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Article

Bacteria with Public Health Relevance in the Head Section of Common Cat Fleas

Georgios Dougas [1,*], Stavroula Beleri [2], Eleni Patsoula [2], Athanassios Tsakris [1] and Joseph Papaparaskevas [1]

[1] Department of Microbiology, Medical School, National and Kapodistrian University of Athens, Athens 11527, Greece

[2] Unit of Medical Entomology, Laboratory for the Surveillance of Infectious Diseases (LSID), Division of Infectious, Parasitic Diseases and Zoonoses, Department of Public Health Policy, School of Public Health, University of West Attica, Athens 11521, Greece

* Correspondence: georgiosdougas@yahoo.com

Simple Summary: Cat fleas (*Ctenocephalides felis*) are common parasites of cats and dogs, but they occasionally bite humans too. These tiny insects feed by piercing the skin and drawing blood through their mouthparts, an apparatus located in the head section. Since flea's head comes into direct contact with the host's skin and blood, it may carry bacteria that can affect human health. In this study, we examined heads of cat fleas to identify pathogenic bacteria. We discovered traces of DNA from 119 bacterial species known to cause disease in humans — some of which have been linked to blood infections with no clear source. Most of these bacteria are able to form biofilm, a protective layer that helps them survive in harsh environments therefore increasing the infection potential. Although finding bacterial DNA alone does not confirm live bacteria or that fleas can spread disease, these results raise important questions. The presence of so many disease-related bacteria in the head section of cat fleas suggests the need for further research to find out whether these insects can play a role in transmitting a wider than expected range of infections to humans.

Abstract: Background: The present study investigated the microbiome of dissected cat flea heads for bacteria with clinical relevance for human infections. Methods: A total of 112 cat fleas (*Ctenocephalides felis*) were collected from 30 cats in Attica, Greece. The heads of the insects were aseptically dissected, pooled in groups by host animals and analyzed with Ion Torrent 16S rRNA metagenomic sequencing. Results: Of the 1578 bacterial species detected, 119 (7.5 %) are known human pathogens. These include the classic flea-borne agents *Bartonella henselae, B. clarridgeiae*, and *B. grahamii*. Most pathogens (89 species, 74.8 %) can cause both bloodstream and skin or soft-tissue infections. Ten species are linked to bacteremia of unknown origin; among these, *Haemophilus influenzae*, *Salmonella enterica*, and *Streptococcus pyogenes* are typically associated with community-onset infections, whereas *Enterococcus faecalis, Escherichia coli, Proteus mirabilis*, and *Staphylococcus aureus* display varied epidemiological profiles related to both community-acquired and hospital-acquired bacteremias. Conclusions: The head microbiome of *C. felis* is exceptionally diverse and includes numerous bacteria of clinical importance. While the presence of DNA alone does not prove vector competence, the combination of biofilm capacity and the flea's haematophagous behaviour supports a plausible—yet unproven—role of fleas in the transmission of unconventional bacterial infections.

Keywords: Ctenocephalides; microbiota; metagenomics; bacteremia; insect vectors

1. Introduction

Ctenocephalides felis (cat flea) is the most abundant and cosmopolitan flea species parasitizing cats, dogs and opportunistically other mammals including humans [1]. The insect is an obligatory hematophagous ectoparasite feeding with the blood of the host [2].

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Cat fleas are not only the most common ectoparasites of companion animals worldwide but also an increasingly recognized reservoir and potential vector of bacterial pathogens of veterinary and human concern [3,4]. Their cosmopolitan distribution and broad host range (cats, dogs, wildlife and humans) create opportunities for cross-species transmission of infectious agents. Historically, the public-health focus on *C. felis* centered on classical vector-borne agents such as *Rickettsia felis* and *Bartonella henselae* [4]. More recent molecular surveys, however, have revealed a far richer flea-associated microbiome, including enteric, skin-associated and opportunistic bacteria [5,6].

While most studies analyze whole insects, the head section is by far of particular interest. Head contains mouthparts, the anatomical interface that pierces human skin, ingests blood and injects saliva, therefore coming in direct contact with the dermis and bloodstream. Laciniae, a pair of hard saw-like cutting organs and epipharynx, a stubbing apparatus, are responsible for the perforation of the skin (Fig. 1). Together they form a dorsal food canal through which the flea sucks blood while the opposing surfaces of laciniae shape a separate ventral salivary canal that injects anticoagulant saliva into the wound [7]. Despite this obvious relevance, systematic characterization of the bacterial communities found specifically in the flea head is scarce.

