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Current Distribution of *Rodentibacter* Species Among the Mice and Rats of an Experimental Facility

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The uncertain taxonomy of [*Pasteurella*] *pneumotropica* and other rodent *Pasteurellaceae* has hindered the acquisition of knowledge on the biology and disease for this group of bacteria. Recently, these organisms have been reclassified within the new genus *Rodentibacter*. In this study, we documented which of the new described *Rodentibacter* spp. are present in the mouse and rat microbiologic units of an experimental facility. Screening all of the microbiologic units populated with mice and rats yielded 51 *Rodentibacter* isolates. Molecular and phenotypic diagnosis indicated the colonization of mice by *R. pneumotropicus* and *R. heylii*, whereas *R. ratti* and *R. heylii* were found in rats. Overall, we document the association of laboratory rodents with 3 of the newly described *Rodentibacter*. Diagnostics of the *Rodentibacter* spp. at the species level can decisively contribute to the progress of knowledge on these bacteria.

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Recently, [*Pasteurella*] *pneumotropica* and its closely related rodent *Pasteurellaceae* have been reclassified in 8 distinct species and 2 genomospecies within the new genus *Rodentibacter*.[2] Colonization of mice and rats by *Rodentibacter* spp. typically does not result in clinical disease, and members of *Rodentibacter* are rather regarded as opportunistic pathogens mainly in connection with promoting factors, such as immunodeficiency. Nevertheless, the clonality of isolates causing disease outbreaks in some mouse colonies indicates that a primary role of *R. heylii* might be possible.[1] Similarly, various *R. pneumotropicus* isolates with particular virulence factors profiles seem to possess primary virulence capacity.[11] In addition to effects on animal health or welfare, this group of bacteria might influence the results of experiments that use contaminated animals.[14] The uncertain taxonomy of [*P.*] *pneumotropica* complex in the past has hindered the acquisition of knowledge regarding the epidemiology, pathogenesis, diagnostics, and control of infections caused by these organisms.[7] The [*P.*] *pneumotropica* complex (now *Rodentibacter* spp.) has been credited for the most prevalent infections of laboratory animals. However, which of the *Rodentibacter* spp. that have been included within the taxon [*P.*] *pneumotropica* that are currently found within animals has not been examined.[7] The *Pasteurellaceae* species are usually associated with only one or very few closely related hosts. Overall, it seems that some of the *Rodentibacter* spp. species are host-specific, whereas other isolates affect a group of closely related hosts.[7] The actual taxonomy of the *Rodentibacter* spp. allows further documentation regarding whether *Rodentibacter* spp., under natural conditions, colonize individual or multiple, closely related species.

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Here we sought to isolate and identify the *Rodentibacter* spp. distributed in the microbiologic units of an experimental facility containing mice and rats, to document which of the newly described species are present.

Materials and Methods

Bacterial isolates and identification methods. The bacterial strains included in this study (Table 1) were isolated during the routine microbiologic monitoring of the mouse and rat colonies in the Animal Research Facility of Heinrich–Heine University (Düsseldorf, Germany), as described previously.[5] The animals are housed in either open cages or IVC in several microbiologic units with differing microbiologic status. Colony health is monitored quarterly through a combined, statistically valid sampling strategy of BALB/c dirty-bedding sentinels and of resident mice. Rat colonies, which are mainly on a Wistar background, are housed in open cages, and monitoring is performed directly on resident animals. All animals that appear to be sick are examined thoroughly. Most mouse and rat areas are free of all agents listed in the FELASA recommendations for health monitoring of rodents[12] as well as *Staphylococcus aureus*, *Proteus* spp., *Klebsiella* spp., *Bordetella bronchiseptica*, *Bordetella hinzii*, *Pseudomonas aeruginosa*, *Muribacter muris*, and dermatophytes. Nevertheless, in some microbiologic units, nonpathogenic intestinal flagellates, mouse norovirus, *Helicobacter* spp., *Rodentibacter* spp., *S. aureus*, *Proteus* spp., *Klebsiella* spp. and *M. muris* are diagnosed and tolerated as single infections or as coinfections. For health monitoring, swabs of the nasal cavities, oropharynx, and genital mucosa of various wild-type and transgenic mouse strains were cultured on Columbia blood agar and MacConkey agar plates (BioMerieux, Nuertingen, Germany) for approximately 48 h at 37 °C under aerobic and anaerobic (GasPak EZ, Becton-Dickinson, Franklin Lakes, NJ) conditions. *Pasteurellaceae*-like colonies were subcultured overnight for isolation and underwent further phenotypic and genetic identification. The health monitoring program has been approved by the appropriate supervisory

