DOI: 10.1002/vms3.456

ORIGINAL ARTICLE

WILEY

Characteristics of *Aerococcus viridans* isolated from porcine fetuses in Korean farms

Van Giap Nguyen[1] | Cheong Ung Kim[2] | Hai-Quynh Do[3,4] | Sook Shin[2] | Keum Chan Jang[2] | Yong Ho Park[2] | Bong Kyun Park[3] | Hee Chun Chung[3]

[1]Department of Veterinary Microbiology and Infectious Diseases, Faculty of Veterinary Medicine, Vietnam National University of Agriculture, Hanoi, Vietnam

[2]Department of Veterinary Microbiology, College of Veterinary Medicine and Research Institute for Veterinary Science, Seoul National University, Seoul, Republic of Korea

[3]Department of Veterinary Medicine Virology Lab, College of Veterinary Medicine and Research Institute for Veterinary Science, Seoul National University, Seoul, Korea

[4]Institute of Genome Research, Vietnam Academy of Science and Technology, Hanoi, Vietnam

Correspondence
Yong Ho Park, Department of Veterinary Microbiology, College of Veterinary Medicine and Research Institute for Veterinary Science, Seoul National University, Seoul 151-742, Republic of Korea.
Email:yhp@snu.ac.kr (Y. H. P.)

Bong Kyun Park and Hee Chun Chung, Department of Veterinary Medicine Virology Lab, College of Veterinary Medicine and Research Institute for Veterinary Science, Seoul National University, Seoul 08826, Korea.
Emails: parkx026@snu.ac.kr (B. K. P.); heeskyi@snu.ac.kr (H. C. C.)

Funding information
This work was supported by the Korea Science and Engineering Foundation (KOSEF) grant funded by the Korea government (No. 2020R1I1A1A01054539).

Abstract

Swine abortion caused by viruses as well as bacteria has caused many economic losses in domestic farms over the years; however, bacterial abortion has not yet been studied in Korea. Several bacterial species were isolated from aborted fetuses (n = 103) for which the cause of death was not viral abortion. Among them, we focused on *Aerococcus viridans*, which had the highest positive rate within three provinces (Gangwon, Jeonnam and Gyeongnam). A total of 16 isolates were identified as *A. viridans* by matrix-assisted laser desorption ionization-time of flight mass spectrometry (MALDI-TOF MS), and 13 were characterized by both antibiotic resistance and 16S rRNA gene analysis. Based on antibiotic susceptibility testing result, eight antimicrobials could not effectively eliminate the present isolation (more than 40% of isolates can resist these antibiotics), while all except two strains were susceptible to trimethoprim/sulfamethoxazole. Molecular analysis indicated genetic variation among these strains. This study is the first report detecting *A. viridans* from aborted fetuses in Korean domestic farms.

KEYWORDS

Aerococcus viridans, antibiotic resistance, phylogeny, porcine fetus

Van Giap Nguyen Cheong Ung Kim and Hai-Quynh Do contributed equally to this work

1 | INTRODUCTION

Abortion is a significant factor for economic losses in domestic farms. The causative agents of abortion in porcine fetuses can be divided into noninfectious and infectious causes. Viral infections such as porcine reproductive and respiratory syndrome virus (PRRSV), porcine parvovirus (PPV), porcine circoviruses, encephalomyocarditis virus (EMCV), Japanese encephalitis virus (JEV), Aujeszky disease virus (ADV) and swine influenza virus (SIV) have normally been highlighted as the major infectious causes (Koenen & Vanderhallen, 1997; Zhang et al., 2015). However, infections with several bacterial species could be considered reasons for reproductive failure in swine. Bacteria associated with abortion in pig herds include *Leptospira* spp., *Brucella suis* and other opportunistic agents such as *Escherichia coli*, *Streptococcus* spp., *Staphylococcus* spp. and *Erysipelothrix rhusiopathiae* (Givens & Marley, 2008; Vannier, 1999). A previous study indicated that pure bacterial factors accounted for approximately 24.5% of total infectious agents detected in fetuses from 2011 to 2013 (Salogni et al., 2016).

