



NOTE

Laboratory Animal Science

Isolation of *Streptococcus cuniculi* from corneal lesion in laboratory-raised mice

Vunjia TIONG¹⁾, Shih-Keng LOONG¹⁾, Haryanti Azura MOHAMAD WALI²⁾,
Kim-Kee TAN¹⁾, Pui-Fong JEE⁴⁾, Fang-Shiang LIM¹⁾, Jing-Jing KHOO¹⁾,
Pooi-Fong WONG^{2,3)}, Li-Yen CHANG⁴⁾ and Sazaly ABUBAKAR^{1,4)*}

¹⁾Tropical Infectious Diseases Research & Education Centre, University of Malaya, 50603 Kuala Lumpur, Malaysia

²⁾Animal Experimental Unit, Faculty of Medicine, University of Malaya, 50603 Kuala Lumpur, Malaysia

³⁾Department of Pharmacology, Faculty of Medicine, University of Malaya, 50603 Kuala Lumpur, Malaysia

⁴⁾Department of Medical Microbiology, Faculty of Medicine, University of Malaya, 50603 Kuala Lumpur, Malaysia

J. Vet. Med. Sci.

83(2): 280–284, 2021

doi: 10.1292/jvms.20-0070

Received: 7 February 2020

Accepted: 14 September 2020

Advanced Epub:

13 January 2021

ABSTRACT. Corneal lesions appearing as white mass beneath intact epithelium, with ocular discharge in one mouse, was observed in a batch of laboratory-raised BALB/c mice (n=9 of 56). The affected mice remained active, well-groomed and had normal appetite. Isolates recovered from swab cultures of the external and internal contents of the eye had partial 16S rRNA gene sequence of 99.1% similarity to *Streptococcus cuniculi*. No previous report of *S. cuniculi* infection in laboratory rodents has been presented. The isolate was susceptible to all antibiotics tested. We suggest *S. cuniculi* is an opportunistic bacteria in laboratory mice but are uncertain of its source. Our findings revealed that *S. cuniculi* is able to colonize laboratory mice and should be considered when mice present with eye lesion or ocular discharge.

KEY WORDS: eye, infectious disease, laboratory mouse, *Streptococcus cuniculi*

Emerging infectious agents continue to be identified in laboratory rodents despite improvements in animal housing and health monitoring programs [6, 10]. While some of these infections can cause overt disease, most are usually subclinical or asymptomatic. Regardless, their presence in laboratory animals can alter the physiological response of the animals used to investigate disease presentations, immunological responses and oncological parameters [3, 17, 28]. Infected rodents could also pose health risks to researchers and the animal facility husbandry staff [4, 10, 23]. Here, we report the isolation and identification of *Streptococcus cuniculi* from corneal lesions presented in specific-pathogen-free (SPF) laboratory-raised BALB/c mice. Thus far, *S. cuniculi* has only been reported to be isolated from nasal and tonsil swabs of healthy wild rabbits [27] and the oral microbiome of wood mouse, *Apodemus sylvaticus* (NCBI BioSample database under accession number SAMN11126842).

A batch of 58 five-week old SPF female BALB/c mice was purchased from a licensed vendor overseas. A health report was issued by the vendor which confirmed the SPF status of the mice purchased. The health report indicated that the mice tested negative for various murine pathogens including 19 types of murine viruses, 15 genera of bacteria/fungi (including β -hemolytic *Streptococcus* and *Streptococcus pneumoniae*) and 17 genera of parasites. The mice were transported in approved standard animal transport containers [9] by the vendor in an air-conditioned van to the Animal Experimental Unit (AEU), Faculty of Medicine, Universiti Malaya, Malaysia. Upon arrival at the AEU, the mice were received by the veterinarian and appeared normal upon inspection. The mice were housed in groups of five or six per individually ventilated cage (IVC) (Tecniplast, Via Maggio, Italy) under SPF conditions for a two-week quarantine period. All IVCs, animal feed, water and bedding (corn cob) were sterilized by autoclaving before use. The animals were maintained at a room temperature of 19 to 23°C and relative humidity of 54 to 65% under a 12:12 hr light:dark cycle. The AEU, an AAALAC-accredited facility, has an animal health monitoring program for all rodents entering the facility. The mice were monitored daily by the veterinarian. They were otherwise healthy and showed no signs of infection or illness during the entire quarantine period. Additionally, no other animals were kept in the same room during this period. Rabbits in the AEU are housed on a separate floor. Every floor requires a change of personal protective clothing, such as isolation gown, gloves, shoe covers, surgical mask and hair net, before entering. Handling of mice and changing of bedding were performed in a laminar airflow hood with HEPA-filtered air supply. The animals purchased and the animal handling procedures were approved by the Institutional Animal Care and Use Committee, University of Malaya (2014-01-07/MMB/R/JPF).

