.** Agarose electrophoresis result of *tetM*, *ermB*, *lnuB*, and *intTn* gene at figure **a**, **b**, **c**, and **d,** respectively. **M**, indicated as a marker, 1 kb DNA ladder; **N**, indicated as negative control; **1**, **2**, **3**, and **4,** indicated as isolate ID number MKPNH0068, MKPNH0069, MKPNH0070, and MKPNH0071, respectively. **a.** The isolate MKPNH0071 successfully amplified the *tetM* gene with the expected PCR product size 657 bp, **b.** Among four isolates, only isolate MKPNH0071 successfully amplified the *ermB* gene with expected PCR product size 639 bp, **c.** The isolate MKPNH0071 did not amplify the *lnuB* gene with the expected PCR product size 944 bp, **d.** Isolate MKPNH0071 also successfully amplified the *intTn* gene with an expected PCR product size 473 bp.

**Antibiotic susceptibility pattern of SS2**

Based on the data obtained from the 2021 Clinical Laboratory Standard Institute (CLSI) antibiotic susceptibility testing results using the VITEK 2 Compact Automatic System (BioMérieux®) with the VITEK® 2 AST-ST03 card, it was observed that all samples (100%) showed that they were still susceptible to the following antibiotics: Benzylpenicillin/BZP; Ampicillin/AMP; Cefotaxime/CTX; Ceftriaxone/CRO; Levofloxacin/LVX; Linezolid/LNZ; Vancomycin/VAN. Conversely, all sample (100%) exhibited resistant toward Tetracycline/TCY. It is noteworthy that a sample MKPNH0071, which exhibited resistant to Tetracycline/TCY, also demonstrated resistant to Erythromycin/ERY and Clindamycin/CLI (Figure 1).

**The *intTn* gene was detected in the co-resistance tetracycline and erythromycin mechanism**

When a test is carried out to detect the antibiotic resistance gene, namely tetracycline (*tetM*), erythromycin (*ermB*), and clindamycin (*lnuB*), the results obtained were that all samples (100%) amplified the tetracycline resistance gene (*tetM*) (Figure 2a) and only one sample (MKPNH0071) amplified the erythromycin resistance gene (*ermB*) (Figure 2b). None of the samples amplify the clindamycin resistance gene (*lnuB*) (Figure 2c). In line with the discovery of the resistance gene to tetracycline (*tetM*) and erythromycin (*ermB*) on one sample (MKPNH0071), followed by gene detection *intTn* on that sample. The results showed that this sample also amplified the gene *intTn* (Figure 2d).

All target genes (*tetM*, *ermB*, and *intTn*) in sample MKPNH0071 were subjected to sequencing. The nucleotide sequences from the sequencing result were

aligned with references sequence using the BLAST® tool at NCBI. The aligning result showed that *tetM*, *ermB*, and *intTn* gene have 100% identity similarity with *S. suis* GX1 *tet(M)* gene for tetracycline resistance (GN_0482244.1), 100% identity similarity with *Streptococcus pyogenes* pDB101 *erm(B)* gene for 23S rRNA (NG_242280.1), and 99% identity similarity with *Streptococcus agalactiae* strain PHEGBS0082 transposon Tn916 (OP715838.1), respectively.

## DISCUSSION

All isolates exhibited resistance to tetracycline, as indicated by the antibiotic susceptibility data and validated by the amplified tetracycline resistance gene (*tetM*) (100%). Both the *tetM* and *tetO* genes, which encode ribosomal protection protein, were prevalent in the tetracycline-resistant *S. suis* bacterium. Additionally, other studies have documented a higher carrier rate of the *tetM* gene in comparison to the *tetO* gene.[23,24] Subsequently, our research aligns with that of Uruen *et al*., who compiled publication data regarding the prevalence of antimicrobial resistance (AMR) across various antibiotic classes in *S. suis* isolates originating from Europe, Asia, and America. Notably, their study documented the highest rate of tetracycline resistance.[14] The escalating prevalence of tetracycline resistance can be attributed to the widespread use of this antibiotic to treat infectious diseases in food production animals, especially in intensive pig farming.[14] Free access to antibiotics without a doctor's prescription in Indonesia has led to unrestricted usage; consequently, become an AMR threat in Indonesia.[25,26]

