Uruburuella suis gen. nov., sp. nov., isolated from clinical specimens of pigs

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Five strains of an unusual Gram-negative, catalase-positive, oxidase-positive, coccobacillus-shaped bacterium isolated from the lungs and heart of pigs with pneumonia and pericarditis were characterized by phenotypic and molecular genetic methods. On the basis of cellular morphology and biochemical criteria, the isolates were tentatively assigned to the family *Neisseriaceae*, although they did not appear to correspond to any recognized genus or species. Comparative 16S rRNA gene sequencing showed that the five unidentified strains were phylogenetically highly related to each other and represent a hitherto unknown subline within the family *Neisseriaceae*. On the basis of both phenotypic and phylogenetic evidence, it is proposed that the unknown isolates from pigs be classified as a novel genus and species within the family *Neisseriaceae*, for which the name *Uruburuella suis* gen. nov., sp. nov. is proposed. The type strain of *U. suis* is 1258/02^T (=CCUG 47806^T = CECT 5685^T).

The family *Neisseriaceae* was proposed by Prévot in 1933. As circumscribed by Bøvre (1984), it accommodated a group of Gram-negative, oxidase- and catalase-positive, aerobic or facultatively anaerobic, non-spore-forming, rod- or coccoid-shaped organisms and embraced four genera: *Neisseria*, *Kingella*, *Acinetobacter* and *Moraxella* (Bøvre, 1984). The family *Neisseriaceae* has undergone various revisions over the years, and numerous other genera have been assigned to it [e.g. *Psychrobacter* (Juni & Heym, 1986), *Mesophilobacter* (Nishimura *et al.*, 1989), *Alysiella* (Rossau *et al.*, 1989) and *Eikenella* and *Simonsiella* (Dewhirst *et al.*, 1989)]. On the basis of rRNA cistron similarity studies, Rossau *et al.* (1986) recommended that the genera *Acinetobacter*, *Moraxella* and *Branhamella* be excluded from the family *Neisseriaceae*. Subsequent studies by Rossau *et al.* (1989) showed that the genera *Neisseria*, *Kingella*, *Eikenella*, *Simonsiella* and *Alysiella* were closely related and should be classified together in the family *Neisseriaceae*, which comprises a major branch of the β -*Proteobacteria*. Comparative 16S rRNA gene sequencing studies by Dewhirst *et al.* (1989) confirmed the results of Rossau *et al.* (1989) and led to the recommendation that the genera *Eikenella* and *Simonsiella* be transferred to the family *Neisseriaceae*. In

recent years, other genera, e.g. *Laribacter* (Yuen *et al.*, 2001) and *Microvirgula* (Patureau *et al.*, 1998), have been described and assigned to the family *Neisseriaceae*.

Organisms within the family *Neisseriaceae* can be isolated from a range of environmental sources (Moss & Bryant, 1982) as well as from different human and animal mucosal surfaces (Kuhn, 1981; Snell, 1984). Only two species, *Neisseria gonorrhoeae* and *Neisseria meningitidis*, are well-established human pathogens (Morse & Knapp, 1991). Other species of the *Neisseriaceae* have been implicated as opportunistic human pathogens in a number of different clinical processes (Wong & Janda, 1992; Dolter *et al.*, 1998). In veterinary medicine, too, neisseriae have been implicated as pathogens in a number of different clinical processes (Elad *et al.*, 1990; Yeruham *et al.*, 2001). In this study, we have characterized five *Neisseria*-like organisms isolated from the heart and lungs of pigs with respiratory infections. On the basis of phenotypic and molecular taxonomic evidence, we present the description of a novel genus and species within the family *Neisseriaceae*, for which we propose the name *Uruburuella suis* gen. nov., sp. nov.

The bacterial strains were isolated from the lungs (strains 1258/02^T and 221/01) and hearts (strains 959/02, 1039/02 and 238/01) of different animals with lesions of pneumonia and pericarditis, respectively. The pigs were between 5 and 6 months old and were kept under intensive management conditions. Samples from affected zones of lung and heart were collected under aseptic conditions and kept under refrigeration until being processed in the laboratory (within

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An extended dendrogram including a wider sample of reference species is available as a supplementary figure in IJSEM Online.