After a two-week quarantine period and prior to planned experimentation, a whitish mass was observed in the eyes of 9 out of 58

*Correspondence to: AbuBakar, S.: sazaly@um.edu.my

©2021 The Japanese Society of Veterinary Science



This is an open-access article distributed under the terms of the Creative Commons Attribution Non-Commercial No Derivatives (by-nc-nd) License. (CC-BY-NC-ND 4.0: <https://creativecommons.org/licenses/by-nc-nd/4.0/>)

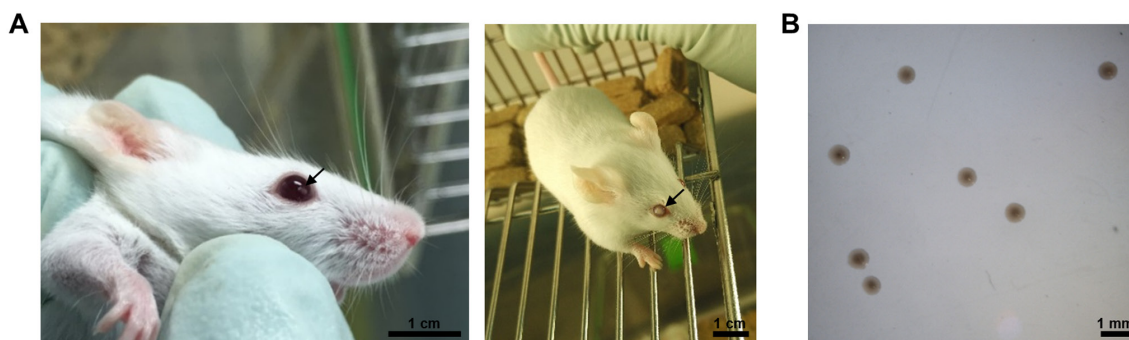


Fig. 1. (A) Arrows pointing to corneal lesion characterized by circumscribed white mass beneath an intact epithelium observed in the eyes of BALB/c mice. (B) *Streptococcus cuniculi* UM-25 colonies on Mueller-Hinton agar, isolated from the eye lesions.

mice and suspected to be an infection. The condition affected only one eye in 6/9 mice and both eyes in 3/9 mice. The white mass was of varying size and beneath intact epithelium (Fig. 1A). All mice were active, well-groomed and had normal appetite, as determined by the veterinarian. Ocular discharge was observed in only one of the affected mice. The eye condition was observed in mice housed in three of the six IVCs and not all mice in those IVCs were affected. The affected batch of mice was not used in subsequent experimentation and those not affected did not develop symptoms of eye infection thereafter. To identify any microorganisms associated with the lesion, the mice were fully anesthetized by intraperitoneal injection of ketamine (80–120 mg/kg) and xylazine (10–16 mg/kg). In a biosafety cabinet, the area around the eye was carefully swabbed with 70% ethanol before swabbing the external surface of the eye for culture using sterile cotton buds pre-wetted with sterile phosphate-buffered saline. The eye swabs were then inoculated onto Columbia agar supplemented with 5% sheep blood and nutrient agar followed by incubation at 37°C for up to 72 hr. After swabbing the eyes, the mice were euthanized by intraperitoneal injection with an overdose of ketamine (240–360 mg/kg) and xylazine (30–48 mg/kg). Following that, the eyes from the mice were extracted for culture of the internal contents.

