

Specialist information

from the Committee for Hygiene

Changes in taxonomy of microorganisms relevant to laboratory animals

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Contents

1.	Introduction	3
2.	Pasteurellaceae	3
2.1.	“Pasteurella pneumotropica”	3
2.2.	“Actinobacillus muris”	5
2.3.	Pasteurellaceae in guinea pigs (<i>Cavia porcellus</i>)	6
2.4.	Pasteurellaceae in the golden hamster (<i>Mesocricetus auratus</i>) and European field hamster (<i>Cricetus cricetus</i>)	7
3.	“Bordetella hinzii”	7
4.	“CAR bacillus”	8
5.	<i>Streptobacillus</i> spp.	8

1. Introduction

In recent years, several microorganisms that are relevant to laboratory animals have undergone systematic and taxonomic revision. While the classification of bacteria was based on phenotypic criteria up to about 1970, molecular genetic criteria are additionally used today in taxonomic classification. This has inevitably led to changes in familiar names and sometimes to a segregation into several species. In other cases, microorganisms that were hitherto not specifically named or described have been characterized and taxonomically classified. With the taxonomic description of bacterial species, the sequence data and type strains of the bacterial species are made accessible at the same time via recognized strain collections. Thus, every laboratory can carry over the results of the studies and thereby achieve a standardization of study results among different laboratories.

With the new descriptions, the designations hitherto used are no longer valid, i.e. are obsolete, and should therefore no longer be used (Table 1).

2. Pasteurellaceae

2.1. “*Pasteurella pneumotropica*”

Background: The most important taxonomic change concerns the family of *Pasteurellaceae* and here in particular the new classification of “*Pasteurella pneumotropica*”. These bacteria are very frequently mentioned in the literature and are regularly listed in health certificates. They were first described by Jawetz (Jawetz 1948). Some years later, a variant was found (Heyl 1963). Both variants were later described as different “biotypes”. Further differences were also observed in phenotypic traits or phenotypes which made it difficult to establish an unequivocal, homogeneous identity. In particular, the commercial biochemical identification systems generally used are not able to reliably determine “*Pasteurella pneumotropica*” and other *Pasteurellaceae* that occur in rodents. Over time, a confusing diversity of different names have been used for *Pasteurellaceae* in rodents. In some cases, the name “*Pasteurella pneumotropica*” has also been used as a collective term for completely different *Pasteurellaceae* that occur in laboratory rodents. It is therefore impossible to offer any reliable statements on the importance of these bacteria.

When it comes to the identification of the same isolates in different laboratories, differing diagnoses are often made, with the result that *Pasteurellaceae* are frequently not listed in health certificates owing to the lack of clarity in the classification. Since “*Pasteurella pneumotropica*” were not unambiguously defined, several molecular biologic methods of detection (PCR) were described, which covered a different bacterial spectrum, however, and likewise led very frequently to confusion. Also, the long-since obsolete assignment of growth factor-dependent *Pasteurellaceae* to the genus “*Haemophilus*” has resulted in these bacteria not normally being detected in tests and hence also not being listed in health certificates. Because of the unclear taxonomy and difficulties in identification, FELASA recommended testing for *Pasteurellaceae* in 2002 (Nicklas et al. 2002). In the revised version (Mähler et al. 2014) and also in the recommendation of a working group of AALAS-FELASA (Pritchett-Corning et al. 2014), however, it is now recommended again to test for “*Pasteurella pneumotropica*”.

When it came to establishing the family *Pasteurellaceae* (Mannheim 1984) the known species were incorporated into three genera (*Haemophilus*, *Actinobacillus*, *Pasteurella*) in 1981. Today more than 25 genera are known to belong to the family *Pasteurellaceae*.

The new taxonomy is based primarily on genetic differences and includes a variety of genes. Early molecular genetic studies by Dewhirst et al. (1993) already showed in 1993 that "*Pasteurella pneumotropica*" does not belong to the genus *Pasteurella* in the stricter sense. On the basis of various studies, the second species frequently seen in mice, "*Actinobacillus muris*", also does not belong to the genus *Actinobacillus* and is more closely related to "*Pasteurella pneumotropica*".