Aerococcus viridans is a Gram-positive cocci bacteria belonging to the *Aerococcaceae* family, *Lactobacillales* order. This species is considered an opportunistic bacteria that is relevant to several human infections, such as endocarditis, urinary tract infections, arthritis and meningitis (Ezechukwu et al., 2019; Nathavitharana et al., 1983; Taylor & Trueblood, 1985; Zhou et al., 2013). This *species* was also detected in clinical specimens from livestock (Martín et al., 2007; Pan et al., 2017; SAISHU et al., 2015) and wild animals (Colombo et al., 2021) with acute infections. In this study, several bacteria were isolated from samples collected over 2 years from aborted fetuses that did not have abortion-causing viruses detected. Among of them, we focused on *A. viridans* and characterized it through molecular biological analyses.

2 | MATERIALS AND METHODS

2.1 | Sample collection and bacterial isolation and identification

From January 2019 to May 2020, 103 fetal samples from different sows (2 fetuses per sow; 2019; $n = 50$ and 2020; $n = 53$) were separately collected from 25 farms (2019; $n = 13$ and 2020; $n = 12$) in six provinces (Gyeongnam, Gyeongbuk, Gangwon, Gyeonggi, Jeonnam and Chungnam) throughout South Korea (Figure S1). Pooled organs were homogenized and diluted 10-fold (w/v) with phosphate-buffered saline. These samples were confirmed as negative with eight reproductive-related viruses including: porcine circovirus type 2 (PCV2), PCV3, PRRSV, PPV, SIV, EMCV, JEV and ADV using the published primers (Nguyen et al., 2018) or commercial kits according to the methods of a previous study (Oh et al., 2017). Therefore, bacteria were isolated and purified on blood agar plus 5% defibrinated sheep's blood. Pure colonies were maintained in stock containing glycerol at −70°C and then reactivated in brain–heart infusion (BHI) medium supplemented with fetal calf serum (5%) for 24 hr at 37°C for identification.

Pure isolated bacterial proteins were extracted for identification using matrix-assisted laser desorption ionization-time of flight mass spectrometry (MALDI-TOF MS; Bruker Daltonics, Germany) based on an ethanol/formic acid protocol (Hijazin et al., 2012). The obtained spectra were compared with the patented manufacturer's library. Bacteria were identified based on similarity log scores according to the standard Bruker interpretative criteria: a score ≥2.0 was accepted for species assignment, and a score ≥1.7 and ≤2.0 was accepted for genus identification.

2.2 | Antibiotic susceptibility profiling of 13 isolated strains of *A. viridans*

We conducted antimicrobial susceptibility testing of 13 isolated *A. viridans* fetus strains. The antimicrobial susceptibility to different commonly used antimicrobials was determined by the disk diffusion method using commercial disks (Oxoid, Ltd.). Nine antimicrobial disks were prepared: lincomycin/spectinomycin (109 μg), ceftiofur (30 μg), gentamicin (10 μg), oxytetracycline (30 μg), penicillin G (10 U), ampicillin (10 μg), trimethoprim/sulfamethoxazole (25 μg), amoxicillin/clavulanic acid (20/10 μg) and tiamulin (30 μg; Table 2). For the susceptibility test, 13 inoculated strains were prepared from a 48-hr Columbia blood agar plate by suspending four colonies in 5 ml of Mueller-Hinton broth and adjusting the solution to a 0.5 McFarland standard. The disk diffusion test was performed as described by the Clinical and Laboratory Standards Institute (CLSI; formerly the National Committee for Clinical Laboratory Standards; Shryock, 2002) using Mueller-Hinton plates (Oxoid) supplemented with 5% defibrinated sheep's blood (Martín et al., 2007). The inhibition zone diameter (IZD) breakpoint used was that recommended following the protocol for testing *Staphylococcus* (Martín et al., 2007; Standards, 2002), and the IZD breakpoint used was recommended by the CLSI (Lalitha, 2004; Watts, 1999).

2.3 | 16S rRNA sequencing of 13 isolated strains of *A. viridans*

DNA extraction was performed using a DNA/RNA extraction kit (iNtRON Biotechnology, Inc.). We amplified part of the 16S rRNA gene (Kozitskaya et al., 2005) using PlsF and PlsR primers. The PCR reaction mixture consisted of 2 μl of template DNA, 1 μl of each primer (10 μM) and 16 μl of Master mix solution (iNtRON Biotechnology, Inc.). The PCR thermal profile was initially denaturized for 5 min at 95°C, followed by 40 cycles of 30 s at 95°C, 2 min at 55°C, 1 min 20 s at 72°C and final extension for 7 min at 72°C. At approximately 1,400 bp, amplifying products were sent to a commercial facility (Macrogen Co., Ltd.) for sequencing.

