



Article

# Characterization of Multidrug-Resistant *Trueperella* (*Arcanobacterium*) pyogenes Isolates from Vertebral Osteomyelitis in Slaughtered Pigs

In-Haeng Lee <sup>1,†</sup>, Gun Lee <sup>2,†</sup>, Hyeon Jeong Moon <sup>2</sup>, Dae-Young Kim <sup>1</sup>, Jong-Woog Choi <sup>1</sup>, Yeong-Bin Baek <sup>3</sup>, Sang-Ik Park <sup>2</sup>, Dae-Sung Yoo <sup>4</sup>, Jun Bong Lee <sup>5</sup>, Bock-Gie Jung <sup>6</sup>, Kwang-Jun Lee <sup>7,\*</sup> and Jun-Gyu Park <sup>8,\*</sup>

- Health and Environment Research Institute of Gwangju, Gwangju 61954, Republic of Korea; slayer19@korea.kr (I.-H.L.); daeyoung.keem@gmail.com (D.-Y.K.); lovnat@korea.kr (J.-W.C.)
- Department of Veterinary Pathology, College of Veterinary Medicine and BK21 FOUR Program, Chonnam National University, Gwangju 61186, Republic of Korea; udlrjs77@naver.com (G.L.); dals203@naver.com (H.J.M.); sipark@jnu.ac.kr (S.-I.P.)
- Department of Veterinary Pathology, College of Veterinary Medicine, Chonnam National University, Gwangju 61186, Republic of Korea; ybbaek@jnu.ac.kr
- Department of Veterinary Public Health, College of Veterinary Medicine, Chonnam National University, Gwangju 61186, Republic of Korea; shanuar@inu.ac.kr
- Department of Food and Environmental Hygiene, College of Veterinary Medicine, Chonnam National University, Gwangju 61186, Republic of Korea; jblee12@jnu.ac.kr
- Department of Veterinary Microbiology, College of Veterinary Medicine, Chonnam National University, Gwangju 61186, Republic of Korea; bjung@jnu.ac.kr
- Division of Zoonotic and Vector-Borne Disease Research, Center for Infectious Diseases Research, National Institute of Health, Cheongju 28159, Republic of Korea
- Department of Veterinary Zoonotic Diseases, College of Veterinary Medicine, Chonnam National University, Gwangju 61186, Republic of Korea
- \* Correspondence: kwangjun@korea.kr (K.-J.L.); kingsalt@jnu.ac.kr (J.-G.P.)
- † These authors contributed equally to this work.

#### Simple Summary

Slaughterhouses offer a valuable opportunity to detect hidden animal diseases that may not be observed during life. In this study, we examined fully condemned pig carcasses with spinal abscesses and identified *Trueperella pyogenes* as a key pathogen. By analyzing samples collected postmortem, we uncovered high levels of antibiotic resistance and evidence of potential virulence, providing critical insights into both animal health and food safety. This research demonstrates how slaughterhouse monitoring can support the early detection of emerging pathogens, track antimicrobial resistance, and guide farm-level interventions. It highlights the epidemiological value of routine meat inspection data for improving animal production systems and protecting public health.

#### Abstract

Slaughterhouses serve as critical surveillance hubs for identifying subclinical and economically important diseases in food-producing animals. *Trueperella (Arcanobacterium) pyogenes*, an opportunistic pathogen commonly found on the mucous membranes of livestock, is associated with mastitis, abortion, and suppurative infections such as abscesses. In this study, we investigated 30 pig carcasses fully condemned due to vertebral osteomyelitis (VO) at two slaughterhouses in Gwangju, Republic of Korea, between November 2023 and May 2024. From abscess lesions, 11 *T. pyogenes* strains were isolated and characterized morphologically, biochemically, and genetically. The hemolytic exotoxin pyolysin (*plo* gene), a major virulence factor, was detected in five isolates (45.46%). Phylogenetic analysis of partial 16S rDNA sequences confirmed close



Academic Editor: Jesús Hernández

Received: 22 August 2025 Revised: 2 October 2025 Accepted: 8 October 2025 Published: 14 October 2025

Citation: Lee, I.-H.; Lee, G.; Moon, H.J.; Kim, D.-Y.; Choi, J.-W.; Baek, Y.-B.; Park, S.-I.; Yoo, D.-S.; Lee, J.B.; Jung, B.-G.; et al. Characterization of Multidrug-Resistant *Trueperella* (*Arcanobacterium*) pyogenes Isolates from Vertebral Osteomyelitis in Slaughtered Pigs. *Animals* 2025, 15, 2970. https://doi.org/10.3390/ani15202970

Copyright: © 2025 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (https://creativecommons.org/licenses/by/4.0/).

Animals **2025**, 15, 2970 2 of 14

clustering with known *T. pyogenes* reference strains. All 11 isolates exhibited multidrug resistance, showing resistance to 8–14 antimicrobial agents per strain. Complete resistance (11/11, 100%) was observed against amikacin (AMI), nalidixic acid (NAL), chloramphenicol (CHL), florfenicol (FFN), and trimethoprim/sulfamethoxazole (SXT). High resistance rates were also detected for erythromycin (ERY) and clindamycin (CLI) (10/11, 90.9%), ceftazidime (TAZ), ceftriaxone (AXO), ciprofloxacin (CIP) (7/11, 63.6%), and tetracycline (TET) and streptomycin (STR) (5/11, 45.5%), while gentamicin (GEN) resistance was found in three isolates (27.3%). In contrast, none of the isolates showed resistance to ampicillin, cefoxitin, or cefotaxime. These findings underscore the epidemiological value of abattoir-based monitoring in detecting emerging pathogens and tracking antimicrobial resistance. The results provide important baseline data to inform disease control strategies, guide antimicrobial stewardship, and support One Health approaches, including the development of preventive measures such as vaccines.

**Keywords:** *Trueperella pyogenes*; slaughterhouse epidemiology; vertebral osteomyelitis; antimicrobial resistance; virulence factors

#### 1. Introduction

Trueperella pyogenes is a Gram-positive, non-motile, non-capsulated, non-spore-forming facultatively anaerobic short rod-shaped bacterium either singly, in pairs, or in clusters [1,2]. Initially classified as Bacillus pyogenes, this organism has undergone several taxonomic reclassifications from Corynebacterium pyogenes to Actinomyces pyogenes, Arcanobacterium pyogenes, and finally, T. pyogenes [3]. Although its growth requirements are not complex, it requires media enriched with blood or serum for successful culture [1]. Early identification of T. pyogenes relies on its cell morphology, colony characteristics (such as  $\beta$ -hemolysis on blood agar), and a negative catalase test; subsequent biochemical testing including CAMP test, urease activity, nitrate reduction, oxidase test, gelatin and esculin hydrolysis, and fermentation of various carbohydrates (e.g., glucose, lactose, mannitol, maltose, sucrose, and xylose) aids in species confirmation [4]. As an opportunistic pathogen, this bacterium commonly colonizes the skin and mucosal surfaces of domestic animals like cattle, swine, sheep, goats, and chicken where it can cause infections such as mastitis, wound infections, pneumonia, and liver abscesses [1,5]. Infections in humans are rare and tend to occur sporadically, primarily in immunocompromised individuals with exposure to farm animals [1,2]. Exposure to this pathogen possibly induces vertebral osteomyelitis (VO), secondary to tail-biting or any wounds causing entry of bacterial pathogens [6], sepsis [7], endocarditis [3,8], pneumonia [9], and skin ulcers [10]. These various clinical manifestations and pathogenicity are known to be related with various virulence factors including pyolysin (plo), fimbrial proteins (fimA, fimC, fimE, fimG), neuraminidases (nanH, nanP), collagen-binding protein (cbpA), and superoxide dismutase (sodA) [2,11]. Among them, the plo gene encodes a cholesterol-dependent cytolysin that disrupts host cell membranes and is regarded as a key virulence determinant [12,13].

