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Description of *Kingella potus* sp. nov., an Organism Isolated from a Wound Caused by an Animal Bite

Paul A. Lawson,[1]* Henry Malnick,[2] Matthew D. Collins,[1] Jayesh J. Shah,[2] Marie A. Chattaway,[4] Richard Bendall,[3] and John W. Hartley[3]

School of Food Biosciences, University of Reading, Reading RG6 6AP,[1] Laboratory of HealthCare Associated Infection, Centre for Infections, Health Protection Agency, Central Public Health Laboratory, London, NW9 5HT,[2] Department of Clinical Microbiology, Royal Cornwall Hospital, Penventinnie Lane, Treliske, Truro, Cornwall TR1 3LQ,[3] and National Collection of Type Cultures, Health Protection Agency, London,[4] United Kingdom

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We report the isolation and characterization of a hitherto unknown gram-negative, rod-shaped *Neisseria*-like organism from an infected wound resulting from a bite from a kinkajou. Based on both phenotypic and phylogenetic evidence, it is proposed that the unknown organism be classified as a new species, *Kingella potus* sp. nov.

Animal bites represent a significant source of wound infections in humans. Most commonly the animals involved are cats and dogs, and a variety of gram-negative bacteria from these wound infections have been described (1, 3, 17, 19, 20). In this article we report a novel, gram-negative-staining *Neisseria*-like organism isolated from a wound infection caused by the bite of a kinkajou, an arboreal mammal found in the rain forests of Central and South America. Based on the phenotypic characteristics of the novel organism and the results of comparative 16S rRNA gene sequencing, we describe a hitherto unknown *Kingella* species, *Kingella potus* sp. nov.

A previously healthy 53-year-old female zookeeper was referred to hospital with an infected wound on her right forearm. Three days earlier she had sustained a bite to the area from a kinkajou (*Potus flavus*). She was allergic to penicillin but otherwise had no history of note. The wound was cleaned following the bite, and she had been prescribed oral erythromycin. At presentation, she complained of pain over the anterior aspect of the right wrist and palm. She was apyrexial, her C-reactive protein level was 66.5 mg/liter, and her white blood cell count was 10.6×10^9 with 7.41×10^9 neutrophils. There were three puncture wounds noted on the volar surface of the right wrist surrounded by a 5- by 5-cm area of erythema and swelling. Pus was exuding from the bite, and she had tenderness over the wound and carpal tunnel. Infection of the flexor tendons and deep spaces of the wrist was suspected and urgent exploration undertaken. At operation, the sinuses extending from the bite wounds were excised and extended. There was a collection of pus deep into the fascia, which was sampled for culture. The wound was debrided and washed out. The tendon of flexor carpi radialis was frayed, and the belly of flexor pollicis longus was traumatized. Exploration of the carpal tunnel revealed thickened synovium but no pus. The flexor tendon sheaths were explored and washed out. She was treated with clarithromycin, ciprofloxacin, and metronidazole for 14 days, by which time the wound had healed. Culture of the pus, debrided tissue, and a swab from a tendon sheath yielded an alpha-hemolytic streptococcus, mixed anaerobic bacteria, and heavy growth of a gram-negative, rod-shaped organism.

The dominant gram-negative-staining isolate was recovered and grown on chocolatized Columbia blood agar base (Oxoid) supplemented with 5% horse blood. Plates were incubated at 37°C under an aerobic atmosphere with 5% added CO_2. The strain, designated 3/SID/1128^T, has been deposited in the National Collection of Type Cultures and the Culture Collection of the University of Göteborg under accession numbers NCTC 13336^T and CCUG 49773^T, respectively. Observations on cellular and colonial morphology were based on a 2-day incubation. Biochemical tests were carried out using API NH (BioMérieux, La Balme les Grottes, France), fermentation tests using phenol red broth base sugars (BBL Microbiological Systems, Cockeysville, Md.), and other tests by the methods of Cowan and Steel (5). The isolate was grown on chocolatized Trypticase soy agar with 5% sheep blood. Plates were incubated at 37°C under an aerobic atmosphere with 5% CO_2 added. Long-chain cellular fatty acids were extracted and analyzed by gas chromatography (MIDI Sherlock, Newark, N.J.) as described previously (15). The mol% G+C content of DNA was determined by high-performance liquid chromatography according to the work of Mesbah et al. (10). Antibiotic sensitivity testing was carried out by using the E-test system (AB Biodisk, Sweden) with Diagnostic Sensitivity Test agar (Oxoid) supplemented with 5% saponin-lysed horse blood. For phylogenetic analysis, the 16S rRNA gene of the strain was amplified by PCR and directly sequenced using a *Taq* dye-Deoxy terminator cycle sequencing kit (Applied Biosystems) and an automatic DNA sequencer (model 373A; Applied Biosystems). The closest known relatives of the new isolate were determined by performing GenBank/EMBL database searches using the Fasta program (14). These sequences and those of other known related strains were retrieved from GenBank/EMBL

* Corresponding author. Present address: Department of Botany and Microbiology, University of Oklahoma, Norman, OK 73019-6131. Phone: (405) 325-4321. Fax: (405) 325-7619. E-mail: paul.lawson@ou.edu.