Swabs from eyes with no visible corneal lesion or discharge did not result in bacteria growth on the agar medium. Meanwhile, culture of the affected eye's external surfaces and internal contents resulted in growth of small, round colonies that were white-yellowish in color (Fig. 1B). All colonies were morphologically similar and Gram staining revealed the presence of gram positive cocci. The colonies were non-motile, catalase-negative and produced acid from fermentation of mannitol, maltose, sucrose, lactose, but not glycerol. Hydrolysis of arginine and urea was also present. Two isolates from different mice housed in different IVCs were selected for further analyses, designated UM-25 (cultured from the eye's internal content) and UM-29 (cultured from the eye's external surface), respectively. Nucleic acid amplification and sequencing of the 16S ribosomal RNA gene were performed [14] on the two isolates. The amplified nucleic acid fragments were purified, sequenced and compared against available sequences from the NCBI GenBank database. BLAST results of the partial 16S ribosomal RNA gene (543 bp) produced matches to known *Streptococcus* species: 99.1% (538/543), 97.6% (530/543) and 97.2% (528/543) to *S. cuniculi* NED12-00049-6B, *S. azizii* 12-5202 and *S. acidominimus* LMG17755, respectively. A phylogenetic tree constructed to visualize the genetic relationships of isolates UM-25 and UM-29 with other known species of the genus *Streptococcus* from the NCBI database (Fig. 2) revealed that isolates UM-25 and UM-29 clustered together with *S. cuniculi*, with bootstrap support value of 85%. Analysis of partial *rpoB*, *sodA* and *recN* showed 91.07%, 87.44% and 82.74%, respectively sequence similarity between isolates UM-25 and

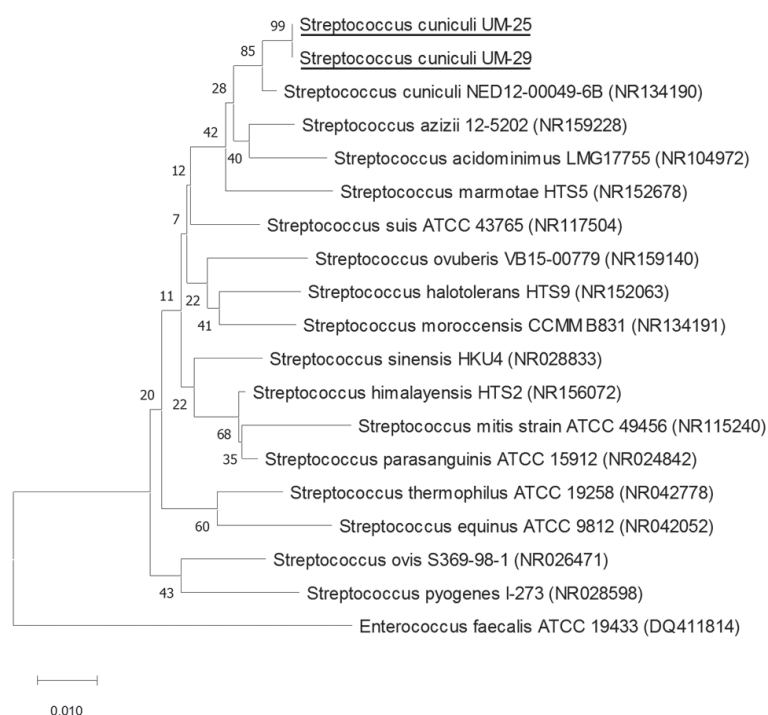


Fig. 2. Neighbor-joining phylogenetic tree of selected *Streptococcus* sp. 16S ribosomal RNA gene. Isolates underlined (*Streptococcus cuniculi* UM-25 and UM-29) are those reported in this study. *Enterococcus faecalis* ATCC 19433 was used as an outgroup. Numbers at nodes indicate bootstrap values (%) for 1,000 replicates. The scale bar indicates nucleotides substitution per site.

UM-29 and *S. cuniculi* NED12-00049-6B.