In this study, the bacterial flora of cat-flea heads is investigated for pathogenic species and the potential role of fleas to act as vectors of unconventional pathogens is discussed.

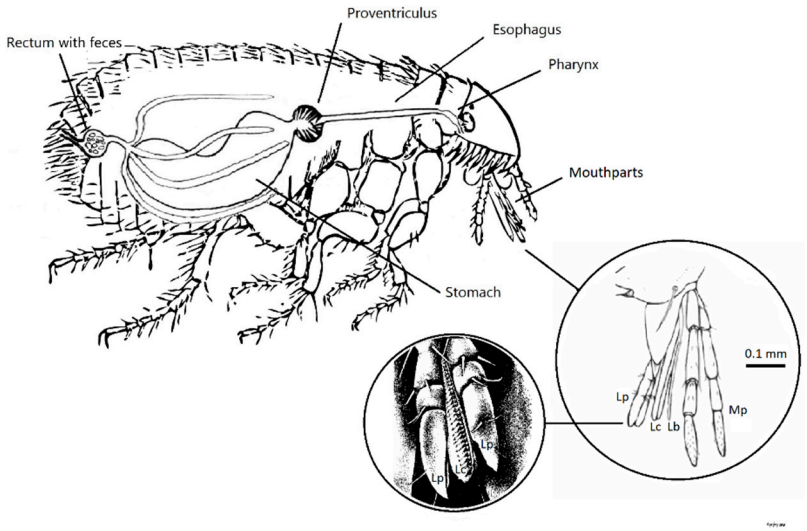

Figure 1. *Ctenocephalides felis* schematic representation of mouthparts and digestive system. Lc: Laciniae, Lb: Labrum, Mp: Maxillary palps, Lp: Labial palps. Adapted from Dougas G. et al., Trop. Med. Infect. Dis. 2021, 6(1), 37; CC BY 4.0.

2. Materials and Methods

2.1. Sample Collection and Preparation

Fleas were collected from cats visiting local veterinary clinics in the region of Attica, Greece, along with animal-related data (sex, age, ownership status). The insects were preserved at room temperature for a maximum of 48 hours before dissection and DNA extraction. The genus and species of fleas were identified by standard anatomic keys [7,8] using stereo microscope under 10–40 X magnification (NIKON SMZ645, Nikon Instruments Inc., Surrey, UK). *C. felis* heads were separated with fine-tipped forceps and dissecting scalpel at 40 X magnification and pooled in groups by host-cat (flea-groups). (Fig. 2). The heads of each flea-group were thoroughly homogenized with a pestle and DNA was extracted with the QIAamp DNA Mini Kit (Qiagen, Hilden, Germany) according to manufacturer's instructions. All manipulations were performed under sterile conditions.

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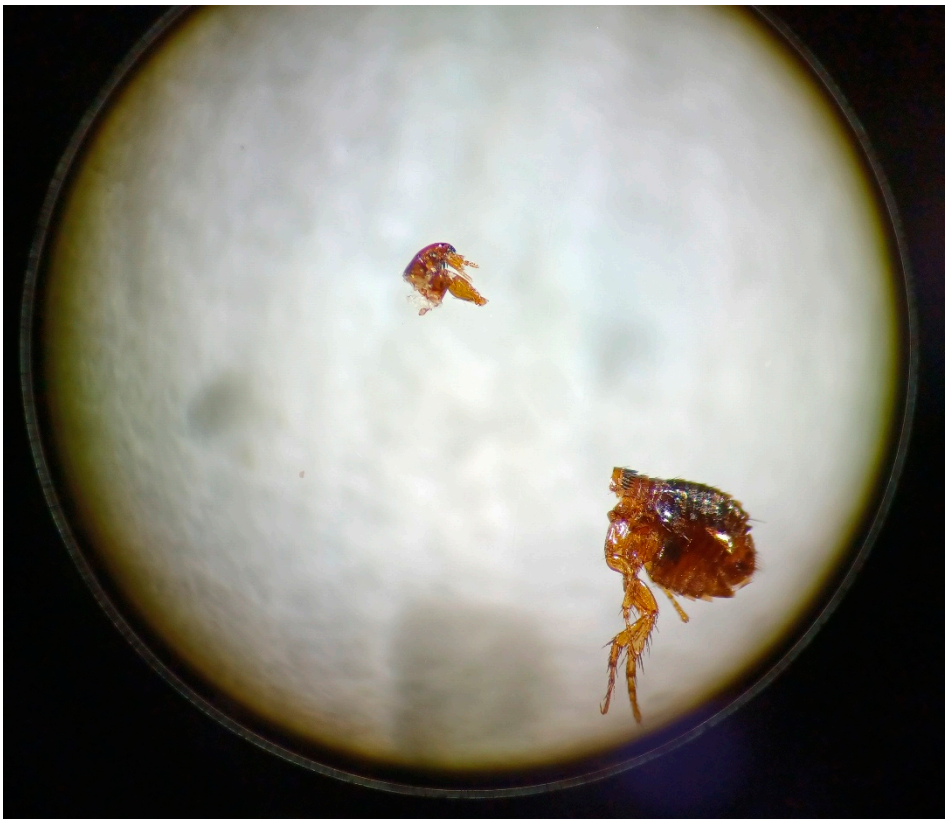

