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Characterization of *Fusobacterium* isolates from the respiratory tract of white-tailed deer (*Odocoileus virginianus*)

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Abstract. A total of 23 clinical isolates of *Fusobacterium* spp. were recovered at necropsy over a 2-year period from the respiratory tract of white-tailed deer (*Odocoileus virginianus*). Isolates were identified as *Fusobacterium varium* (18/23), *Fusobacterium necrophorum* subsp. *funduliforme* (3/23), and *Fusobacterium necrophorum* subsp. *necrophorum* (2/23). Using polymerase chain reaction–based detection of virulence genes, all *F. necrophorum* isolates were positive for the promoter region of the leukotoxin operon and the hemagglutinin-related protein gene, while all *F. varium* isolates were negative. The presence of the leukotoxin gene in *F. necrophorum* isolates and the absence of this gene in *F. varium* isolates were confirmed by Southern hybridization using 2 separate probes. Toxicity to bovine polymorphonuclear leukocytes was observed with all *F. necrophorum* isolates, but was not observed in any *F. varium* isolates. Susceptibility to antimicrobials was markedly different for *F. varium* as compared to *F. necrophorum*. In summary, no evidence of leukotoxin production was detected in any of the 23 *F. varium* isolates used in the current study. The data suggests that *F. varium*, the most common species isolated, may be a significant pathogen in deer with a different virulence mechanism than *F. necrophorum*.

Key words: *Fusobacterium necrophorum*; *Fusobacterium varium*; leukotoxin; white-tailed deer.

Introduction

Fusobacterium spp. are Gram-negative, non-spore-forming, anaerobic, rod-shaped bacteria of the *Bacteroidaceae* family. *Fusobacterium necrophorum*, which is currently recognized as 2 subspecies, *F. n.* subsp. *necrophorum* and *F. n.* subsp. *funduliforme*, is one of the most common anaerobic bacteria isolated from abscesses, respiratory tract infections, and other necrotizing infections in domestic livestock, wild mammals, and human beings.^{6,15,23} While the role of *F. necrophorum* in hepatic abscessation, necrotizing laryngitis, and foot rot in cattle, small ruminants, and camelids is well recognized, the association with severe respiratory tract infections in deer is infrequently recognized in the literature and the pathogenesis is poorly characterized.^{3,21} Although less extensively investigated, *Fusobacterium varium* has been associated with ulcerative colitis, colonic neoplasia, decubitus ulcers, and respiratory tract infections in human beings, and with suppurative or ulcerative lesions of the gastrointestinal tract or oral cavity in animals.^{4,11,19} Various *Fusobacterium* spp. have been isolated from wild ruminants affected by necrobacillosis, a common disease of farm raised deer characterized by purulent necrotic lesions most commonly affecting the mouth, pharynx, lung, liver, or feet.^{7,21} Some of the most frequently isolated pathogens in necrobacillosis cases are *F. necrophorum* and *F. varium*, with coinfections commonly associated with *Trueperella (Arcanobacterium) pyogenes*.^{4,8} The infection often spreads to multiple organs resulting in death, as antibiotic therapy is often not successful.¹² The objective of the current study was to phenotypically and genotypically characterize clinical isolates of *Fusobacterium* spp. from the respiratory tract of white-tailed deer (*Odocoileus virginianus*).

Materials and methods

Collection and identification of isolates

Twenty-three clinical isolates of *Fusobacterium* spp. were used in the current study. All isolates were obtained at necropsy at the Animal Diagnostic Laboratory at The Pennsylvania State University (University Park, Pennsylvania) from the respiratory tract (lungs or larynx) of white-tailed deer from Pennsylvania. The selected isolates were presumptively identified as *Fusobacterium* sp. and further analyzed as described below (Table 1). Additionally, 3 reference strains, *F. varium* (American Type Culture Collection [ATCC] 8501), *F. n.* subsp. *necrophorum* (ATCC 25286), and *F. n.* subsp. *funduliforme* (ATCC 51357), were included in the study.^a Tissue samples were cultured on prerduced *Brucella* agar plates^b supplemented with 5% sheep blood, hemin (5mg/l), and vitamin K (10 mg/l), and then incubated at 37°C for 48 hr in an anaerobic chamber. The 3-way antibiotic disk diffusion susceptibility test and nitrate test were conducted on *Brucella* agar plates with vancomycin, kanamycin, colistin, and nitrate disks^c Nitrate-negative isolates showing resistance to vancomycin and susceptibility to kanamycin and colistin were presumptively determined to be *Fusobacterium* sp.⁹ Following presumptive identification, the indole test was performed by placing 1 drop of indole reagentc onto an antibiotic disk. A pre-reduced McClung–Toabe egg yolk agar plateb was inoculated and incubated anaerobically at 37°C for 48–72 hr to determine lipase activity. Bile tolerance was determined by plating colonies onto pre-reduced *Bacteroides fragilis* isolation agar platesb containing 20% bile and incubating anaerobically at 37°C for 48–72 hr. Overnight cultures in prerduced anaerobically sterilized brain–heart infusion broth were observed for growth and sedimentation. All isolates were analyzed with commercial kits^{d,e} according to the manufacturers’ instructions.