2.4 | Phylogenetic tree construction and analysis

Including the 13 *A. viridans* strains from this study, the 16S rRNA genes from a total of 104 *A. viridans* strains were collected from GenBank to conduct a phylogenetic analysis. Using IQ-TREE v1.6.12 (Nguyen et al., 2015), the genetic relationships between *A. viridans* were inferred by the maximum likelihood (ML) method. The "-m TEST" option was used to help select data that automatically best fit the nucleotide substitution model. Branch support values were estimated by ultrafast bootstrap approximation (Hoang et al., 2018) implemented in IQ-TREE via the "-bb 1,000" option. The reconstructed phylogenies were displayed and the midpoint rooted by FigTree v1.4.3. To detect any potential recombination sites on the 16S rRNA gene of each *A. viridans* strain, Recombination Detection Program (RDP; version 4.460) was used (Martin & Rybicki, 2000) to compare the 13 *A. viridans* strains from this study with the 16S rRNA genes of 104 *A. viridans* strains collected from GenBank.

3 | RESULTS

3.1 | Bacterial isolation and phenotypic characteristics of *A. viridans*

In this study, 113 isolates of 46 different species were detected (Table 1). Of these, *A. viridans* was the most abundant species collected from fetuses (16 isolates) followed *Enterococcus faecalis* (14 isolates). Other commonly prevalent bacteria were *Staphylococcus simulans* (7 isolates), *Camobacterium maltsomaticum* (6 isolates), *Lactobacillus sakei* (5 isolates), *Lactobacillus curvatus* (4 isolates), *Clostridium perfringens* (4 isolates) and *Streptococcus thoraltensis* (4 isolates). Several other types of bacteria were also found (Table 1).

Focusing on *A. viridans*, this species was detected most commonly in Gangwon province (13 samples) followed by Jeonnam (three samples) and Gyeongnam (one sample). There were no *A. viridans*-positive samples in the remained three provinces. *A. viridans* growing on sheep blood agar showed circular yellowish colonies of a very small size of 1 mm, circular and several were α-haemolytic (Figure 1a). The microscopic characteristics of these isolates indicated that they were Gram-positive cocci measuring 1 μm in size that formed pairs or small groups (Figure 1b).

3.2 | Antibiotic susceptibility profiling of 13 isolated strains of *A. viridans*

Based on the IZD results, evaluation of these present isolates revealed that a high number of strains tolerated tiamulin (92.3%), amoxicillin/clavulanic acid (84.6%), lincomycin/spectinomycin (84.6%), ampicillin (76.9%), oxytetracycline (61.5%) and gentamicin (53.8%; Table 2). Nearly half of these isolates were resistant to ceftiofur (46.2%) and penicillin (46.2%; Table 2). On the other hand, trimethoprim/sulfamethoxazole tended to be effective for treating *A. viridans*, as only 15.4% of the strains were tolerant (Table 2).

TABLE 1 Bacteria isolates from 103 fetuses from Korean domestic farms from 2019 to 2020

Species identification	2019 ($n = 50$)	2020 ($n = 53$)	Total	Prevalence
Aerococcus viridans	7	9	16	15.5%
Enterococcus faecalis	6	8	14	13.4%
Staphylococcus simulans	1	6	7	6.8%
Camobacterium maltsomaticum	2	4	6	5.8%
Lactobacillus sakei	5	0	5	4.9%
Streptococcus thoraltensis	1	3	4	3.9%
Lactobacillus cuvatus	4	0	4	3.9%
Clostridium perfingen	1	3	4	3.9%
Escherichia coli	0	3	3	2.9%
Enterococcus avium	0	3	3	2.9%
Enterococcus pseudoavium	0	2	2	1.9%
Staphylococcus aureus	0	2	2	1.9%
Staphylococcus hyicus	0	2	2	1.9%
Staphylococcus haemolyticus	1	1	2	1.9%
Corynebacterium xerosis	2	0	2	1.9%
Vagococcus fluvialis	2	0	2	1.9%
Clostridium tertium	0	2	2	1.9%
Lactobacillus paracasei	0	2	2	1.9%
Streptococcus dysgalactiae	0	2	2	1.9%
Other bacteria	11	18	29	28.2%
Total	43	70	113	