6 h). Strains were isolated on Columbia blood agar plates (bioMérieux) and incubated for 24 h at 37 °C under aerobic and anaerobic conditions. The strains were biochemically characterized using the API 20NE, API NH and API ZYM systems (bioMérieux) according to the manufacturer's instructions. The production of DNase was determined on DNA medium (bioMérieux). Fatty acid methyl esters were prepared and analysed as described by Kämpfer & Kroppenstedt (1996). The G+C content of the DNA (mol%) was determined by HPLC according to Mesbah *et al.* (1989). For 16S rRNA gene sequence analysis, a large fragment (around 1450 bases) of the 16S rRNA gene of the isolates was amplified by using a PCR and was directly sequenced using a *Taq* DyeDeoxy terminator cycle sequencing kit (Applied Biosystems) and an automatic DNA sequencer (model 373A; Applied Biosystems). The nearest described relatives of the new isolates were determined by performing searches of GenBank using the program FASTA (Pearson, 1994). These closely related sequences were retrieved from GenBank and aligned with the newly determined sequences using the program DNATools (Rasmussen, 1995). A phylogenetic tree was reconstructed according to the neighbour-joining method (Saitou & Nei, 1987), with the programs DNATools and TREEVIEW (Page, 1996), and the stability of the groupings was estimated by bootstrap analysis (1000 replications).

The five unidentified isolates consisted of Gram-negative, non-motile, non-spore-forming, coccobacillus-shaped cells. The organisms were facultatively anaerobic and catalase- and oxidase-positive. When cultured aerobically on Columbia blood agar plates, the isolates formed small colonies (approx. 1–2 mm diameter, after 24 h incubation at 37 °C) that were circular, smooth, entire, non-haemolytic and yellow-pigmented. They did not grow on MacConkey or Thayer–Martin agars. Swimming motility was absent. In the miniaturized biochemical kits, the isolates displayed very similar, though not identical, phenotypic profiles. All of the isolates reduced nitrate and nitrite, produced acid from glucose and fructose and gave a positive reaction for proline arylamidase. All isolates gave negative results in the tests for indole production, hydrolysis of gelatin and aesculin, arginine dihydrolysis and ornithine decarboxylation. Three of the five strains (1258/02^T, 959/02 and 1039/02) hydrolysed urea. None of the isolates produced acid from sucrose or maltose. Assimilation of glucose, arabinose, mannitol, gluconate and malate was observed, but citrate, maltose, caprate, phenylacetate and adipate were not assimilated. Two strains (221/01 and 238/01) did not assimilate mannose. Using the API ZYM system, positive reactions for esterase C4, ester lipase C8, leucine arylamidase and acid phosphatase, and a weak reaction for alkaline phosphatase, were detected. All other tests were negative. In terms of their overall cellular morphology and biochemical characteristics, the unidentified isolates somewhat resembled members of the family *Neisseriaceae*. To clarify the association of the unknown pig isolates with the family *Neisseriaceae*, two representative strains, 1258/02^T and 959/02,

were subjected to cellular fatty acid analysis. The cellular lipid composition of the isolates was characterized by the absence of branched fatty acids, which is consistent with their classification in the family *Neisseriaceae* (Bøvre, 1984). The major fatty acids of the strains were C16:1 *cis*-9, C16:0 and C18:1 *cis*-11 (mean amounts, 30.6, 33.9 and 17.9 %, respectively). The isolates also contained moderate or small amounts of C12:0 (6.1 %), 3-OH C12:0 (4.2 %), C14:0 (2.3 %), 3-OH C14:0 (1.6 %), C18:2 *cis*-9 (1.3 %) C18:0 (0.7 %), C16:1 *cis*-7 (0.6 %) and C18:1 *cis*-9 (0.6 %).

To determine the phylogenetic position of the unknown isolates, their 16S rRNA gene sequences were determined by direct sequencing of *in vitro*-amplified rRNA gene products. The sequence of a large fragment (>1400 nt) from strains 1258/02^T and 959/02, and approximately 1000 nt of the other three strains were determined; comparative analysis revealed 99.9–100 % 16S rRNA gene sequence similarity among the strains, thereby demonstrating high genealogical homogeneity. Sequence searches of GenBank showed that the isolates were most closely related to species of the family *Neisseriaceae* (data not shown). The pig isolates exhibited similar levels of sequence similarity with *Neisseria* (approx. 92–96 %), *Simonsiella* (94–95 %), *Kingella* (94–95 %), *Alysiella* (94 %) and *Eikenella* (94 %) species. It is pertinent to note that the five isolates possessed an adenine and a uracil at positions 585 and 756, respectively (numbering relative to the *Escherichia coli* sequence), in their 16S rRNA. These signatures are characteristic of members of the family *Neisseriaceae* (Dewhirst *et al.*, 1989). Treeing analysis confirmed this affinity, and the unknown bacterium (as exemplified by strain 1258/02^T) formed a distinct subline within a large rRNA cluster that included the genera *Neisseria*, *Kingella*, *Alysiella*, *Eikenella* and *Simonsiella*. Bootstrap resampling analysis, however, showed that the unknown bacterium did not possess significant affinity with any recognized species. A dendrogram depicting the phylogenetic relationships of the unidentified bacterium is shown in Fig. 1.