Current status: (Adhikary et al. 2017)

The bacteria previously described as "*Pasteurella pneumotropica*" now form a genus of their own, *Rodentibacter*, which today comprises eight species and two additional genomospecies, which are clearly distinguished from other isolates in the group on the basis of genetic criteria, but cannot be reliably differentiated with regard to their phenotypic characteristics. The two variants formerly regarded as "biotypes" now form their own species. ***Rodentibacter (R.) pneumotropicus*** (formerly biotype Jawetz) and ***Rodentibacter heylii*** (formerly biotype Heyl) are predominantly found in mice, but *R. heylii* in particular can also colonize other animal species, such as rats or golden hamsters, if they are housed together with mice. Both bacterial species normally grow independently of growth factors (X and V factors). However, growth factor-dependent isolates (formerly "*Haemophilus*") are known in both species.

A further important species in laboratory rodents is ***Rodentibacter ratti***. This species consists of growth factor-dependent and independent bacteria and is primarily found in rats, but occasionally also in mice. This bacterium is widespread because it was not sufficiently taken into account until recently.

Rodentibacter trehalosifermentans has been detected in rats and culture is possible only in the presence of growth factors (V factor, NAD).

The isolates of ***Rodentibacter heidelbergensis*** known to date were isolated from rats, and their growth is likewise predominantly dependent on growth factors (V factor, NAD), but isolates independent of growth factors are also known. Isolates of ***Rodentibacter rarus*** were cultured from rats. This species has only very rarely been detected to date and grows independently of growth factors.

Rodentibacter mrazii has only been isolated from mice of the genus *Apodemus* to date and is therefore unlikely to be of any importance in laboratory mice or rats. This also applies to ***Rodentibacter myodis***. This species occurs in the bank vole (*Myodes glareolus*).

For two further genomospecies, no phenotypic criteria were found that allow an unequivocal differentiation from other *Rodentibacter* species, in particular from *R. pneumotropicus* and *R. heylii*. ***Rodentibacter* genomospecies 1** was predominantly isolated from mice of the genus *Apodemus*, but also from house mice (*Mus musculus*) and laboratory rats (*Rattus norvegicus*). The isolates from ***Rodentibacter* genomospecies 2** were isolated from mice of the genus *Apodemus*.

There is no information as yet on the importance of individual species, because a differentiation of the species has not been undertaken to date

Literature

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2.2. “*Actinobacillus muris*”

Background: This bacterium was first described by Bisgaard (1986) and is widespread in laboratory mice. However, the frequent practice of subsuming it under “*Pasteurella pneumotropica*” means that it has only rarely been taken into account and only very rarely listed in health certificates. It has occurred in many phenotypic variants and is therefore difficult to identify. A reliable determination using commercial identification systems is almost impossible, because it is not included in the standard databases. Identification errors are often made, which result e.g. in the designation “*Mannheimia haemolytica*” or “*Pasteurella multocida*”. For this bacterium, too, Dewhirst et al. (1993) have already shown that it is more closely related with “*Pasteurella pneumotropica*” and not with the genus *Actinobacillus*.

Current status: (Nicklas et al. 2015)

These bacteria form a genus of their own, *Muribacter*, of which only the species ***Muribacter muris*** has been described to date. It can be assumed that further species will be added. The bacteria are almost exclusively found in mice. The colony forms and phenotypic (biochemical) characteristics are highly variable. It can be easily cultured on blood agar, but there are very rarely strains that are also dependent on growth factors. Very little is known about these bacteria.

Literature

- Bisgaard M. 1986. *Actinobacillus muris* sp. nov. isolated from mice. Acta Path Microbiol Scand Sect. B 94:1-8.
- Dewhirst FE, Paster BJ, Olsen I, and Fraser GJ. Phylogeny of the *Pasteurellaceae* as determined by comparison of 16S ribosomal ribonucleic acid sequences. Zentralbl Bakteriol 1993; 279: 35-44.
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2.3. *Pasteurellaceae* in guinea pigs (*Cavia porcellus*)

Background: Various authors have described differing *Pasteurellaceae* in guinea pigs, but most of them have not been characterized in any detail or even unequivocally classified in taxonomic terms. A few isolates were assigned provisional names (e.g. named after authors, such as Stewart and Letscher, abbreviations such as SP Group, or phenotypic groups were simply consecutively numbered [e.g. Bisgaard Taxa 5, 7, 8]). In the meantime, some of these bacteria have been shown to form separate genera or species.

