

ORIGINAL ARTICLE

Isolation and identification of respiratory tract and intestinal microflora of *Meriones meridianus* in a conventional animal facility

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Abstract

Background: Culturable bacterial species from the respiratory tract and ileocecal junction of *Meriones meridianus* (midday gerbils) captured in the Xinjiang Luntai area were isolated and identified to confirm the microflora and develop approaches for biological purification of laboratory animals and relevant microbial precautions.

Methods: Bacteria from respiratory tracts and ileocecal junctions of 30 wild *M. meridianus* were harvested and isolated by inoculation into culture media. Isolated strains were confirmed by mass spectrometry and 16S rRNA sequencing.

Results: Thirty-nine bacterial species from 20 families and 27 genera were identified and isolated from wild *M. meridianus*. Typical bacteria were Enterobacteriaceae, *Enterococcus*, and *Staphylococcus aureus*, and the most common microflora were *Vibrio*, *Staphylococcus aureus*, and *Pseudomonas aeruginosa*.

Conclusion: Wild *M. meridianus* carries multiple bacteria, most of which are pathogenic or conditional pathogens. This study provides a basis for the development of animal models and laboratory animals from wild *M. meridianus*.

KEYWORDS

bacteria, culture, isolate, *M. meridianus*, pathogenic bacteria

1 | INTRODUCTION

Meriones meridianus (Pallas, 1773; Rodentia, Cricetidae, Meriones, Gerbillinae, Gerbillus) is one of the most widely distributed gerbil species¹ and it is credited with being a plague vector,² as well as a distributor of Lester bacterial disease, leishmaniasis, tick-borne relapsing fever, and brucellosis. Because of its strong disease resistance and rapid breeding, *M. meridianus* is of interest as a novel experimental animal resource. At present, there are no reports of

microbial flora by *M. meridianus*, so we investigated the bacterial species residing in *M. Meridianus* in Xinjiang to establish an assay for identifying microbial flora of this animal. These studies may support the use of *M. meridianus* as a new model species and offer reference values for microbiological studies.

2 | MATERIALS AND METHODS**2.1 | Animal source**

During the season when the food is scarce in the wild (April-May), we placed a rodent cage containing fried peanuts fixed to the cage

Yimei Xu and Shen Shi contributed equally to this work.

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with wire in a hole frequented by gerbils, and checked it after 8 hours. A total of more than 30 midday gerbils were captured using this method.

2.2 | Animal samples and environmental facilities

Wild *M. meridianus* (males 80–100 g; females 60–80 g) were captured in Xinjiang Luntai, China and housed in the animal facilities of Xinjiang Laboratory Animal Research Center (SYXK [new] 2011-0002). Animals were housed at 17–26°C and 30%–65% relative humidity with a 12/12 hour light/dark cycle and automatic fan ventilation throughout the day. Experiments and procedures were approved by the animal welfare committee of the Laboratory Animal Research Center, Xinjiang, China.

2.3 | Culture medium and reagents

Columbia blood agar (batch number: 20140822), SS agar (batch number: 20140927), Staphylococcus selective agar (batch number: 20150416), NAC agar (batch number: 20140329), and modified PSB (batch number: 20140923), CIN-1 medium base (batch number: 20150812) and additives, anaerobic culture gas bags and oxygen indicators, and porcelain beads species preserved tubes (batch number: 20150522) were purchased from Qingdao Hope Biotechnology Co., Ltd, China. The Bacterial DNA Extraction Kit was from Tiangen Biotechnology Co., Ltd, China. The Vitek MS-DS target board and MS-CHCA matrix liquid were from bioMerieux, France. Taq DNA (batch number: 04124) polymerase and the agarose gel recovery kit were from Tiangen Biochemical Technology.

2.4 | Equipment

The following equipment was used: BPH-9028 precision constant temperature incubator (Yi heng Scientific Instrument Co., Ltd, Shanghai, China), NU-437-600E biological safety cabinet (NuAire, USA), Vitek MS automated microbial mass spectrometry detection system (bioMerieux, France), T100 PCR T100 Thermal Cycler for PCR (Bio-Rad, USA).

