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The aerobic bacterial flora of laboratory rats from a danish breeding centre

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Introduction

Through the last thirty years infections being of importance in animal experimentation have been eliminated from colonies of laboratory rodents. However, this does not mean that today laboratory rodents never suffer from disease, in which microorganisms play a primary or secondary etiological part. Some experiments might stress the animals and give opportunistic bacteria a chance of producing disease (*Urano & Maejima* 1982, *Matsumoto* 1980, *Matsumoto* 1982, *Brook et al.* 1984, *Banerjee et al.* 1988, *Detmer et al.* 1990). Other bacteria might be apathogenic in one species, while showing pathogenicity in other species. E.g. this is the case for *Klebsiella pneumoniae,* which is pathogenic for mice (*Webster* 1930, *Ehrenwort & Baer* 1956), but not for rats (*Weisbroth* 1979).

Diagnosis of bacterial disease discovered during an experiment is normally achieved by culturing bacteria from the diseased organ. In order to interpret the results of such investigations correctly, it is essential to know the bacterial flora observed in healthy animals and the normal prevalences and isolation sites of the observed organisms.

Sometimes users have specific needs for knowledge whether the animals used in their experiment are harbouring certain bacteria or not. To be able to make such an investigation with an acceptable confidence limit, normally $p < 0.05$, it is necessary to know the normally observed prevalence of that bacterium in infected colonies.

At our laboratory bacteriology on healthy laboratory rats is performed as a part of the health monitoring programme. In the following report the prevalences of the various bacteria found are stated in order to give a picture of the bacterial flora of healthy rats and the question whether the observed microorganisms might influence the outcome of animal experiments or not is discussed.

Materials and methods

Ten rats were investigated bacteriologically every three months. Of these two were weanlings, 4 were 8–15 weeks, while 4 were retired breeders. The rats were anasthesized by carbon dioxide and blood was sampled from the heart. Hereafter the rats were killed by carbon dioxide.

The results include such investigations of nose, trachea, lungs and genitalia of 515 rats and the ceacum of 110 rats from 12 different units. Some of the colonies were only run for a part of the observation period. As animals were sampled every three months this lead to a lower sample size, the lowest being a total sample size of 20 rats for three of the colonies, while the highest total sample size was 80 rats for two of the colonies. In a single sampling it was necessary to half the sampling size due to the lack of rats.

From the nose, the trachea, the lungs and the genitalia were inoculated on 10 % horse blood agar (Gibco, Paisly, Scotland) by the use of a platin needle. All agar plates were incubated aerobically at 37°C. After 24 hours of incubation all morphologically different colonies were reinoculated on blood agar and reincubated at 37°C for 24 hours, whereupon all cultures were described by their colony pigmentation and size, Gram stain, catalase and cytochrome oxidase reaction as described by *Aalbæk et al.* (1981).

Gram positive, catalase positive cocci were tested by the use of a combined protein A and bound coagulase kit, Staphaurex (Wellcome Diagnostics, Dartford, England), and

identified as either *Staphylococcus aureus* or coagulase negative micrococcaceal species. Occassionally, *micrococci* and *staphylococci* were identified by the use of the fermentation kit, API STAPH (BioMerieux, Meyrin-Geneve, France). Gram positive, catalase negative cocci were tested for sensitivity to optochin and by latex agglutination for the presence of Lancefield's antigens A, B, C, D, F and G (Streptex, Wellcome Diagnostics, Dartford, England).

Gram positive rods were identified according to their bacterial morphology, presence of spores in spore staining and catalase reaction. If necessary, fermentation tests were made in order to identify *Corynebacterium kutscheri* or *Listeria Monocytogenes*, using the kits API 20E or API STREP (BioMerieux, Meyrin-Geneve, France), respectively.
Gram negative bacteria were identified by the fermentation test kits API 20 E and API 20 NE (BioMerieux, Meurin-Geneve, Fran-

Table 1. Prevalence rates of aerobic or facultative anaerobic bacteria isolated from the nose, trachea, lung, genital organs or caecum of laboratory rats in an investigation of 12 different colonies. Total number of rats examined was 625.