Current status: (Christensen et al. 2011a,b; Adhikary et al. 2018)

Few isolates from guinea pigs have been taxonomically classified to date. In the case of bacteria that were previously named Bisgaard Taxon 25, a close relationship has been found with the genus *Mannheimia*. These bacteria form a species of their own ***Mannheimia caviae***. Other bacteria which were originally named as SP Group form a genus of their own and have been renamed as ***Necropsobacter rosorum***. The isolates from guinea pigs named previously as Bisgaard Taxa 5 and 7 also form genera of their own and have been classified as ***Caviibacterium pharyngocola*** and ***Conservatibacter flavescens***.

Literature

- Christensen H, Bojesen AM, Bisgaard M. 2011 *Mannheimia caviae* sp. nov., isolated from epidemic conjunctivitis and otitis media in guinea pigs. Int J Syst Evol Microbiol 61:1699-1704.
- Christensen H, Korczak BM, Bojesen, AM, Kuhnert P, Frederiksen W, Bisgaard M. 2011. Classification of organisms previously reported as the SP and Stewart–Letscher groups, with descriptions of *Necropsobacter* gen. nov. and of *Necropsobacter rosorum* sp. nov. for organisms of the SP group. Int J Syst Evol Microbiol 61:1829–1836.
- Adhikary S, Bisgaard M, Nicklas W, Christensen H. 2018. Reclassification of Bisgaard taxon 5 as *Caviibacterium pharyngocola* gen. nov., sp. nov. and Bisgaard taxon 7 as *Conservatibacter flavescens* gen. nov., sp. nov. Int J Syst Evol Microbiol 68:643-650.

2.4. *Pasteurellaceae* in the golden hamster (*Mesocricetus auratus*) and European field hamster (*Cricetus cricetus*)

Background Bacteria of the genus *Rodentibacter* are occasionally found in golden hamsters, but phenotypic variants are also known that do not belong to this genus. European field hamsters are used less often as laboratory animals, but in these animals, too, *Pasteurellaceae* have likewise been described that were originally not assigned a definitive taxonomic classification. As with isolates from other animal species, provisional names were assigned here as well.

Current status: (Christensen et al. 2014)

From both animal species, the first species of bacteria have now been characterized in more detail. Isolates from European field hamsters that were kept as laboratory animals proved to be a genus of their own and have been named *Cricetibacter osteomyelitidis*. Isolates from golden hamsters are not closely related with these and form a genus of their own. These bacteria have been classified as *Mesocricetibacter intestinalis*.

Literature

Christensen H, Nicklas W, Bisgaard M. 2014. Investigation of taxa of *Pasteurellaceae* isolated from Syrian and European hamsters and proposal of *Mesocricetibacter intestinalis* gen. nov., sp. nov. and *Cricetibacter osteomyelitidis* gen. nov., sp. nov. Int J Syst Evol Microbiol 64(Pt 11):3636–3643.

3. “*Bordetella hinzii*”

Background *Bordetella* (*B.*) *hinzii* was originally isolated from poultry. Human isolates also exist. The literature mentions “*Bordetella hinzii*” only sporadically as an infectious pathogen in laboratory mice, but it has been detected more frequently in other rodents and also in rabbits (Hayashimoto et al. 2012, Jiyipong et al. 2013). In both natural and experimental infections in the mouse, the bacterium can cause respiratory symptoms and histopathological lesions in the respiratory tract (Clark et al. 2016, Hayashimoto et al. 2008). It is usually identified using commercial identification systems (e.g. Api 20 NE) or also through sequencing of the 16S rRNA gene. In the sequencing of other genes (Spilker et al. 2014, Loong et al. 2016) it was found that isolates from mice (*Bordetella* genogroup 16) are clearly distinct from typical *Bordetella hinzii*.

Current status: (Ivanov et al. 2016)

In detailed taxonomic studies, it was shown that isolates from mice that were originally classified as *B. hinzii* form a species of their own which is designated *Bordetella pseudohinzii*. Isolates from poultry, humans and rabbits studied in the same investigation were classified as *B. hinzii*.