DNA extraction and 16S ribosomal

DNA sequencing

Chromosomal DNA was extracted from all isolates. Briefly, cultures were grown anaerobically on pre-reduced blood agar plates at 37°C for 48–72 hr. The DNA was isolated using a commercial kit^f according to the manufacturer's instructions. The extracts were stored at –20°C.

Universal primers (p515FPL forward

GCGGATCCTCTAGACTGCAGTGCCAGCAGCCGCGGTAA, and p13B reverse

CGGGATCCCAGGCCCGGGAACGTATTCAC) were used to amplify a region of the 16S

ribosomal RNA gene by polymerase chain reaction (PCR).²² Amplified products were

electrophoresed in 1.5% agarose gels and purified using a commercial kit^g The purified PCR

product was submitted to a commercial laboratory^h for sequence analysis. For each isolate,

sequences with >99% agreement were calculated using commercially available softwareⁱ and

selected for further analysis. Sequence alignment and phylogenetic analysis were conducted

using Web-based software^j and the neighbor-joining method.²⁴ Partial 16S ribosomal DNA

(rDNA) sequences of all clinical isolates from the present study were deposited in GenBank and

assigned accession numbers KC407916 through KC407943.

Lkt promoter and hemagglutinin gene PCR

Primers specific for the promoter region of the *lktB* gene and the hemagglutinin-related protein

gene were used. The “lktpXmXh” primers detect the *lkt* promoter in both subspecies, whereas

the “fund” primers detect the *lkt* promoter of only the *funduliforme* subspecies.²⁷ The “haem”

primer detects the gene that codes for hemagglutinin-related protein.¹ Primers were as follows:

lktpXmXh forward 5'-TCTCCCGGGCTCGAGGAAATCTTTAAAGCAC-3', lktpXmXh

reverse 5'-TCTCCCGGGCATAATTTCTCCCAATTTTATT-3', fund forward 5'-
CTCAATTTTTGTTGGAAGCGAG-3', fund reverse 5'-
CATTATCAAATAACATATTTCTCAC-3', haem forward 5'-
CATTGGGTTGGATAACGACTCCTAC-3', and haem reverse 5'-
CAATTCTTTGTCTAAGATGGAAGCGG-3'.^{1,27} Amplification was performed in a thermal
cycler using a modification of previously described conditions.²⁷ The PCR conditions were as
follows: initial denaturation at 94°C for 3 min, followed by 35 cycles of denaturation at 94°C for
1min, annealing at 45°C for 30 sec, and 62.4°C for 30 sec, extension at 72°C for 1 min, and the
final extension at 72°C for 4 min. The annealing temperature was reduced from 62.4°C for
lktpXmXh primers to 55.1°C for haem primers and 50.8°C for fund primers. The amplified
products were electrophoresed in a 1.5% agarose gel containing ethidium bromide.

Leukotoxin gene detection by Southern hybridization

Southern blot hybridization was performed as previously described with modifications.¹⁷ Briefly,
chromosomal DNA was isolated using a commercial kitk as per the manufacturer's instructions.
Chromosomal DNA (1.5 µg) was digested to completion using *Hae*III and electrophoresed in a
1% agarose gel overnight. The resolved DNA fragments were transferred to a positively charged
nylon membrane and immobilized by baking. Two DNA probes were prepared using a PCR-
based digoxigenin (DIG)-labeled probe synthesis kit¹ following the manufacturer's instructions.
One probe was 1,200 base pairs (bp) and corresponded to a conserved *lktA* gene region of *F.*
necrophorum. The second probe was 571 bp and corresponded to the promoter region of the *lktA*
gene of *F. necrophorum*. The immobilized DNA was probed at 43.6°C for the 1,200-bp probe,
and 40°C for the 571-bp probe. Colorimetric detection of hybridized signals was accomplished
following the manufacturer's instructions.

