Commensal Aerobic Bacterial Flora of the Gastrointestinal Tract of Pipistrellus nathusii (Chiroptera: Vespertilionidae): Lack of Escherichia coli in Fecal Samples

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Source: Acta Chiropterologica, 4(1) : 99-106

Published By: Museum and Institute of Zoology, Polish Academy of Sciences

URL: https://doi.org/10.3161/001.004.0112

Commensal aerobic bacterial flora of the gastrointestinal tract of *Pipistrellus nathusii* (Chiroptera: Vespertilionidae): lack of *Escherichia coli* in fecal samples

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Key words: *Pipistrellus nathusii*, oral cavity, feces, commensal flora, *Escherichia coli*, Poland

INTRODUCTION

The relationships between the macro-organism and commensal bacterial flora are influenced by many factors, including the host specificity and food composition (Jabłoński, 1979). Nevertheless, even basic knowledge about bacterial microflora of wild mammals is insufficient (Akatov, 1984; see also Souza *et al.*, 1999), and bats are not an exception.

These animals seem to be especially interesting for the study of macro- and micro-organism relationships and host specificity of bacteria due to many unusual features, e.g., hypothermia during winter. The recent study of Różalska *et al.* (1998) showed lack of one of the most common fecal bacteria, *Escherichia coli*, in the gastrointestinal track of *Myotis myotis* and *Barbastella barbastellus*. The aim of the present work was to gain information on the composition of the bacterial microflora of *Pipistrellus nathusii.*

MATERIALS AND METHODS

Samples were taken from the oral cavity and feces of *P. nathusii* found in day roosts on Vistula Split (northern Poland) in the 2nd half of October 2000 by using sterile swabs moistened with 0.9% NaCl before sampling. They were inoculated on solid media (blood agar, MacConkey agar, Chapman agar, Sabouraud agar), and incubated 24 hrs at 37°C and then 24 hrs at room temperature. After incubation, micrococci and staphylococci were distinguished according to the method described by Schumacher (1984). Staphylococcal cultures were identified to species level by using the ID 32 staph kit (Bio-Merieux). The gram-negative bacteria were identified by using ID 32 GN kit (BioMerieux). Antibiotic susceptibility was investigated by the disk-diffusion method on Mueller-Hinton agar. A total of 93 bacterial strains were recovered from 25 oral and 26 fecal samples processed in the Department of Medical Microbiology, Medical University of Gdańsk, Poland.

RESULTS

Most (40%) of all bacterial strains found in fecal samples ($n = 57$) belonged to the family Enterobacteriaceae (*Enterobacter cloacae, E. agglomerans, Serratia liquefaciens, S. fonticola, Hafnia alvei, Klebsiella pneumoniae,* and *Citrobacter freundii*); 35% of the strains were coagulase-negative staphylococci (*Staphylococcus warneri, S. xylosus, S. sciuri, S. lentus,* and *S. equorum*) and the remaining strains (25%) belonged to the genera *Micrococcus*, *Enterococcus*, and non-fermenting rods (Fig. 1).

Of the strains isolated from oral cavity ($n = 36$), 43% were staphylococci (*S. equorum, S. warneri*), 27% micrococci, 18% enterococci, and the remaining strains (12%) belonged to the genus B*acillus* and the family Enterobacteriaceae (*E. cloacae, E. agglomerans*; Fig. 1). There was no dominant species among rods of the family Enterobacteriaceae. However, the staphylococcal flora was dominated by *S. equorum* (15% of all isolates versus up to 3.2% isolates for other species; Table 1).

TABLE 1. The percentage of isolates of different species of bacteria (93 strains) from *P. nathusii*

Gram-negative	Isolates (%)	Gram-positive	Isolates (%)
Enterobacteriaceae		*Bacillus* sp.	1.1
Seratia liquefaciens	6.5	Cocci	
Hafnia alvei	4.3	*Staphylococcus equorum*	15.1
Enterobacter cloacae	4.3	*S. lentus*	3.2
E. agglomerans	3.2	*S. xylosus*	3.2
Serratia fonticola	2.1	*S. warneri*	2.1
Citrobacter freundii	1.1	*S. sciuri*	1.1
Klebsiella pneumoniae	1.1	*Staphylococcus* (k-) sp.	9.7
Unidentified	3.2	*Enterococcus* sp.	10.8
Non-fermenting rods		*Micrococcus* sp.	18.3
Pseudomonas fluorescens	2.1	*M. luteus*	2.1
P. putida	2.1	*M. roseus*	1.1
Unidentified	2.1		

As to the frequency of occurrence of different groups of bacteria, the most common isolates in feces of *P. nathusii* ($n = 26$) were gram-negative rods of the family Enterobacteriaceae (62%), and gram-positive cocci of the genera *Staphylococcus* (k-) (58%) and *Enterococcus* (12%) (Fig. 2). In oral cavity samples the gram-positive cocci (*Staphylococcus*, 42%, and *Micrococcus*, 30%) dominated. Enterococci were found in 18% of oral samples (Fig. 2).

