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First Isolation of Morganella morganii from Endometritis in a Chinese Rhesus Monkey (Macaca mulatta): Molecular and Antimicrobial Characterization

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ABSTRACT

A bacterial isolate was obtained from the uterine discharge of a rhesus macaque suffering from Endometritis and subsequently characterized. On Columbia blood agar incubated at 37 °C for 24 h, the organism formed circular, elevated, gray-white colonies with smooth surfaces, typically 1–2 mm in diameter. When cultured on salmonella–shigella agar (S.S.) under the same conditions, the colonies appeared flat, transparent, and round. Microscopic examination following Gram staining revealed gram-negative, blunt-ended rods lacking spores. Sequencing of the 16S rRNA gene demonstrated more than 96.9% identity with published Morganella morganii sequences listed in GenBank. Integrating morphological and molecular analyses confirmed the isolate (RM2023) from the rhesus monkey's cervical secretions as M. morganii. Antibiotic susceptibility profiling showed RM2023 was responsive to ceftriaxone, amikacin, gentamicin, cefazolin, cefuroxime, ceftazidime, levofloxacin, cotrimoxazole, norfloxacin, and tetracycline; had intermediate response to ampicillin; and was non-susceptible to penicillin, vancomycin, ciprofloxacin, and clindamycin. These results enhance understanding of infectious diseases in rhesus monkeys and supply useful data for molecular epidemiological work.

Keywords: Rhesus macaque, Morganella morganii, Bacterial isolation, Molecular characterization

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Introduction

Because of their close resemblance to humans in physiology and metabolism, rhesus monkeys are key species in experimental medicine [1]. The reliability of such studies depends heavily on the use of healthy and well-maintained animals. Yet, those raised in open or semi-natural conditions frequently develop bacterial infections—one of the principal causes of uterine inflammation. During delivery or the postpartum stage, the cervix dilates, allowing microbes from the surroundings, skin, or feces to ascend into the uterus through the vaginal canal. When the uterine lining is damaged or the animal's immune function declines, normal defense mechanisms weaken, hindering bacterial clearance and resulting in metritis or Endometritis [2].

M. morganii is a bacterium commonly present in soil, water, and the intestinal tracts of many species. It behaves as both an opportunistic and zoonotic organism [3]. Human–animal transmission has been documented in several situations: M. morganii has been detected in snakes' mouths and may infect people through bites or scratches [4, 5]; isolates recovered from captive dolphins were shown by Park *et al*. [6] to cause lethal zoonotic infection and exhibit antibiotic resistance; and as a histamine-producing bacterium, M. morganii can contaminate seafood or dairy products, causing foodborne histamine poisoning [7, 8]. Reported infections include cellulitis, osteomyelitis, endocarditis, arthritis, diarrhea, and systemic septicemia in humans and animals [8–10]. Although typically

opportunistic, the pathogen's growing antimicrobial resistance and virulence have complicated clinical therapy [10, 11].

In the present case, a rhesus macaque experienced Endometritis unresponsive to penicillin. A bacterial agent was isolated from the uterine exudate and identified as M. morganii using morphological inspection and molecular sequencing. Drug susceptibility results guided treatment with ceftriaxone and gentamicin, which effectively cleared the infection. While M. morganii infections have been documented in multiple species, this report represents, to our knowledge, the first confirmed case in macaques, offering a new reference for disease prevention in primate colonies.

Materials and Methods

Sample collection

The study animal originated from the State Key Laboratory of Primate Biomedical Research, Kunming University of Science and Technology [SYXK (Yunnan) K2022-0001]. The female exhibited abnormal reproductive cycles, excessive menstrual bleeding, reduced appetite during estrus, cervical looseness, cervical dilation, and copious foul-smelling discharge approximately six months after giving birth. After disinfecting the external cervical region with iodine, sterile cotton swabs were used to collect secretions, which were then placed into a sterile 15 mL tube containing roughly 2 mL of sterile saline. The specimen was immediately sent to the laboratory for microbiological analysis.