Leukotoxicity assay by flow cytometry

Leukotoxicity was determined by incubation of bovine polymorphonuclear leukocytes (PMNs) with sterile culture supernatant and propidium iodide (PI) as previously described.¹⁶

Fusobacterium isolates were grown in prereduced anaerobically sterilized brain–heart infusion at 37°C to an absorbance of 0.7–0.8 at 600 nm, placed on ice, and pelleted by centrifugation at 10,000 × *g* for 10 min at 4°C as previously described.²⁵ Supernatants were filter sterilized with 0.22-μm membrane filters and stored at –80°C. Bovine peripheral PMNs were prepared as previously described.²⁵ Purified leukocytes were washed twice and resuspended in 0.01 M phosphate buffered saline. Viable cell concentration was determined with a hemocytometer by the trypan blue dye exclusion method.²⁵ Approximately 2 × 10⁶ PMNs were placed into sterile 5-ml polystyrene culture tubes and incubated with an equal volume of filter-sterilized culture supernatant. Cells were incubated for 45 min at 37°C and 5% CO₂ washed twice, and resuspended in phosphate buffered saline. Five microliters of 1 mg/ml PI was added to each tube and the tubes incubated at room temperature for 5–10 min in the dark. Samples were analyzed on a flow cytometer.^m The proportion of cells stained with PI was determined by the detection of red fluorescence of cells in the PMN gate.

Antimicrobial susceptibility determination

The broth microdilution method of antimicrobial susceptibility was performed by a commercial system according to the manufacturer's instructions. Colonies were selected from 48-hr cultures on blood agar plates, inoculated into Mueller–Hinton broth tubesⁿ and transferred into supplemented *Brucella* broth tubesⁿ adjusted to 1 × 10⁶ colony forming units/ml. Aliquots (100 μl) were transferred to wells of a 96-well antimicrobial susceptibility plate.^o Plates were covered

by perforated adhesive seals, incubated anaerobically at 37°C for 48 hr, and the minimal concentration of antimicrobial agents that inhibited bacterial growth was determined by visual examination. Breakpoints were interpreted by Clinical and Laboratory Standards Institute (CLSI) guidelines.²⁶ Because no CLSI guidelines are available for *Fusobacterium* spp., the CLSI breakpoints for other veterinary pathogens were used (Table 2). Specifically, minimal inhibitory concentration (MIC) breakpoints for bovine respiratory disease pathogens were used when available, otherwise MIC breakpoints for swine respiratory pathogens, *Enterobacteriaceae*, or undefined veterinary pathogens were used.²⁶ No CLSI approved MIC breakpoints were available for neomycin or tylosin, therefore breakpoints were obtained from the product data sheet provided by the manufacturer for Gram-negative bacteria.

Statistical analysis

Flow cytometry data was evaluated by one-way analysis of variance with comparisons for each pair using the Student *t*-test. Standard statistical software^p was used. For all analyses, a value of $P < 0.05$ was considered significant.

Results

Species identification of isolates

A single *Fusobacterium* sp. was isolated from each animal included in the study. The isolates included 18 (78.3%) *F. varium*, 3 (13.0%) *F. n.* subsp. *funduliforme*, and 2 (8.7%) *F. n.* subsp. *necrophorum*. Results are summarized in Table 1. Determination of species or subspecies was made by standard phenotypic methods and analysis of biochemical characteristics through the use of 2 commercial kits with confirmation by 16S rDNA sequence analysis. The kits performed

similarly in the identification of *F. n. subsp. necrophorum*; however, *F. n. subsp. funduliforme* was incorrectly identified as *F. nucleatum* by kit 2^c and some isolates of *F. varium* were incorrectly identified as *F. nucleatum* by kit 1.^d

The sequences of the 5 *F. necrophorum* isolates clustered together and the 2 subspecies were not distinguishable by 16S rDNA sequences alone (Fig. 1). All *F. necrophorum* isolates also clustered apart from the previously reported *F. equinum* sequences, thus excluding the potential misidentification of *F. necrophorum* as *F. equinum*.²⁸ Similarly, all *F. varium* isolates clustered apart from *F. necrophorum*. Previously deposited sequences for *F. nucleatum* and *F. equinum* were distinct from the clinical isolates evaluated in the current study.