To date, no effective vaccine is available to prevent *T. pyogenes* infections [2]. For this reason, antimicrobial therapy remains the only available option for the prevention and treatment of VO caused by *T. pyogenes*. Commonly used antimicrobial agents include aminoglycosides, β-lactams, tetracyclines, macrolides, and fluoroquinolones [2]. However, the misuse and overuse of antibiotics in conventional pig farming systems have led to the emergence of antibiotic-resistant strains, thereby compromising the effectiveness of antimicrobial treatments [14]. Numerous studies have investigated the antimicrobial resistance (AMR) profiles of *T. pyogenes* across different countries, time periods, and animal species. Reported resistance rates vary, with tetracycline resistance ranging from 53.12% to 81.65%, streptomycin resistance

Animals 2025, 15, 2970 3 of 14

from 32.6% to 72.48%, and erythromycin (ERY) resistance up to 21.10%. Lower resistance rates have been observed for ciprofloxacin (CIP) (around 12.5%), kanamycin (approximately 11.6%), and enrofloxacin (up to 21.87%). In contrast, all isolates in these studies remained susceptible to gentamicin (GEN), penicillin, and cephalothin [2,14–16].

In this study, we examined 30 pig carcasses fully condemned due to VO at two slaughterhouses in Gwangju, Republic of Korea, between November 2023 and May 2024. Using a combination of bacterial culture, molecular identification, PCR-based toxin gene detection, and antimicrobial susceptibility testing, we characterized 11 *T. pyogenes* isolates. This study was conducted to better understand the etiological role of *T. pyogenes* in swine VO, a condition that leads to significant carcass condemnation and economic loss. Our aim was to identify the pathogen's AMR patterns and virulence profiles to support early detection and control strategies at the slaughterhouse level. In addition, we sought to assess its potential as a zoonotic pathogen, given the risk of transmission to humans and its implications for public health.

#### 2. Materials and Methods

#### 2.1. Bacteria Isolation and Culture Conditions

In 2024, a total of 470,414 pigs were slaughtered in the Gwangju area, among which 0.033% of carcasses were condemned. As part of our monitoring between November 2023 and May 2024, 235,430 pigs were examined, and among them, 30 pigs were diagnosed with vertebral osteomyelitis (VO). The affected pigs included 14 females and 16 castrated males. Of these, 29 pigs had been raised in Jeollanam-do, while 1 was raised in Gyeongsangbuk-do, a province located in the southeastern region of the country. Swab samples were collected directly from the abscesses after recording the sex and carcass weight of each animal (Table 1). The abscesses were in the vertebrae, including the cervical, thoracic, and/or lumbar regions. As the lesions were identified postmortem, no clinical signs were observed prior to slaughter.

Sample	Weight (kg)	Relative Body Weight to Control Pigs (%)	Sex	Collection Date		
H2311HS_GJ01	103 kg	91.57%	Female	16 November 2023		
IH2311.1MA_GJ02	113 kg	96.2%	Castrated	16 November 2023		
IH2311.2MA_GJ03	112 kg	99.58%	Female	23 November 2023		
IH2312KC_GJ05	100 kg	88.91%	Female	12 December 2023		
IH2312GM_GJ06	82 kg	69.81%	Castrated	12 December 2023		
IH2403HN_GJ16	114 kg	101.35%	Female	20 March 2024		
IH2403JH_GJ17	111 kg	98.69%	Female	21 March 2024		
IH2403YG_GJ18	115 kg	102.24%	Female	22 March 2024		
IH2403GY_GJ19	115 kg	102.24%	Female	26 March 2024		
IH2404GS_GJ20	104 kg	92.47%	Female	1 April 2024		
IH2404MA_GJ21	119 kg	101.3%	Castrated	4 April 2024		

**Table 1.** Information of pigs used in this study.

#### 2.2. Virulence Factor Analysis

To confirm the presence of toxins in the isolated *T. pyogenes* strains, PCR analysis was performed. Briefly, a single colony of the pure isolate was selected and mixed with 1 mL of sterile PBS, followed by vortex. The mixture was then heat-treated at 100 °C for 15 min and centrifuged at 12,000 rpm for 15 min. The supernatant was used as the DNA template. PCR was conducted with the primer of F: 5′-GGC CCG AAT GTC ACC GC-3′ (positions 823–839) and R: 5′-AAC TCC GCC TCT AGC GC-3′ (positions 1092–1076), with an annealing temperature 55 °C, yielding a product size of 270 bp to detect the hemolytic exotoxin pyolysin (*plo* gene), a major virulence factor of *T. pyogenes* [17]. After PCR, the products were analyzed using 1.5% agarose gel electrophoresis at 100 V for 30 min using

Animals 2025, 15, 2970 4 of 14

Bio-Rad Mini-Sub Cell GT System (Bio-Rad, Hercules, CA, USA), and the presence of bands was used to determine positive results [18].

#### 2.3. Antimicrobial Susceptibility Test

The antimicrobial susceptibility of *T. pyogenes* isolates was assessed by determining the minimum inhibitory concentrations (MICs) using the broth microdilution method with Sensititre panel (TREK Diagnostic System, Cleveland, OH, USA). Briefly, *T. pyogenes* colonies grown on blood agar plate (Synergy inovation, Seongnam, Republic of Korea) were suspended in 5 mL of sterile saline to obtain a McFarland standard of 0.5. Bacterial cell suspensions were diluted with 11 mL of Tryptic soy broth (BD Difco, Sparks, MD, USA) supplemented with 5% lysed horse blood (MBcell, Seoul, Republic of Korea) and dispensed onto the Sensititre panel. The panels were incubated at 37 °C with anaerobic condition for 96 h, and susceptibility was interpreted according to the criteria used in this study.

Using the Sensititre panel, 17 antibiotics including ampicillin (AMP, 1–64  $\mu$ g/mL), cefoxitin (FOX, 4–8  $\mu$ g/mL), ceftazidime (TAZ, 1–16  $\mu$ g/mL), cefotaxime (FOT, 1–32  $\mu$ g/mL), ceftriaxone (AXO, 0.25–64  $\mu$ g/mL), tetracycline (TET, 2–128  $\mu$ g/mL), GEN (1–64  $\mu$ g/mL), streptomycin (STR, 2–128  $\mu$ g/mL), amikacin (AMI, 4–64  $\mu$ g/mL), CIP (0.03–16  $\mu$ g/mL), nalidixic acid (NAL, 2–128  $\mu$ g/mL), colistin (COL, 2–16  $\mu$ g/mL), chloramphenicol (CHL, 2–32  $\mu$ g/mL), florfenicol (FFN, 0.03–64  $\mu$ g/mL), azithromycin (AZI, 2–32  $\mu$ g/mL), trimethoprim/sulfamethoxazole (SXT, 0.12/2.38–4/76  $\mu$ g/mL), clindamycin (CLI, 0.03–16  $\mu$ g/mL), and ERY (0.03–64  $\mu$ g/mL) were tested. The MIC breakpoint used to classify isolates as susceptible or resistant to each antibiotic was identified using previous reports, TET, GEN, STR [19], TAZ, FOT, AMI, CIP, CHL, FFN, AZI, CLI antibiotics [20]; CLSI document M100 (34th edition, 2024) for AXO, FOX, NAL antibiotics; CLSI document VET06 (CLSI, 2017) for AMP, SXT and ERY. *Escherichia coli* ATCC 25922 and *Staphylococcus aureus* ATCC 25923 were used as internal quality control strains for antimicrobial susceptibility test. Based on the standards outlined in Table 2, isolates were classified as susceptible (S) or resistant (R) [19–22].