Figure 2. Dissected head of a *Ctenocephalides felis* flea in stereo microscope (40 X magnification).

2.2. Molecular Investigation

DNA extracts were analyzed using a 16S metagenomics assay, employing the Ion 16S Metagenomics Kit on the PGM Ion Torrent platform (Thermo Fisher Scientific, Waltham, MA, USA). Sequencing data were processed with QIIME version 2, using the MicroSEQ v2013.1 and GreenGenes v13.5 16S rDNA reference databases for taxonomic classification. Only bacteria identified at species level were included in the analysis.

2.3. Clinical Relevance and Infection Dynamics

Pathogenic bacteria were those enlisted as infectious agents in the ICD-11 International Classification of Diseases, 11th Revision [9] or in the communicable diseases case definitions, according to the European Centre for Disease Prevention and Control [10]. The type of infection was categorized as blood stream infection (BSI) or skin and soft tissue infection (SSTI) according to the ECDC case definitions and for those not covered by ECDC, according to available literature.

2.4. Assessment of Infection Potential

Flea-head microbiome was assessed for pathogens associated with bacteremia of unknown origin (BUO). According to ECDC, BUO (also called "primary" or "cryptogenic" bacteremia) is a primary BSI of unknown origin, not related to vascular-catheter infection with no identifiable source [11]. The microbiome was cross examined with commonly cited BUO bacteria recovered from BSI when no infection source is obvious: *Staphylococcus aureus*, coagulase-negative Staphylococci, β-haemolytic or Viridans-group Streptococci, *Enterococcus faecalis/faecium*, *Escherichia coli*, *Klebsiella pneumoniae*, *Enterobacter cloacae* complex, *Serratia marcescens*, *Proteus mirabilis*, *Pseudomonas aeruginosa*, *Acinetobacter baumannii*, *Bacteroides fragilis* group, *Clostridium perfringens*, *Salmonella enterica*, *Haemophilus influenzae* [12–20].

To further evaluate the potential role of fleas to transmission of BUO flea-head bacteria, the common epidemiological context of infection (hospital-acquired vs community-onset) was reviewed.

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Suspicion for flea implication further increased with community-dominant BUO agents, rather than with those cited as hospital-acquired.

3. Results

In total, 112 *C. felis* were collected from 30 cats. Host animals had a male-to-female ratio of 1.14:1, median age 18 months (IQR: 6-39), and were 87% stray. The examined fleas had a female-to-male ratio of 4.9:1. Flea-groups comprised a median of 3 insect heads per host cat (IQR: 2–4).

Molecular investigation of the flea-groups' DNA extracts revealed 1578 bacterial species that belonged to 14 phyla, 168 families, and 511 genera. The bacterial species associated with human diseases were, in total, 119 (7.5%), corresponding to four phyla, 18 families, and 23 genera; of them, 83 (69.7%) were characterized as pathogenic according to ECDC, 28 (23.5%) according to ICD-11 whereas eight (6.7%) were classified as pathogens based on both sources. Among the identified pathogens, 74.8% were associated with both BSI and SSTI, 16.0% with BSI, and 9.2% with SSTI. Flea groups had a median of 3.5 (IQR: 1.25-6.00) pathogenic species and a minimum of one disease-related species per group. The standard flea-borne pathogens *B. henselae and B. clarridgeiae* were among the detected species. Pathogenic genera included *Acinetobacter, Actinomyces, Aerococcus, Bacillus, Bartonella, Clostridium, Corynebacterium, Enterobacter, Enterococcus, Escherichia, Haemophilus, Micrococcus, Nocardia, Pasteurella, Propionibacterium, Proteus, Rickettsia, Salmonella, Serratia, Staphylococcus, Streptobacillus, Streptococcus* and *Streptomyces*.