Table 1. Identification of mice and rats *Rodentibacter* isolates

Isolate	Source	Multiplex PCR analysis	16S rRNA identification (% similarity to closest species / GenBank no. of strain sequence)
212/17	M, pharynx	*R. pneumotropicus*	*R. pneumotropicus* (99.6% / BBIX01000006)
213/17	M, nose	*R. pneumotropicus*	*R. pneumotropicus* (99.6% / BBIX01000006)
214/17	M, prepuce	*R. pneumotropicus*	*R. pneumotropicus* (99.5% / BBIX01000006)
215/17	M, nose	*R. heylii*	*R. heylii* (100% / KR364783)
218/17	M, nose	*R. pneumotropicus*	*R. pneumotropicus* (99.8% / BBIX01000006)
220/17	M, pharynx	*R. heylii*	*R. heylii* (99.8% / AF012090)
223/17a	M, nose	*R. heylii*	*R. heylii* (99.7% / KR364783)
223/17b	M, nose	*R. pneumotropicus*	*R. pneumotropicus* (99.6% / BBIX01000006)
438/17	M, nose	*R. heylii*	*R. heylii* (99.9% / AF012090)
439/17	M, nose	*R. heylii*	*R. heylii* (99.9% / AF012090)
440/17	M, prepuce	*R. heylii*	*R. heylii* (99.5% / AF012090)
442/17	M, pharynx	*R. heylii*	*R. heylii* (99.9% / AF012090)
443/17a	M, pharynx	*R. heylii*	*R. heylii* (99.9% / KR364783)
443/17b	M, pharynx	*R. pneumotropicus*	*R. pneumotropicus* (99.5% / BBIX01000006)
517/17	M, nose	*R. heylii*	*R. heylii* (99.9% / AF012090)
518/17	M, nose	*R. pneumotropicus*	*R. pneumotropicus* (99.0% / BBIX01000006)
519/17	M, nose	*R. pneumotropicus*	*R. pneumotropicus* (99.7% / BBIX01000006)
521/17	M, prepuce	*R. pneumotropicus*	*R. pneumotropicus* (99.9% / BBIX01000006)
529/17	M, nose	*R. pneumotropicus*	*R. pneumotropicus* (99.7% / BBIX01000006)
530/17	M, nose	*R. pneumotropicus*	*R. pneumotropicus* (99.8% / BBIX01000006)
532/17	M, nose	*R. pneumotropicus*	*R. pneumotropicus* (99.5% / BBIX01000006)
555/17	M, prepuce	*R. heylii*	*R. heylii* (99.9% / KR364783)
556/17	M, nose	*R. heylii*	*R. heylii* (99.8% / KR364783)
557/17	M, prepuce	*R. heylii*	*R. heylii* (99.7% / KR364783)
558/17	M, nose	*R. pneumotropicus*	*R. pneumotropicus* (99.7% / BBIX01000006)
559/17	M, prepuce	*R. pneumotropicus*	*R. pneumotropicus* (99.7% / BBIX01000006)
560/17	M, nose	*R. pneumotropicus*	*R. pneumotropicus* (99.8% / BBIX01000006)
594/17	M, nose	*R. heylii*	*R. heylii* (99.6% / KR364783)
596/17	M, vagina	*R. heylii*	*R. heylii* (99.8% / KR364783)
597/17	M, prepuce	*R. heylii*	*R. heylii* (99.7% / AF012090)
33/18	M, nose	*R. heylii*	*R. heylii* (98.4% / KR364783)
251/17	R, nose	*R. heylii*	*R. heylii* (99.3% / KR364783)
341/17	R, nose	*Pasteurellaceae*	*R. ratti* (98.1% / KX858074)
342/17	R, pharynx	*Pasteurellaceae*	*R. ratti* (97.7% / KX858074)
343/17	R, nose	*Pasteurellaceae*	*R. ratti* (97.7% / KX858074)
345/17	R, pharynx	*Pasteurellaceae*	*R. ratti* (97.5% / KX858074)
637/17	R, nose	*Pasteurellaceae*	*R. ratti* (97.5% / KX858074)
638/17	R, pharynx	*Pasteurellaceae*	*R. ratti* (97.9% / KX858074)
639/17	R, pharynx	*Pasteurellaceae*	*R. ratti* (98.5% / KX858074)
794/17	R, vagina	*R. heylii*	*R. heylii* (99.0% / KX858063)
1002/17	R, nose	*Pasteurellaceae*	*R. ratti* (97.5% / KX858074)
1012/17	R, nose	*Pasteurellaceae*	*R. ratti* (99.1% / KX858335)
1359/17	R, nose	*R. heylii*	*R. heylii* (99.0% / KX858218)
1360/17	R, pharynx	*Pasteurellaceae*	*R. ratti* (97.2% / KX858242)
1361/17	R, pharynx	*Pasteurellaceae*	*R. ratti* (98.2% / KX858074)
257/18	R, pharynx	*Pasteurellaceae*	*R. ratti* (97.4% / KX858074)
258/18	R, pharynx	*Pasteurellaceae*	*R. ratti* (97.6% / KX858150)
259/18	R, pharynx	*R. heylii*	*R. heylii* (99.1% / KR364783)
260/18	R, pharynx	*Pasteurellaceae*	*R. ratti* (97.2% / KX858242)
261/18	R, pharynx	*Pasteurellaceae*	*R. ratti* (98.5% / KX858074)
262/18	R, pharynx	*Pasteurellaceae*	*R. ratti* (97.6% / KX858242)