2.5 | Tissue sample methods

All cultures except for the CIN-1 agar plates (26°C) were incubated at 36°C. All cultures were incubated for 48 hours, except for the SS agar plate, which was incubated for 24 hours, and the NAC agar plate, which was incubated for 72 hours. Columbia blood agar plates were incubated in anaerobic conditions.

Animal cages were sterilized by autoclave (132°C, 8 minutes). Bacteria sampling was done according to published methods (GB/T14926, 42-2001). *M. meridianus* were anesthetized with carbon dioxide (50 mg/kg) and killed by cervical vertebra dislocation. The animals were then placed on a laminar flow cabinet and the body surface was disinfected with 75% alcohol. A "T"-shaped incision was made in the trachea and larynx and respiratory secretions from

a ring of tissue were inoculated into medium. Next, the abdominal cavity was opened and the ileum and cecum were isolated. The ileocecal valve was cut and the contents were inoculated onto plates.

2.6 | Inoculation of samples from the respiratory tract of animals

Samples from animal respiratory secretions were inoculated and cultured in media as described above. After incubation, colony morphology and Gram staining was performed. All colonies were inoculated onto Columbia blood agar plates as described and final purified culture media were transferred to porcelain bead strain preservation tubes and stored at –80°C until analysis. The analysis results are shown in Table 1.

2.7 | Inoculation of samples from ileocecal junctions

Samples from ileocecal junctions were inoculated and cultured in media as described. Samples inoculated with CIN-1 culture medium were initially inoculated into modified PBS for 10 days at 4°C, and then inoculated onto the CIN-1 agar plate. NAC agar plates were cultured for up to 5 days. After incubation, colony morphology and Gram stains were assessed and classified. All colonies were inoculated onto Columbia blood agar plates under the same conditions and final purified culture media were transferred to porcelain bead strain preservation tubes and stored at –80°C until analysis. The analysis results are shown in Table 2.

2.8 | Identification of isolated bacterial strains by mass spectrometry

Frozen strains were inoculated onto Columbia blood agar plates and cultured as described. Strain colonies were selected and added to the target plate of the Vitek MS microbial mass spectrometry system. The supporting matrix liquid was applied and allowed to dry under ambient temperatures after loading the target plate for testing.

2.9 | 16S rRNA sequencing³

For the microbial mass spectrometry detection system, the identification results cannot be given, and the samples with lower confidence levels are subjected to 16SrRNA sequencing. Bacterial

TABLE 1 Culture medium and culture condition of respiratory tract secretion

Culture medium	Culture condition(°C)	Culture time(h)
Columbia blood agar plate	36	48
Columbia blood agar plate	36anaerobic	48
SS agar plate	36	24

TABLE 2 The culture medium and culture condition of the content of ileocecal

Culture medium	Culture condition(°C)	Culture time(h)
Columbia blood agar plate	36	48
Columbia blood agar plate	36anaerobic	48
SS agar plate	36	24
Staphylococcus selective agar plate	36	48
NACAgar plate	36	72
CIN-1Agar plate	26	48

genome DNA was extracted using a bacterial DNA extraction kit. The 16S rRNA gene was amplified by PCR with a 5'-AGAGTTT-GATCCTGGCTCAG-3' forward primer and a 5'-ACGGCTACCTGT-TACGACTT-3' reverse primer. Amplification conditions were as follows: 94°C denaturation, 5 minutes; 94°C, 30 seconds; 53°C, 30 seconds; 72°C, 90 seconds for 35 cycles, with a final extension at 72°C, for 10 minutes. Next, 5 µl samples of PCR product were separated using SDS-PAGE, and the target strips were sequenced by Beijing BoMaiDe Gene Fragment Purification Technology Co. Ltd. The sequences were submitted to Genbank for a BLAST analysis.