Table 2. Antimicrobial agents used in this study and their minimal inhibitory concentration breakpoint.

Antibiotic Class	Antibiotic Name (Abbreviation)	Minimal Inhibitory Concentration Breakpoint (μg/mL)				
	_	Susceptible (S)	Resistant (R)			
β-lactams	Ampicillin (AMP)	<u>&lt;1</u>	>2			
•	Cefoxitin (FOX)		$\begin{array}{c} \geq 2 \\ \geq 8 \\ \geq 8 \\ \geq 16 \\ \geq 1 \\ \geq 4 \\ \geq 16 \\ \geq 8 \\ \geq 16 \\ \geq 4 \\ \geq 32 \\ \geq 1 \\ \geq 1 \\ \geq 8 \\ \geq 8 \\ \geq 8 \end{array}$			
	Ceftazidime (TAZ)	$\overline{\leq}4$	$\geq 8$			
	Cefotaxime (FOT)	≤8	≥16			
	Ceftriaxone (AXO)	≤0.5	≥1			
Tetracyclines	Tetracycline (TET)	≤2	$\geq 4$			
Aminoglycosides	Gentamicin (GEN)	≤8	≥16			
	Streptomycin (STR)	$\leq 4$	≥8			
	Amikacin (AMI)	≤8	≥16			
Fluoroquinolones	Ciprofloxacin (CIP)	≤2	$\geq 4$			
Quinolone	Nalidixic acid (NAL)	≤16	≥32			
Phenicols	Chloramphenicol (CHL)	≤0.5	≥1			
	Florfenicol (FFN)	≤0.5	≥1			
Macrolides	Azithromycin (AZI)	$\leq 4$	≥8			
	Erythromycin (ERY)	$\leq 4$	≥8			
Sulfonamides	Trimethoprim/Sulfamethoxazole (SXT)	≤0.12	≥0.25			
Lincosamides	Clindamycin (CLI)	$\leq 4$	≥8			

### 2.4. Analysis of 16S rRNA Gene Partial Sequences

The phylogenetic relationship of *T. pyogenes* isolates was analyzed in comparison to reference strains by targeting the species-specific region of the 16S rRNA intergenic

Animals **2025**, 15, 2970 5 of 14

spacer region (ISR). The 16S rRNA gene was partially amplified using primers F8 (5′-GAGTTTGATCCTGGCTCAG-3′) and 1492R (5′-GGACTACCAGGGTATCTAAT-3′), as previously described [23,24]. The amplified fragments were purified with the Illustra GFX<sup>™</sup> PCR DNA and Gel Band Purification Kit (GE Healthcare, Chicago, IL, USA) and the amplified partial 16S rRNA gene was sequenced using an Applied Biosystems automated sequencer (ABI 3730XL) at Macrogen Co., Ltd. (Seoul, Republic of Korea). The resulting sequences were analyzed using BLAST version 2.13.0+ against the GenBank nucleotide non-redundant database. The 16S rRNA sequences generated in this study have been submitted to NCBI GenBank. The 16S rRNA gene sequences were aligned and used to construct a phylogenetic tree. The tree was generated using the neighbor-joining (NJ), kimura 2-parameter model in MEGA (version 12), with 1000 bootstrap replications performed as previously described [25].

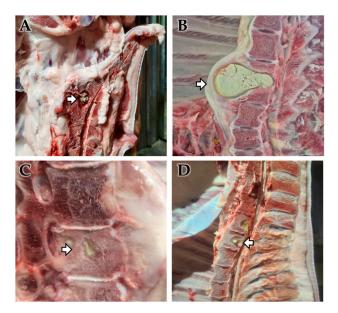
#### 2.5. Statistical Analysis

Welch's *t*-test was conducted using Prism software version 10.4.1 (GraphPad, San Diego, CA, USA) to compare the mean body weights between pigs with VO and healthy pigs. Statistical significance was defined as follows: p < 0.05 (\*), p < 0.01 (\*\*), and p < 0.001 (\*\*\*).

#### 3. Results

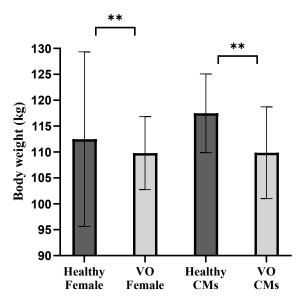
#### 3.1. Characteristics of Vertebral Osteomyelitis Cases

Bacterial culture revealed that *T. pyogenes* was isolated from 11 of 30 VO samples (isolation rate: 36.7%). Among the 11 samples where *T. pyogenes* was identified, 8 were from females (out of 14 total female samples; 57.1%) and 3 were from castrated males (out of 16 total castrated male samples; 18.8%) (Figure 1, Table 1). The average body weight of pigs with VO was 4.8% lower in females (107.07  $\pm$  5.51 kg, n = 14) compared to healthy female controls (112.48  $\pm$  16.844 kg, n = 100,356), and 5.6% lower in castrated males (110.87  $\pm$  8.54 kg, n = 16) compared to healthy castrated-male controls (117.47  $\pm$  7.60 kg, n = 135,074) at the time of slaughter (Figure 2, Table 1).



**Figure 1.** Gross observation of vertebral osteomyelitis (VO) cases. Gross pathological findings of a VO in pigs. Abscesses (arrow) were found from the intervertebral disks (**A**,**B**) or inside of thoracic vertebrae (**C**,**D**). Each lesion revealed extensive suppurative inflammation and tissue destruction in the thoracic vertebral bone and spinal column, with lesions filled with necrotic debris and infiltration into surrounding tissues, indicating a chronic suppurative process associated with bacterial infection.

Animals 2025, 15, 2970 6 of 14



**Figure 2.** Weight differences between vertebral osteomyelitis (VO) and healthy pigs. The mean body weights (kg) of healthy females, VO females, healthy castrated males (CMs), and VO-CMs are shown as bar graphs. Statistical analysis was performed using Welch's t-test. A statistically significant difference was observed between the healthy-CMs and VO-CMs groups (p < 0.01), indicated by \*\* and between the healthy-Female and VO-Female groups (p < 0.01), also indicated by \*\* Significance levels are represented as follows: p < 0.05 (\*), p < 0.01 (\*\*), and p < 0.001 (\*\*\*).