Literature

Clark S E, Purcell JE, Sammani S, Steffen EK, Crim MJ, Livingston RS, Besch-Williford C, Fortman JD. 2016. *Bordetella pseudohinzii* as a confounding organism in murine models of pulmonary disease. Comp Med 66(5):361–366.

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- Spilker T, Leber AL, Marcon MJ, Newton DW, Darrah R, Vandamme P, Lipuma JJ. 2014. A simplified sequence-based identification scheme for *Bordetella* reveals several putative novel species. *J Clin Microbiol* 52:674-677.

4. “*CAR bacillus*”

Background “*CAR bacillus*” (cilia-associated respiratory bacillus) was first detected in 1980 in rats, where it was found to act as a pathogen causally involved in chronic respiratory disease (CRD). Similar bacteria were later detected in mice and rabbits, but also in cows and pigs. They were originally cultured in embryonated chicken eggs and later in cultures of mammalian cells. Because of difficulties in cultivation and the lack of reference bacteria in strain collections, the bacterium was hardly accessible for laboratories.

Current status: (Ike et al. 2016)

A bacterial strain originally isolated from a rat with CRD was studied in more detail and, together with other isolates from rodents, was described as a genus of its own. The official name is now *Filobacterium rodentium*.

Literature

- Ike F, Sakamoto M, Ohkuma M, Kajita A, Matsushita S, Kokubo T. 2016. *Filobacterium rodentium* gen. nov., sp. nov., a member of *Filobacteriaceae* fam. nov. within the phylum *Bacteroidetes*; includes a microaerobic filamentous bacterium isolated from specimens from diseased rodent respiratory tracts. *Int J Syst Evol Microbiol* 66:150-157.

5. *Streptobacillus* spp.

Background *Streptobacillus moniliformis* is a major zoonotic pathogen that has been found predominantly in the pharynx of rats, but also in other animal species and causes a form of so-called rat-bite fever in humans. It has to be assumed that infections with *Streptobacillus moniliformis* occur more frequently than they are diagnosed (Regnath et al. 2015). Due to the

demanding requirements of culture conditions and the slow growth rate, the bacterium is difficult to cultivate, but the culture succeeds under aerobic conditions at increased CO₂ concentration. In contrast, it has been reported that isolates from guinea pigs require anaerobic conditions for growth.

Current status:

It has been shown that isolates from guinea pigs are not related to *Streptobacillus* (*S.*) *moniliformis* at genus level. They form a genus of their own and have been described as ***Caviibacter abscessus*** (Eisenberg et al. 2016b). In addition, in-depth studies in a large number of isolates of *Streptobacillus moniliformis* have shown that several species exist here. Besides *Streptobacillus moniliformis*, the classical pathogen for rat-bite fever, several species have been described to date, but these are likely to be less relevant to laboratory rodents and hence also to questions concerning laboratory animal science. These are

- ***S. felis***, isolated from a cat; host unknown (Eisenberg et al. 2015b)
- ***S. notomytis***, host: spinifex hopping mouse (*Notomys alexis*) and house rat (*Rattus rattus*) (Eisenberg et al. 2015a, Michel et al. 2018)
- ***S. hongkongensis***, host: human (Woo et al. 2014)

Differentiation of these species is not possible using biochemical methods, but is achieved by means of MALDI-TOF mass spectrometry as a phenotypic method and also based on molecular genetic sequence comparisons (Eisenberg et al. 2016a). Only very few isolates of the newly described species are known to date, so it is not possible to draw conclusions as to the potential for zoonoses or the host spectrum. Apart from *S. hongkongensis* found in humans in the context of clinical symptoms, the only other one of the newly described species to be found in humans is *S. notomytis* in rat-bite fever (Fukushima et al. 2018, Ogawa et al. 2018).