Bacterial species	Colonies infected with the organism infected/examined	Prevalence observed within infected colonies		Minimal sample size for detection of the organism in routine microbiological monitoring	Primary isolation site
		mean	s. d.		
Micrococcae					
Staphylococcus aureus	12/12	52.2	14.1	4	Nose
Protein A/Bound coagulase-negative spp.	12/12	42.5	18.4	5	Nose
Streptococcae					
Group B	3/12	8.3	4.2	35	Genitalia
Group G	3/12	8.2	1.5	35	Nose
Other streptococci	8/12	10.5	4.5	27	Trachea
Enterococci*	9/12	22.3	11.2	12	Nose
Lactobacillus spp.	4/12	8.5	9.5	34	Trachea
Bacillus spp.	7/12	7.0	4.0	41	Trachea
Corynebacterium spp.*	1/12	3.0		8	Trachea
Enterobacteriaceae					
Escherichia coli	11/12	69.3	34.0	3	Caecum
Enterobacter cloacae	4/12	28.6	14.8	9	Genitalia
Proteus mirabilis	7/12	24.0	43.4	11	Caecum
Morganella morganii	1/12	7.5	6.9	38	Genitalia
Citrobacter diversus	1/12	4.4		67	Trachea
Klyuvera spp.	1/12	5.0		58	Nose
Klebsiella pneumoniae	2/12	20.0	26.5	13	Caecum
Other enterobacteriacea spp.	7/12	23.8	21.6	11	Genitalia/ Caecum
Neisseriaceae					
Acinetobacter iwoffii	2/12	3/5	1.0	84	Nose
Pasteurellaceae					
"Pasteurella pneumotropica"**	1/12	19.6		14	Nose
Haemophilus spp.	6/12	7.0	5.0	41	Genitalia
Pseudomonadaceae					
Pseudomonas aeruginosa	1/12	5.0		58	Nose/Trachea
Xanthomonas maltophilia	2/12	3.1	1.9	95	Nose

* Previously designated group D *streptococci* (*Schleifer* 1987).
** Taxonomically no longer regarded as *Pasteurella* spp., but rather as an *Actinobacillus* spp. (*Mutters* 1985).

ce). Gram negative rods, from which pure cultures could only be grown by the addition of the V-factor, was designated *Haemophilus* spp. and not identified any further.

Interpretation of results from API kits was done by the computer software APILAB-PLUS (BioMerieux, Meyrin-Geneve, France), except for the results of incubation of *Corynebacterium* spp. in API 20E, which was interpreted by comparison with the profile given by *Kunstyr et al.* (1989).

From the ceacum was inoculated into Salmonella Enrichment Broth ad mod. Rappaport (Merck, Darmstadt, Germany) and Yersinia Enrichment Broth (Merck, Darmstadt, Germany). Salmonella Enrichment Broth was incubated for 24 hours at 37°C, whereafter reinoculation on Brilliantgreen Phenolred Lactose Saccarose agar (Merck, Darmstadt, Germany) was performed. Red colonies were tested for the presence of the enzymes phenylalanine deaminase, β-galactosidase, lipase, esterase and fast-violet blue reaction (Rapidec Z, BioMerieux, Meyrin-Geneve, France) and oxydase-activity. If necessary further testing was performed as described for gram negative rods. Yersinia Enrichment Broth was incubated at 22°C temperature for 48 hours, whereupon reinoculation on Yersinia Selective Agar (Merck, Darmstadt, Germany) at 30°C for 48 hours was performed. Yersinia suspect