## 3.2. Bacteria Screening and Isolation

A total of 30 spinal abscess samples were cultured on MacConkey agar, Baird-Parker agar, XLD agar, and EMB agar. All 11 *T. pyogenes*-positive isolates formed pinpoint, shiny, β-hemolytic colonies on blood agar. No colony growth was observed on MacConkey, Baird-Parker, or XLD agar. Although colonies were present on EMB agar, none exhibited the characteristic metallic green sheen, which is typically associated with *E. coli* rather than *T. pyogenes*.

# 3.3. Virulence Related Gene Examination

To investigate bacterial pathogenicity in the 11 *T. pyogenes* isolates, the *plo* toxin gene, a key virulence factor, was examined using PCR analysis. As a result, 5 out of 11 *T. pyogenes* strains (IH2311HS\_GJ01, IH2311.1MA\_GJ02, IH2311.2MA\_GJ03, IH2312KC\_GJ05, and IH2312GM\_GJ06) showed positive for *plo* gene (Table 3).

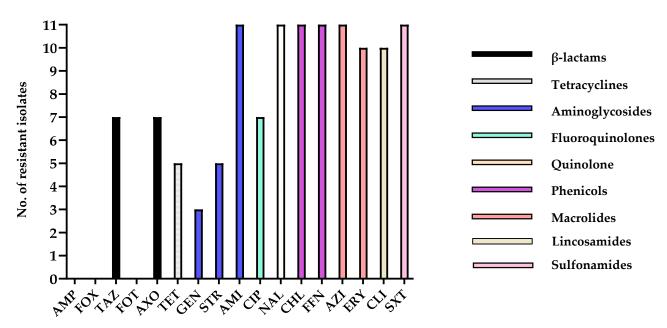
<b>Table 3.</b> Detection of	<i>plo</i> toxin gene	e from T. pyogenes-po	sitive colonies.
------------------------------	-----------------------	-----------------------	------------------

Sample	plo Gene PCR Result				
H2311HS_GJ01	+				
IH2311.1MA_GJ02	+				
IH2311.2MA_GJ03	+				
IH2312KC_GJ05	+				
IH2312GM_GJ06	+				
IH2403HN_GJ16	_				
IH2403JH_GJ17	_				
IH2403YG_GJ18	_				
IH2403GY_GJ19	_				
IH2404GS_GJ20	_				
IH2404MA_GJ21	_				

Animals **2025**, 15, 2970 7 of 14

#### 3.4. Antimicrobial Susceptibility Test

Through the antimicrobial susceptibility test, all 11 isolates exhibited multidrug resistance, with resistance observed to between 8 and 14 antimicrobial agents per strain (Table 4). When grouped by antibiotic classes, most  $\beta$ -lactams exhibited susceptibility across isolates, with the exception of TAZ, to which 7 of 11 isolates were resistant. In the tetracycline class, 5 out of 11 isolates displayed resistance. Among aminoglycosides, GEN resistance was limited to three isolates, while STR resistance was identified in 5 of 11 isolates, and all isolates were resistant to AMI. In contrast, high resistance rates were observed across other antibiotic classes, with all isolates resistant to fluoroquinolones (CIP), quinolones (NAL), phenicols (CHL, FFN), macrolides (AZI, ERY), Sulfonamides (SXT), and lincosamides (CLI) (Table 4 and Figure 3).



**Figure 3.** Number of antimicrobial-resistant isolates. This graph shows the number of resistant isolates for each antimicrobial agent tested. The x-axis represents the antimicrobial agents, and the y-axis represents the number of isolates that showed resistance to a specific antimicrobial agent based on the CLSI (or other relevant standards) breakpoints.

# 3.5. Analysis of 16S rRNA Gene Partial Sequences

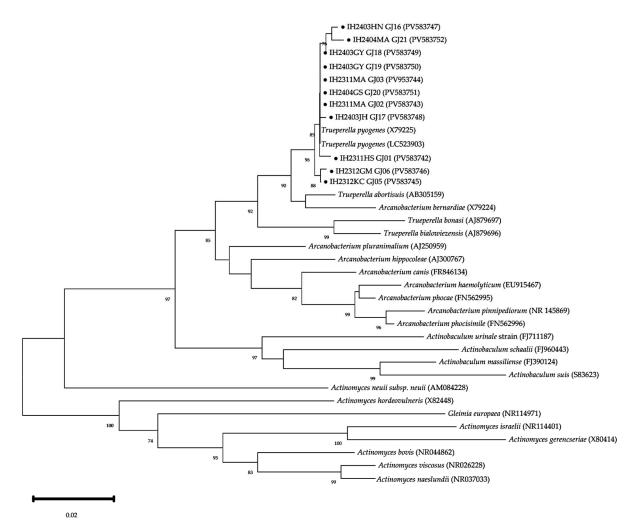
The phylogenetic analysis based on 16S rRNA gene sequences showed that isolates IH2311HS\_GJ01 (accession No. PV583742), IH2311.1MA\_GJ02 (accession No. PV583743), IH2311.2MA (accession No. PV583744), IH2312KC\_GJ05 (accession No. PV583745), IH2312GM\_GJ06 (accession No. PV583746), IH2403HN\_GJ16 (accession No. PV583747), IH2403JH\_GJ17 (accession No. PV583748), IH2403YG\_GJ18 (accession No. PV583749), IH2403GY\_GJ19 (accession No. PV583750), IH2404GS\_GJ20 (accession No. PV583751), and IH2404MA\_GJ21 (accession No. PV583752) clustered closely with reference strains of *T. pyogenes* (e.g., X79225 and LC523903) in the phylogenetic tree (Figure 4). The nucleotide sequence homology varied from 98.27% to 99.66% for LC523903 and from 98.52% to 99.59% for X79225. Sequence analysis that the isolates in this study belong to *T. pyogenes*, as they showed lower homology (97.05–98.07%) with other *Trueperella* species, including *T. abortisuis*, the closest known relative (Figure 4).