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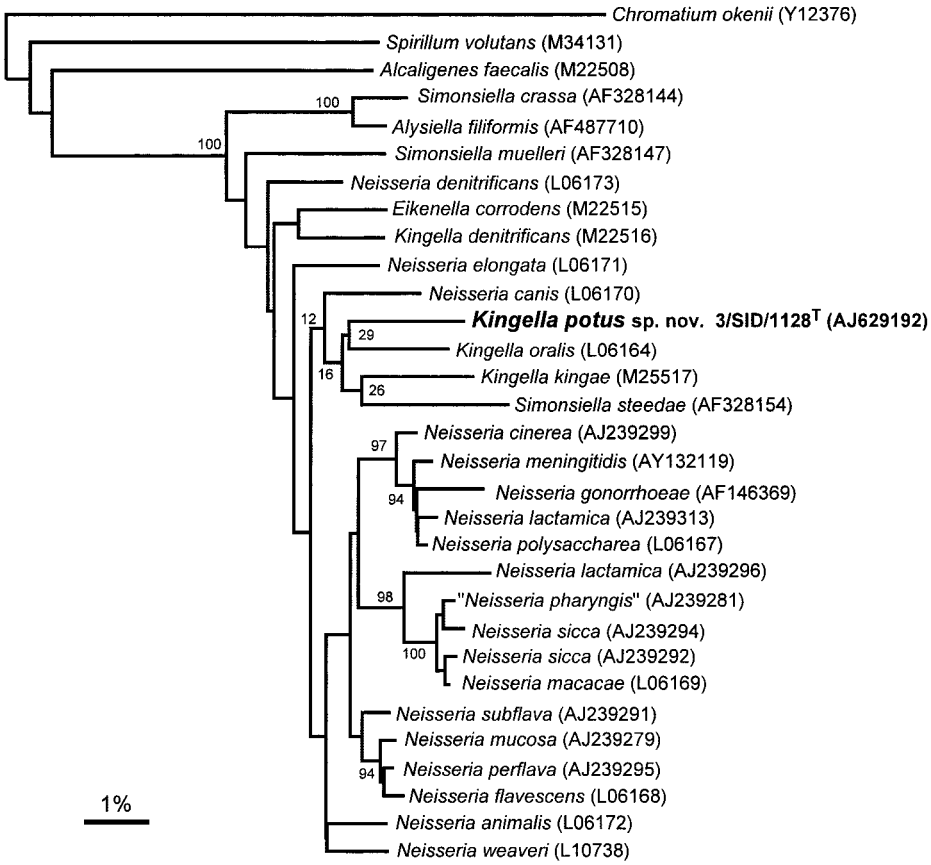

FIG. 1. Unrooted tree based on 16S rRNA showing the phylogenetic relationships of *Kingella potus* sp. nov. Bootstrap values (expressed as percentages of 1,000 replications) are given at the branching points. Bar, 1% sequence divergence.

and aligned with the newly determined sequence by using the SEQtools program (16). The resulting multiple sequence alignment had approximately 100 bases at the 5′ end of the rRNA omitted from further analysis, because of alignment uncertainties due to the highly variable region V1, by using the GeneDoc program (12). A phylogenetic tree was reconstructed according to the neighbor-joining method with the SEQtools and TREEVIEW (13) programs, and the stability of the groupings was estimated by bootstrap analysis (1,000 replications) using the same programs.