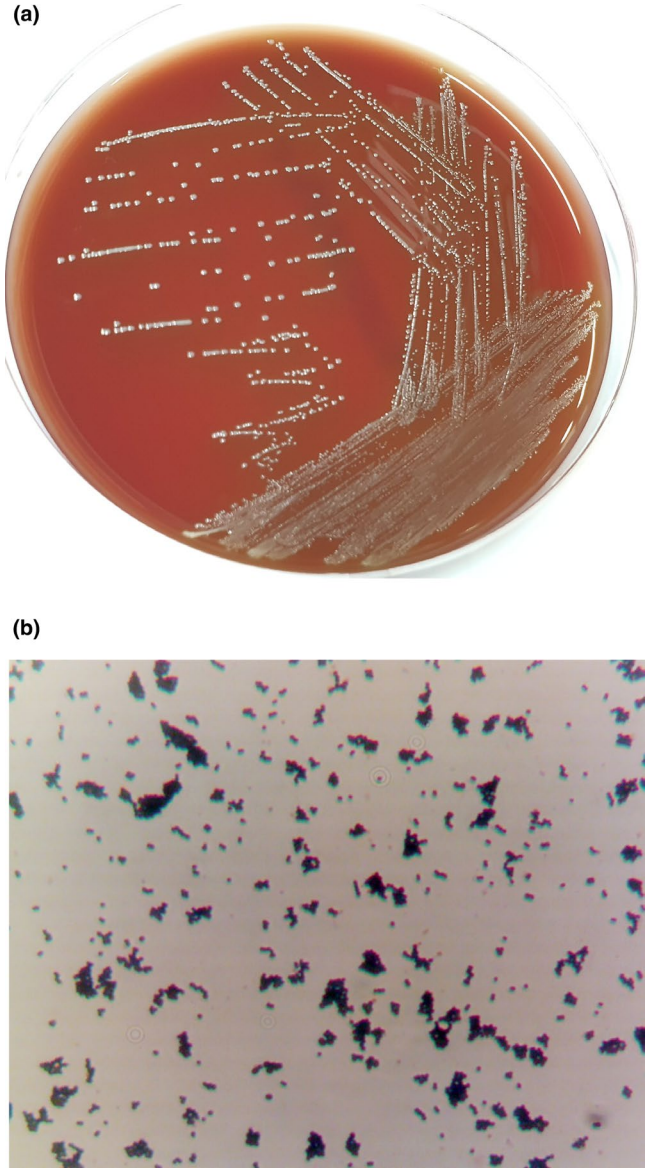

FIGURE 1 *Aerococcus viridans* identification. (a) Gram's stain the *A. viridans* fetus of E14_Korea_2020_Pig colonies showing gram positive by 400× microscope. (b) The E14 strain of *A. viridans* grown for 24 hr in 5% sheep blood agar. Small colonies with clear α-haemolysis can be observed

Interestingly, whereas almost all of the isolated strains were resistant to at least five different types of antibiotics, isolates E9 and E13 were only tolerant to one or two types of antibiotics.

3.3 | Phylogenetic tree analysis and potential recombinant origin of Korean *A. viridans* strains

A total of 13 strains of *A. viridans* were obtained accounting for approximately 1,400 bp of the partial 16S rRNA gene: W254 to W258, E3, and E8 to E14 (GenBank accession number. MT921600 to MT921611, MT928815). In the phylogenetic tree, the Korean strains showed genetic variation (Figure 2). Most of our isolates were close to the human-related strains or environmental origin strains, while W256 and E14 formed a separated branch in the phylogenetic tree. Eight strains (E3, E8, E10, E11, W254, W255, W267 and W25) were close to reference strains A.V.04_China_2010_Human and A.V.04_China_2015_Human. The E9_Korea_2020_Pig strain was genetically related to I17_China_2015_Air strain. In addition, the E12 and E13_Korea_2020_Pig strains were grouped with Clone_C040_Spain_2015_Sediment and were significantly different from the Korean isolates. The remaining two Korean strains (W256 and E14) each formed a different branch (Figure 2). We also detected several potential recombinant regions in the 16S RNA sequence of *A. viridans* isolates in this study (Table S1).