Isolation and morphology of bacteria

A small quantity of the mixed secretion sample was pipetted into a sterile 1.5 mL microtube. Using a sterile loop, the suspension was streaked onto Columbia blood agar (HKM, Guangzhou, China) and S.S. agar (Hopebio, Qingdao, China) plates, then incubated at 37 °C for 24 h. Colonies displaying distinct morphological traits were subcultured on fresh media and incubated again for 18–24 h under identical conditions to observe colony behavior. Once purified, isolated colonies were smeared on glass slides containing saline, air-dried, and Gram-stained with a commercial kit (Solarbio, Beijing, China). Microscopic examination was carried out using a Nikon optical microscope (Tokyo, Japan) at 1000× magnification.

Amplification and sequencing of bacterial 16S rRNA

The bacterial 16S rRNA gene was amplified using the primers 27F (5′-AGAGTTTGATCCTGGCTCAG-3′) and 1492R (5′-CGGCTACCTTGTTACGACTT-3′). Each PCR reaction contained 12.5 μL of 2× Taq PCR Master Mix (Tiangen, Beijing, China), 1 μL of each primer (10 μmol/L), and 9.5 μL of sterile distilled water. A trace amount of bacterial material obtained from a single colony on Columbia blood agar using a sterile tip was used as the DNA source. The amplification program began with an initial denaturation at 95 °C for 3 min, followed by 35 amplification cycles (95 °C for 30 s, 55 °C for 30 s, and 72 °C for 1 min), ending with a final elongation at 72 °C for 5 min. PCR products (5 μL) were separated via 1.5% agarose gel electrophoresis for 30 min at 120 V, stained with ethidium bromide, and visualized under UV light (Aplege, Pleasanton, CA, USA). The desired DNA fragment was excised and purified using a DNA extraction kit (Trelief, Beijing, China) before sequencing at Beijing Tsingke Biotech Co., Ltd.

Sequence alignment and phylogenetic tree construction

The resulting sequence was analyzed for homology through BLAST comparison with entries in NCBI GenBank using MegAlign 7.0.26. Sequence alignment and phylogenetic relationships of Morganella morganii isolates from multiple hosts were subsequently examined. The evolutionary tree was generated in MEGA11 to evaluate genetic proximity among the strains.

Antibiotic sensitivity testing

A total of fifteen commonly used antibiotic discs (BKMAM, Changde, China) were employed to evaluate drug susceptibility of the isolated M. morganii strains by the Kirby–Bauer disc diffusion technique. The inhibition zones were measured, and resistance, intermediate, or sensitive reactions were determined according to standardized antibiotic reference ranges.

Results and Discussion

Samples from cervical swabs were cultured on Columbia blood agar and S.S. media, both yielding morphologically identical colonies. After 24 h incubation on Columbia agar, colonies appeared circular, convex, smooth, and grayish-white, measuring 1–2 mm in diameter **(Figure 1a)**. Growth on S.S. plates produced flat, translucent colonies **(Figure 1b)**. Gram staining revealed Gram-negative bacilli with rounded ends and no spores **(Figure 1c)**.

a) b) c)

Figure 1. Morphology of M. morganii isolated from a rhesus monkey: (a) growth on Columbia blood agar; (b) colonies on Salmonella–Shigella agar; (c) Gram-stained microscopic appearance.

PCR amplification yielded a single band between 1000 and 2000 bp, consistent with the expected size for the 16S rRNA gene **(Figure 2)**. The final sequence length obtained was 1010 bp.

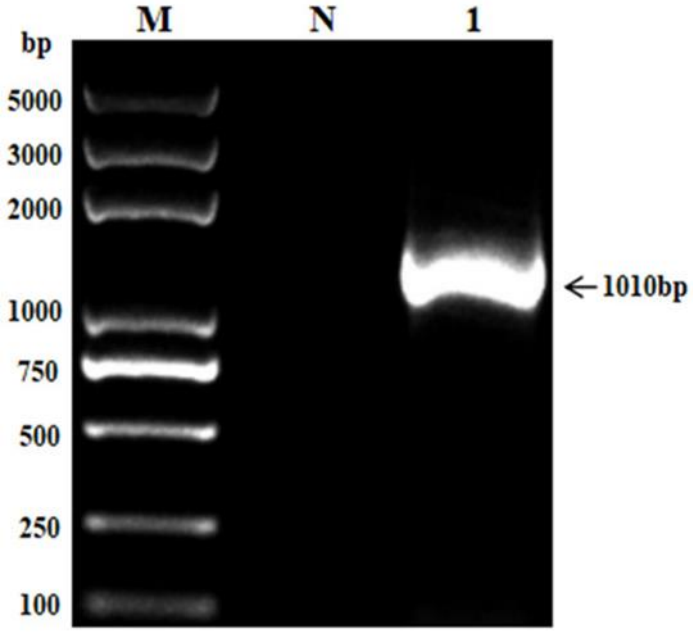

Figure 2. Identification of M. morganii 16S rRNA gene in rhesus monkey. (M: DL-2K marker; 1: sample; N: negative control).