The family *Neisseriaceae* comprises a major branch of the β -*Proteobacteria*. Phylogenetically, the family encompasses the genera *Neisseria*, *Kingella*, *Eikenella*, *Simonsiella* and *Alysiella* (Dewhirst *et al.*, 1989; Rossau *et al.*, 1989). It is clear from the results of the present study that the unidentified bacterium from pigs is phylogenetically a member of this family grouping. Phylogenetically, the pig bacterium forms a distinct subline and does not display a close relationship with any described organism (Fig. 1). The generic placement of the unidentified bacterium is problematic given the phylogenetic intermixing of some species of the genera *Neisseria*, *Kingella*, *Eikenella* and *Simonsiella*. Upon treeing analysis, the swine bacterium displays a loose association with *Neisseria canis*, although this relationship was not supported by bootstrap resampling. Species currently assigned to the genus *Neisseria* are phylogenetically not homogeneous, although some *Neisseria* species form a robust cluster with *N. gonorrhoeae*, the type species of the

Fig. 1. Unrooted tree based on 16S rRNA showing the phylogenetic relationships of *U. suis* sp. nov. Bootstrap values (each expressed as a percentage of 1000 replications) are given at branching points. A full version of the dendrogram, including a wider sample of *Streptococcus* species, is available as a supplementary figure in IJSEM Online. Bar, 1% sequence divergence.

genus. The unidentified pig bacterium, however, is far removed from *N. gonorrhoeae* and its close relatives and therefore cannot be considered a legitimate member of this genus. The unknown bacterium also fails to display specific phylogenetic affinities with other related genera (*Alysella*, *Kingella*, *Eikenella* and *Simonsiella*) within the *Neisseriaceae*. Support for the separateness of the unknown bacterium is also evident from phenotypic considerations. The pig bacterium is similar to *Eikenella* species and some *Kingella* species in being facultatively anaerobic, but differs markedly from species of the genera *Neisseria*, *Alysella* and *Simonsiella*, which are strictly aerobic. However, the unknown bacterium is catalase-positive, whereas members of the genera *Eikenella* and *Kingella* are catalase-negative. Similarly, the unknown bacterium differs further from members of the genera *Eikenella* and *Kingella* in not displaying twitching motility. The coccobacillus-shaped pig bacterium can also be readily distinguished from species of the genera *Alysella* and *Simonsiella*, which form flat multicellular filaments (which may reach 2–10 µm in width and 50 µm or more in length and possess bilateral symmetry). Members of the latter two genera also display gliding motility, which is absent in the unidentified bacterium. Given these phenotypic differences and the distinct phylogenetic subline formed by the pig bacterium, we consider that this organism merits classification in a novel genus within the *Neisseriaceae*. The five isolates showed very high 16S rRNA gene sequence relatedness, as well as relatively uniform

morphological and biochemical characteristics, which, together with their recovery from similar sources, is consistent with them/their belonging to a single species. Therefore, on the basis of the relatively high homogeneity of the isolates and their distinctiveness from other members of the *Neisseriaceae*, we propose that these organisms from pigs be classified as *Uruburuella suis* gen. nov., sp. nov. Tests that are useful in distinguishing *Uruburuella* from other closely related genera are shown in Table 1.

Description of *Uruburuella* gen. nov.

Uruburuella (U.ru.bu.ru.el'la. L. dim. ending -ella; N.L. fem. dim. n. *Uruburuella* in memory of the Spanish microbiologist Federico Uruburu, for his contributions to the promotion and strengthening of the Spanish Type Culture Collection).