The draft genome sequencing of the *S. cuniculi* UM-25 was performed as previously described [12, 25] with minor modification. Genome libraries corresponding to 200 bp sequencing were prepared using E-Gel® SizeSelect™ Agarose Gel, 2% (Thermo Fisher Scientific, Waltham, MA, USA). Genome sequencing was undertaken using the IonTorrent PGM sequencer (Life Technologies, Carlsbad, CA, USA) using Ion PGM™ Hi-Q™ sequencing kit. The draft genome of *S. cuniculi* UM-25 was 2,330,972 bp in length, comprising 109 contigs with N50 of 54,985 bp (Table 1). The G+C content of the genome was approximately 40.9% with a total of 2,071 protein-coding genes, comparable to *S. respiraculi* [20]. The draft genome sequence was deposited at the European Nucleotide Archive under the sample accession number SAMEA7010696 and study accession number PRJEB36355. The 16S rRNA sequence for isolates UM-25 and UM-29 were deposited under the same study with accession number LR877007 and LR877008, respectively.

Transmission electron microscopy performed following the protocols described by Loong *et al.* (2017) [13] revealed morphological similarity of *S. cuniculi* UM-25 to *S. azizii*, a phylogenetically close relative found to be associated with meningoencephalitis in C57BL/6 laboratory mice [5]. The diameter of a *S. cuniculi* cell was approximately 700 nm and surrounded by protein fibrils (Fig. 3).

The antimicrobial susceptibility profile of *S. cuniculi* UM-25 was determined using the disc diffusion method on Mueller-Hinton agar with 5% sheep blood incubated at 37°C with 5% CO₂ for 24 hr. The assay was performed to standards according to the Clinical and Laboratory Standards Institute (CLSI) guidelines [8]. Results were interpreted following the standards for *Streptococcus* spp. viridans group according to the CLSI guidelines. Antimicrobial susceptibility analysis revealed that *S. cuniculi* UM-25 was susceptible to ceftriaxone, chloramphenicol, clindamycin, erythromycin, levofloxacin and vancomycin (BBL™ Sensi-Disc™, Becton Dickinson, Franklin Lakes, NJ, USA) (Table 2).

During the same time the eye lesion in mice was observed, swabs of work surfaces, which included bench tops, table tops, taps, door handles and IVCs from the quarantine room, animal holding room and animal experimentation room, were taken. The autoclaved food pellet and water were also sampled. All the samples were found negative for *S. cuniculi*, suggesting that the observed white mass in the cornea did not originate from the AEU. Additionally, *S. cuniculi* was never recovered in any sentinel animals used for bacterial surveillance at the Faculty of Medicine, University of Malaya [15].

Surveys of various animal resource centers [6, 11] and data from a major commercial rodent diagnostic laboratory that has evaluated large numbers of mouse samples [22] indicate that *Streptococcus* spp. is not a common infection of laboratory mice and rats [6, 22]. *Streptococcus* spp. are generally commensals found on the skin, upper respiratory and digestive tracts of mammals [7]. However, under opportunistic circumstances, they can cause infection and disease. Bacteria such as *Staphylococcus* spp., *Streptococcus* spp., *Pseudomonas* spp. that are normally found on the eye lids or the periocular skin is commonly the source of eye infection [1]. *S. pneumoniae* and *S. viridans* are responsible for the majority of post-cataract surgery endophthalmitis and keratitis cases in humans [18], while the *S. viridans* group of bacterial have been implicated in corneal ulcers in dogs [21]. *Streptococcus* spp. is able to infect both immunocompetent and immunocompromised laboratory-raised mice [7, 15]. In animal facilities, infection in laboratory-raised mice may be contracted from other animal occupants or humans working with these animals [15]. Although most infections do not produce noticeable symptoms, there are a number of *Streptococcus* spp. that can cause disease in mice [2, 5, 7, 24].

Table 1. General genome feature of *Streptococcus cuniculi* UM-25

Attributes	Chromosome
Genome size	~2.3 megabases
G+C content	~41%
Contigs	109
ORFs	2,071
RNA (tRNA and rRNA)	44

Table 2. Antimicrobial susceptibility results for *Streptococcus cuniculi* UM-25

Antibiotic	Sensitivity testing results by disc diffusion assay ^{a)}
Ceftriaxone (30 µg)	S (29 mm)
Vancomycin (30 µg)	S (20 mm)
Erythromycin (15 µg)	S (21 mm)
Chloramphenicol (30 µg)	S (22 mm)
Clindamycin (2 µg)	S (20 mm)
Levofloxacin (5 µg)	S (19 mm)

^{a)} Zone diameter values are in parentheses and interpreted according to CLSI breakpoints. S; sensitive.