Virulence gene analysis

Using the *lktp* primers for the promoter region of *lktB*, all *F. varium* isolates were negative for the *lkt* promoter. Using these same primers, all *F. n. subsp. necrophorum* isolates amplified the expected product of approximately 571 bp, while all *F. n. subsp. funduliforme* isolates amplified a slightly smaller product of approximately 449 bp as expected. With the fund primers specific for the *lkt* promoter region of *F. n. subsp. funduliforme*, all *F. varium* isolates were negative. With these same primers, all *F. n. subsp. necrophorum* were negative and 1 out of 3 (33%) *F. n. subsp. funduliforme* isolates amplified the expected 337-bp product. Using the haem primers for the hemagglutinin-related protein gene, all *F. varium* isolates were negative. With the haem primers, all *F. n. subsp. necrophorum* strains amplified the expected 311-bp product, while all *F. n. subsp. funduliforme* isolates were negative. Using 2 different probes for the detection of the *lktA* gene and the leukotoxin promoter, it was determined by Southern hybridization that the leukotoxin gene was absent from all isolates of *F. varium* and present in all isolates of *F.*

necrophorum. When probed with the 1,200-bp DIG-labeled *F. n. subsp. necrophorum lktA*, isolates of *F. n. subsp. necrophorum* produced a hybridizing band (approximately 23 kb), and *F. n. subsp. funduliforme* produced a hybridizing band of a slightly larger size (Fig. 2A). When probed with the 571-bp DIG-labeled *F. n. subsp. necrophorum lkt* promoter, isolates of *F. n. subsp. necrophorum* produced a hybridizing band (approximately 23 kb), and *F. n. subsp. funduliforme* isolates showed no hybridization (Fig. 2B). No *F. varium* isolates produced a hybridizing band with either probe, indicating the absence of the leukotoxin gene.

Leukotoxin activity by flow cytometry

The toxicity of culture supernatant to bovine PMNs, as determined by PI staining, was highly dependent on fusobacterial species (Fig. 3). The uptake of PI by PMNs was highest in cells incubated with supernatant of *F. n. subsp. necrophorum* (mean: 67.0%; standard error [SE]: 11.5), intermediate with *F. n. subsp. funduliforme* (mean: 18.9%; SE: 9.1), and very low for *F. varium* (mean: 2.3%; SE: 0.1), which approximated the negative control (mean: 1.2%; SE: 0.4). Leukotoxicity, as determined by mean PI uptake, for *F. n. subsp. necrophorum* differed significantly from the means of *F. n. subsp. funduliforme* ($P < 0.0001$) and *F. varium* ($P < 0.0001$).

Leukotoxicity for *F. n. subsp. funduliforme* differed significantly from *F. n. subsp. necrophorum* ($P < 0.0001$) and from the mean of *F. varium* ($P < 0.002$). The means of *F. varium* and the negative control were not significantly different ($P > 0.05$).

Antimicrobial susceptibility

The results of the broth microdilution antimicrobial susceptibility tests are summarized in Table 2. While *F. n. subsp. necrophorum* and *F. n. subsp. funduliforme* showed similar patterns of susceptibility, the susceptibility of *F. varium* was markedly different for many antimicrobials.

All isolates (23/23) of all 3 fusobacterial species were susceptible to ampicillin, florfenicol, and trimethoprim–sulfamethoxazole. Fewer *F. varium* isolates were susceptible to chlortetracycline, clindamycin, oxytetracycline, tiamulin, tilmicosin, tulathromycin, and tylosin as compared with *F. necrophorum*. Antimicrobials to which isolates demonstrated intermediate or variable susceptibilities included ceftiofur, danofloxacin, neomycin, penicillin, spectinomycin, and sulfadimethoxine. Nearly all isolates (22/23) were resistant to enrofloxacin and gentamycin.