3 | RESULTS

In order to investigate the pathogenicity of the isolates, reference was made to the relevant literature on *M. meridianus* (Table 3 for details). A total of 182 bacterial strains were isolated and cultured in each medium. The Vitek MS microbiological mass spectrometry system identified 141 isolates to the species level (77.5%). The other strains were sequenced by 16S rRNA sequencing. The results of both methods were statistically analyzed and compared with published results (Table 4). Mass spectrometer and 16S rRNA sequencing analysis showed that the respiratory and intestinal tracts of wild *M. meridianus* had a total of 39 bacterial species from 20 families and 27 genera, of which 23 bacterial species were isolated from the intestine, 11 were from the respiratory tract, and 5 were found in both intestinal and respiratory tracts (Table 4 for details). Staining combined with microscopy analysis found that 23 species were Gram-positive, 17 were Gram-negative, 18 were coccus and 22 were bacillus. *Escherichia coli*, which constitutes normal intestinal flora, had the highest detection rate (80%). The most commonly detected pathogenic bacteria were river *Vibrio*, *Staphylococcus aureus*, and *Pseudomonas aeruginosa*, with *Staphylococcus aureus* detected with the highest rate (40%) in both the respiratory and intestinal tracts. The conditional pathogens with higher detection rates were *Staphylococcus sciuri* (23.3%), *Staphylococcus xylosus* (40%) *Staphylococcus lentus* (20%), *Proteus mirabilis* (10%), *Oligella urethralis* (20%), *Enterococcus durans* (10%), *Enterococcus faecalis* (16.6%), *Haemophilus parahaemolyticus* (16.6%) and swine *Actinobacillosis* (10%).

4 | DISCUSSION

An investigation of the bacteria carried by new species of laboratory animal, *M. meridianus*, was carried out. The purpose of this study was to describe the general composition of the bacteria in the respiratory and intestinal tracts of *M. meridianus* and the key pathogenic of microbial flora. *M. meridianus* is currently under study for biological purification for use as an experimental animal but there are no microbiological data available for this species. We measured culturable bacteria in this animal and noted that they were diverse and most were pathogenic (conditional pathogens). Among them, 25 kinds of bacteria such as *Staphylococcus* and *Enterococcus*⁴ were human pathogenic bacteria. Based on the Laboratory Animal—Microbiological Standards and Monitoring guidelines (GB 14922.2-2010),⁵ specific pathogen free (SPF) rodents should not carry pathogens such as *Staphylococcus aureus* and *Pseudomonas aeruginosa*, so these pathogens should be removed before this species is experimentally viable. Du et al⁶ and Solomon et al⁷ isolated *Staphylococcus aureus* in *M. meridianus*, which is consistent with the results of this study. Six strains isolated from wild Mongolian gerbils were the same as in this study, which may be related to the habitat and feeding habits of *M. meridianus*.

In this study, we detected *Actinobacillus hominis*, an important swine conditional pathogen in pigs, in the respiratory tract of three *M. meridianus*.⁸ Feng et al⁹ and Du et al⁶ isolated this bacterial strain and other pathogenic strains in *M. meridianus*. Although this type of bacteria is not pathogenic to humans, we should consider the potential threat to other experimental animals in experimental animal facilities, and should focus on monitoring and eliminating the strain as part of future biological purification programs. We isolated *Vibrio fluvialis*, which has some pathogenicity in humans,¹⁰ from the intestine of one *M. meridianus* animal. This species too should be considered in future detection and purification work, since it is clear that the bacteria are sporadically or permanently present in animal populations.

The data from this study were measured within 3 days of the capture of the wild gerbils. The animals were kept in a normal animal experimental facility, and were given sterile water and feed, a regime that maintains the characteristics of the bacterial communities in the wild species. The bacterial strains were identified using a variety of media for culture and isolation, with the aim of improving the detection rate. However, we should recognize the limitations of the culture and isolation methods. Studies have shown that most bacteria

TABLE 3 Analysis of pathogenicity of flora in *Meriones meridianus*

Classification	Pathogenic bacteria	Conditional pathogenic bacteria	Non-pathogenic bacteria
Species	3	26	10
Mean detection rate (%) ^a	15.53	10.61	14.63
Percentage (%) ^b	43.3(13/30)	70(21/30)	83.3(25/30)

^aThe average value of the detection rate of each bacteria.