The Sanger sequencing results were deposited in GenBank under accession number PP741825. Sequence alignment showed 99% identity with M. morganii reference sequences. Comparative analysis of the rhesus monkey strain (RM2023) indicated over 96.9% similarity with M. morganii strains from various hosts, with the highest similarity (99.5%) observed for isolates from chicken (Gallus domesticus, LC385634) and water monitor lizard (Varanus salvator, OQ644263) **(Figure 3)**.

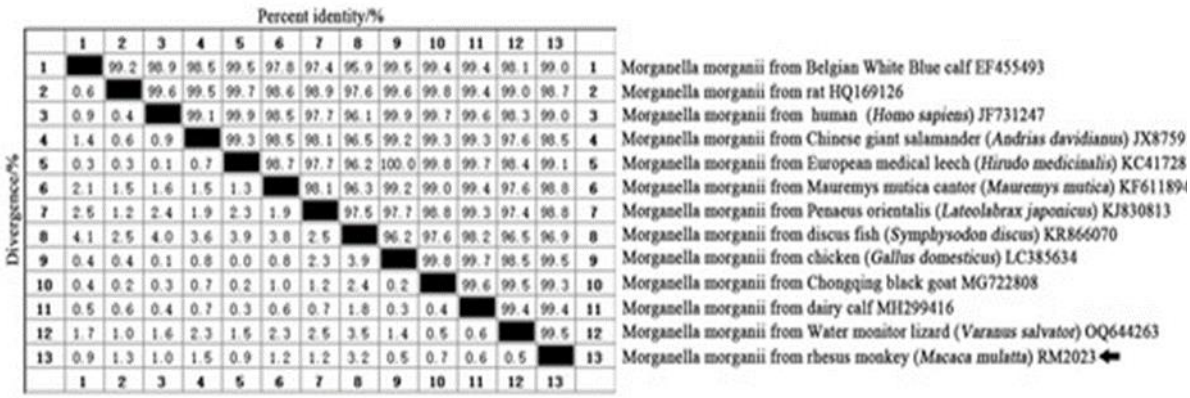

Figure 3. Comparative similarity of M. morganii (RM2023) from rhesus monkey (Macaca mulatta) with other M. morganii strains.

Phylogenetic evaluation revealed that RM2023 formed a unique branch closely clustering with M. morganii from Varanus salvator (OQ644263) **(Figure 4)**. It also grouped within a larger cluster containing isolates from Hirudo medicinalis (KC417281), Homo sapiens (JF731247), Gallus domesticus (LC385634), Rattus norvegicus (HQ169126), Andrias davidianus (JX875917), Chongqing black goat (MG722808), Bos taurus (MH299416), Mauremys mutica (KF611894), and Lateolabrax japonicus (KJ830813). Conversely, the Symphysodon discus isolate (KR866070) was positioned distantly. These findings verified that the bacterium isolated from the rhesus monkey's cervical secretion was Morganella morganii, genetically closest to the Varanus salvator strain and most divergent from Symphysodon discus.