After a 48-h incubation, the gram-negative rod formed colonies that were yellow pigmented, low convex, 1.5 to 2 mm in diameter, and friable. The unidentified bacterium was oxidase positive, catalase negative, and casein and DNase positive. It gave negative results in the following tests: nitrate and nitrite reduction; hydrolysis of esculin, Tween 80, and tyrosine; malonate and phenylalanine deamination; and indole production. Arginine dehydrolase, lysine and ornithine decarboxylases, and urease were absent. The organism failed to produce acid from glucose, fructose, mannose, mannitol, maltose, lactose, and sucrose. The API NH kit profile number was 0001, indicating that the only positive result for this test system was gamma glutamyl transferase. No acid production was observed, and alkaline phosphatase, β-glucuronidase, β-galactosidase, α-glucosidase, and indole were not detected. Antibiotic E-test results showed the isolate was sensitive to ampicillin, amoxicillin, co-amoxiclav, penicillin, cefotaxime, ciprofloxacin, ceftriaxone, imipenem, and meropenem and resistant to erythromycin, clarithromycin, clindamycin, gentamicin, tetracycline, chloramphenicol, and trimethoprim. The long-chain cellular fatty acids of the organism were predominantly of the straight-chain saturated and monounsaturated types, with $C_{16:0}$ and $C_{18:1\omega7c}$ as the major components. The quantitative fatty acid profile of the isolate consisted of 3-OH $C_{10:0}$ (3.0%), $C_{11:0}$ (2.0%), $C_{12:0}$ (5.2%), 3-OH $C_{12:0}$ (3.6%), $C_{13:1}$ (0.1%), $C_{13:0}$ (0.1%), $C_{14:0}$ (2.7%), $C_{15:0}$ (2.1%), 3-OH $C_{15:0}$ (0.2%), $C_{16:0}$ (34.8%), 3-OH $C_{16:0}$ (1.5%), $C_{17:0}$ (0.9%), $C_{17:1\omega6c}$ (0.2%), $C_{18:0}$ (1.0%), $C_{18:1\omega7c}$ (18.5%), and $C_{18:1\omega9c}$ (2.4%). In addition, the profile also contained several summed features consisting of $C_{12:0\ ALDE}$/unknown 10.928 and $C_{16:1\ iso\ I}/C_{14:0\ 3OH}$ (3.0%), $C_{16:1\omega7c}$ /$C_{15:0\ iso\ 2OH}$ (14.4%), and $C_{18:2\omega6,9c}/C_{18:0\ ANTE}$ (3.3%). The cellular lipid composition of the isolate was consistent with its classification within the family *Neisseriaceae* (4). The MIDI database indicated that the fatty acid profile of the isolate was closest to those of *Neisseria elongata* subsp. *glycolytica* and *N. weaveri*. To further investigate the phylogenetic position of the unidentified organism, its 16S rRNA genes were amplified by PCR and sequenced. The almost-complete gene sequence (>1,400 nucleotides) was determined. Sequence database searches showed that the unknown organism displayed the highest 16S rRNA sequence similarity to members of the family *Neisseriaceae* (data not shown). Tree analysis confirmed this

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TABLE 1. Characteristics that differentiate *Kingella potus* sp. nov. from other *Kingella* and *Neisseria* species[a]

Species	Finding											
	Cocci	Rods or coccobacilli	Pigment	Glucose	Maltose	Fructose	Sucrose	NO_3^-	NO_2^-	DNase	Cat	β-GAL
K. potus sp. nov.	−	+	+	−	−	−	−	−	−	+	−	−
K. kingae	−	+	−	+	+	−	−	−	−	−	−	−
K. oralis	−	+	−	+	−	−	−	−	−	−	−	−
K. denitrificans	−	+	−	+	−	−	−	V	V	−	−	−
N. meningitidis	+	−	−	+	+	−	−	−	V	−	+	−
N. lactamica	+	−	−	+	+	−	−	−	V	−	+	+
N. cinerea	+	−	−	−	−	−	−	−	+	−	+	−
N. polysaccharea	+	−	−	+	+	−	−	−	V	−	+	−
N. kochii	+	−	−	+	−	−	−	−	−	−	+	−
N. flavescens	+	−	+	−	−	−	−	−	−	−	+	−
N. sicca	+	−	V	+	+	+	+	−	+	−	+	−
N. subflava											+	−
Biovar *subflava*	+	−	+	+	+	−	−	−	+	−	+	−
Biovar *flava*	+	−	+	+	+	+	−	−	+	−	+	−
Biovar *perflava*	+	−	+	+	+	+	+	−	+	−	+	−
N. mucosa	+	−	+	+	+	+	+	+	+	−	+	−
N. elongata												
subsp. *elongata*	−	+	+	−	−	−	−	−	+	−	V	−
subsp. *glycolytica*	−	+	+	(+)	−	−	−	−	+	−	+	−
subsp. *nitroreducens*	−	+	+	V	−	−	−	+	+	−	+	−
N. animalis	+	−	−	+	−	(+)	+	−	+	−	+	−
N. canis	+	−	+	−	−	−	−	+	−	(+)	+	−
N. caviae	+	−	−	−	−	−	−	+	+	(+)	+	−
N. cuniculi	+	−	−	−	−	−	−	−	−	+	+	−
N. denitrificans	+	−	V	+	−	+	+	−	+	+	+	−
N. macacae	+	−	+	+	+	+	+	−	+	+	+	−
N. ovis	+	−	−	−	−	−	−	+	−	+	+	−
N. weaveri	−	+	+	−	−	ND	−	−	+	ND	+	−
N. iguanae	+	−	−	(+)	−	−	(+)	+	V	−	+	−