^bThe percentage of bacteria in this animal was determined.

TABLE 4 The body of *Meriones meridianus* bacteria detection statistics

Strain classification microscopy			Microscopic examination	Detection rate(n = 30)	Separation site		Literature pathogenicity
					Respiratory tract	Intestine	
<i>Vibrionaceae</i>	<i>Vibrio</i>	<i>Vibrio fluvialis</i>	G-Bacillus	3.3(1/30)	0	1	pathogenic bacteria
<i>Staphylococcaceae</i>	<i>Staphylococcus</i>	<i>Staphylococcus aureus</i>	G+coccus	40(12/30)	3	9	pathogenic bacteria
		<i>Staphylococcus pasteurii</i>	G+coccus	3.3(1/30)	0	1	Conditional pathogenic bacteria ¹³
		<i>Staphylococcus cohnii</i>	G+coccus	3.3(1/30)	0	1	Conditional pathogenic bacteria
		<i>Staphylococcus sciuri</i>	G+coccus	23.3(7/30)	1	6	Conditional pathogenic bacteria
		<i>Staphylococcus xylosus</i>	G+coccus	40(12/30)	1	11	Conditional pathogenic bacteria
		<i>Staphylococcus lentus</i>	G+coccus	20(6/30)	3	3	Conditional pathogenic bacteria
		<i>Staphylococcus epidermidis</i>	G+coccus	6.6(2/30)	1	1	Conditional pathogenic bacteria
<i>Pseudomonadaceae</i>	<i>Pseudomonas</i>	<i>Pseudomonas aeruginosa</i>	G-Bacillus	3.3(1/30)	0	1	Pathogenic bacteria
		<i>Pseudomonas putida</i>	G-Bacillus	6.6(2/30)	0	2	Pathogenicity is weak
<i>Burkholderiaceae</i>	<i>Ralstonia</i>	<i>Ralstonia mannitolilytica</i>	G-Bacillus	3.3(1/30)	0	1	Conditional pathogenic bacteria
	<i>Burkholderia</i>	<i>Burkholderia cepacia</i>	G-Bacillus	3.3(1/30)	0	1	Conditional pathogenic bacteria
<i>Moraxellaceae</i>	<i>Acinetobacter</i>	<i>Acinetobacter baumannii</i>	G-Bacillus	3.3(1/30)	0	1	Conditional pathogenic bacteria
<i>Aerococcaceae</i>	<i>Aerococcus</i>	<i>Aeroecoccus viridans</i>	G+coccus	40(12/30)	1	4	Conditional pathogenic bacteria
<i>Enterobacteriaceae</i>	<i>Serratia</i>	<i>Serratia plymuthica</i>	G-Bacillus	3.3(1/30)	0	1	Conditional pathogenic bacteria
	<i>Proteus</i>	<i>Proteus mirabilis</i>	G-Bacillus	10(3/30)	1	2	Conditional pathogenic bacteria
	<i>Providencia Ewing</i>	<i>Providencia rettgeri</i>	G-Bacillus	3.3(1/30)	0	1	Conditional pathogenic bacteria ¹⁴
	<i>Escherichia</i>	<i>Escherichia coli</i>	G-Bacillus	80(24/30)	2	22	Intestinal normal flora
	<i>Morganella</i>	<i>Morganella morganii</i>	G-Bacillus	3.3(1/30)	0	1	Intestinal normal flora
<i>Alcaligenaceae</i>	<i>Alcaligenes</i>	<i>Alcaligenes faecalis</i>	G-Bacillus	16.6(5/30)	0	5	Nonpathogen
	<i>Oligella</i>	<i>Oligella urthralis</i>	G-Bacillus	20(6/30)	3	3	Conditional pathogenic bacteria
<i>Flavobacteriaceae</i>	<i>Flavobacterium</i>	<i>Myroides odoratus</i>	G-Bacillus	3.3(1/30)	0	1	Conditional pathogenic bacteria ¹²
<i>Enterococcaceae</i>	<i>Enterococcus</i>	<i>Enterococcus hirae</i>	G+coccus	6.6(2/30)	0	2	Conditional pathogenic bacteria
		<i>Enterococcus gallinarum</i>	G+ coccus	3.3(1/30)	0	1	Conditional pathogenic bacteria
		<i>Enterococcus casseliflavus</i>	G+ coccus	6.6(2/30)	1	1	Conditional pathogenic bacteria
		<i>Enterococcus mundtii</i>	G+ coccus	3.3(1/30)	0	1	Conditional pathogenic bacteria
		<i>Enterococcus durans</i>	G+coccus	10(3/30)	0	3	Conditional pathogenic bacteria
		<i>Enterococcus faecalis</i>	G+coccus	16.6(5/30)	0	5	Conditional pathogenic bacteria