Cells are Gram-negative, non-spore-forming, non-motile coccobacilli. Facultatively anaerobic and catalase- and oxidase-positive. Non-diffusible yellow pigments are produced. Grows in 3% (w/v) NaCl. Produces acid from glucose and some other sugars. Reduces nitrate and nitrite. Long-chain fatty acids are of the straight-chain saturated and monounsaturated types, with C16:0, C16:1 *cis*-9 and C18:1 *cis*-11 predominating. The G + C content of the DNA of the type species is 55 mol%. The genus *Uruburuella* is a member of the family *Neisseriaceae* in the β -subclass of the

Table 1. Characteristics that differentiate *Uruburuella* gen. nov. from its closest phylogenetic relatives

Taxa: 1, *Uruburuella suis* gen. nov., sp. nov.; 2, *Neisseria*; 3, *Kingella*; 4, *Eikenella*; 5, *Simonsiella*; 6, *Alysiella*. Data for reference genera were obtained from Vedros (1984), Jackson & Goodman, (1984), Larkin (1989), Morse & Knapp (1991), Dewhirst *et al.* (1993), Barrett & Sneath (1994) and Hedlund & Staley (2002). Symbols: +, positive; –, negative; v, variable; ND, not determined.

Characteristic	1	2	3	4	5	6
Cell morphology						
Cocci	–	+	–	–	–	–
Rods	+	+	+	+	+	+
Facultatively anaerobic	+	–	+	+	–	–
Catalase production	+	+	–	–	+	+
Twitching motility	–	– ^a	+	+	–	–
Gliding motility	–	–	–	–	+	+
Growth in 3% (w/v) NaCl	+	– ^c	–	ND	–	–
Acid from glucose	+	+	+	–	+	+
Urease	v	–	–	–	–	–
Nitrate reduction	+	– ^f	– ^g	+	+	–
Nitrite reduction	+	+	– ^g	–	+	–

**Simonsiella* and *Alysiella* species form flat multicellular filaments that may reach 2–10 µm in width and 50 µm or more in length and possess bilateral symmetry.

†Discrepant results are indicated by: *a*, *Neisseria elongata* can be negative; *b*, *N. gonorrhoeae* and *N. meningitidis* may demonstrate twitching motility; *c*, *N. canis*, *Neisseria animalis* and *Neisseria weaveri* may show growth; *d*, *N. canis*, *Neisseria ovis*, *Neisseria cuniculi*, *Neisseria caviae*, *Neisseria cinerea*, *Neisseria flavescens* and *N. elongata* may be negative; *e*, *Simonsiella steedae* may be negative; *f*, *N. canis*, *N. ovis*, *N. caviae* and *Neisseria mucosa* may reduce nitrate; *g*, *Kingella denitrificans* may reduce nitrate and nitrite; *h*, *Simonsiella muelleri* may not reduce nitrate and/or nitrite; *i*, *N. gonorrhoeae*, *N. canis*, *N. ovis*, *N. cuniculi* and *N. caviae* may not reduce nitrite.

Proteobacteria. The type species of the genus is *Uruburuella suis*.

Description of *Uruburuella suis* sp. nov.

Uruburuella suis (su'is. L. fem. n. *sus*, *suis* pig, hog; L. gen. n. *suis* of the hog).

Displays the following properties in addition to those given in the genus description. Colonies are circular, yellow-pigmented, smooth, entire and approximately 1–2 mm diameter on Columbia blood agar after 24 h incubation at 37 °C. Colonies are non-haemolytic. No growth occurs on MacConkey agar or Thayer–Martin agar. Aesculin and gelatin are not hydrolysed. Hydrolysis of urea is variable. Indole is not produced. Acid is produced from fructose and glucose but not from maltose or sucrose. Assimilation of glucose, arabinose, mannitol, gluconate and malate is observed but citrate, maltose, caprate, phenylacetate and adipate are not assimilated. Mannose may or may not be

assimilated. Activity for alkaline phosphatase (weak reaction), ester lipase C8, esterase C4, leucine arylamidase, acid phosphatase and proline arylamidase is detected. Arginine dihydrolase, lipase C14, valine arylamidase, cystine arylamidase, trypsin, α-chymotrypsin, naphthol-AS-BI-phosphohydrolase, α-glycosidase, β-galactosidase, β-glucuronidase, α-glucosidase, β-glucosidase, N-acetyl-β-glucosaminidase, α-mannosidase, α-fucosidase and ornithine decarboxylase are not produced.

Isolated from the lungs and heart of pigs with respiratory disease. The type strain is 1258/02^T (=CCUG 47806^T = CECT 5685^T).

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