Animals **2025**, 15, 2970 8 of 14

**Table 4.** Antimicrobial susceptibility profiles of *T. pyogenes* isolates based on Sensititre plate assay.

	AMP	FOX	TAZ	FOT	AXO	TET	GEN	STR	AMI	CIP	NAL	CHL	FFN	AZI	ERY	CLI	SXT	No. of Resistant
Sample	Minimum Inhibitory Concentrations (MICs, μg/mL)/ Susceptibility (S: Susceptible, R: Resistant)									Antimicrobial Agents								
IH2311HS_GJ01	<1/	<4/ S	4/ S	<1/	2/ R	64/ R	>64/ R	>128/ R	64/ R	4/ R	>128/ R	32/ R	8/ R	>32/ R	>64/ R	>16/ R	0.5/ R	13
IH2311.1MA_GJ02	<1/	<4/ S	4/ S	<1/	2/ R	<2/ S	4/ S	4/ S	16/ R	2/ S	>128/ R	8/ R	2/ R	>32/ R	>64/ R	>16/ R	1/ R	9
IH2311.2MA_GJ03	<1/	<4/ S	8/ R	<1/	2/ R	64/ R	>64/ R	>128/ R	64/ R	4/ R	>128/ R	8/ R	2/ R	>32/ R	>64/ R	>16/ R	0.25/ R	14
IH2312KC_GJ05	<1/	<4/ S	8/ R	<1/	2/ R	<2/ S	8/ S	4/ S	16/ R	1/ S	>128/R	8/ R	2/ R	>32/ R	>64/ R	>16/ R	2/ R	10
IH2312GM_GJ06	<1/	<4/ S	8/ R	<1/	1/ R	<2/	4/ S	4/ S	16/ R	2/ S	>128/R	8/ R	1/ R	>32/ R	0.06/ S	0.12/ S	0.5/ R	8
IH2403HN_GJ16	<1/	<4/	8/ R	<1/	1/ R	<2/	4/ S	4/ S	16/ R	4/ R	>128/ R	8/ R	2/ R	>32/ R	>64/ R	>16/ R	1/ R	11
IH2403JH_GJ17	<1/	<4/ S	8/ R	<1/	1/ R	<2/ S	4/ S	4/ S	16/ R	4/ R	>128/ R	8/ R	2/ R	>32/ R	>64/ R	>16/ R	1/ R	11
H2403YG_GJ18	<1/	<4/ S	<1/	<1/	1/ S	64/ R	8/ S	>128/ R	32/ R	4/ R	>128/ R	8/ R	2/ R	>32/ R	>64/ R	>16/ R	0.5/ R	11
IH2403GY_GJ19	<1/	<4/ S	4/ S	<1/	0.25/ S	64/ R	8/ S	>128/ R	32/ R	4/ R	>128/ R	8/ R	2/ R	>32/ R	>64/ R	>16/ R	0.5/ R	11
IH2404GS_GJ20	<1/	<4/ S	16/ R	<1/	0.25/ S	<2/	<4/ S	4/ S	16/ R	4/ R	>128/ R	8/ R	2/ R	>32/ R	>64/ R	>16/ R	1/ R	10
IH2404MA_GJ21	<1/s	<4/ S	16/ R	<1/s	0.5/ S	32/ R	>64/ R	>128/ R	64/ R	2/ S	>128/ R	32/ R	16/ R	>32/ R	>64/ R	>16/ R	0.25/ R	12

Animals **2025**, 15, 2970 9 of 14



**Figure 4.** Phylogenic analysis of *T. pyogenes* based on 16S rRNA gene sequences. The phylogenetic tree was constructed using the neighbor-joining method with the Kimura 2-parameter model in MEGA version 12, based on partial 16S rRNA gene sequences. Black circles (•) indicate isolates used in this study. These isolates clustered closely with reference strains of *T. pyogenes* (accession No. NCTC5225, LC259203), confirming their identification. Bootstrap values (≥70%) from 1000 replicates are shown at the corresponding nodes. The scale bar represents 0.02 nucleotide substitutions per site.

## 4. Discussion

*T. pyogenes* is widely recognized as an opportunistic bacterium that colonizes the skin and mucosal surfaces of the upper respiratory, gastrointestinal, and urogenital tracts of animals [1,2,26]. Although it has been known for decades, the mechanisms underlying its pathogenicity, transmission dynamics, and environmental reservoirs remain only partially understood. The organism is implicated in various suppurative infections such as mastitis, metritis, pneumonia, and abscess formation that result in significant economic losses in livestock production systems.

In 2024, a total of 470,414 pigs were slaughtered in the Gwangju area, among which 154 (0.033%) carcasses were condemned. This result is consistent to the previous study conducted in Korea that the occurrence of VO in slaughtered pigs ranged from 0.03% to 0.07% [26]. Notably, 68 cases (44.2%) were attributed to VO cases. The next most common causes of carcass condemnation were rectal stricture (33.7%), abnormal odor (12.9%), and peritonitis (5.8%), in decreasing order of frequency (Unpublished data). Among the pathogens implicated in VO, *T. pyogenes* is particularly notable for its ability to cause deep tissue infections in swine and ruminants [10,26–28].

Animals 2025, 15, 2970 10 of 14

This study reinforces the value of slaughterhouses as sentinel sites for animal disease surveillance. Postmortem inspection provides a unique opportunity to detect chronic, subclinical, or localized infections that may not manifest overt clinical signs on farms. As such, slaughterhouse-based monitoring not only reflects the health status of regional livestock populations but also offers a cost-effective strategy for detecting zoonotic pathogens and tracking AMR trends [29,30]. These data are critical for both veterinary public health and food safety systems, aligning with global One Health priorities.

AMR is an escalating global health concern, and food-producing animals are increasingly recognized as reservoirs for resistant bacteria [29–31]. Despite the widespread presence of *T. pyogenes*, no commercial vaccine is currently available, making antimicrobial treatment the primary therapeutic strategy [1,11,18]. However, the efficacy of such treatments is increasingly jeopardized by the emergence of resistant strains, often driven by the routine and sometimes excessive use of antibiotics in agriculture [32–39]. Previous studies have reported variable antimicrobial resistance rates of *T. pyogenes* depending on the host species and clinical conditions. For instance, resistance to streptomycin ranged from 32.6% to 81.25%, tetracycline from 32.6% to 53.12%, and erythromycin from 28.12% to 85.20%, with generally high resistance observed in isolates from cows with mastitis or metritis. In contrast, most isolates remained susceptible to penicillin, gentamicin, and cephalexin in certain cases, while resistance to ampicillin, ciprofloxacin, and enrofloxacin was considerably higher in bovine and caprine isolates. Notably, the current study revealed high resistance rates to several antibiotics including amikacin, florfenicol, chloramphenicol, azithromycin, clindamycin, and trimethoprim-sulfamethoxazole in swine-derived isolates, suggesting an emerging multidrug-resistant profile (Table 5) [2,11,40,41]. In the current study, all 11 T. pyogenes isolates from VO cases exhibited AMR, with resistance patterns ranging from 8 to 14 antibiotics per isolate. Notably, resistance was observed against antibiotic classes commonly used in swine farms, including tetracyclines (TET), phenicols (CHL, FFN), aminoglycosides (STR, AMI, kanamycin), and macrolides (tylosin, ERY). Resistance to these classes is particularly concerning, as they represent frontline drugs frequently employed for both therapeutic and prophylactic purposes in pig production. The widespread resistance to tetracyclines and macrolides may reflect long-term and repeated use in feed additives, while resistance to aminoglycosides and phenicols indicates the limited efficacy of these traditionally relied-upon treatments. These results not only indicate the rise in multidrug-resistant strains but also suggest that commonly used antibiotics may no longer be effective for treating bacterial infections in livestock. This underscores the urgent need for continuous AMR surveillance, stricter regulation of antimicrobial use, and the promotion of prudent veterinary-guided antibiotic selection to preserve the effectiveness of existing drugs and ensure sustainable livestock production.

Zoonotic risk associated with *T. pyogenes* is significant, as this bacterium serves as a common commensal in various animal species, acting as a reservoir for AMR genes. Importantly, resistant strains may cross species barriers and be transmitted to humans, where they pose a therapeutic challenge [3,42,43]. Although human cases of *T. pyogenes* infections are relatively rare, they have been documented in clinical settings, including severe manifestations such as sepsis [7,44], endocarditis [3,8,45], ophthalmic and central nervous system infection [46], soft tissue infection [10,47], and skin ulcers [1,3,8,18,46], suggesting its zoonotic potential and occurrence across different regions of the world. In animals, it has been associated with reproductive, respiratory, and dermatological conditions, further amplifying its impact on productivity and animal welfare [8,48]. Therefore, continuous monitoring and a deeper understanding of its transmission pathways are essential to safeguard both human and animal health.