Discussion

Respiratory tract infection is one of the primary causes of mortality in farm-raised white-tailed deer.^{3,8} The definitive cause of such infections remains undetermined, but one of the most commonly isolated pathogens is *Fusobacterium*.^{4,8} It is important for the deer farming industry and the animal health community that the etiology and pathogenesis of this disease are more fully characterized in order to develop appropriate treatment and prevention measures. The current study shows that *F. varium* comprises a large proportion of the clinical isolates from cases of fusobacterial respiratory tract infections in deer. Standard phenotypic methods with the inclusion of lipase activity, bile tolerance, and sedimentation were sufficient for subspecies level identification of the clinical *Fusobacterium* isolates used in the current study. However, such methods are labor intensive and time consuming and may be insufficient for speciating or subtyping some *Fusobacterium* spp. isolates. Commercial bacterial identification kits were useful as part of a diagnostic scheme, but were inadequate as the sole means of identification of many *Fusobacterium* spp. to the species or subspecies level. Although *F. n.* subsp. *necrophorum* was consistently correctly identified at the species level with both test kits, *F. n.* subsp. *funduliforme* and *F. varium* were sometimes misidentified as *F. nucleatum*. Genotypic analysis by PCR or ribosomal gene sequencing may be preferable for diagnosis or confirmation.

Fusobacterium necrophorum possesses several virulence factors, and leukotoxin is considered to be the major virulence factor involved in animal infections.^{15,23} Virulence factors of *F. varium*, however, have not been well characterized. The *F. necrophorum* leukotoxin operon consists of 3 genes, *lktB*, *lktA*, and *lktC* corresponding respectively to an outer membrane protein with putative transporter function, the leukotoxin protein, and a protein of unknown function.²³ The *lkt* operon promoter region is distinct for each of the 2 subspecies of *F. necrophorum* and allows for differentiation of the 2 subspecies.²⁷ Amplification by PCR of the *lkt* promoter and the hemagglutinin gene differentiated *F. necrophorum* subspecies from other *Fusobacterium* spp.¹⁰ In the current study, the inconsistency in the band produced by *F. n.* subsp. *funduliforme* with fund primers may represent polymorphisms in the sequence of this virulence gene. No evidence of the leukotoxin gene or leukotoxin production by any isolate of *F. varium* was detected by PCR analysis, Southern blotting, or the leukocyte viability assay. Although it is fundamentally difficult to prove the absence of a gene,² the sum of these analyses collectively supports the conclusion that *F. varium* does not produce leukotoxin. It is, therefore, possible that the pathogenicity of *F. varium* is not associated with leukotoxin production but with some other virulence factor. One study suggests that the leukotoxin gene is much less prevalent in non-bovine, invasive animal and human strains and, thus, should not be considered the primary virulence factor in all hosts.¹³ For example, lysis of erythrocytes by hemolysin has been proposed as a major fusobacterial virulence factor.¹⁴ Additionally, butyric acid has been identified as a potential virulence factor in *F. varium* based on cytotoxicity to Vero (African green monkey kidney epithelial) cells and production of colonic lesions in mice.^{18,19} Butyric acid has been demonstrated to induce apoptosis of colonic epithelium and to induce secretion of

inflammatory cytokines.¹⁹ Under certain conditions, some commensal organisms such as *F. varium* may be capable of invading epithelial cells and activating early host inflammatory responses.¹⁹ Few studies on antimicrobial susceptibility of *F. varium* have been reported, although the susceptibility of other *Fusobacterium* species has been investigated. The results of the current study suggest that susceptibility to antimicrobials is markedly different among the *Fusobacterium* species evaluated, and are consistent with previous studies.^{5,11,20} It has been reported that most *Fusobacterium* spp. are sensitive to β -lactams and clindamycin, but that *F. varium* tends to be more resistant.¹¹ The data of the current study supports the increased resistance of *F. varium* to clindamycin as well as chlortetracycline, oxytetracycline, tiamulin, tilmicosin, tulathromycin, and tylosin in comparison to *F. necrophorum*. This finding is of clinical relevance to managers of captive deer herds and wild ruminant populations because traditional treatment and prevention efforts have been directed toward *F. necrophorum* infections, selecting antibiotics and formulating vaccines accordingly, as most fusobacterial infections in animals have been attributed to *F. necrophorum*. However, in the dataset used in the current study, the majority (78.3%) of clinical isolates were *F. varium*, which suggests that most cases of necrobacillosis may be caused by a different species of *Fusobacterium* with a different antibiotic resistance profile and, potentially, a different antigenic profile. Therefore, in order to properly diagnose and provide critical information for treatment and preventive measures, it is essential that clinical *Fusobacterium* isolates are identified to the species or subspecies level. Additional studies are required to determine virulence factors of *F. varium* and the pathogenicity and host immune response to *F. varium* infection in order to develop effective treatment and prevention strategies.