4 | DISCUSSION

Viruses have been considered the main causes of swine abortion worldwide (Balasuriya & Carossino, 2017; Mak et al., 2018), but reports of bacterial abortion are scarce. This study was performed by collecting aborted fetuses in which the abortions were not caused by viral infection. Reproductive problems caused by bacteria are normally related to *Brucella suis* and *Leptospira* spp. (Vannier, 1999). However, in our study, *A. viridans* was highly prevalent, followed by *Enterococcus faecalis* and *S. simulans* (Table 1). A previous study classified these viruses into group I, which included facultative pathogens (Vannier, 1999). This study also suggested that, despite few reports focusing on opportunistic bacterial pathogens, the roles of this group in reproductive problems in pigs may be more common than is generally realized. For example, while *Erysipelothrix* spp. appeared to be quite common in healthy pigs, this genus still caused acute bacterial disease and induced reproductive diseases in sows (Opriessnig et al., 2020). Applying a vaccine strategy against this virus resulted in increased total born and live-born litter sizes in a Hungarian breeding unit (Hoffmann & Bilkei, 2002). Similarly, *Staphylococcus* spp. were isolated from the internal organs and fluids of aborted swine fetuses, suggesting its potential role in abortion in sows (Onet & Pommer, 1991; Salogni et al., 2016). Considering the absence of reproduction-related viral infection, isolated opportunistic pathogens were suggested to be the causative agents of swine reproductive disorders in the investigated farms.

In our study, the two most common bacteria detected in fetuses were *A. viridans* and *Enterococcus faecalis*. These species are commonly found in the urinary tracts of animals and may cause urinary/genital tract infection (Colombo et al., 2021; Ezechukwu et al., 2019; Strateva et al., 2016). However, the roles of these species in reproductive disorders are still under debate. Several species belonging to the *Aerococcus* genus were isolated in fetal organs after abortion (El-Arabi et al., 2008; Twomey et al., 2008; Wolf-Jäckel et al., 2020). Focusing on *A. viridans*, this species has been reported to be associated with infection in humans (Ezechukwu et al., 2019; Nathavitharana et al., 1983; Taylor & Trueblood, 1985; Zhou et al., 2013) and animals (Devriese et al., 1999; Martín et al., 2007). A previous study indicated that this species was only isolated in the

TABLE 2 Nine antimicrobial agents tested against 13 *Aerococcus viridans*-positive isolates using the disk diffusion method

Antimicrobials	IZD[+] breakpoints	*A. viridans*-positive strains (in this study)											Resistant strains (%)
		W254	W255	W256	W257	W258	E3	E8	E9	E10	E11	E12	E13	E14	
Lincomycin/Spectinomycin (109 μg)	≤14	+(0)	−(16)	+(0)	+(13)	+(11)	+(0)	+(0)	+(0)	+(0)	+(0)	−(23)	+(0)	+(0)	84.6
Ceftiofur (30 μg)	≤17	−(29)	−(27)	−(29)	+(17)	+(16)	+(0)	+(0)	−(25)	+(20)	+(16)	−(25)	−(27)	-(25)	46.2
Gentamicin (10 μg)	≤12	+(0)	+(0)	+(0)	+(0)	-(13)	+(0)	+(0)	-(18)	-(16)	+(0)	-(20)	-(20)	-(13)	53.8
Oxytetracycline (30 μg)	≤15	+(13)	+(10)	+(12)	-(16)	-(26)	-(25)	+(0)	-(18)	+(0)	+(12)	+(0)	-(18)	+(11)	61.5
Penicillin G (10 U)	≤14	-(25)	-(26)	-(25)	-(20)	-(21)	+(13)	+(0)	-(25)	+(0)	+(0)	+(0)	-(25)	+(0)	46.2
Ampicillin (10 μg)	≤16	+(15)	+(15)	+(14)	-(20)	+(14)	+(10)	+(0)	-(24)	+(0)	+(9)	+(0)	-(24)	+(0)	76.9
Trimethoprim/sulfamethoxazole (25 μg)	≤12	-(27)	-(25)	-(25)	+(9)	-(13)	-(17)	+(0)	-(15)	-(22)	-(16)	-(20)	-(15)	-(20)	15.4
Amoxicillin/Clavulanic acid (20/10 μg)	≤13	+(0)	+(0)	+(0)	+(0)	+(0)	+(0)	+(0)	-(23)	+(0)	+(0)	+(0)	-(25)	+(0)	84.6
Tiamulin (30 μg)	≤16	+(0)	+(10)	+(0)	+(9)	+(14)	+(10)	+(0)	+(15)	+(13)	+(9)	+(14)	-(19)	+(16)	92.3
Resistant/Total		6/9	5/9	6/9	6/9	5/9	7/9	9/9	2/9	7/9	8/9	5/9	1/9	6/9	

[+]Inhibition zone diameter (IZD) breakpoints.