M, mouse; R, rat

Table 2. Biochemical characteristics of rat *Rodentibacter* isolates according to API 20E tests

Isolate	ONPG	ADH	LDC	ODC	Cit	H_2S	Ure	TDA	Ind	VP	Gel	Glu	Man	Ino	Sor	Rha	Sac	Mel	Amy	Ara	Ox
341/17	+	—	—	—	—	—	+	—	+	+	—	+	—	—	w	—	—	—	—	—	+
342/17	+	—	—	—	—	—	+	—	+	+	—	+	—	—	w	—	—	—	—	w	+
343/17	+	—	—	—	—	—	+	—	+	+	—	+	—	—	w	—	—	—	—	w	+
345/17	+	—	—	—	—	—	+	—	+	+	—	+	—	—	—	—	—	—	—	—	+
637/17	+	—	—	—	—	—	+	—	+	+	—	+	—	—	—	—	—	—	—	—	+
638/17	+	—	—	—	—	—	+	—	+	+	—	+	—	—	+	—	—	—	—	w	+
639/17	+	—	—	—	—	—	+	—	+	+	—	+	—	—	w	—	—	—	—	w	+
1002/17	+	—	—	—	—	—	+	—	+	+	—	+	—	—	—	—	—	—	—	—	+
1012/17	+	—	—	—	—	—	+	—	+	+	—	+	—	—	—	—	—	—	—	—	+
1360/17	+	—	—	—	—	—	+	—	+	+	—	+	—	—	—	—	—	—	—	—	+
1361/17	+	—	—	—	—	—	+	—	+	+	—	+	—	—	—	—	—	—	—	—	+
257/18	+	—	—	—	—	—	+	—	+	+	—	+	—	—	—	—	—	—	—	—	+
258/18	+	—	—	—	—	—	+	—	+	+	—	+	—	—	—	—	—	—	—	—	+
260/18	+	—	—	—	—	—	+	—	+	+	—	+	—	—	w	—	—	—	—	—	+
261/18	+	—	—	+	—	—	+	—	+	+	—	+	—	—	w	—	—	—	—	w	+
262/18	+	—	—	—	—	—	+	—	+	+	—	+	—	—	—	—	—	—	—	—	+