Animals 2025, 15, 2970 11 of 14

**Table 5.** Comparison of antimicrobial resistance rates of *T. pyogenes* isolates among different animal species.

Antibiotic Class	Antibiotic	Pig (This Study)	Pig (Pneumonia) [40]	Cow (Mastitis) [41]	Cow (Metritis) [41]	Sheep/ Goat [2,11]
β-lactams	Amoxicillin	_	0%	87.50%	100%	_
·	Amoxicillin/ Clavulanic acid	_	_	_	_	38.40%
	AMP	0%	_	90.62%	100%	_
	Cefalexin	-	_	84.37%	97.56%	_
	Cefazolin	_	7.40%	O4.57 70	77.5070	_
	FOT	0%	7.4070	_	_	_
	FOX	0%	_	_	_	_
	TAZ	63.60%	_	_	_	_
	AXO	63.60%	_	_	_	_
	Penicillin	_	0%	100%	97.56%	0.00-5.80%
	AXO	63.60%	_	_	_	_
Tetracyclines	TET	45.50%	_	53.12%	48.78%	32.60-53.12%
Aminoglycosides	GEN	27.30%	77.80%	100%	97.56%	21.87
0,7	STR	45.50%	_	81.25%	56.09%	32.60-56.25%
	AMI	100%	74.10%	_	_	_
Fluoroquinolones	CIP	63.60%	0%	53.12%	70.73%	_
-	Enrofloxacin	_	0%	59.37%	73.17%	78.13
Quinolone	NAL	100%	_	_	_	_
Phenicols	FFN	100%	0%	_	_	_
	CHL	100%	_	_	_	14.00%
Macrolides	AZI	100%	85.20%	37.50%	46.34%	_
	Tylosin	_	_	62.50%	63.41%	_
	ERY	90.90%	85.20%	53.12%	39.02%	28.12-44.20%
	Clindamycin	90.90%	_	_	_	4.70%
Sulfonamides	SXT	100%	_	87.50%	70.73%	37.50%
	Sulfisoxazole	_	_	_	_	37.20%
Lincosamides	Lincomycin	_	_	43.75%	58.53%	_
Rifamycins	Rifampicin	-	_	59.37%	68.29%	_

The diverse clinical manifestations associated with *T. pyogenes* infections are mediated by a range of virulence factors, including plo, neuraminidases (nanP, nanH), and fimbrial proteins (fimA, fimC, fimE). A recent study from China reported that all T. pyogenes isolates (total 86 strains) obtained from goats and sheep harbored the plo gene, regardless of the clinical manifestation, which included pneumonia, subcutaneous abscesses, mastitis, ulcerated skin, and metritis [11]. In contrast, only 45.5% of the isolates in the present study, which were collected from pigs with VO, were positive for the *plo* gene and no clear association was observed between the presence of the plo gene and antimicrobial susceptibility profiles (Tables 3 and 4). This discrepancy suggests that the presence of plo alone may not be sufficient to explain specific tissue tropism, such as VO, and that the genotypic profiles of virulence factors vary across host species. However, despite collecting VO cases for one year, only 11 isolates were available for analysis, which limits the representativeness of the findings. Furthermore, the exclusive focus on the plo gene does not capture the broader virulence gene repertoire, and the association between virulence markers and clinical manifestations remains unclear. Vertebral infections in pigs are often attributed to ascending infections from tail wounds caused by fighting between animals, suggesting that both the infection route and specific gene expression patterns may influence disease localization. To address these gaps, future studies should include larger sample sizes, investigate multiple virulence factors beyond plo, and employ functional approaches such as in vivo models to clarify the pathogenic mechanisms and better define the clinical relevance of virulence gene expression in VO.

# 5. Conclusions

This study identified *T. pyogenes* as the primary pathogen responsible for vertebral abscesses that led to carcass condemnation in pigs at slaughter. The isolates demon-

Animals 2025, 15, 2970 12 of 14

strated multidrug resistance, indicating the ongoing antimicrobial pressure within livestock production environments. These results reinforce the importance of slaughterhouses as practical surveillance points, where postmortem examination allows for the detection of both clinically apparent and subclinical infections. Such surveillance efforts play a key role in tracking antimicrobial resistance trends and understanding pathogen dynamics in food animals, providing valuable insights for animal health management and food safety monitoring.

**Author Contributions:** Conceptualization, I.-H.L., G.L.; methodology, D.-Y.K., B.-G.J.; software, J.-W.C.; validation, Y.-B.B., D.-S.Y.; formal analysis, S.-I.P., J.B.L.; investigation, I.-H.L., H.J.M., D.-Y.K., J.-W.C., J.B.L., B.-G.J.; resources, H.J.M.; data curation, H.J.M., D.-S.Y.; writing—original draft preparation, I.-H.L., G.L.; writing—review and editing, G.L., J.B.L., K.-J.L., J.-G.P.; visualization, Y.-B.B.; supervision, K.-J.L., J.-G.P.; project administration, S.-I.P.; funding acquisition, J.-G.P., S.-I.P. All authors have read and agreed to the published version of the manuscript.

Funding: This research was supported by National Institute of Health (NIH) research project (grant No. 2024ER210101 and 2024ER210300), Korea Institute of Planning and Evaluation for Technology in Food, Agriculture and Forestry (IPET) and Korea Smart Farm R&D Foundation (KosFarm) through Smart Farm Innovation Technology Development Program, funded by Ministry of Agriculture, Food and Rural Affairs (MAFRA) and Ministry of Science and ICT (MSIT), Rural Development Administration (RDA) (RS-2025-0221738140982119420101).

**Institutional Review Board Statement:** This study did not require Institutional Review Board (IRB) approval, as it did not involve experimental procedures on live animals. All samples were obtained postmortem from pig carcasses at slaughterhouses with official permission from the facility operators.

Informed Consent Statement: Not applicable.

Data Availability Statement: All data in this study are included in the article.

Conflicts of Interest: The authors declare they have no conflicts of interest.