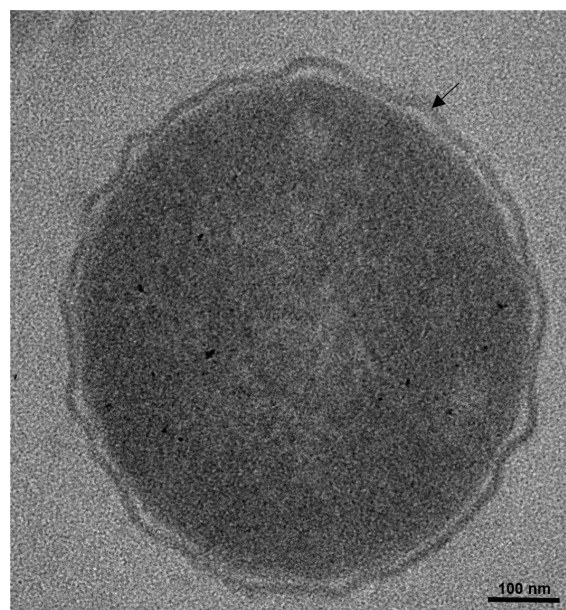


Fig. 3. Transmission electron microscopy of an ultra-thin section of *Streptococcus cuniculi* UM-25. Arrow indicates the protein fibrils surrounding the bacterial cell. Bar is 100 nm.

Thus far, *S. cuniculi* has not been reported to be isolated from laboratory mice. In our experience, mice purchased from the same vendor in the past two years have been clinically healthy and did not show signs of the eye lesions or discharge reported here. It is currently unclear why the condition was observed only in a small number of mice. We note that only *S. cuniculi* grew from the eye contents cultured on agar. We suggest that the *S. cuniculi* isolated in this study was opportunistic, but are currently unable to determine if it is commensal bacteria or acquired externally. Although the mouse strain is different, *Streptococcus* spp. was not one of the 14 bacteria species isolated from the resident bacterial flora on the skin of C57BL/6 mice housed under SPF conditions [26]. We were unable to determine the condition of the transport vehicle or obtain surface swabs. The vendor is known to supply other laboratory-raised animals, such as rabbits. A possible source of infection could be from a crossover from rabbits to the mice, during transport or handling of the animals, either from sharing the same transport vehicle or inadequate decontamination of the van interior between trips. This seems to be the most plausible explanation considering that *S. cuniculi* was susceptible to all the tested antimicrobials, suggesting that it originated from an antibiotic-free environment [16] such as the laboratory-raised rabbit's microflora. Internal organs were not screened so it is unclear if *S. cuniculi* was present elsewhere other than the eyes. Future re-infection studies can be performed to determine if *S. cuniculi* is causative of corneal lesion in mice and histopathological analysis could reveal the extent of colonization. In mice, infection associated with α -hemolytic *Streptococcus* is rare and disease presentation is more associated with β -hemolytic *Streptococcus* [7]. For instance, mice infected with *S. pyogenes* exhibited ruffled hair coats and inactivity before death [19]. Infection with *S. pneumoniae* remains the most common streptococci infection in animal experimental models using laboratory rodents [2]. Here, we report the isolation of *S. cuniculi* from corneal lesion in mice and suggest that monitoring of *S. cuniculi* should be considered even after the quarantine period and also in health surveillance programs, as the impact of *S. cuniculi* colonization on mouse physiology is still not fully elucidated. Lastly, as it is possible that the infection was acquired from rabbits, there must be good practices in place to handle, house and transport laboratory rodents to minimize the introduction of infectious agents from one species to the other.

POTENTIAL CONFLICTS OF INTEREST. The authors have nothing to disclose.

ACKNOWLEDGMENTS. This study was supported in parts by research grants from the University of Malaya, Malaysia, under the Research University grant (RU002-2019 and RU005-2020), the UMCoe Top 100 Research Grant (UM.00000188/HGA.GV) and the Pharmaceutical & Nutraceutical R&D Initiatives Fund (09-05-IFN-MEB-005).