+, positive; –, negative; ADH, arginine dihydrolase; Amy, amygdalin; Ara, L-arabinose; Cit, citrate utilization; H_2S, H_2S production; Gel, gelatinase; Glu, D-glucose; Ind, indole production; Ino, inositol; LDC, lysine decarboxylase; Man, D-mannitol; Mel, D-melibiose; ODC, ornithine decarboxylase; ONPG, β-galactosidase; Ox, cytochrome oxydase; Sac, D-saccharose; Sor, D-sorbitol; Rha, L-rhamnose; TDA, tryptophan deaminase; Ure, urease; VP, acetoin production; w, weak reaction.

authority as part of the breeding program. All procedures were performed in accordance with German legislation for the care and use of laboratory animals.

Molecular identification by PCR analysis and 16S rDNA sequencing. Template DNA was extracted from isolated colonies picked from agar plates and amplified by using the multiplex PCR assay for rodent *Pasteurellaceae* described previously, which is able to detect and differentiate among the main *Pasteurellaceae* found in laboratory mice.[4] For 16S rDNA amplification and sequencing, we followed a previously published method.[6]

For identification, the 16S rDNA sequences we obtained were compared with the 16S rDNA sequences from EzTaxon (https://www.ezbiocloud.net/identify).[16] The isolates showing less than 0.5% sequence difference between 2 species in EzTaxon and thus a low separation limit to the next species[10] underwent BLAST analysis (https://blast.ncbi.nlm.nih.gov/Blast.cgi)[3] against all *Rodentibacter* 16S rDNA sequences currently available.[2] This process applied to all *R. ratti* isolates included in this study.

Phenotypic identification of rat isolates. To verify the results obtained by using molecular methods, we obtained the biochemical profiles of the rat isolates by using the API 20E Kit (BioMérieux), which incorporates several assays previously proven to be useful in differentiating among *Rodentibacter* spp.[2] The phenotypic characteristics obtained were compared with the profiles previously proposed to uniquely identify *Rodentibacter* spp.[2]

Results

Identification of *Rodentibacter* isolates by using molecular methods. The screening of approximately 50 microbiologic units populated with mice and rats from our facility yielded 51 *Rodentibacter* isolates. By using multiplex PCR analysis,[4] 16 of the 31 mouse isolates were identified as *R. heylii*; the remaining 15 isolates were *R. pneumotropicus*. Among the 20 rat isolates, 16 were classified as *Pasteurellaceae*, with the remaining 4 belonging to *R. heylii* (Table 1).

The results provided by the multiplex PCR tests were confirmed and complemented by means of 16S rRNA gene sequencing analysis. Sequence fragments of approximately 1100 bp and thus covering the V1–V6 variable regions of the 16S rRNA gene were obtained from all but the strains 521/17, 530/17, and 1361/17, for which the fragment length was 900 bp and covered the V1–V5 variable regions (GenBank accession nos., MH990266 through MH990316). All *R. pneumotropicus* isolates and all *R. heylii* isolates except 33/18 were assigned to the respective species at sequence similarity values exceeding 99.0%. In contrast, only 1 of the 16 rat *Rodentibacter* sequences obtained showed greater than 99% similarity to a *R. ratti* strain; the remaining 15 isolates were 97.2% to 98.5% similar to *R. ratti* (Table 1). Despite the relatively low similarity of some isolates to published sequences, a demarcation level of at least 0.6% to *R. mrazii* and *R. heildelbergensis* was recorded for all isolates.