#### References

- 1. Rzewuska, M.; Kwiecien, E.; Chrobak-Chmiel, D.; Kizerwetter-Swida, M.; Stefanska, I.; Gierynska, M. Pathogenicity and Virulence of *Trueperella pyogenes*: A Review. *Int. J. Mol. Sci.* **2019**, 20, 2737. [CrossRef]
- 2. Gulaydin, O.; Kayikci, C.; Gulaydin, A. Determination of antimicrobial susceptibility and virulence-related genes of *Trueperella pyogenes* strains isolated from various clinical specimens in animals. *Pol. J. Vet. Sci.* **2024**, 27, 193–202. [CrossRef]
- 3. Deliwala, S.; Beere, T.; Samji, V.; McDonald, P.J.; Bachuwa, G. When Zoonotic Organisms Cross Over-*Trueperella pyogenes* Endocarditis Presenting as a Septic Embolic Stroke. *Cureus* **2020**, *12*, e7740. [CrossRef] [PubMed]
- 4. Ding, H.; Lammler, C. Evaluation of the API Coryne test system for identification of *Actinomyces pyogenes*. *J. Vet. Med. Ser. B* **1992**, 39, 273–276. [CrossRef] [PubMed]
- 5. da Silva, A.P.; Shivaprasad, H.L.; Jerry, C.; Stoute, S. An Uncommon Case of *Trueperella pyogenes* Infection in an Adult Backyard Rooster and a Retrospective Study; 2000–20. *Avian Dis.* **2021**, *65*, 171–176. [CrossRef] [PubMed]
- 6. Vieira-Pinto, M.; Azevedo, J.; Poeta, P.; Pires, I.; Ellebroek, L.; Lopes, R.; Veloso, M.; Alban, L. Classification of Vertebral Osteomyelitis and Associated Judgment Applied during Post-Mortem Inspection of Swine Carcasses in Portugal. *Foods* **2020**, *9*, 1502. [CrossRef]
- 7. Levy, C.E.; Pedro, R.J.; Von Nowakonski, A.; Holanda, L.M.; Brocchi, M.; Ramo, M.C. *Arcanobacterium pyogenes* sepsis in farmer, Brazil. *Emerg. Infect. Dis.* **2009**, *15*, 1131–1132. [CrossRef]
- 8. Stuby, J.; Lardelli, P.; Thurnheer, C.M.; Blum, M.R.; Frei, A.N. *Trueperella pyogenes* endocarditis in a Swiss farmer: A case report and review of the literature. *BMC Infect. Dis.* **2023**, 23, 821. [CrossRef]
- 9. Hermida Amejeiras, A.; Romero Jung, P.; Cabarcos Ortiz De Barron, A.; Trevino Castallo, M. One case of pneumonia with *Arcanobacterium pyogenes*. *An. Med. Interna* **2004**, *21*, 334–336. [CrossRef]
- 10. Kavitha, K.; Latha, R.; Udayashankar, C.; Jayanthi, K.; Oudeacoumar, P. Three cases of *Arcanobacterium pyogenes*-associated soft tissue infection. *J. Med. Microbiol.* **2010**, *59*, 736–739. [CrossRef]
- 11. Wei, Y.; Wang, B.; Wu, K.; Wang, C.; Bai, X.; Wang, J.; Yang, Z. Prevalence, Virulence Genes, Drug Resistance and Genetic Evolution of *Trueperella pyogenes* in Small Ruminants in Western China. *Animals* **2024**, *14*, 2964. [CrossRef]

Animals 2025, 15, 2970 13 of 14

12. Zhang, W.; Wang, H.; Wang, B.; Zhang, Y.; Hu, Y.; Ma, B.; Wang, J. Replacing the 238th aspartic acid with an arginine impaired the oligomerization activity and inflammation-inducing property of pyolysin. *Virulence* **2018**, *9*, 1112–1125. [CrossRef]

- 13. Liang, H.; Wang, B.; Wang, J.; Ma, B.; Zhang, W. Pyolysin of *Trueperella pyogenes* Induces Pyroptosis and IL-1beta Release in Murine Macrophages Through Potassium/NLRP3/Caspase-1/Gasdermin D Pathway. *Front. Immunol.* 2022, 13, 832458. [CrossRef]
- 14. Kwiecien, E.; Stefanska, I.; Chrobak-Chmiel, D.; Salamaszynska-Guz, A.; Rzewuska, M. New Determinants of Aminoglycoside Resistance and Their Association with the Class 1 Integron Gene Cassettes in *Trueperella pyogenes*. *Int. J. Mol. Sci.* **2020**, 21, 4230. [CrossRef]
- 15. Fujimoto, H.; Shimoji, N.; Sunagawa, T.; Kubozono, K.; Nakajima, C.; Chuma, T. Differences in phenotypic and genetic characteristics of *Trueperella pyogenes* detected in slaughtered cattle and pigs with septicemia. *J. Vet. Med. Sci.* **2020**, *82*, 626–631. [CrossRef]
- 16. Fujimoto, H.; Shimoji, N.; Sunagawa, T.; Sanga, G.; Chuma, T. Differences in antimicrobial resistance-related genes of *Trueperella pyogenes* between isolates detected from cattle and pigs. *J. Vet. Med. Sci.* **2024**, *86*, 1119–1123. [CrossRef] [PubMed]
- 17. Jost, B.H.; Post, K.W.; Songer, J.G.; Billington, S.J. Isolation of *Arcanobacterium pyogenes* from the porcine gastric mucosa. *Vet. Res. Commun.* **2002**, *26*, 419–425. [CrossRef] [PubMed]
- 18. Wen, X.; Cheng, J.; Liu, M. Virulence factors and therapeutic methods of *Trueperella pyogenes*: A review. *Virulence* **2025**, *16*, 2467161. [CrossRef]
- 19. Fujimoto, H.; Nakamura, T.; Sato, A.; Chuma, T. Antimicrobial susceptibility of *Trueperella pyogenes* isolated from cattle and pigs with septicemia in southern Kyushu, Japan. *J. Vet. Med. Sci.* 2023, *85*, 379–382. [CrossRef]
- 20. Fessler, A.T.; Schwarz, S. Antimicrobial Resistance in *Corynebacterium* spp., *Arcanobacterium* spp., and *Trueperella pyogenes*. *Microbiol. Spectr.* **2017**, *5*, 395–408. [CrossRef] [PubMed]
- 21. Jorgensen, J.H.; Hindler, J.F. New consensus guidelines from the Clinical and Laboratory Standards Institute for antimicrobial susceptibility testing of infrequently isolated or fastidious bacteria. *Clin. Infect. Dis.* 2007, 44, 280–286. [CrossRef]
- 22. Aino, M.; Oka, S.; Haruguchi, K.; Aino, M.; Hashimura, S.; Kurosawa, K. Comparison of spinal column alignment and autonomic nervous activity using the intersegmental tenderness test in the segment above. *J. Phys. Ther. Sci.* **2021**, *33*, 570–575. [CrossRef]
- Rogovskyy, A.S.; Lawhon, S.; Kuczmanski, K.; Gillis, D.C.; Wu, J.; Hurley, H.; Rogovska, Y.V.; Konganti, K.; Yang, C.Y.; Duncan, K. Phenotypic and genotypic characteristics of Trueperella pyogenes isolated from ruminants. *J. Vet. Diagn. Investig.* 2018, 30, 348–353. [CrossRef]
- Rzewuska, M.; Stefanska, I.; Osinska, B.; Kizerwetter-Swida, M.; Chrobak, D.; Kaba, J.; Bielecki, W. Phenotypic characteristics and virulence genotypes of *Trueperella* (Arcanobacterium) pyogenes strains isolated from European bison (*Bison bonasus*). Vet. Microbiol. 2012, 160, 69–76. [CrossRef] [PubMed]
- 25. Kim, H.J.; Park, J.G.; Moon, K.S.; Jung, S.B.; Kwon, Y.M.; Kang, N.S.; Kim, J.H.; Nam, S.J.; Choi, G.; Baek, Y.B.; et al. Identification and characterization of a marine bacterium extract from *Mameliella* sp. M20D2D8 with antiviral effects against influenza A and B viruses. *Arch. Virol.* 2024, 169, 41. [CrossRef] [PubMed]
- 26. Han, S.J.; Kim, Y.H.; Lee, D.K.; Im, C.W.; Kim, S.J.; Han, J.H. Investigation of characteristics and classification for swine vertebral osteomyelitis in South Korea. *BMC Vet. Res.* **2025**, *21*, 192. [CrossRef]
- 27. Baird, R.M.; Lee, W.H. Media used in the detection and enumeration of Staphylococcus aureus. *Int J Food Microbiol* **1995**, *26*, 15–24. [CrossRef] [PubMed]
- 28. Facklam, R.; Elliott, J.; Shewmaker, L.; Reingold, A. Identification and characterization of sporadic isolates of *Streptococcus iniae* isolated from humans. *J. Clin. Microbiol.* **2005**, *43*, 933–937. [CrossRef]
- 29. Despotovic, M.; de Nies, L.; Busi, S.B.; Wilmes, P. Reservoirs of antimicrobial resistance in the context of One Health. *Curr. Opin. Microbiol.* **2023**, 73, 102291. [CrossRef]
- 30. Garcia-Diez, J.; Saraiva, S.; Moura, D.; Grispoldi, L.; Cenci-Goga, B.T.; Saraiva, C. The Importance of the Slaughterhouse in Surveilling Animal and Public Health: A Systematic Review. *Vet. Sci.* **2023**, *10*, 167. [CrossRef]
- Apenteng, O.O.; Aarestrup, F.M.; Vigre, H. Modelling the effectiveness of surveillance based on metagenomics in detecting, monitoring, and forecasting antimicrobial resistance in livestock production under economic constraints. Sci. Rep. 2023, 13, 20410.
   [CrossRef] [PubMed]
- 32. Ashrafi Tamai, I.; Mohammadzadeh, A.; Zahraei Salehi, T.; Mahmoodi, P. Genomic characterisation, detection of genes encoding virulence factors and evaluation of antibiotic resistance of *Trueperella pyogenes* isolated from cattle with clinical metritis. *Antonie Van Leeuwenhoek* 2018, 111, 2441–2453. [CrossRef]
- 33. Liu, M.C.; Wu, C.M.; Liu, Y.C.; Zhao, J.C.; Yang, Y.L.; Shen, J.Z. Identification, susceptibility, and detection of integron-gene cassettes of *Arcanobacterium pyogenes* in bovine endometritis. *J. Dairy Sci.* **2009**, *92*, 3659–3666. [CrossRef]
- 34. Rzewuska, M.; Czopowicz, M.; Gawrys, M.; Markowska-Daniel, I.; Bielecki, W. Relationships between antimicrobial resistance, distribution of virulence factor genes and the origin of *Trueperella pyogenes* isolated from domestic animals and European bison (*Bison bonasus*). *Microb. Pathog.* **2016**, *96*, 35–41. [CrossRef]