FIGURE 2 The phylogeny classification of *Aerococcus viridans*. Selected maximum likelihood trees of 16S RNA gene with bootstrap 1,000, automatically best fitting model selected by IQ-TREE. In this figure, strains ($n = 13$) are highlighted with red color; the W254, W255, W256, W257, W258, E3, E8, E9, E10, E11, E12, E13 and E14 strains and the posterior supported values are represented in the branch labels

herds with PRRSV (Martín et al., 2007). However, our study showed contrasting results that indicated that this species could be isolated in the clinical specimens without PRRSV infection. Nevertheless, our study showed high genetic diversity of *A. viridans* isolates (Figure 2), in agreement with the findings of previous studies (Martín et al., 2007; Nathavitharana et al., 1983). Environmental factors may have played a significant role in the divergence among hosts. The appearance in high frequency of this species in fetal organs might suggest the potential role of this species in swine reproductive diseases.

Our study has a number of limitations. First, this study did not analyse the relationship between symptoms of the sows and detection of abnormalities in fetal tissues. Additionally, environmental samples and cords from healthy piglets from the same farm were not collected. Thus, the possibility of *A. viridans* causing abortion in swine still needs to be verified. Abortions caused by environmental bacteria are normally affected by environment influences and divergent among hosts. The lack of data about the presence of

microbiological ecosystems in herds could limit the effects of these bacteria involved in abortion cases. Therefore, further case–control studies are needed to demonstrate the effect of this species in swine diseases.

In conclusion, *A. viridans* might be a potential pathogen related to abortion in pigs, but further studies focusing on its epidemiology and challenge experiments in sows must be pursued, and antimicrobial susceptibility testing of *A. viridans* in domestic pigs of all ages should be performed.

ACKNOWLEDGEMENTS

The authors would like to thank Eun Ok Kim and Jung Ah Kim for their excellent technical assistance.

CONFLICT OF INTEREST

The authors declare there is no conflict of interest.

AUTHOR CONTRIBUTION

Van Giap Nguyen: Data curation. **Cheong Ung Kim:** Conceptualization; Writing-original draft. **Quynh Do Hai:** Data curation; Writing-review & editing. **Sook Shin:** Data curation; Formal analysis. **Keum Chan Jang:** Formal analysis. **Yong Ho Park:** Conceptualization. **Bong-Kyun Park:** Conceptualization. **Hee Chun Chung:** Conceptualization; Supervision; Writing-original draft; Writing-review & editing.

PEER REVIEW

The peer review history for this article is available at https://publons.com/publon/10.1002/vms3.456.

ORCID

Van Giap Nguyen https://orcid.org/0000-0001-5250-1825
Cheong Ung Kim https://orcid.org/0000-0002-8245-3315
Hai-Quynh Do https://orcid.org/0000-0001-7950-2174
Sook Shin https://orcid.org/0000-0003-3577-9651
Keum Chan Jang https://orcid.org/0000-0003-0091-5502
Yong Ho Park https://orcid.org/0000-0002-4168-6884
Bong Kyun Park https://orcid.org/0000-0002-4301-8740
Hee Chun Chung https://orcid.org/0000-0003-4666-5393

REFERENCES

Balasuriya, U. B., & Carossino, M. (2017). Reproductive effects of arteriviruses: Equine arteritis virus and porcine reproductive and respiratory syndrome virus infections. *Current Opinion in Virology*, *27*, 57–70.

Colombo, S. A., Silva, B. A., Gómez, S. Y. M., Santos, R. D. L., Tinoco, H. P., Coelho, C. M., Teixera, J. P., & Leal, C. A. G. (2021). Genital infection by *Aerococcus viridans* in a captive african elephant (*Loxodonta africana*). *Ciência Rural*, *51*(1). https://doi.org/10.1590/0103-8478cr20200513

Devriese, L., Vandamme, P., Collins, M., Alvarez, N., Pot, B., Hommez, J., Butaye, P., & Haesebrouck, F. (1999). Streptococcus pluranimalium sp. nov., from cattle and other animals. *International Journal of Systematic and Evolutionary Microbiology*, *49*, 1221–1226.