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Sources and manufacturers

- a. American Type Culture Collection, Manassas, VA.
- b. Remel Inc., Lenexa, KS.
- c. Anaerobe Systems, Morgan Hill, CA.
- d. Rapid ANAII, Remel Inc., Lenexa, KS.
- e. RapID 32A, BioMerieux SA, Marcy l'Etoile, France.
- f. DNeasy Blood and Tissue Kit, Qiagen GmbH, Hilden, Germany.
- g. QIAquick Gel Extraction Kit, Qiagen GmbH, Hilden, Germany.
- h. Davis Sequencing, Davis, CA.
- i. Vector NTI Advance 11.0 Align X, Invitrogen Corp., Carlsbad, CA.
- j. Mega version 4 by Clustal W, <http://www.megasoftware.net/>
- k. Wizard genomic DNA purification kit, Promega Corp., Madison, WI.
- l. Roche Diagnostics Corp., Indianapolis, IN.
- m. Cytomics FC500, Beckman Coulter, Fullerton, CA.
- n. Sensititre System, ThermoFisher Scientific; formerly TREK Diagnostics; Cleveland, OH.
- o. BOPO6F, ThermoFisher Scientific; formerly TREK Diagnostics; Cleveland, OH.
- p. JMP, SAS Institute Inc., Cary, NC.

Declaration of conflicting interests

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Tables

Table 1. Identification of *Fusobacterium* spp. isolates from white-tailed deer (*Odocoileus virginianus*).

Table 2. Antimicrobial susceptibility of *Fusobacterium* spp. from white-tailed deer (*Odocoileus virginianus*).

Figures

Fig. 1. Phylogenetic analysis (neighbor-joining method) of the 16S rRNA gene sequence of clinical *Fusobacterium* spp. isolates aligned with previously deposited *Fusobacterium* spp. sequences.

Fig. 2. Southern hybridization patterns of *Hae*III-digested genomic DNA probed with DIG-labeled *F. necrophorum lktA* gene conserved region (a), or *F. necrophorum lktA* promoter (b). Lane description: M, DIG-labeled marker; 1, *F. n.* subsp. *necrophorum* ATCC no. 25286; 2, *F. n.* subsp. *funduliforme* ATCC no. 51357; 3, *F. varium* ATCC no. 8501; 4-9, clinical *F. varium* isolates.

Fig. 3. Leukocyte viability assay. Bovine PMNs treated with culture supernatant were stained with PI and analyzed by flow cytometry. Control, no culture supernatant.

Table 1. Identification of *Fusobacterium* spp. isolates from white-tailed deer (*Odocoileus virginianus*). *

Kit 1 Result	Kit 2 Result	16S rRNA Result	Sed	Lip	Final ID	No. of isolates
<i>F. necrophorum</i> (99.8)	<i>F. necrophorum</i> (70.7)	<i>F. necrophorum</i>	-	+	<i>F.n. necrophorum</i>	2
<i>F. necrophorum</i> (60)	<i>F. nucleatum</i> (62)	<i>F. necrophorum</i>	+	+	<i>F.n. funduliforme</i>	3
<i>F. varium</i> (>99.9)	<i>F. varium</i> (11.3)	<i>F. varium</i>	-	-	<i>F. varium</i>	9
<i>F. nucleatum</i> (>99.9)	<i>F. varium</i> (92.5)	<i>F. varium</i>	-	-	<i>F. varium</i>	4
<i>F. varium</i> (98.9)	<i>F. varium</i> (99.9)	<i>F. varium</i>	-	-	<i>F. varium</i>	2
<i>F. varium</i> (>99.9)	<i>F. varium</i> (99.9)	<i>F. varium</i>	-	-	<i>F. varium</i>	2
<i>F. nucleatum</i> (99.4)	<i>F. varium</i> (92.5)	<i>F. varium</i>	-	-	<i>F. varium</i>	1

* Kit 1 (Rapid ANAII, Remel Inc., Lenexa, KS) and kit 2 (RapID 32A, bioMerieux SA, Marcy l'Etoile, France). ID = identification; rDNA = ribosomal DNA; Sed = sedimentation in broth; Lip = lipase activity; Bile = bile tolerance. Numbers in parentheses are quality of ID percentage. † Highest quality ID (72%) for *Clostridium haemolyticum* was inconsistent with negative Gram staining. Result shown is second highest quality ID.