The phenotypic findings derived from the VITEK 2 data indicated that a single sample exhibited resistance to tetracycline and erythromycin/clindamycin. This was corroborated by the amplification of the tetracycline resistance gene (*tetM*) and the erythromycin resistance gene (*ermB*), but not the clindamycin resistance gene (*lnuB*). The observed co-resistance of *S. suis* serotype 2 to tetracyclines and macrolides/lincosamides, is still infrequent. Similar findings were also reported by other researchers.[27,28,29] *Streptococcus* sp. from clinical isolates that are resistant to macrolide frequently possess resistance genes encoded by ribosomal methylase (*erm*) and efflux (*mef*) genes.[21] Our research aligns with the findings of Ye *et al*., who identified the *ermB* gene in every erythromycin-resistant isolate, thus validating its prevalence in *S. suis* type 2 in China.[23]

Furthermore, one sample of co-resistance to erythromycin and tetracycline also exhibits amplification of the *intTn* gene. The literature indicates that the *intTn* gene, which is a member of the conjugate transposon Tn916 family, was horizontally media gene transfer transferred for tetracycline resistance gene (*tetM*) and erythromycin resistance gene (*ermB*) onto plasmids *S. suis* serotype 2. This plasmid contains the tetracycline resistance gene (*tetM*) and the erythromycin resistance gene (*ermB*), which frequently results in co-resistance to both antibiotics.[4,16] Furthermore, the existence of components associated with Tn916 and *tetM* in *S. suis* serotype 2 may significantly contribute to the pathogenic nature of this bacterial pathogen.[16] In contrast to the *tetM* and *ermB* genes, the *lnuB* gene was exclusively found in the TnGBS2 family and not the Tn916 family.[4] The peculiar aspect is that instances of co-resistance to tetracycline and erythromycin antibiotics are predominantly documented in pigs.[16] It is possible that the transmission of the antibiotic resistance gene to humans occurred via contaminated meat harboring *S. suis*.

In contrast, while none of the isolates exhibited amplification of the clindamycin resistance gene (*lnuB*), one isolate demonstrated phenotypic resistance to clindamycin. This phenomenon can be delineated through the examination of two clindamycin-specific resistance mechanisms: the first pertains to an ABC transporter that is encoded by the *lsaE* gene, and the second concerns the target's modification by a nucleotidyl-transferase, which is either encoded by the *lnuB* or *lnuC* genes. In order to validate the resistance of our isolate to clindamycin, it is necessary to examine the remaining two clindamycin-resistant genes, namely *lsaE* and *lnuC*.[4] Clindamycin resistance was uncommon in this study, which is a relatively low number in comparison to the resistance rates observed with tetracycline and erythromycin.

## CONCLUSIONS

This is the first investigation into the antibiotic resistance genotype and phenotype of *S. suis* serotype 2 (SS2) obtained from human sources in Bali, Indonesia. According to the findings, the isolate of SS2 (MKPNH0071) which demonstrated the occurrence tetracycline co-resistance with erythromycin might be facilitated by the horizontal acquisition of the genetic element Tn916. Further characterization of the mobile resistome of *S. suis* is imperative due to its potential to function as a reservoir of resistance genes for other species inhabiting the same habitats. This pathogen poses a threat to the health of both animals and humans and may also facilitate the transmission of antimicrobial resistance (AMR) genes between these species. In the coming years, this will be one of the global challenges that must be addressed in order to preserve essential antimicrobial activity.

## ACKNOWLEDGEMENTS

This research was supported by Universitas Udayana with Grant No. B/1.647/UN14.4.A/PT.01.03.2023 under the Udayana Excellence Research Grant Scheme. Ni Komang Ratih Anggreni and Kadek Bella Karunia Sari are thanked for their aid in the sample preparation at the laboratory.

## REFERENCES

1. Segura M. *Streptococcus suis*: An emerging human threat. J Infect Dis. 2009;199(1):4–6. https://academic.oup.com/jid/article-lookup/doi/10.1086/594371
2. Feng Y, Zhang H, Wu Z, Wang S, Cao M, Hu D, et al. *Streptococcus suis* infection: An emerging/reemerging challenge of bacterial infectious diseases? Virulence. 2014;5(4):477–97. http://www.tandfonline.com/doi/abs/10.4161/viru.28595