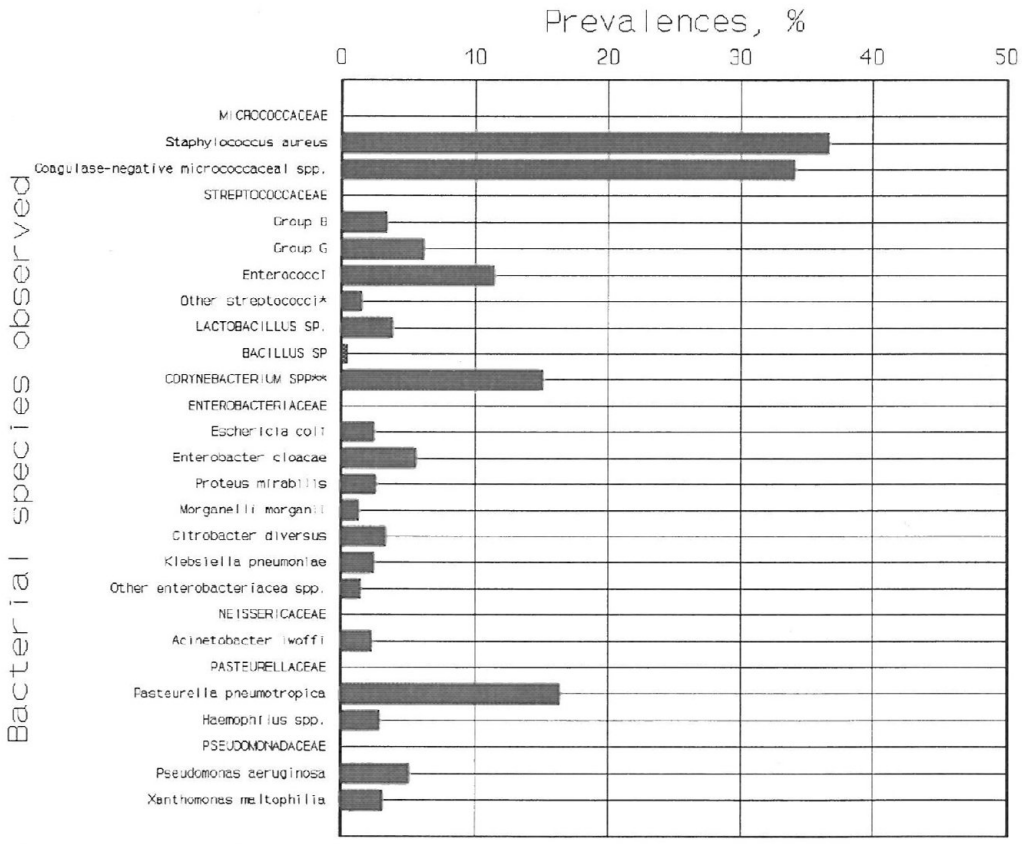

Figure 1. Prevalences of bacteria isolated from the nose of laboratory rats. * Streptococcal species not reacting with antibodies to antigens of Lancefield's group A, B, C, D, E or G. ** Not C. kutscheri.

colonies were identified as described for gram negative rods.
Two types of prevalence were calculated for each bacterial species identified:
– "Colonies infected with the organism" gives the number out of the 12 colonies in which the bacterial species in question was found.
– "Prevalence observed within infected colonies" gives the mean percentage of animals infected with the bacterial species in question, but only for those colonies, from which this bacteria was isolated at least once.
The minimal sample size for each bacteria was calculated from the formula

$$S = \frac{\log 0.05}{\log (1\text{-prevalence})}$$

Results
The prevalence for all bacteria found are given i table 1, as well as the optimal sample size and the primary isolation site. The prevalences for each of the five organs examined are given in figure 1, 2, 3, 4 and 5. Coagulase negative *micrococcaceae* identified have been *S. xylosus, S. simulans, S. hominis, S. cohnii* and *S. lugdunensis.* Identified *Lactobacillus* species includes only *Lactobacillus lactis* subspp. *lactis.* Fermentation