El-Arabi, A., Taylor, D., Fishwick, G., & Logue, D. (2008). Aerococcus urinae in ovine reproductive disease. *The Veterinary Record*, *163*, 607.

Ezechukwu, I., Singal, M., & Igbinosa, O. (2019). *Aerococcus viridans*: Case report, microbiology, and literature review. *The American Journal of Case Reports*, *20*, 697–700.

Givens, M. D., & Marley, M. S. D. (2008). Infectious causes of embryonic and fetal mortality. *Theriogenology*, *70*, 270–285.

Hijazin, M., Alber, J., Lämmler, C., Weitzel, T., Hassan, A. A., Timke, M., Kostrzewa, M., Prenger-Berninghoff, E., & Zschöck, M. (2012). Identification of Trueperella (Arcanobacterium) bernardiae by matrix-assisted laser desorption/ionization time-of-flight mass spectrometry analysis and by species-specific PCR. *Journal of Medical Microbiology*, 457–459.

Hoang, D. T., Chernomor, O., von Haeseler, A., Minh, B. Q., & Vinh, L. S. (2018). UFBoot2: Improving the ultrafast bootstrap approximation. *Molecular Biology and Evolution*, *35*, 518–522.

Hoffmann, C. W., & Bilkei, G. (2002). Case study: Chronic erysipelas of the sow – A subclinical manifestation of reproductive problems. *Reproduction in Domestic Animals = Zuchthygiene*, *37*, 119–120.

Koenen, F., & Vanderhallen, H. (1997). Comparative study of the pathogenic properties of a Belgian and a Greek encephalomyocarditis virus (EMCV) isolate for sows in gestation. *Journal of Veterinary Medicine, Series B*, *44*, 281–286.

Kozitskaya, S., Olson, M. E., Fey, P. D., Witte, W., Ohlsen, K., & Ziebuhr, W. (2005). Clonal analysis of Staphylococcus epidermidis isolates carrying or lacking biofilm-mediating genes by multilocus sequence typing. *Journal of Clinical Microbiology*, *43*, 4751–4757.

Lalitha, M. K. (2004). Manual on antimicrobial susceptibility testing. *Performance Standards for Antimicrobial Testing: Twelfth Informational Supplement*, *56238*, 454–456.

Mak, C. K., Yang, C., Jeng, C.-R., Pang, V. F., & Yeh, K.-S. (2018). Reproductive failure associated with coinfection of porcine circovirus type 2 and porcine reproductive and respiratory syndrome virus. *Canadian Veterinary Journal*, *59*, 525–530.

Martin, D., & Rybicki, E. (2000). RDP: Detection of recombination amongst aligned sequences. *Bioinformatics*, *16*, 562–563.

Martín, V., Vela, A. I., Gilbert, M., Cebolla, J., Goyache, J., Domínguez, L., & Fernández-Garayzábal, J. F. (2007). Characterization of *Aerococcus viridans* isolates from swine clinical specimens. *Journal of Clinical Microbiology*, *45*, 3053–3057.

Nathavitharana, K. A., Arseculeratne, S. N., Aponso, H. A., Vijeratnam, R., Jayasena, L., & Navaratnam, C. (1983). Acute meningitis in early childhood caused by *Aerococcus viridans*. *British Medical Journal*, *286*, 1248.

Nguyen, L.-T., Schmidt, H. A., von Haeseler, A., & Minh, B. Q. (2015). IQ-TREE: A fast and effective stochastic algorithm for estimating maximum-likelihood phylogenies. *Molecular Biology and Evolution*, *32*, 268–274.

Nguyen, V. G., Chung, H. C., Huynh, T. M. L., Cao, T. B. P., Vu, T. N., Le, V. T., & Pham, H. Q. (2018). Molecular Characterization of Novel Porcine Circovirus 3 (PCV3) in Pig Populations in the North of Vietnam. *Archives of Gene and Genome Research*, *1*.

Oh, W., Nguyen, V.-G., Moon, H. J., Lee, J., Kim, H., Park, S., Chung, H.-C., & Park, B.-K. (2017). Investigation on infectious agents of aborted pig fetuses and its correlation with PRRSV MLV vaccine. *Journal of Agricultural Science & Technology A*, *7*.