A detailed breakdown of the pathogenic bacteria detected in cat flea heads is presented in Table A1 (Appendix).

Of the 119 pathogenic species of flea-head microbiome, ten are well associated with BUO cases; three are community-dominant, four are both community-onset and hospital-acquired and three are solely associated with hospital infections (Table 1). Most flea-groups (21; 70%) harbored at least one BUO species with a median of two species (IQR: 2.0, 3.0) per group.

Table 1. The common epidemiological setting and clinical context of Bacteremia of Unknown Origin (BUO) associated with bacteria in the head section of Ctenocephalides felis (cat fleas).

Genus species	Common epidemiologic setting	Typical clinical context
Acinetobacter baumannii	Nosocomial-dominant	Ventilator-associated pneumonia, ICU bacteraemia, wounds
Enterococcus faecalis	Both	CA & HA UTIs; endocarditis; line-related BSI
Enterococcus faecium	Nosocomial-dominant	VRE catheter- or line-associated BSI, post-op wound
Escherichia coli	Both	Uncomplicated CA-UTI; ESBL/VRE-unit BSIs
Haemophilus influenza	Community-dominant	CAP, COPD exacerbations, paediatric invasive disease
Proteus mirabilis	Both	CA pyelonephritis; crystalline CAUTI in long-term care
Salmonella enterica	Community-dominant	Food-borne enteritis, typhoid bacteraemia
Serratia marcescens	Nosocomial-dominant	NICU/ICU outbreaks, device & water-borne clusters
Staphylococcus aureus	Both	Skin/SSTI (CA); device/endovascular BSI (HA)

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Streptococcus pyogenes	Community-dominant	Pharyngitis, cellulitis, necrotising fasciitis

CA: Community-acquired

NICU / ICU: Neonatal Intensive Care Unit / Intensive Care Unit

HA: Hospital-acquired (nosocomial)

SSTI: Skin and Soft-Tissue Infection

UTI / CA-UTI / CAUTI: Urinary-tract infection / Community-acquired UTI / Catheter-associated UTI

CAP: Community-acquired Pneumonia

BSI: Blood-stream infection

COPD: Chronic Obstructive Pulmonary Disease

VRE: Vancomycin-resistant Enterococcus

Post-op: Post-operative

ESBL: Extended-spectrum β-lactamase

4. Discussion

This study demonstrates that the head section of *C. felis* harbors a diverse array of bacterial species, including a substantial proportion classified as human pathogens under ICD-11 and ECDC definitions. Notably, nearly 75% of these pathogens were associated with both bloodstream and skin/soft tissue infections, underscoring the potential for clinically significant exposure via the flea's piercing mouthparts.

The identification of the classical flea-borne agents such as *Bartonella* species supports existing literature [3,4] and confirms the validity of flea head sampling as a method for vector surveillance. Furthermore, the detection of *B. henselae* and *B. clarridgeiae* in flea heads—rather than in whole bodies or feces of fleas—represents a finding that could support hypotheses of mechanical or salivary transmission during feeding. While vector competence has not been established for these bacteria via flea bite, their presence in the anatomical structures of the head strengthens the argument for a transmission potential. The detection of the rodent-specific *B. grahamii* in cat fleas agrees with previous reports [21], supporting a wider vector range.

The detection, however, of a much broader panel of potential pathogens, including agents linked to Bacteremia of Unknown Origin, adds novel insight. Among the 119 pathogenic species identified, ten are widely cited in the literature as causative agents of BUO—bacteremia without a clear source of infection. The presence of these bacteria in flea heads may represent a hypothetical but plausible transmission mechanism, especially given their compatibility with a cutaneous or hematogenous route.

Of particular interest is the epidemiological context of these BUO-associated bacteria. While species such as *E. faecium*, *S. marcescens*, and *A. baumannii* are primarily nosocomial pathogens [22], others such as *Streptococcus pyogenes*, *H. influenzae*, and *S. enterica* are more commonly associated with community-acquired infections [23], making their presence in cat flea heads particularly relevant from a One Health perspective.