REFERENCES

- Abbott, R. L., Zegans, M. and Kremer, P. A. 2011. Chapter 18 bacterial corneal ulcers. In: Duane's Ophthalmology, 2011 ed. (Duane, T. D., Tasman, W., Jaeger, E. A. eds.), Lippincott Williams & Wilkins, Philadelphia.
- Baker, D. G. 1998. Natural pathogens of laboratory mice, rats, and rabbits and their effects on research. *Clin. Microbiol. Rev.* **11**: 231–266. [Medline] [CrossRef]
- Besselsen, D. G., Franklin, C. L., Livingston, R. S. and Riley, L. K. 2008. Lurking in the shadows: emerging rodent infectious diseases. *ILAR J.* **49**: 277–290. [Medline] [CrossRef]
- Bleich, A. and Nicklas, W. 2008. [Zoonoses transmitted by mouse and rat maintained as laboratory or pet animals]. *Berl. Munch. Tierarztl. Wochenschr.* **121**: 241–255 [Article in German]. [Medline]
- Braden, G. C., Arbona, R. R., Lepherd, M., Monette, S., Toma, A., Fox, J. G., Dewhirst, F. E. and Lipman, N. S. 2015. A novel α -hemolytic *Streptococcus* species (*Streptococcus azizii* sp. nov.) associated with meningoencephalitis in naïve weanling C57BL/6 mice. *Comp. Med.* **65**: 186–195. [Medline]
- Carty, A. J. 2008. Opportunistic infections of mice and rats: Jacoby and Lindsey revisited. *ILAR J.* **49**: 272–276. [Medline] [CrossRef]
- Clifford, C. B. and Pritchett-Corning, K. R. 2012. Chapter 3.3 bacterial infections of laboratory mice. pp. 481–501. In: *The Laboratory Mouse*, 2nd ed. (Hedrich, H. ed), Elsevier, London.
- Clinical and Laboratory Standards Institute. 2016. Performance Standards for Antimicrobial Susceptibility Testing, 26th ed. CLSI supplement M100S. Clinical and Laboratory Standards Institute, Wayne.
- Committee on Guidelines for the Humane Transportation of Laboratory Animals. 2006. Guidelines for the humane transportation of research animals, the National Academies Press, Washington, D.C.
- Hankenson, F. C., Johnston, N. A., Weigler, B. J. and Di Giacomo, R. F. 2003. Zoonoses of occupational health importance in contemporary laboratory animal research. *Comp. Med.* **53**: 579–601. [Medline]
- Jacoby, R. O. and Lindsey, J. R. 1998. Risks of infection among laboratory rats and mice at major biomedical research institutions. *ILAR J.* **39**: 266–271. [Medline] [CrossRef]
- Loong, S. K., Tan, K. K., Zulkif, N. I. and AbuBakar, S. 2019. Draft genome of *Paraburkholderia fungorum* sequence type 868 recovered from human synovial tissues. *Data Brief* **25**: 104159. [Medline] [CrossRef]
- Loong, S. K., Tan, K. K., Zainal, N., Phoon, W. H., Zain, S. N. M. and AbuBakar, S. 2017. Draft genome of the emerging pathogen, *Kocuria marina*, isolated from a wild urban rat. *Mem. Inst. Oswaldo Cruz* **112**: 857–859. [Medline] [CrossRef]
- Loong, S. K., Mahfodz, N. H., Wali, H. A., Talib, S. A., Nasrah, S. N., Wong, P. F. and Abubakar, S. 2016. Molecular and antimicrobial analyses of non-classical *Bordetella* isolated from a laboratory mouse. *J. Vet. Med. Sci.* **78**: 715–717. [Medline] [CrossRef]
- Loong, S. K., Mohamad-Wali, H. A., Che-Mat-Seri, N. A. A., Mahfodz, N. H., Mohd-Ghazali, D., Wong, P. F. and AbuBakar, S. 2018. Effectiveness of sentinel rodents for surveillance of exposure to undocumented bacterial pathogens in animal research facility. *Wetehasan Sattawaphaet* **48**: 671–679.
- Loong, S. K., Lee, H. Y., Khoo, J. J., Lim, F. S., Ahmad-Nasrah, S. N., Azman, A. S., Suntharalingam, C., Panchadcharam, C. and AbuBakar, S. 2019. Microbiological analysis of raw milk unveiled the presence of a dairy contaminant, *Corynebacterium lipophiloflavum*. *J. Appl. Biol. Biotechnol.* **7**: 41–44. [CrossRef]