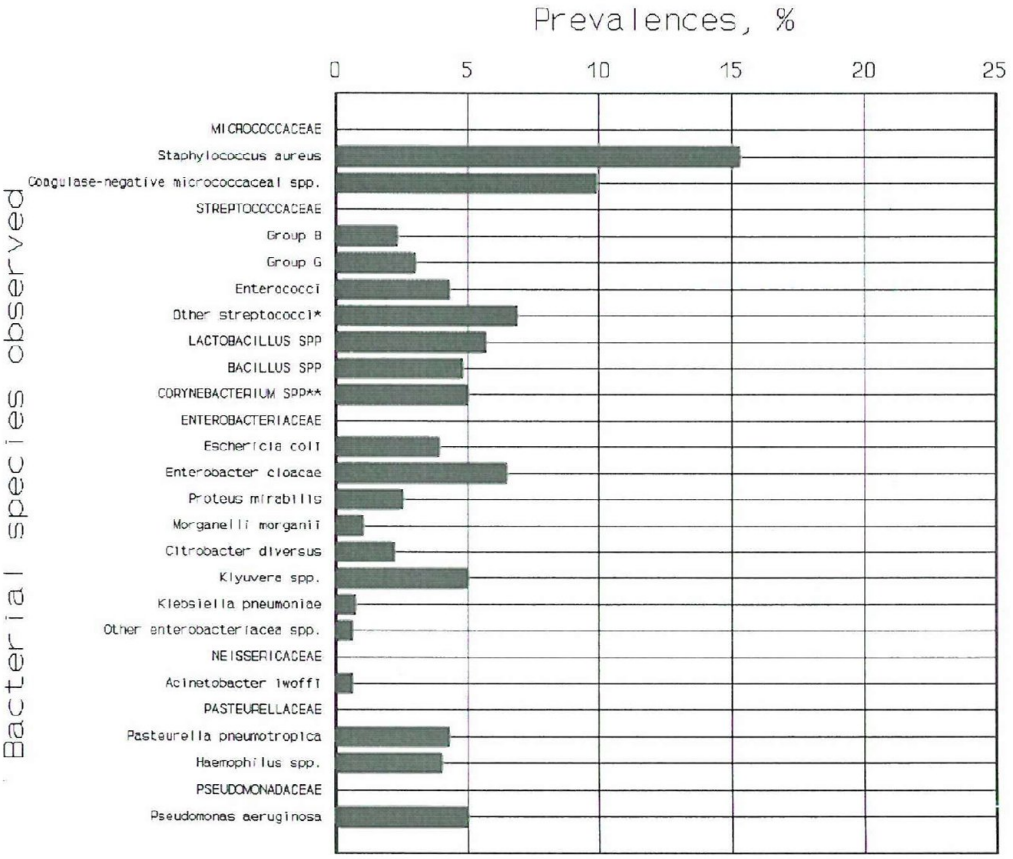

Figure 2. Prevalences of bacteria isolated from the trachea of laboratory rats. * Streptococcal species not reacting with antibodies to antigens of Lancefield's group A, B, C, D, E or G. ** Not C. kutscheri.