Animals 2025, 15, 2970 14 of 14

35. Trinh, H.T.; Billington, S.J.; Field, A.C.; Songer, J.G.; Jost, B.H. Susceptibility of *Arcanobacterium pyogenes* from different sources to tetracycline, macrolide and lincosamide antimicrobial agents. *Vet. Microbiol.* **2002**, *85*, 353–359. [CrossRef]

- 36. Werckenthin, C.; Alesik, E.; Grobbel, M.; Lubke-Becker, A.; Schwarz, S.; Wieler, L.H.; Wallmann, J. Antimicrobial susceptibility of Pseudomonas aeruginosa from dogs and cats as well as *Arcanobacterium pyogenes* from cattle and swine as determined in the BfT-GermVet monitoring program 2004-2006. *Berl. Munch. Tierarztl. Wochenschr.* 2007, 120, 412–422. [PubMed]
- 37. Yoshimura, H.; Kojima, A.; Ishimaru, M. Antimicrobial susceptibility of *Arcanobacterium pyogenes* isolated from cattle and pigs. *J. Vet. Med. B Infect. Dis. Vet. Public Health* **2000**, 47, 139–143. [CrossRef]
- 38. Zhang, D.; Zhao, J.; Wang, Q.; Liu, Y.; Tian, C.; Zhao, Y.; Yu, L.; Liu, M. *Trueperella pyogenes* isolated from dairy cows with endometritis in Inner Mongolia, China: Tetracycline susceptibility and tetracycline-resistance gene distribution. *Microb. Pathog.* **2017**, *105*, 51–56. [CrossRef] [PubMed]
- 39. Zhao, K.L.; Liu, Y.; Zhang, X.Y.; Palahati, P.; Wang, H.N.; Yue, B.S. Detection and characterization of antibiotic-resistance genes in *Arcanobacterium pyogenes* strains from abscesses of forest musk deer. *J. Med. Microbiol.* **2011**, *60*, 1820–1826. [CrossRef]
- 40. Dong, W.L.; Liu, L.; Odah, K.A.; Atiah, L.A.; Gao, Y.H.; Kong, L.C.; Ma, H.X. Antimicrobial resistance and presence of virulence factor genes in *Trueperella pyogenes* isolated from pig lungs with pneumonia. *Trop. Anim. Health Prod.* **2019**, *51*, 2099–2103. [CrossRef]
- 41. Rezanejad, M.; Karimi, S.; Momtaz, H. Phenotypic and molecular characterization of antimicrobial resistance in *Trueperella pyogenes* strains isolated from bovine mastitis and metritis. *BMC Microbiol.* **2019**, *19*, 305. [CrossRef]
- 42. Trinchera, M.; De Gaetano, S.; Sole, E.; Midiri, A.; Silvestro, S.; Mancuso, G.; Catalano, T.; Biondo, C. Antimicrobials in Livestock Farming and Resistance: Public Health Implications. *Antibiotics* **2025**, *14*, 606. [CrossRef]
- 43. Chilanga, F.; Kasozi, K.I.; Mazeri, S.; Paterson, G.K.; Muwonge, A. A systematic review of antimicrobial resistance transmission inferences at the human-livestock interface in Africa. *NPJ Antimicrob. Resist.* **2025**, *3*, 58. [CrossRef] [PubMed]
- 44. Hoshina, D.; Tsujiwaki, M.; Furuya, K.; Kawamura, T. Erythema multiforme associated with *Trueperella pyogenes* bacteremia. *J. Dermatol.* **2017**, 44, e83–e84. [CrossRef]
- 45. Chesdachai, S.; Larbcharoensub, N.; Chansoon, T.; Chalermsanyakorn, P.; Santanirand, P.; Chotiprasitsakul, D.; Ratanakorn, D.; Boonbaichaiyapruck, S. *Arcanobacterium pyogenes* endocarditis: A case report and literature review. *Southeast Asian J. Trop. Med. Public Health* **2014**, 45, 142–148.
- 46. Calo, L.; Scarano, E.; Brigato, F.; Di Cintio, G.; Galli, J. Bilateral cavernous sinus and ophthalmic vein thrombosis secondary to *Trueperella pyogenes* rhinosinusitis. *Indian J. Otolaryngol. Head Neck Surg.* **2024**, *76*, 2840–2843. [CrossRef]
- 47. Meili, Z. *Trueperella pyogenes* pharyngitis in an immunocompetent 40-year-old man. *BMJ Case Rep.* **2020**, *13*, e236129. [CrossRef] [PubMed]
- 48. Marchionatti, E.; Kittl, S.; Sendi, P.; Perreten, V. Whole genome-based antimicrobial resistance, virulence, and phylogenetic characteristics of *Trueperella pyogenes* clinical isolates from humans and animals. *Vet. Microbiol.* **2024**, 294, 110102. [CrossRef] [PubMed]

**Disclaimer/Publisher's Note:** The statements, opinions and data contained in all publications are solely those of the individual author(s) and contributor(s) and not of MDPI and/or the editor(s). MDPI and/or the editor(s) disclaim responsibility for any injury to people or property resulting from any ideas, methods, instructions or products referred to in the content.