Table 2. Antimicrobial susceptibility of *Fusobacterium* spp. from white-tailed deer (*Odocoileus virginianus*).*

Antimicrobial	<i>F. n. necrophorum</i> (n = 2)			<i>F. n. funduliforme</i> (n = 3)			<i>F. varium</i> (n = 18)			MIC breakpoints (µg/ml)		
	No. sus	No. int	No. res	No. sus	No. int	No. res	No. sus	No. int	No. res	Sus	Int	Res
Ampicillin	2	0	0	3	0	0	18	0	0	≤8	16	≥32
Ceftiofur	1	0	1	3	0	0	5	6	7	≤2	4	≥8
Chlortetracycline	2	0	0	3	0	0	10	1	7	≤2	4	≥8
Clindamycin	2	0	0	3	0	0	0	4	14	≤0.5	1–2	≥4
Danofloxacin	0	2	0	0	3	0	0	18	0	≤0.25		
Enrofloxacin	0	1	1	0	0	3	0	0	18	≤0.25	.5–1	≥2
Florfenicol	2	0	0	3	0	0	18	0	0	≤2	4	≥8
Gentamicin	0	0	2	0	1	2	0	0	18	≤4	8	≥16
Neomycin	0	2	0	0	3	0	0	18	0	≤8		
Oxytetracycline	2	0	0	3	0	0	1	5	12	≤2	4	≥8
Penicillin	2	0	0	3	0	0	0	18	0	≤0.12	.25–2	≥4
Spectinomycin	2	0	0	3	0	0	1	17	0	≤32	64	≥128
Sulfadimethoxine	1	0	1	3	0	0	17	0	1	≤256		≥512
Tiamulin	2	0	0	3	0	0	0	0	18	≤16		≥32
Tilmicosin	2	0	0	3	0	0	0	5	13	≤8	16	≥32
Trimethoprim–sulfamethoxazole	2	0	0	3	0	0	18	0	0	≤2		≥4
Tulathromycin	2	0	0	3	0	0	1	3	14	≤16	32	≥64
Tylosin	2	0	0	3	0	0	0	0	18	≤5		≥10

* *F. n. necrophorum* = *Fusobacterium necrophorum* subsp. *necrophorum*; *F. n. funduliforme* = *Fusobacterium necrophorum* subsp. *funduliforme*; MIC = minimal inhibitory concentration; Sus = susceptible; Int = intermediate; Res = resistant. MIC breakpoints were derived from Clinical Laboratory Standards Institute guidelines²⁶ for other veterinary aerobic pathogens except for neomycin and tylosin (for these 2 agents the product technical insert guidelines for Gram-negative bacteria were used).

Figure 1.