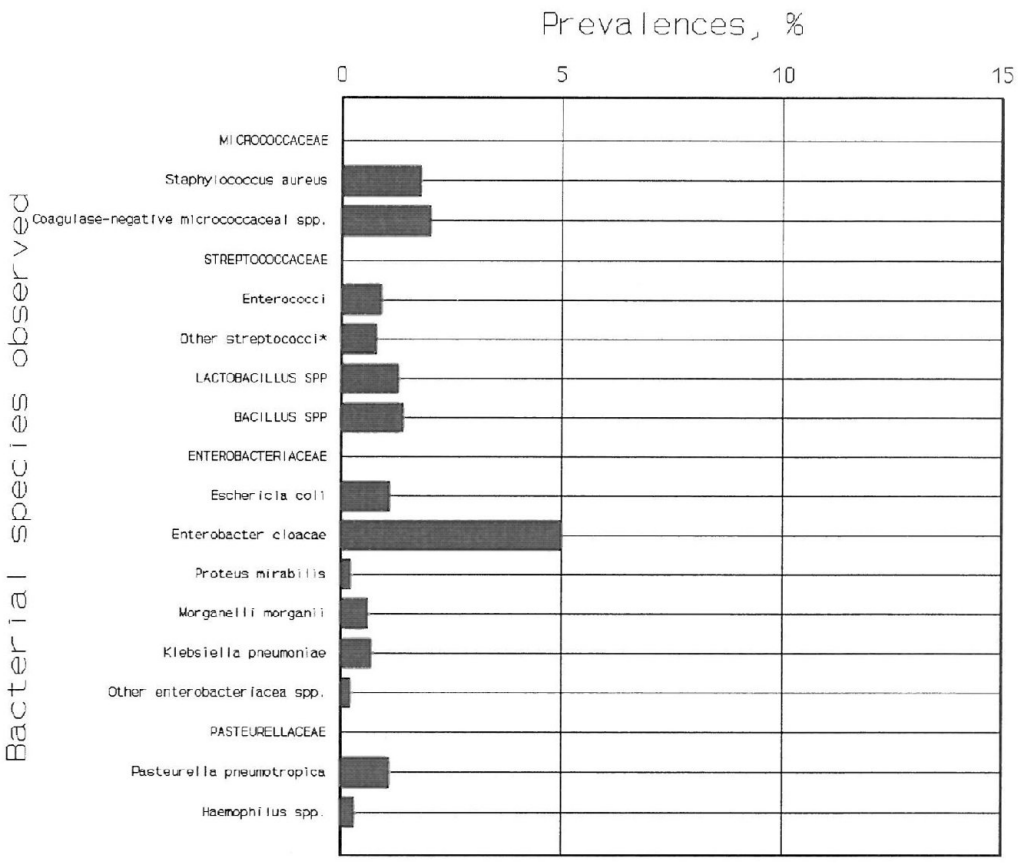

Figure 3. Prevalences of bacteria isolated from the lungs of laboratory rats. * Streptococcal species not reacting with antibodies to antigens of Lancefield's group A, B, C, D, E or G.

tests of *Corynebacterium* spp. showed that these were not identical with *C. kutscheri* and further identification was not done. Attempts to identify *Haemophilus* and *Bacillus* species further than to the genus level have not been made. Bacteria designated as "other *enterobacteriacea* spp" include only species, which did not give any clear identification results in the systems used. *Salmonella* spp. or *Yersinia* spp. was not isolated.

Discussion

The microorganism most commonly observed was *S. aureus*. This is in accordance with observations of others (*Needham* 1980, *Wullenweber-Schmidt et al.* 1989). One of the reasons for this high prevalence might be contamination from the human caretakers, as the phage-types of *S. aureus* observed in the animals of an animal unit are the same as those which can be isolated from the human caretakers (*Wullenweber et al.* 1990). It might be surprising that *S. sciuri* was not identified in these rats, as this bacteria is commonly found in rodent colonies (*Kloos* 1980, *Wullenweber-Schmidt et al.* 1987). However, as this is a rodent specific bacteria, it might have no chance of entering a barrier-facility, if the rats have not been