Identification of the rat isolates according to biochemical profiles. To verify the results of the molecular identification, we used the biochemical tests of the API 20E kit, which contains several tests useful for differentiating *Rodentibacter* spp. All isolates tested were positive for β-galactosidase, urease, indole, and acetoin (Table 2). In addition, isolate 261/18 was ornithine decarboxylase–positive. All isolates were able to use glucose, and several isolates also displayed weak positive reactions for saccharose and arabinose (Table 2).

Discussion

Although diverse in phenotype and genotype, most of the rodent *Pasteurellaceae* isolates typically were classified—until recently—as [*P.*] *pneumotropica*, due to lack of information regarding taxonomy.[7] Consequently, knowledge regarding epidemiology, pathogenicity, and virulence factors was hindered due to the inability to distinctly classify these organisms at the species level. Unfortunately, in the past literature, comparison of previous designations of members of the [*P.*] *pneumotropica* complex with the current taxonomy of *Rodentibacter* is rarely possible. Precise classification of *Rodentibacter* isolates at the

species level is essential to our further understanding of these bacteria.

In the present investigation, we analyzed the distribution of *Rodentibacter* spp. among the mice and rats in our facility. Mice are the main hosts for *R. pneumotropicus* and *R. heylii*,[2] and, indeed, the mice in our facility were colonized exclusively by these *Rodentibacter* species. Nevertheless, whereas mouse *Rodentibacter* isolates were able to infect rats through experimental contact, only a few rat isolates could infect a limited number of mice thus demonstrating the higher species specificity of rat isolates compared with mouse isolates.[13] Similarly, results from hemagglutination tests suggest that the *Pasteurellaceae* of mice and *Mastomys* may be related and differ from those isolated from other rodent species.[9] Overall, it seems that some of the *Rodentibacter* spp. strains are host-specific, whereas other variants can be found in a group of closely related hosts.[15] Interestingly, rats in our facility harbored several isolates of *R. heylii*, suggesting that these bacterial strains crossed the mouse species barrier and colonized rats. Although the mice and rats were located in different microbiologic units, contamination of the rats due to the *R. heylii* infected-mice as a source cannot be definitively excluded because of the open-cage housing system used. A second *Rodentibacter* species found in the rat population at our facility is *R. ratti*. This species belongs with *R. heidelbergensis*, *R. trehalosifermentans*, and *R. rarus* as the species for which rats seem to be the predominant host.[2] According to the 16S rDNA sequence, the demarcation level between *R. ratti* strains isolated and *R. heidelbergensis* was *R. mrazii* was low but exceeded the 0.5% threshold frequently used to differentiate among species.[10] Currently, few 16S rDNA sequences from rat *Rodentibacter* species are available, but *R. ratti* is assumed to be widely spread among the laboratory rats.[2] To further assess the sequencing identification phenotypically, we characterized the *R. ratti* isolates biochemically by using API 20E tests. The indole-positive tests indicate that the isolates tested do not belong to *R. heidelbergensis*, and the meso-inositol–negative and arabinose-positive reactions suggest that the isolates are *R. ratti* rather than *R. mrazii*. Moreover, *Apodemus* spp. are the predominant host for *R. mrazii*, which has never previously been isolated from rats.[2] Overall, the biochemical results strengthened and confirmed the results of the molecular diagnostics. Although the pathogenic potential of *R. ratti* is unknown, this species should be included in the health monitoring of laboratory rats, given that it seems to be the most prevalent rat-specific *Rodentibacter* species. Moreover, the pathogenicity is not the only criteria to be considered when designing health surveillance programs.[8] The sequence variation among the isolates that we obtained suggests that several different isolates—rather than a clonal distribution of the same isolate—have been established in our facility over time.

In conclusion, we here document the association of 3 of the newly described *Rodentibacter* spp. with laboratory rodents and consider that the diagnosis of rodent *Pasteurellaceae* at the species level decisively contributes to our understanding of this group of bacteria.

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