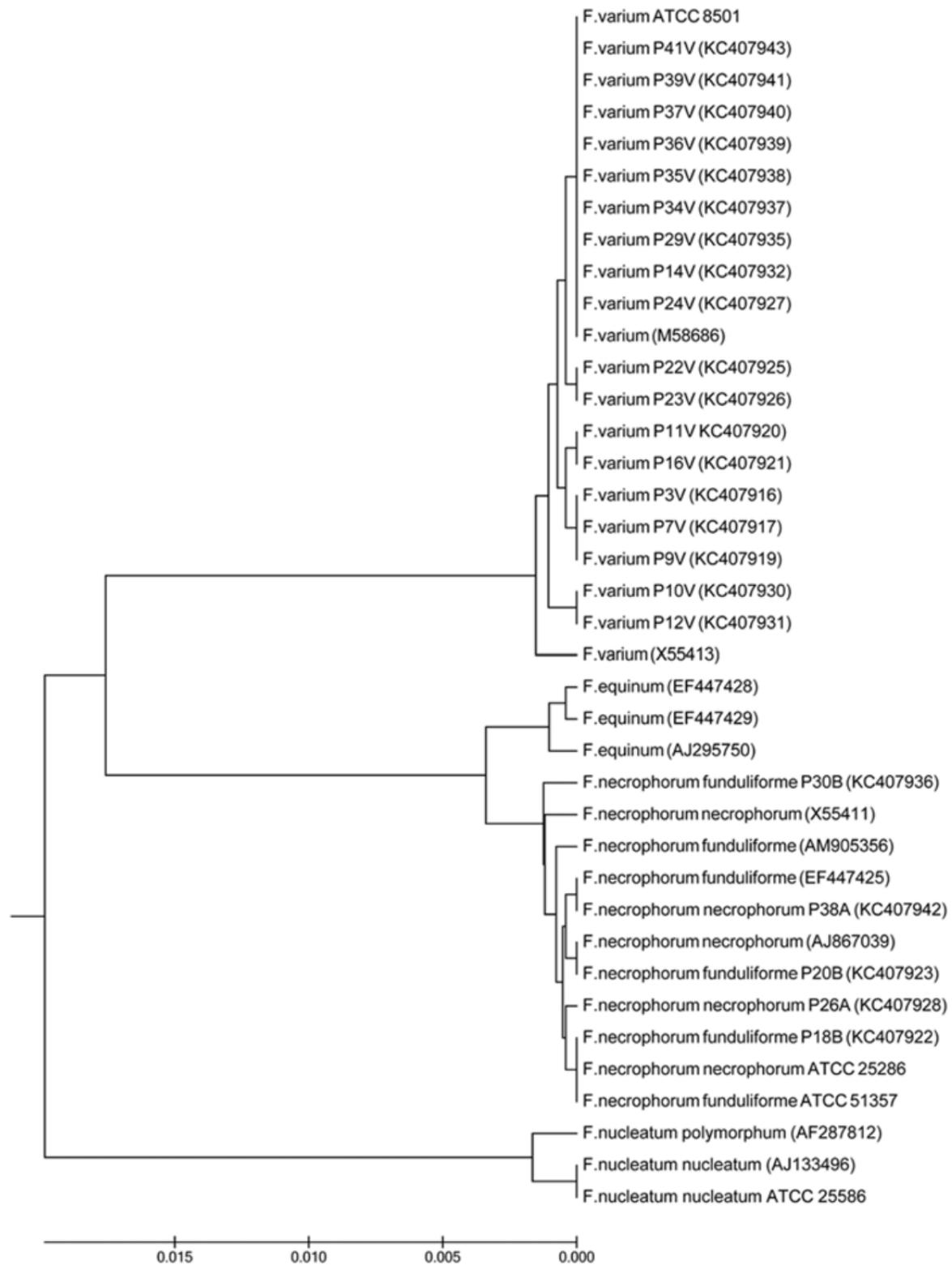


Figure 2.

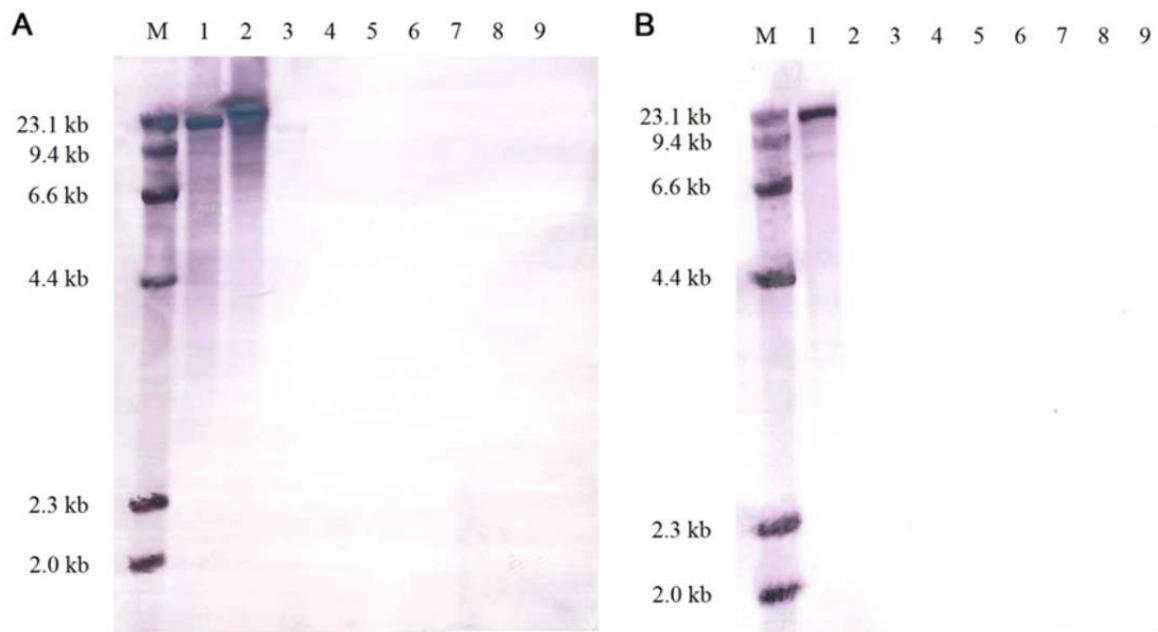


Figure 3.