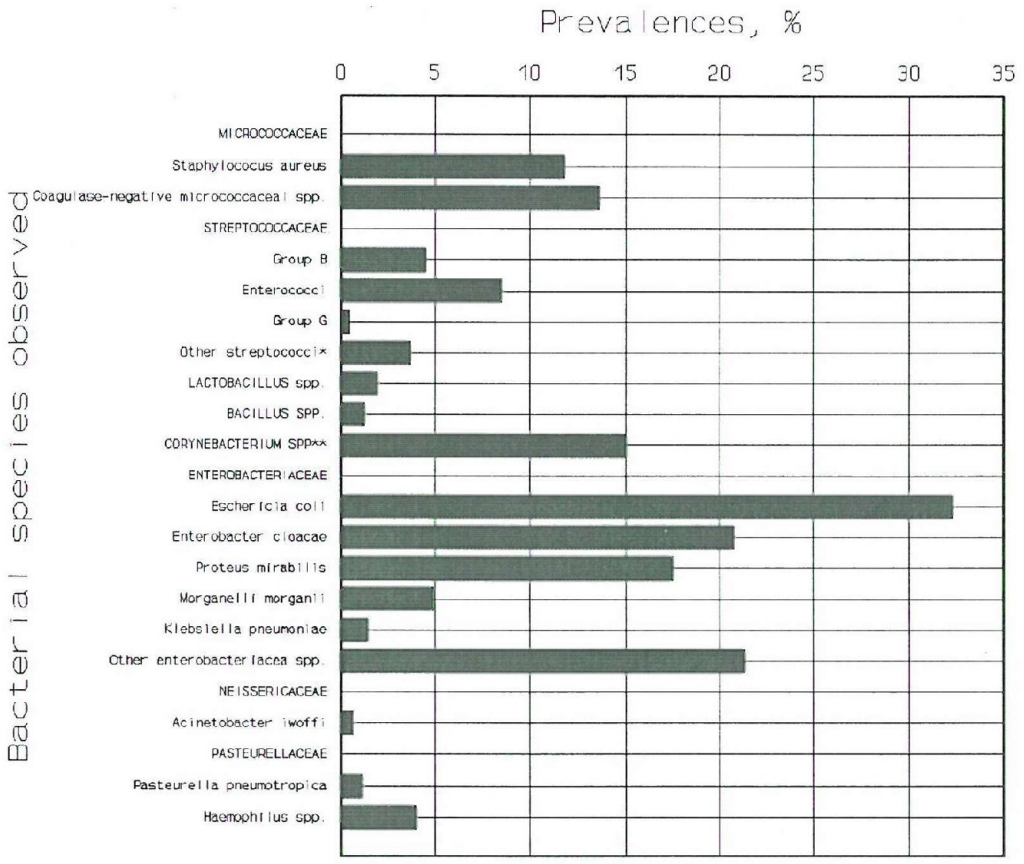

Figure 4. Prevalences of bacteria isolated from the genital organs of laboratory rats. * Streptococcal species not reacting with antibodies to antigens of Lancefield's group A, B, C, D, E or G. ** Not C. kutscheri.

associated with this species after rederivation.

Regarding the Gram negative flora of the respiratory system, it is interesting, that *Enterobacteriaceae* spp. such as E. coli and *E. cloacae* were far more common than non-enteric rods such as *Pasteurellaceae, Neisseriaceae,* and *Bordetella* spp. The prevalence for *P. pneumotropica* observed in this report is low compared to other reports, where the prevalence of carriers within infected colonies varies from 48 to 95 % (*Besch-Williford & Wagner* 1986). *Nakagawa et al.* (1984) found 9.7 % of japanese SPF rat colonies infected, while 70.3 % of Japanese, conventional colonies were infected. In Europe 9 % of rat colonies show antibodies to *P. pneumotropica* in ELISA test (Zentralinstitut für Versuchstierzucht, 1990). *B. bronchiseptica* was not isolated from these rats, although *Fujiwara* (1980) serologically found a high prevalence of infection with this bacteria in rat colonies. Low prevalences have also been found by cultivation (*Sparrow* 1976). The low prevalence of *P. aeruginosa* organism might be due to the acidification of drinking water, which is an efficient method for avoiding growth of *Pseudomonas* spp. in drinking bottles (*Hoag et al.* 1965). *White* (1989)