A major consideration is biofilm formation that facilitates bacterial perseverance under adverse conditions. Almost all species identified in this study, including BUO agents, have documented biofilm-forming capabilities [24]. Only a few species, specifically *Rickettsia australis* and *Streptobacillus moniliformis*, are confirmed non-biofilm builders [25]. Biofilm-forming bacteria may persist in the flea's mouthparts or salivary glands, increasing the transmission likelihood.

It is important to recognize the study's limitations. Detection of bacterial DNA does not equate to viable organisms or vector capacity. Moreover, contamination during sampling, particularly from the host's skin microbiota, cannot be entirely ruled out despite rigorous dissection protocols.

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Nevertheless, several of the species identified—such as *S. enterica*, *H. influenzae*, and *S. pyogenes*—are not typical skin flora and may indicate bloodmeal-derived colonization [23].

Our findings are consistent with previous observations that fleas harbor a complex microbiome [5,6]. However, this study is, to our knowledge, the first to comprehensively characterize the microbial flora of the flea head alone using high-throughput metagenomics, and to relate these findings specifically to BUO-associated pathogens.

Future studies should prioritize the isolation of viable bacteria from dissected mouthparts and salivary glands to assess transmission risk. Approaches combining culture-based techniques and metagenomic data with experimental infection models would allow for a more complete characterization of viable and infectious organisms.

5. Conclusions

In conclusion, the head microbiome of cat flea contains a wide range of human pathogens, including established and emerging species associated with bloodstream and skin or soft tissue infections. A subset of the identified bacteria are agents of community-onset bacteremias of unknown origin. While causality cannot be inferred from detection alone, the anatomical proximity to host entry sites, combined with the known pathophysiology of these bacteria, warrants further investigation into the role of cat fleas as potential vectors of non-classical pathogens.

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Conflicts of Interest: The authors declare no conflicts of interest.

Abbreviations

The following abbreviations are used in this manuscript:

MDPI	Multidisciplinary Digital Publishing Institute
DOAJ	Directory of open access journals
TLA	Three letter acronym
LD	Linear dichroism

Appendix A

Appendix A.1

Table 1. Pathogenic bacterial species (n=119) identified in the heads of Ctenocephalides felis (cat fleas) collected from cats, organized in 30 flea-groups by host cat (n=30), according to ICD-11 classification of diseases and the communicable diseases classification (ECDC, 2018).

Genus	species	Number of flea-groups	%	Pathogenic by ICD-11	Pathogenic by ECDC (2018)
Acinetobacter	*baumannii*	*15*	50.0	Yes	Yes
	baylyi	*5*	16.7	No	Yes
	beijerinckii	*4*	13.3	No	Yes

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	calcoaceticus	*8*	26.7	No	Yes
	guillouiae	*2*	6.7	No	Yes
	gyllenbergii	*4*	13.3	No	Yes
	johnsonii	*23*	76.7	No	Yes
	junii	*15*	50.0	No	Yes
	lwoffii	*24*	80.0	No	Yes
	radioresistens	*5*	16.7	No	Yes
	schindleri	*15*	50.0	No	Yes
	soli	*5*	16.7	No	Yes
	towneri	*2*	6.7	No	Yes
	ursingii	*13*	43.3	No	Yes
Actinomyces	*dentalis*	*1*	3.3	Yes	No
	graevenitzii	*2*	6.7	Yes	No
	naeslundii	*16*	53.3	Yes	No
	odontolyticus	*6*	20.0	Yes	No
	radingae	*1*	3.3	Yes	No
	viscosus	*6*	20.0	Yes	No
Aerococcus	*urinaeequi*	*10*	33.3	No	Yes
Bacillus	*acidiproducens*	*1*	3.3	No	Yes
	badius	*2*	6.7	No	Yes
	cecembensis	*2*	6.7	No	Yes
	cereus	*3*	10.0	No	Yes
	coagulans	*1*	3.3	No	Yes
	decisifrondis	*9*	30.0	No	Yes
	eiseniae	*1*	3.3	No	Yes
	firmus	*2*	6.7	No	Yes
	funiculus	*1*	3.3	No	Yes
	graminis	*6*	20.0	No	Yes
	humi	*2*	6.7	No	Yes
	lentus	*4*	13.3	No	Yes
	nealsonii	*1*	3.3	No	Yes
	niacini	*2*	6.7	No	Yes
	taeanensis	*1*	3.3	No	Yes
	thermoamylovorans	*4*	13.3	No	Yes
	thermocopriae	*1*	3.3	No	Yes
	thermolactis	*1*	3.3	No	Yes
	vietnamensis	*1*	3.3	No	Yes
	weihenstephanensis	*1*	3.3	No	Yes
Bartonella	*clarridgeiae*	*6*	20.0	Yes	No
	grahamii	*2*	6.7	Yes	No
	henselae	*3*	10.0	Yes	No
Clostridium	*perfringens*	*11*	36.7	Yes	No
Corynebacterium	*accolens*	*6*	20.0	No	Yes
	afermentans	*1*	3.3	No	Yes