DISCUSSION

The results of the study show several differences between aerobic gastrointestinal flora of bats from the genus *Pipistrellus* and other species of mammals. The most surprising is the lack of *Escherichia coli* among bacterial flora. This bacterium is believed to be one of the most common in the intestinal track of vertebrates. Furthermore, *E. coli* plays an important role in the synthesis of some vitamins (B, K, C) and utilization of food (Jabłoński, 1979). The presence of *E. coli* can also prevent of colonization of gastrointestinal tract by pathogenic species, like *Staphylococcus aureus* and *Salmonella typhimurium* (Ushijima and Ozaki 1986, 1988). As there is no *E. coli* found in feces of *M. myotis* and *B. barbastellus* (Różalska *et al.*, 1998), the lack of

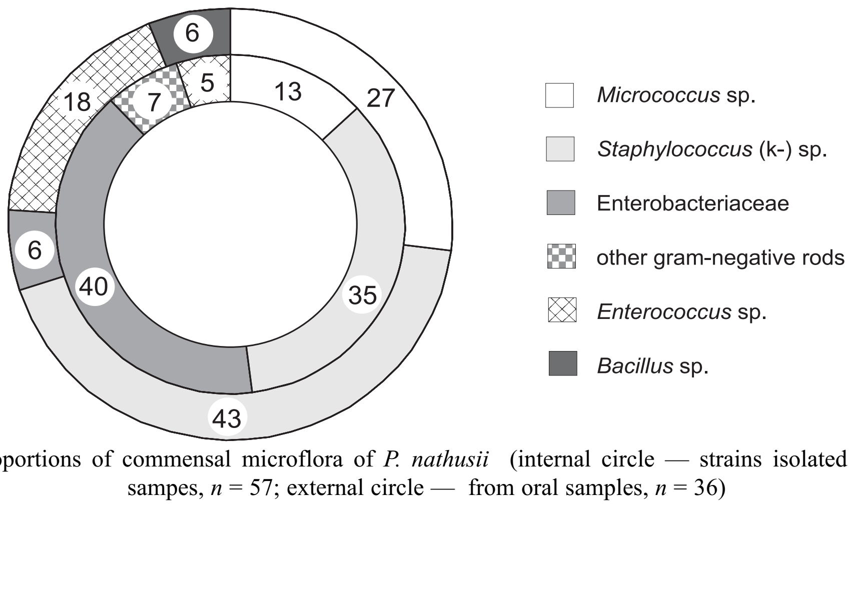

FIG. 1. Proportions of commensal microflora of *P. nathusii* (internal circle — strains isolated from fecal sampes, $n = 57$; external circle — from oral samples, $n = 36$)

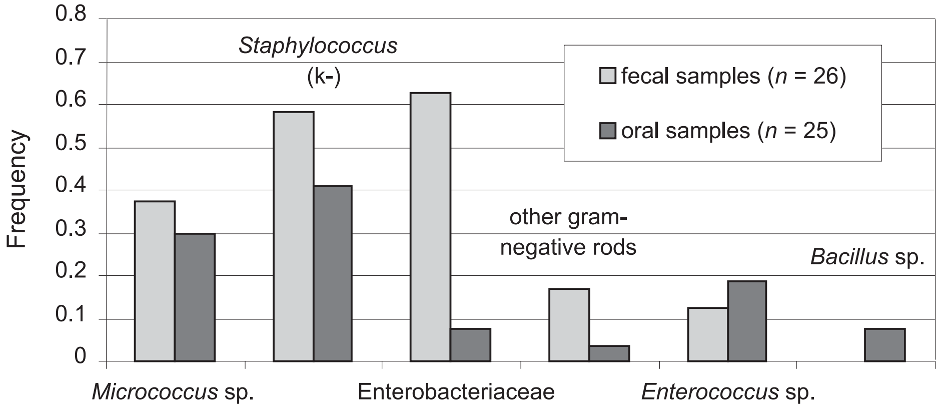

FIG. 2. Frequency of occurrence of different groups of bacteria in samples from *P. nathusii*

this bacterium differs vespertilionid bats from other vertebrates (including pteropids — Heard *et al*., 1997), and the means of regulation of commensal flora in the intestinal tract of these bats remains unknown.

In contrast to Różalska *et al.* (1998), we have not isolated *Bacillus* strains from feces originating from *P. nathusii*. Nevertheless, the staphylococcal flora of *P. nathusii* seems to be more diverse than that of wild birds where only two species of coagulase-negative staphylococci were discovered (Akato *et al*., 1984).

The frequency of recovery of gram-negative rods from feces of *P. nathusii* was similar to that observed in *M. myotis,* but it was higher than that in *B. barbastellus* (Różalska *et al.,* 1998). In contrast to *M. myotis* and *B. barbastellus*, non-fermenting gram-negative rods have been not isolated from the oral cavity samples of *P. nathusii*. On the other hand, the gram-negative rods of the family Enterobacteriaceae were less frequent in *P. nathusii* than in the other bat taxa, whereas enterococci were more frequent.

The observed interspecific differences in the specific composition of bacteria may result from slight differences in bats' diets, although *Myotis, Barbastella*, and *Pipistrellus* are insectivorous. In addition, differences in bacterial flora composition can be observed before and after hibernation (Różalska *et al.,* 1998). Unfortunately, because there are no winter roosts of *P. nathusii* in the study area, it is impossible to make this comparison.

ACKNOWLEDGEMENTS

I thank Prof. J. Galiński and Prof. W. Bogdanowicz, and two anonymous reviewers for their helpful comments on an earlier version of this manuscript.

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Received 5 October 2001, accepted 28 January 2002