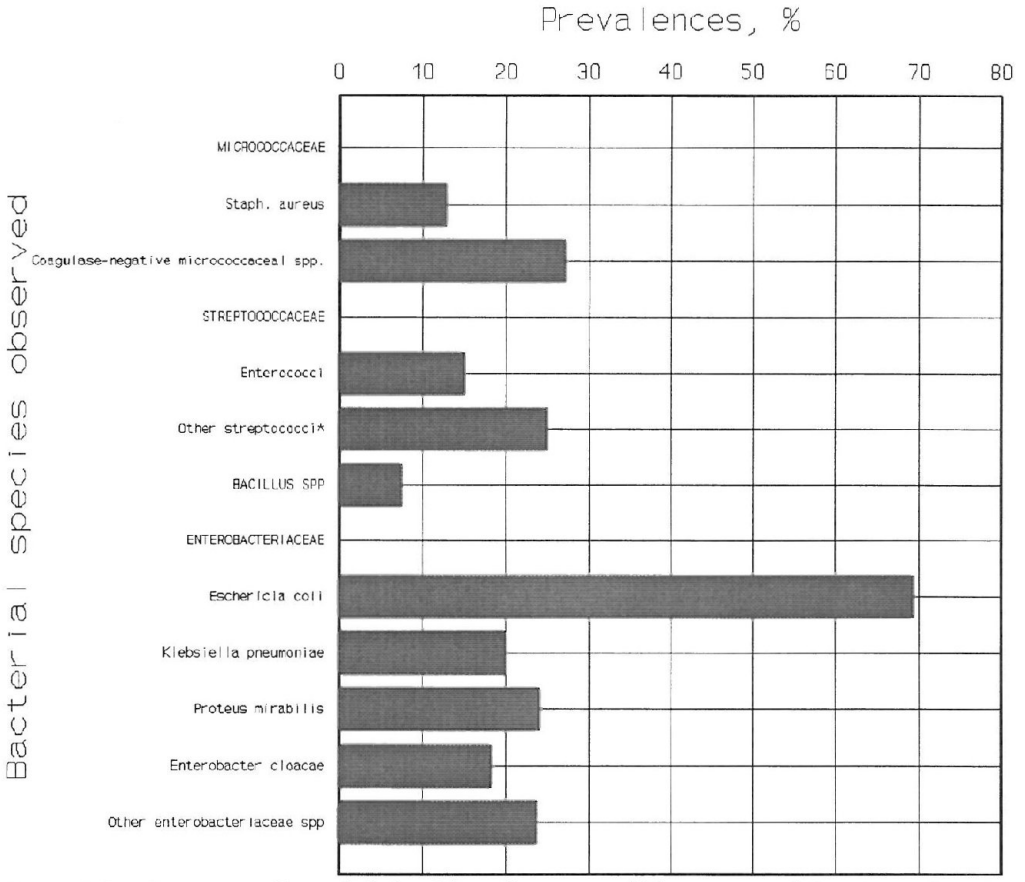

Figure 5. Prevalence rates of bacteria isolated from the caecum of laboratory rats. * Streptococcal species not reacting with antibodies to antigens of Lancefield's group A, B, C, D, E or G. ** Not C. kutscheri.

finds contamination with *K. pneumonia* unavoidable in barrier-maintained rat colonies. This is confirmed by observations made by *Needham* (1980). *Proteus* spp isolated from rats seem to be only *P. mirabilis* and *Morganella morganii* (which in elder systematics also is regarded a *Proteus* spp.).

All these findings are from healthy animals. Such results can be used for interpretation of results of bacteriological examination of diseased rats. For instance, the number of bacteria found in the lungs is low, so isolation of bacteria from the lungs of rats with pneumonia probably indicates the etiology of the disease, although the bacteria might only serve as secondary pathogenes. Some of the observed bacteria might cause disease in rats. Conjunctivitis and respiratory disease might be connected with the isolation of *E. coli, P. aeruginosa, S. aureus* and *S. xylosus* (Unpublished), *P. aeruginosa, E. coli, S. aureus* and *Bacillus fragilis* have experimentally been shown to cause post-surgical wound infections in rodents (*Edlich et al.* 1968, *McRipley & Whitney* 1976, *Moesgaard et al.* 1983, *Panton et al.* 1985), *E. coli* might cause diarrhoea (Unpublished), while *Haemophilus* spp. might be the cause of light histopathological changes in the lungs

(*Nicklas* 1989, *Nicklas* 1990). The rats of the colony infected with *P. pneumotropica* did not suffer from any kind of respiratory disease. Although previously described as a pathogen of rats (*Wheater* 1967, *van der Schaff et al.* 1970, *Young & Hill* 1974), it is the general opinion today that this organism if showing any pathogenicity mostly acts as a secondary pathogen in connection with respiratory disease of mycoplasmal or viral etiology (*Brennan* 1969). Some of the observed bacterial species such as *staphylococci (Brook et al.* 1984, *Detmer et al.* 1990), *streptococci* (*Brook et al.* 1984), *enterobacteriaceae* (*Matsumoto* 1980, *Matsumoto* 1982, *Brook et al.* 1984, *Banerjee et al.* 1988) and *Pseudomonas* (*Taffs* 1974, *Urano* 1978, *Urano & Maejima* 1978) might cause disease and death in immunocompromised animals. Parts of the aerobic flora such as *Streptococci of group D, Lactobacilli* and *Bacillus* spp. might have a symbiotic value for the animal (*Jawetz* 1980, *Tannock* 1987).