Literature

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- Eisenberg, T, Glaeser, S. P., Nicklas, W., Mauder, N., Contzen, M., Aledelbi, K., Kämpfer, P. 2015b. *Streptobacillus felis* sp. nov. isolated from a cat with pneumonia, and emended descriptions of the genus *Streptobacillus* and of *Streptobacillus moniliformis*. *Int J Syst Evol Microbiol* 65:2172–2178.
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- Ogawa Y, Kasahara K, Lee ST, Ito T, Hasegawa H, Hirose S, Santo S, Yoshida A, Nakano R, Yano H, Mikasa K. 2018. Rat-bite fever in human with *Streptobacillus notomytis* infection. *Emerg Infect Dis* 24:1377-1379. <https://doi.org/10.3201/eid2407.171580>
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- Woo PC, Wu AK, Tsang CC, Leung KW, Ngan AH, Curreem SO, Lam KW, Chen JH, Chan JF, Lau SK. 2014. *Streptobacillus hongkongensis* sp. nov., isolated from patients with quinsy and septic arthritis in Hong Kong, and emended descriptions of the genus *Streptobacillus* and the species *Streptobacillus moniliformis*. *Int J Syst Evol Microbiol* 64:3034-3039.

Table 1: Changes in the taxonomy of microorganisms relevant to laboratory animals

New name	Old name	Host species
<i>Rodentibacter pneumotropicus</i>	<i>Pasteurella pneumotropica</i> "Jawetz biotype"	House mouse (<i>Mus musculus</i>)
<i>Rodentibacter heylii</i>	<i>Pasteurella pneumotropica</i> "Heyl biotype"	House mouse (also laboratory rat, golden hamster)
<i>Rodentibacter rattii</i>	---	Laboratory rat (also house mouse)
<i>Rodentibacter trehalosifermentans</i>	---	Laboratory rat (<i>Rattus norvegicus</i>)
<i>Rodentibacter heidelbergensis</i>	---	Laboratory rat (<i>Rattus norvegicus</i>)
<i>Rodentibacter rarus</i>	---	Laboratory rat (<i>Rattus norvegicus</i>)
<i>Rodentibacter mrazii</i>	---	<i>Apodemus</i> sp.
<i>Rodentibacter myodis</i>	---	Bank vole (<i>Myodes glareolus</i>)
<i>Rodentibacter genomospecies 1</i>	---	<i>Apodemus</i> sp. (also house mouse and laboratory rat)
<i>Rodentibacter genomospecies 2</i>	---	<i>Apodemus</i> sp.
<i>Muribacter muris</i>	<i>Actinobacillus muris</i>	House mouse (<i>Mus musculus</i>)
<i>Mannheimia caviae</i>	Bisgaard Taxon 25	Guinea pig (<i>Cavia porcellus</i>)
<i>Necropsobacter rosorum</i>	SP Group	Guinea pig (<i>Cavia porcellus</i>)
<i>Caviibacterium pharyngocola</i>	Bisgaard Taxon 5	Guinea pig (<i>Cavia porcellus</i>)
<i>Conservatibacter flavescens</i>	Bisgaard Taxon 7	Guinea pig (<i>Cavia porcellus</i>)
<i>Cricetibacter osteomyelitis</i>	---	European Field hamster (<i>Cricetus cricetus</i>)
<i>Mesocricetibacter intestinalis</i>	---	Syrian golden hamster (<i>Mesocricetus auratus</i>)
<i>Bordetella pseudohinzii</i>	<i>Bordetella hinzii</i> , <i>Bordetella</i> genogroup 16	House mouse (<i>Mus musculus</i>)
<i>Filobacterium rodentium</i>	CAR bacillus	Laboratory rat (<i>Rattus norvegicus</i>)
<i>Streptobacillus moniliformis</i>	<i>Streptobacillus moniliformis</i>	Laboratory rat, house mouse, turkey, human
<i>Streptobacillus notomytis</i>	<i>Streptobacillus moniliformis</i>	Spinifex hopping mouse (<i>Notomys alexis</i>), house rat, human
<i>Streptobacillus rattii</i>	<i>Streptobacillus moniliformis</i>	House rat (<i>Rattus rattus</i>)
<i>Streptobacillus hongkongensis</i>	---	Human
<i>Streptobacillus felis</i>	---	Cat (<i>Felis catus</i>)
<i>Caviibacter abscessus</i>	<i>Streptobacillus moniliformis</i>	Guinea pig (<i>Cavia porcellus</i>)

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