(Continues)

TABLE 4 (Continued)

Strain classification microscopy			Microscopic examination	Detection rate(n = 30)	Separation site		Literature pathogenicity
					Respiratory tract	Intestine	
Pasteurellaceae	<i>Haemophilus</i>	<i>Haemophilus parahaemolyticus</i>	G-Bacillus	16.6(5/30)	5	0	Conditional pathogenic bacteria
	<i>Actinobacillus</i>	<i>Actinobacillus suis</i>	G-Bacillus	10(3/30)	3	0	Pathogenic bacteria of anima ⁸
Corynebacteriaceae	<i>Corynebacterium</i>	<i>Corynebacterium haemolyticum</i>	G+Bacillus	6.6(2/30)	0	2	Conditional pathogenic bacteria
Bacteroidaceae	<i>Bacteroides</i>	<i>Bacteroides ovatus</i>	G-Bacillus	3.3(1/30)	1	0	Conditional pathogenic bacteria ⁴
Clostridiaceae	<i>Clostridium</i>	<i>Clostridium butyricum</i>	G+Bacillus	3.3(1/30)	0	1	Nonpathogen
Bacillaceae	<i>Bacillus</i>	<i>Lysinibacillus fusiformis</i>	G+Bacillus	3.3(1/30)	1	0	Nonpathogen
Planococcaceae	<i>Sporosarcina</i>	<i>Sporosarcina ureae</i>	G+coccus	10(3/30)	0	3	Nonpathogen
Lactobacillaceae	<i>Lactobacillus</i>	<i>Lactobacillus animalis</i>	G+Bacillus	16.6(5/30)	3	2	Nonpathogen
Planococcaceae	<i>Jeotgalicoccus</i>	<i>Jeotgalicoccus halophilus</i>	G+coccus	3.3(1/30)	0	1	Nonpathogen
Streptococcaceae	<i>Streptococcus</i>	<i>Streptococcus salivarius</i>	G+Streptococcus	6.6(2/30)	2	0	Nonpathogen
Brevibacteriaceae	<i>Brevibacterium</i>	<i>Brevibacterium linens</i>	G+Bacillus	3.3(1/30)	0	1	Nonpathogen

cannot easily be cultured.^{10,11} In future purification studies, the frequency of detection and number of samples should be increased and other detection techniques and methods used to give a more comprehensive picture the microbial flora carried by the animals.

In conclusion, using selected culture and isolation methods, this study found a limited number of bacteria present in *M. meridianus*, but we may not have found all important species.¹² Future work on the purification of *M. meridianus* should increase the frequency of detection and sampling amount to fully characterize this new model species.

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CONFLICT OF INTEREST

None.

AUTHOR CONTRIBUTIONS

YMX conceived and designed the experiments. SS, XHX and JLY performed the experiments. SS and HKY analyzed the data. SS and YMX wrote the manuscript. All authors analyzed the data and were involved in writing the manuscript. And all authors have read and approved the final manuscript.

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