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Osteomyelitis Caused by *Metamycoplasma gateae* after Cat Bite, Japan, 2026

Appendix

Culture Media and Incubation Conditions for Pus and Tissue Specimens

The following media were used for the culture of pus and tissue specimens: 1) sheep blood agar (Shimadzu Diagnostics, https://www.shimadzu.com), 2) chocolate agar (Kyokuto Pharmaceutical Industrial, https://www.kyokutoseiyaku.co.jp), 3) BBL BTB Lactose-Contained Agar (Becton Dickinson, https://www.bd.com), 4) Pourmedia egg yolk agar (Eiken Chemical, https://www.eiken.co.jp), 5) sheep blood agar containing colistin and aztreonam (Kyokuto Pharmaceutical Industrial), 6) Brucella HK agar (Kyokuto Pharmaceutical Industrial) for anaerobic culture, and 7) HK semisolid medium (Kyokuto Pharmaceutical Industrial) for enrichment culture. The culture conditions were as follows: media 1), 2), and 5) were incubated at 35°C for 20 h in a 5% CO_2 atmosphere; media 3) and 4) were incubated aerobically at 35°C for 20 h; medium 6) was incubated anaerobically at 35°C for 48 h; and medium 7) was incubated aerobically at 35°C for 7 days.

16S rRNA Gene Sequencing Protocol

Primers: 518A (5′ CCAGCAGCCGCGGTAATAC 3′), 786B (5′ GACTACCAGGGTATCTAATC 3′), and 907A (5′ AAACTCAAAGGAATTGACGG 3′).

DNA was extracted from clinical specimens by using the QIAamp DNA Blood Mini Kit (Qiagen, https://www.qiagen.com), according to the manufacturer's instructions. An extraction blank, consisting of nuclease-free water processed through the entire DNA extraction procedure, was included to monitor contamination during DNA extraction. PCR amplification of the 16S rRNA gene was performed by using TaKaRa Ex Taq (Takara Bio, https://www.takarabio.com)

in a total reaction volume of 50 μL containing 5.0 μL of 10× Ex Taq buffer, 4.0 μL of dNTP mixture (2.5 mM each), 0.25 μL of Ex Taq DNA polymerase, primers, template DNA, and nuclease-free water. Thermal cycling conditions consisted of an initial denaturation at 95°C for 3 min, followed by 35 cycles of denaturation at 95°C for 30 s, annealing at 55°C for 30 s, and extension at 72°C for 1 min, with a final extension at 72°C for 7 min. A PCR negative control containing nuclease-free water instead of template DNA was included in each PCR run to detect contamination during PCR amplification.

16S rRNA Gene Sequencing Result (GenBank accession no. LC934417)

TGGCTCAGGATGAACGCTGGCTGTGTGCCTAATACATGCATGTCGAGCGAGG
TTCTTTTGAACCTAGCGGCGAATGGGTGAGTAACACGTGCTTAATCTACCCTTTAGA
TTGGAATACCCAATGGAAACATTGGCTAATGCCGGATACGCATGGAACCGCATGGT
TCCGTTGTGAAAGGAGCCTCAAAGCTCCGCTAGAGGATGAGGGTGCGGAACATTAG
TTAGTTGGTAGGGTAATGGCCTACCAAGACTATGATGTTTAGCCGGGTCGAGAGACT
GAACGGCCACATTGGGACTGAGATACGGCCCAAACTCCTACGGGAGGCAGCAGTAG
GGAATATTCCACAATGAGCGAAAGCTTGATGGAGCGACACAGCGTGCACGATGAAG
GTCTTCGGATTGTAAAGTGCTGTTATAGGGAAAGAACACTTGGTTGAGGAAATGCTT
CCAAGCTGACGGTACCCTGTCAGAAAGCGATGGCTAACTATGTGCCAGCAGCCGCG
GTAATACATAGGTCGCAAGCGTTATCCGGAATTATTGGGCGTAAAGCGTTCGTAGGC
TGTTTATTAAGTCTGGAGTTAAATCCCAGGGCTCAACCCTGGCTCGCTTTGGATACT
GGTAAACTAGAGTTAGATAGAGGYAAGCGGAATTCCATGTGAAGCGGTGAAATGCG
TAGATATATGGAAGAACACCAAAGGCGAAGGCAGCTTACTGGGTCTATACTGACGC
TGAGGGACGAAAGCGTGGGGAGCAAACAGGATTAGATACCCTGGTAGTCCACGCCG
TAAACGATGATCATTAGTCGGTGGAGAGTTCACTGACGYAGCTAACGCATTAAATG
ATCCGCCTGAGTAGTATGCTCGCAAGAGTGAAACTTAAAGGAATTGACGGGGACCC
GCACAAGCGGTGGAGCATGTGGTTTAATTTGAAGATACGCGGAGAACCTTACCCAC
TCTTGACATCCTTCGCAATGCTATAGAGATATAGCGGAGGTTAACGGAGTGACAGAT
GGTGCATGGTTGTCGTCAGCTCGTGTCGTGAGATGTTTGGTCAAGTCCTGCAACGAG
CGCAACCCCTATCTTTAGTTACTAACGAGTCATGTCGAGGACTCTAGAGATACTGCC
TGGGTAACCGGGAGGAAGGTGGGGATGACGTCAAATCATCATGCCTCTTACGAGTG

GGGCAACACACGTGCTACAATGGTCGGTACAAAGAGAAGCAATATGGCGACATGGA
GCAAATCTCAAAAAGCCGATCTCAGTTCGGATTGGAGTCTGCAATTCGACTCCATGA
AGTCGGAATCGCTAGTAATCGCAGATCAGCTACGCTGCGGTGAATACGTTCTCGGGT
CTTGTACACACCGCCCGTCACACCATGGGAGCTGGTAATACCCAAAGTCGGTTAGCT
AACCTCGGAGGCGACCGCCTAAGGTAGGACTGGTGACTGGGGTGA

Appendix Table. 16S rRNA gene sequence similarity

Description	Identity, nt*	% Identity	GenBank accession no.
Metamycoplasma gateae strain 3700 chromosome, complete genome	1450/1452	99.86%	CP143578.1
M. gateae strain ATCC 23392 16S ribosomal RNA, partial sequence	1440/1442	99.86%	NR_029180.1
Mycoplasma arginini strain 284F08 16S ribosomal RNA gene, partial sequence	1443/1453	99.31%	GQ409971.1
Mycoplasmopsis arginini strain NCTC 10129 chromosome, complete genome	1442/1453	99.24%	CP143577.1
Mycoplasmopsis arginini strain 19776 16S ribosomal RNA gene, partial sequence	1442/1453	99.24%	ON890822.1

*No. identical nucleotides/total no. nucleotides.