3. Huong VTL, Ha N, Huy NT, Horby P, Nghia HDT, Thiem VD, et al. Epidemiology, clinical manifestations, and outcomes of *Streptococcus suis* infection in humans. Emerg Infect Dis. 2014;20(7):1105–14. http://wwwnc.cdc.gov/eid/article/20/7/13-1594_article.htm
4. Dechêne-Tempier M, Marois-Créhan C, Libante V, Jouy E, Leblond-Bourget N, Payot S. Update on the Mechanisms of Antibiotic Resistance and the Mobile Resistome in the Emerging Zoonotic Pathogen *Streptococcus suis*. Microorganisms. 2021 Aug 18;9(8):1765. https://www.mdpi.com/2076-2607/9/8/1765
5. Wertheim H.F.L., Nghia H.D.T., Taylor W, Schultsz C. *Streptococcus suis*: An Emerging Human Pathogen. Clin Infect Dis. 2009 Mar;48(5):617–25. https://academic.oup.com/cid/article-lookup/doi/10.1086/596763
6. Nugroho W, Cargill CF, Putra IM, Kirkwood RN, Trott DJ, Salasia SIO, et al. Investigations of selected pathogens among village pigs in Central Papua, Indonesia. Trop Anim Health Prod. 2016 Jan 17;48(1):29–36. http://link.springer.com/10.1007/s11250-015-0913-5
7. Susilawathi NM, Tarini NMA, Fatmawati NND, Mayura PIB, Suryapraba AAA, Subrata M, et al. *Streptococcus suis* –Associated Meningitis, Bali, Indonesia, 2014–2017. Emerg Infect Dis. 2019 Dec;25(12):2235–42. http://wwwnc.cdc.gov/eid/article/25/12/18-1709_article.htm
8. Lunha K, Chumpol W, Samngamnim S, Jiemsup S, Assavacheep P, Yongkiettrakul S. Antimicrobial Susceptibility of *Streptococcus suis* Isolated from Diseased Pigs in Thailand, 2018–2020. Antibiotics. 2022;11(3):1–12. https://www.mdpi.com/2079-6382/11/3/410
9. Wilson T, Jeffers J, Rappgabrielson V, Martin S, Klein L, Lowery D, et al. A novel signature-tagged mutagenesis system for *Streptococcus suis* serotype 2. Vet Microbiol. 2007 May 16;122(1–2):135–45. https://linkinghub.elsevier.com/retrieve/pii/S0378113507000077
10. Princivalli MS, Palmieri C, Magi G, Vignaroli C, Manzin A, Camporese A, et al. Genetic diversity of *Streptococcus suis* clinical isolates from pigs and humans in Italy (2003-2007). Euro Surveill. 2009;14(33):1–7. http://dx.doi.org/10.2807/ese.14.33.19310-en
11. Estrada AA, Gottschalk M, Rossow S, Rendahl A, Gebhart C, Marthaler DG. Serotype and genotype (multilocus sequence type) of *Streptococcus suis* isolates from the United States serve as predictors of pathotype. J Clin Microbiol. 2019;57(9). https://journals.asm.org/doi/10.1128/JCM.00377-19
12. Hu P, Yang M, Zhang A, Wu J, Chen B, Hua Y, et al. Comparative Genomics Study of Multi-Drug-Resistance Mechanisms in the Antibiotic-Resistant *Streptococcus suis* R61 Strain. Read TD, editor. PLoS One. 2011 Sep 26;6(9):e24988. https://dx.plos.org/10.1371/journal.pone.0024988
13. Pérez-Trallero E, Montes M, Orden B, Tamayo E, García-Arenzana JM, Marimón JM. Phenotypic and genotypic characterization of *Streptococcus pyogenes* isolates displaying the M.L.S.B. phenotype of macrolide resistance in Spain, 1999 to 2005. Antimicrob Agents Chemother. 2007;51(4):1228–33. https://journals.asm.org/doi/10.1128/AAC.01054-06
14. Uruén C, García C, Fraile L, Tommassen J, Arenas J. How *Streptococcus suis* escapes antibiotic treatments. Vol. 53, Veterinary research. BioMed Central; 2022. 91 p. https://doi.org/10.1186/s13567-022-01111-3
15. Dwijayanti N, Puspita Y, Susilawathi N, Tarini N. Antibiotic Susceptibility Pattern of *Streptococcus suis* Isolate from Clinical Specimens at Sanglah General Hospital, Denpasar, Bali. Int J Med Rev Case Reports. 2023;7(0):1. https://www.mdpub.net/?mno=122982
16. Zhang C, Zhang Z, Song L, Fan X, Wen F, Xu S, et al. Antimicrobial Resistance Profile and Genotypic Characteristics of *Streptococcus suis* Capsular Type 2 Isolated from Clinical Carrier Sows and Diseased Pigs in China. Biomed Res Int. 2015;2015:1–9. http://www.hindawi.com/journals/bmri/2015/284303/
17. Nedbalcova K, Kucharovicova I, Zouharova M, Matiaskova K, Kralova N, Brychta M, et al. Resistance of *Streptococcus suis* Isolates from the Czech Republic during 2018–2022. Antibiotics. 2022;11(9):1–15. https://www.mdpi.com/2079-6382/11/9/1214
18. CLSI. *Performance Standards for Antimicrobial Susceptibility Testing*. 31st ed. CLSI supplement M100. Clinical and Laboratory Standards Institute; 2021. https://clsi.org/about/press-releases/clsi-publishes-m100-performance-standards-for-antimicrobial-susceptibility-testing-31st-edition/
19. Ishida S, Tien LHT, Osawa R, Tohya M, Nomoto R, Kawamura Y, et al. Development of an appropriate PCR system for the reclassification of *Streptococcus suis*. J Microbiol Methods. 2014;107:66–70. http://dx.doi.org/10.1016/j.mimet.2014.09.003
20. Nutravong T, Angkititrakul S, Jiwakanon N, Wongchanthong W, Dejsirilerts S, Nawa Y. Identification of major *Streptococcus suis* serotypes 2, 7, 8 and 9 isolated from pigs and humans in upper northeastern Thailand. Southeast Asian J Trop Med Public Health. 2014;45(5):1173–81. https://pubmed.ncbi.nlm.nih.gov/25417521/
21. Gygax SE, Schuyler JA, Kimmel LE, Trama JP, Mordechai E, Adelson ME. Erythromycin and clindamycin resistance in group B streptococcal clinical isolates. Antimicrob Agents Chemother. 2006;50(5):1875–