Normally, a sample size of eight or ten animals is used for bacteriological examinations. It is noticable, that this sample size only results in an acceptable confidence limit for very few of these bacterial species. This problem might be solved for some of the bacteria by sampling some more animals, e.g. in the case of *K. pneumoniae* and *P. pneumotropica.* For other bacteria the problem might be solved by choosing diagnostic methods with a higher nosographic sensitivity.

It can be concluded that although barrier-maintained rats are mostly free of specific pathogens they harbour a great number of different bacterial species with a certain potential of influencing research and that experimentation might be improved by a careful definition of this flora prior to experimentation.

Summary

The aerobic bacterial flora of barrier-maintained laboratory rats from 12 different units was examined by the use of non-selective bacteriological cultivation. All rats were randomly sampled healthy rats. The number of infected clonies out of the total number of examined colonies is given with the mean prevalence observed within the colonies for each of the bacterial species identified. The minimal sample size for detection of the organisms in routine microbiologicall monitoring is estimated on basis of the prevalences observed. Bacterial species from the groups *Micrococcaceae, Streptococcaceae, Lactobacillus* spp, *Bacillus* spp, *Corynebacterium* spp, *Enterobacteriaceae, Neisseriaceae, Pasteurellaceae* and *Pseudomonadaceae* were found. The most frequently isolated species was *Staphylococcus aureus* in the respiratory organs and *Escherichia coli* in the intestines. The prevalence of the different bacterial species within the colonies varied from 3.1 to 69.3. The minimal sample size for each bacterial species varies from 3 to 95.

Sammendrag

Den aerobe bakterie-flora hos barriere-opfostrede laboratorierotter fra 12 forskellige staldenheder bestemtes ved brug af non-selektiv kultivering. Alle rotter var random-sampled og uden klinisk sygdom. Antallet af inficerede kolonier ud af de 12 undersøgte kolonier angives for hver af de observerede bakterie-arter, ligesom den gennemsnitlige prævalens i de inficerede enheder angives. Ud fra disse værdier beregnes den minimale prøvestørrelse til brug for rutinemæssig sundhedskontrol. Arter fra grupperne *Micrococcaceae, Streptococcaceae, Lactobacillus* spp, *Bacillus* spp, *Corynebacterium* spp, *Enterobacteriaceae, Neisseriaceae, Pasteurellaceae* og *Pseudomonadaceae* isoleredes. *Staphylococcus aureus* var den mest almindelige bakterie i luftvejene, medens *Escherichia coli* var den mest almindelige i tarmen. Den gennemsnitlige prævalens af de enkelte bakterier varierede fra 3,1 til 69,3. Den minimale prøvestørrelse fandtes at variere fra 3 til 95.

Yhteenveto / K. Pelkonen

Tuössä selvitettiin viljelemällä 12 koe-eläinyksiköstä olevista barrieerikasvatetuista satunnaisesti otetuista rotista niiden aerobinen bakteerilloora. Esiintyvyyden perustella annetaan se pienin näytteiden lukimäärä joka tarvitaan ko. organismin havaitsemiseksi rutiinitakastuksessa. Selvityksessä löydettiin bakteereja ryhmistä *Micrococceae, Streptococceae, Lactobacillus* spp., Corynebacterium spp., *Enterobacteriaceae, Neisseriaceae, Pasteurellaceae* ja *Pseudomonaceae.* Yleisimmät eristetyt olivat *Staphyllococcus aureus* hengityselimistöstä ja *Escherichia coli* suolistosta. Keuhkoista eristettyjen bakteerien lukumäärä oli alhainen. Eri bakteerilajien esiintyvyys kolonioissa vaihteli välillä 3.1 ja 69.3. Pienin näytemäärä kaukin bakteerilajia kohti vaihteli välillä 3 ja 95. Monet näistä bakteereista saattavat apatogeenisyydestään

huolimatta liittyä johonkin sairauteen, erityisesti immunosuppressoiduissa eläimissä.

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