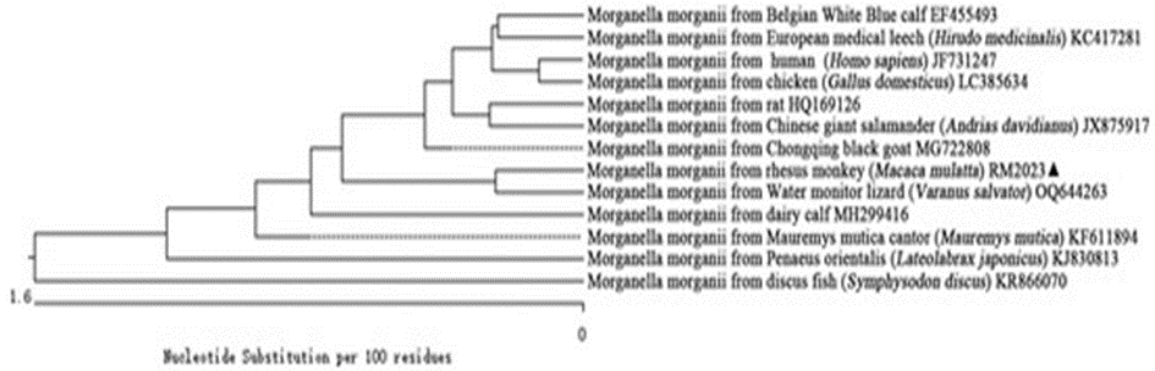

Figure 4. Phylogenetic diagram showing the relationship of 16S rRNA sequences between Morganella morganii isolated from a rhesus monkey (Macaca mulatta) (RM2023) and previously reported M. morganii strains. The triangle symbol ("▲") denotes the isolate obtained in this study.

The antimicrobial profile of the bacterial isolate RM2023 was assessed using the Kirby–Bauer disc diffusion assay against fifteen antibiotics. The strain demonstrated susceptibility to ten drugs—ceftriaxone, amikacin, gentamicin, cefazolin, cefuroxime, ceftazidime, levofloxacin, cotrimoxazole, norfloxacin, and tetracycline—while exhibiting moderate response to ampicillin and resistance to penicillin, vancomycin, ciprofloxacin, and clindamycin **(Table 1)**.

Table 1. Antimicrobial response pattern of M. morganii obtained from a rhesus monkey sample.

Antibiotics (Medication Dose: µg/tablet)	**IZD (mm)**	**Sensitivity**	**Judgment Standard of Inhibition Zone Diameter (mm)**		
			Resistant	Medium Sensitivity	Highly Sensitive
Penicillin (10)	0	R	≤14	—	≥15
Ceftriaxone (30)	30	S	≤13	14–22	≥23
Amikacin (30)	20	S	≤14	15–16	≥17
Gentamicin (10)	18	S	≤12	13–14	≥15

Ampicillin (10)	14	I	≤13	14–16	≥17
Cefazolin (30)	22	S	≤14	15–17	≥18
Cefuroxime (30)	20	S	≤16	17–19	≥20
Ceftazidime (30)	28	S	≤17	18–20	≥21
Levofloxacin (5)	22	S	≤13	14–16	≥17
Vancomycin (30)	0	R	≤9	10–11	≥12
Cotrimoxazole (23.75)	20	S	≤10	11–15	≥16
Ciprofloxacin (5)	24	R	≤27	28–40	≥41
Norfloxacin (10)	24	S	≤12	13–16	≥17
Clindamycin (2)	0	R	≤14	15–20	≥21
Tetracycline (30)	24	S	≤14	15–18	≥19

S: sensitive; I: intermediate; R: resistant.

Morganella morganii is a facultative anaerobe, Gram-negative bacillus classified under the Enterobacteriaceae family (Morganella genus) [4]. It is recognized as an opportunistic microorganism [12] possessing numerous pathogenic determinants, such as fimbrial adhesins, lipopolysaccharide (LPS), IgA protease, hemolysin, urease, insecticidal and apoptotic toxins, iron-acquisition systems, type III secretion machinery, and two-component regulatory elements [10]. These factors contribute to its capacity to induce several infectious diseases, including urinary tract infections, bacteremia, hepatobiliary infections, and lesions of skin and soft tissue [3, 8, 13, 14]. In addition, M. morganii has been associated with diverse clinical manifestations, such as abscesses of the brain and liver, chorioamnionitis, peritonitis, pericarditis, septic arthritis, rhabdomyolysis, necrotizing fasciitis following snakebite, bilateral keratitis, neonatal hemolytic anemia, and non-clostridial gas gangrene [15]. This microorganism can affect a wide range of hosts, including humans [16, 17], pigs (Sus) [18], European medicinal leeches (Hirudo medicinalis) [19], bullfrogs (Rana catesbeiana) [20], South China tigers (Panthera tigris amoyensis) [21], cattle [22], guinea pigs (Cavia porcellus), rabbits (Leporidae), and reptiles [19]. Despite its presence in multiple species, information on M. morganii derived from monkeys remains limited. Thus, the present study offers an initial systematic analysis of the bacterial morphology and genetic identity of M. morganii isolated from a rhesus monkey (Macaca mulatta).