7. https://journals.asm.org/doi/10.1128/AAC.50.5.1875-1877.2006
22. Agersø Y. The identification of a tetracycline resistance gene tet(M), on a Tn916-like transposon, in the *Bacillus cereus* group. FEMS Microbiol Lett. 2002 Sep 10;214(2):251–6. http://doi.wiley.com/10.1016/S0378-1097(02)00883-2
23. Ye C, Bai X, Zhang J, Jing H, Zheng H, Du H, et al. Spread of *Streptococcus suis* Sequence Type 7, China. Emerg Infect Dis. 2008 May;14(5):787–91. http://wwwnc.cdc.gov/eid/article/14/5/07-0437_article.htm
24. Tian Y, Aarestrup FM, Lu CP. Characterization of *Streptococcus suis* serotype 7 isolates from diseased pigs in Denmark. Vet Microbiol. 2004 Oct;103(1–2):55–62. https://linkinghub.elsevier.com/retrieve/pii/S0378113504002652
25. Siahaan, Selma, Herman, Max J., Fitri, Nyoman. Antimicrobial Resistance Situation in Indonesia: A Challenge of Multisector and Global Coordination. *J of Trop Med*. 2022; 2783300, 10 pages. https://doi.org/10.1155/2022/2783300
26. Kurniawan K, Posangi J, Rampengan N. Association between public knowledge regarding antibiotics and self-medication with antibiotics in Teling Atas Community Health Center, East Indonesia. Med J Indones. 2017 May;26(1):62-9. https://mji.ui.ac.id/journal/index.php/mji/article/view/1589
27. Vela AI, Moreno MA, Cebolla JA, González S, Latre M V., Domínguez L, et al. Antimicrobial susceptibility of clinical strains of *Streptococcus suis* isolated from pigs in Spain. Vet Microbiol. 2005 Jan;105(2):143–7. https://linkinghub.elsevier.com/retrieve/pii/S0378113504003815
28. Chu YW, Cheung TKM, Chu MY, Tsang VYM, Fung JTL, Kam KM, et al. Resistance to tetracycline, erythromycin and clindamycin in *Streptococcus suis* serotype 2 in Hong Kong. Int J Antimicrob Agents. 2009 Aug;34(2):181–2. https://linkinghub.elsevier.com/retrieve/pii/S0924857909000454
29. Marie J. Antimicrobial susceptibility of *Streptococcus suis* isolated from swine in France and from humans in different countries between 1996 and 2000. J Antimicrob Chemother. 2002 Aug 1;50(2):201–9. https://academic.oup.com/jac/article-lookup/doi/10.1093/jac/dkf099