17. Mansfield, K. G., Riley, L. K. and Kent, M. L. 2010. Workshop summary: detection, impact, and control of specific pathogens in animal resource facilities. *ILAR J.* **51**: 171–179. [[Medline](#)] [[CrossRef](#)]
18. Miller, D. and Alfonso, E. C. 2004. Comparative *in vitro* activity of levofloxacin, ofloxacin, and ciprofloxacin against ocular streptococcal isolates. *Cornea* **23**: 289–293. [[Medline](#)] [[CrossRef](#)]
19. National Research Council (US) Committee on Infectious Diseases of Mice and Rats. 1991. Infectious Diseases of Mice and Rats. National Academies Press (US), Washington, D. C.
20. Niu, L., Hu, S., Lu, S., Lai, X. H., Yang, J., Jin, D., Rao, L., Lu, G. and Xu, J. 2018. Isolation and characterization of *Streptococcus respiraculi* sp. nov. from *Marmota himalayana* (Himalayan marmot) respiratory tract. *Int. J. Syst. Evol. Microbiol.* **68**: 2082–2087. [[Medline](#)] [[CrossRef](#)]
21. Prado, M. R., Brito, E. H. S., Girão, M. D., Sidrim, J. J. C. and Rocha, M. F. G. 2006. Identification and antimicrobial susceptibility of bacteria isolated from corneal ulcers of dogs. *Arq. Bras. Med. Vet. Zootec.* **58**: 1024–1029. [[CrossRef](#)]
22. Pritchett-Corning, K. R., Cosentino, J. and Clifford, C. B. 2009. Contemporary prevalence of infectious agents in laboratory mice and rats. *Lab. Anim.* **43**: 165–173. [[Medline](#)] [[CrossRef](#)]
23. Sewell, D. L. 1995. Laboratory-associated infections and biosafety. *Clin. Microbiol. Rev.* **8**: 389–405. [[Medline](#)] [[CrossRef](#)]
24. Song, H. Y., Qiu, B. F., Liu, C., Zhu, S. X., Wang, S. C., Miao, J., Jing, J. and Shao, Y. X. 2015. Identification of causative pathogens in mouse eyes with bacterial keratitis by sequence analysis of 16S rDNA libraries. *Exp. Anim.* **64**: 49–56. [[Medline](#)] [[CrossRef](#)]
25. Tan, K. K., Tan, Y. C., Chang, L. Y., Lee, K. W., Nore, S. S., Yee, W. Y., Mat Isa, M. N., Jafar, F. L., Hoh, C. C. and AbuBakar, S. 2015. Full genome SNP-based phylogenetic analysis reveals the origin and global spread of *Brucella melitensis*. *BMC Genomics* **16**: 93. [[Medline](#)] [[CrossRef](#)]
26. Tavakkol, Z., Samuelson, D., deLancey Pulcini, E., Underwood, R. A., Usui, M. L., Costerton, J. W., James, G. A., Olerud, J. E. and Fleckman, P. 2010. Resident bacterial flora in the skin of C57BL/6 mice housed under SPF conditions. *J. Am. Assoc. Lab. Anim. Sci.* **49**: 588–591. [[Medline](#)]
27. Vela, A. I., Sánchez Del Rey, V., Zamora, L., Casamayor, A., Domínguez, L. and Fernández-Garayzábal, J. F. 2014. *Streptococcus cuniculi* sp. nov., isolated from the respiratory tract of wild rabbits. *Int. J. Syst. Evol. Microbiol.* **64**: 2486–2490. [[Medline](#)] [[CrossRef](#)]
28. Working Group on Hygiene of the Gesellschaft für Versuchstierkunde –Society for Laboratory Animal Science (GV-SOLAS). 1999. Implications of infectious agents on results of animal experiments. Report of the Working Group on Hygiene of the Gesellschaft für Versuchstierkunde--Society for Laboratory Animal Science (GV-SOLAS). *Lab. Anim.* **33** Suppl 1: S39–S87. [[Medline](#)]