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	ammoniagenes	2	6.7	No	Yes
	amycolatum	8	26.7	No	Yes
	appendicis	1	3.3	No	Yes
	aquatimens	1	3.3	No	Yes
	aquilae	1	3.3	No	Yes
	aurimucosum	3	10.0	No	Yes
	auriscanis	4	13.3	No	Yes
	bovis	1	3.3	No	Yes
	callunae	2	6.7	No	Yes
	coyleae	15	50.0	No	Yes
	deserti	1	3.3	No	Yes
	doosanense	1	3.3	No	Yes
	durum	7	23.3	No	Yes
	efficiens	1	3.3	No	Yes
	flavescens	2	6.7	No	Yes
	genitalium	3	10.0	No	Yes
	glucuronolyticum	2	6.7	No	Yes
	glutamicum	1	3.3	No	Yes
	halotolerans	2	6.7	No	Yes
	jeikeium	2	6.7	No	Yes
	kroppenstedtii	11	36.7	No	Yes
	maris	1	3.3	No	Yes
	massiliense	1	3.3	No	Yes
	mastitidis	6	20.0	No	Yes
	matruchotii	5	16.7	No	Yes
	minutissimum	1	3.3	No	Yes
	mucifaciens	14	46.7	No	Yes
	mustelae	3	10.0	No	Yes
	pilbarense	9	30.0	No	Yes
	pyruviciproducens	1	3.3	No	Yes
	resistens	3	10.0	No	Yes
	simulans	1	3.3	No	Yes
	singulare	2	6.7	No	Yes
	stationis	4	13.3	No	Yes
	suicordis	5	16.7	No	Yes
	terpenotabidum	6	20.0	No	Yes
	tuberculostearicum	24	80.0	No	Yes
	tuscaniense	2	6.7	No	Yes
	ureicelerivorans	17	56.7	No	Yes
	variabile	9	30.0	No	Yes
	vitaeruminis	1	3.3	No	Yes
	xerosis	1	3.3	No	Yes
Enterobacter	*asburiae*	1	3.3	Yes	No
	hormaechei	1	3.3	Yes	No

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	kobei	*3*	10.0	Yes	No
Enterococcus	*faecalis*	*9*	30.0	Yes	Yes
	faecium	*6*	20.0	Yes	Yes
	gallinarum	*2*	6.7	Yes	No
	hirae	*1*	3.3	Yes	No
	mundtii	*2*	6.7	Yes	No
Escherichia	*coli*	*1*	3.3	Yes	Yes
Haemophilus	*influenzae*	*1*	3.3	Yes	Yes
Micrococcus	*flavus*	*3*	10.0	No	Yes
	lylae	*12*	40.0	No	Yes
Nocardia	*takedensis*	*1*	3.3	Yes	No
	transvalensis	*1*	3.3	Yes	No
	vinacea	*3*	10.0	Yes	No
Pasteurella	*canis*	*1*	3.3	Yes	No
	multocida	*11*	36.7	Yes	No
	stomatis	*7*	23.3	Yes	No
Propionibacterium	*acidifaciens*	*1*	3.3	No	Yes
	acnes	*30*	100.0	Yes	Yes
	granulosum	*17*	56.7	No	Yes
	propionicum	*2*	6.7	No	Yes
Proteus	*mirabilis*	*8*	26.7	Yes	No
Rickettsia	*australis*	*1*	3.3	Yes	No
Salmonella	*enterica*	*2*	6.7	Yes	Yes
Serratia	*marcescens*	*2*	6.7	Yes	
Staphylococcus	*aureus*	*2*	6.7	Yes	Yes
Streptobacillus	*moniliformis*	*1*	3.3	Yes	No
Streptococcus	*pyogenes*	*4*	13.3	Yes	No
Streptomyces	*cacaoi*	*1*	3.3	Yes	No

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