Onet, G. E., & Pommer, J. L. (1991). Staphylococcus hyicus abortion in a sow. *Journal of the American Veterinary Medical Association*, *199*, 362–363.

Opriessnig, T., Forde, T., & Shimoji, Y. (2020). Erysipelothrix spp.: Past, present, and future directions in vaccine research. *Frontiers in Veterinary Science*, *7*, 174.

Pan, Z., Ma, Y., Ma, J., Dong, W., & Yao, H. (2017). Acute meningitis of piglets and mice caused by co-infected with *Streptococcus suis* and *Aerococcus viridans*. *Microbial Pathogenesis*, *106*, 60–64.

Saishu, N., Morimoto, K., Yamasato, H., Ozaki, H., & Murase, T. (2015). Characterization of *Aerococcus viridans* isolated from milk samples

from cows with mastitis and manure samples. *Journal of Veterinary Medical Science*, *77*, 1037–1042.

Salogni, C., Lazzaro, M., Giacomini, E., Giovannini, S., Zanoni, M., Giuliani, M., Ruggeri, J., Pozzi, P., Pasquali, P., Boniotti, M. B., & Alborali, G. L. (2016). Infectious agents identified in aborted swine fetuses in a high-density breeding area: A three-year study. *Journal of Veterinary Diagnostic Investigation*, *28*, 550–554.

Shryock, T. R., & National Committee for Clinical Laboratory Standards. (2002). Performance standards for antimicrobial disk and dilution susceptibility tests for bacteria isolated from animals. Approved standard M31-A2 (2nd ed). National Committee for Clinical Laboratory Standards, Wayne, PA.

Standards., N. C. f. C. L. (2002). Performance standards for antimicrobial susceptibility testing. 12th informational supplement. Approved standard M100-S12. National Committee for Clinical Laboratory Standards Wayne.

Strateva, T., Atanasova, D., Savov, E., Petrova, G., & Mitov, I. (2016). Incidence of virulence determinants in clinical *Enterococcus faecalis* and *Enterococcus faecium* isolates collected in Bulgaria. *Brazilian Journal of Infectious Diseases*, *20*, 127–133.

Taylor, P. W., & Trueblood, M. C. (1985). *Septic arthritis* due to *Aerococcus viridans*. *The Journal of Rheumatology*, *12*, 1004–1005.

Twomey, D., Wessels, M., Hogg, R., Edwards, G., Willmington, J., Anscombe, J., Mitchell, E., Parmar, D., & Holliman, A. (2008). Aerococcus urinae in bovine reproductive disease. *Veterinary Record*, *163*, 492.

Vannier, P. (1999). Infectious causes of abortion in swine. *Reproduction in Domestic Animals*, *34*, 367–376.

Watts, J. L., & National Committee for Clinical Laboratory Standards. (1999). Performance standards for antimicrobial disk and dilution susceptibility test for bacteria isolated from animals: approved standard. NCCLS documents M31-A, National Committee for Clinical Laboratory Standards, Wayne, PA.

Wolf-Jäckel, G. A., Hansen, M. S., Larsen, G., Holm, E., Agerholm, J. S., & Jensen, T. K. (2020). Diagnostic studies of abortion in Danish cattle 2015–2017. *Acta Veterinaria Scandinavica*, *62*, 1.

Zhang, M., Xie, Z., Xie, L., Deng, X., Xie, Z., Luo, S., Liu, J., Pang, Y., & Khan, M. I. (2015). Simultaneous detection of eight swine reproductive and respiratory pathogens using a novel GeXP analyser-based multiplex PCR assay. *Journal of Virological Methods*, *224*, 9–15.

Zhou, W., Nanci, V., Jean, A., Salehi, A. H., Altuwaijri, F., Cecere, R., & Genest, J. (2013). *Aerococcus viridans* native valve endocarditis. *The Canadian Journal of Infectious Diseases & Medical Microbiology*, *24*, 155–158.

SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

How to cite this article: Nguyen VG, Kim CU, Do H-Q, et al. Characteristics of *Aerococcus viridans* isolated from porcine fetuses in Korean farms. *Vet Med Sci*. 2021;7:1325–1331. https://doi.org/10.1002/vms3.456