[a] Data were obtained from references 2, 3, 6, 7, 9, 11, and 20. Abbreviations: β-GAL, β-galactosidase; Cat, catalase; +, positive reaction; −, negative reaction; (+), weak positive; V, variable reaction; ND, not determined.

association, with the unidentified organism forming a distinct rRNA subline within the family. A tree depicting the placement of the unknown organism within the family *Neisseriaceae* is shown in Fig. 1.

The overall morphological and biochemical features and fatty acid composition of the unidentified organism from a wound infection are consistent with its assignment to the family *Neisseriaceae*. This family comprises a major branch of the beta group of the *Proteobacteria* and phylogenetically encompasses the genera *Neisseria*, *Kingella*, *Eikenella*, *Simonsiella*, and *Alysiella*. It is evident from the results of comparative 16S rRNA sequencing that the unidentified rod-shaped organism represents a hitherto unknown species within the family *Neisseriaceae*. The phylogeny of the *Neisseriaceae* is presently unsatisfactory, and it is now known that some genera within the family are not monophyletic (8). In particular, it is recognized that species currently assigned to the genus *Neisseria* are phylogenetically heterogeneous, although some *Neisseria* species form a robust cluster with *N. gonorrhoeae*, the type species of the genus. The unidentified wound bacterium is, however, far removed from *N. gonorrhoeae* and its close relatives and therefore cannot be considered a legitimate member of this genus. In addition, unlike the great majority of *Neisseria* species, the unknown organism is rod-shaped and catalase negative. The nearest phylogenetic relatives of the unknown bacterium correspond to *Kingella* species (94.8 to 95.9% sequence similarity) and *N. canis* (95.4% sequence similarity). Upon tree analysis, the unknown organism was most closely associated with *Kingella oralis*, although bootstrap resampling analysis showed that this association was not particularly significant. Like *Neisseria*, the genus *Kingella* is also not monophyletic (6). However, despite the evident heterogeneity within the genus *Kingella*, this is currently the most appropriate home for the unidentified organism. Phenotypically the unidentified organism has many properties in common with *Kingella* species, but it can be readily distinguished biochemically from all described members of this genus. In addition, the 16S rRNA sequence divergence of >4% strongly supports the recognition of the unidentified organism as a novel species. It is now established that organisms displaying more than 3% sequence divergence belong to different species (18). Therefore, based on both phenotypic and phylogenetic evidence, we consider that the unidentified rod-shaped organism should be classified as a novel species within the genus *Kingella*; the name *Kingella potus* is proposed. Though this isolate was the most abundant organism in the infected area, it is not clear whether the isolate was truly pathogenic or opportunistic or whether its growth was favored over that of other bacteria by the laboratory conditions used to process the specimens. Tests that are useful in distinguishing *Kingella potus* from other *Kingella* species and members of the genus *Neisseria* are shown in Table 1.

Description of *Kingella potus* sp. nov. (po.tus. L. gen. masc. n. *potus*, of the drink or drinking, pertaining to *Potus flavus*, the generic name of the South American kinkajou, the animal

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from which the organism originated.) Cells are gram negative, non-spore-forming, nonmotile rods. Aerobic, oxidase positive, and catalase negative. Colonies are circular, low convex, yellow-pigmented, smooth, entire, approximately 1.5 to 2 mm in diameter, and friable on Columbia blood agar after 48 h of incubation at 37°C. Colonies are nonhemolytic. Nondiffusible yellow pigments are produced. Long-chain fatty acids are of the straight-chain saturated and monounsaturated types, with $C_{16:0}$ and $C_{18:1\omega7c}$ predominating. Nitrate and nitrite are not reduced. Esculin and urea are not hydrolyzed. Indole is not produced. Acid is not produced from fructose, glucose, mannose, mannitol, maltose, lactose, or sucrose. No alkaline phosphatase, α-glycosidase, β-galactosidase, or β-glucuronidase activity is detected. Isolated from the human wound caused by a bite by a kinkajou. The G+C content of DNA is 58.4 mol%. The type strain is 3/SID/1128^T (NCTC 13336^T, CCUG 49773^T).

Nucleotide sequence accession number. The 16S rRNA gene sequence of strain 3/SID/1128^T has been deposited in GenBank under accession number AJ629192.

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