Morphological observation serves as a cornerstone in bacterial taxonomy and diagnostic microbiology, as it aids in recognizing microbial types and guides antibiotic sensitivity evaluation. Endometritis develops when bacteria ascend from the vaginal canal or cervix into the uterus. Research indicates that colonization of the endometrium leads to damage of its lining, infiltration of neutrophils, production of inflammatory cytokines, and persistence of chronic inflammation. Bacteriological culturing remains one of the principal diagnostic tools for detecting chronic Endometritis [23, 24].

In this investigation, bacterial colonies cultured on Columbia blood agar at 37 °C for 24 h appeared round, convex, gray-white, smooth, and measured roughly 1–2 mm in diameter. When grown on S.S. agar under identical conditions, colonies were circular, flat, and semi-transparent. These phenotypic observations matched well with the known features of M. morganii.

M. morganii is a Gram-negative, facultatively anaerobic, short bacillus that produces flat, non-pigmented colonies about 2–3 mm in diameter when cultured on MacConkey or blood agar, while on S.S. agar it forms circular, transparent colonies [12]. These growth characteristics, however, are not unique to this bacterium. In some clinical specimens, low bacterial concentrations prevent visible colony formation, while in others, pale or whitish colonies may develop when growth is more pronounced. So far, no selective culture medium has been specifically established for M. morganii [25].

Because the 16S rRNA gene retains a highly conserved structure and consistent function, it serves as a key molecular marker in bacterial taxonomy, offering accurate, sensitive, and rapid identification. Differences among bacterial species mainly occur in the variable domains of the 16S rRNA sequence, which makes it ideal for classification and species-level distinction.

In the present research, a 16S rRNA gene fragment measuring roughly 1010 base pairs was amplified and sequenced. Comparison of this sequence with records from the NCBI GenBank database showed over 99.3%

similarity to M. morganii isolates previously identified from chicken (Gallus domesticus) (LC385634), water monitor lizard (Varanus salvator) (OQ644263), dairy calf (MH299416), and Chongqing black goat (MG722808). On the basis of morphological observations and molecular homology analysis, the recovered isolate was confirmed to be M. morganii. Phylogenetic analysis revealed that this strain clustered most closely with M. morganii from Varanus salvator (OQ644263), implying a close evolutionary link, whereas it diverged into a distinct branch from the strain found in discus fish (Symphysodon discus) (KR866070), reflecting greater evolutionary distance.

Earlier investigations have shown that M. morganii commonly displays extensive antimicrobial resistance and tends to develop multidrug resistance rapidly [26, 27]. Therefore, bacteriophages have been considered a potential substitute for antibiotic therapy in infections caused by this bacterium [7]. In contrast, the strain isolated in this study was highly sensitive to ceftriaxone, amikacin, gentamicin, cefazolin, cefuroxime, ceftazidime, levofloxacin, cotrimoxazole, norfloxacin, and tetracycline. Administration of ceftriaxone combined with gentamicin successfully resolved the infection in rhesus monkeys. The observed drug responsiveness may relate to environmental influences or strain-specific factors, and variations in antibiotic profiles can occur among M. morganii isolates originating from different hosts.

In summary, this report describes a case of Endometritis caused by M. morganii, marking the first recorded isolation of this organism from a rhesus monkey. The findings provide a useful reference for future work on bacterial disease prevention and characterization in this species.

Conclusion

Using morphological examination and 16S rRNA gene sequencing, this study confirmed the occurrence of M. morganii in cervical secretions from a rhesus monkey diagnosed with Endometritis. The isolate showed susceptibility to ceftriaxone, amikacin, gentamicin, cefazolin, cefuroxime, ceftazidime, levofloxacin, cotrimoxazole, norfloxacin, and tetracycline. These outcomes offer important insights for the recognition, management, and therapeutic approach to M. morganii-associated Endometritis in rhesus monkeys.

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Conflict of Interest: None

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Ethics Statement: None

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