A case of multiple lung abscesses caused by *Actinomyces graevenitzii* mimicking acute pulmonary coccidioidomycosis

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Abstract

Actinomyces graevenitzii is a newly recognized *Actinomyces* species, that is seldom isolated from clinical specimens. A case of multiple pulmonary abscesses mimicking acute pulmonary coccidioidomycosis is described in this study, and the findings indicate that this organism as an opportunistic human pathogen.

(41 words)

Case Report:

A 38-year-old apparently healthy woman complained of fever and dry cough after visiting Los Angeles, California. Her medical history did not reveal any specific illness, including acquired-immune deficiency syndrome. She did not smoke or consume alcohol. During her 3-day stay, she visited the beach in California. On her way to the beach, she encountered a dust storm and inhaled a large amount of dust. Seven days after she returned to Japan (9 days after encountering the dust storm), she was admitted to a local hospital in Nagasaki owing to a progressive dry cough (clinical day 8). On admission, the vital signs of the patient were as follows: body temperature, 37.5℃; blood

pressure, 99/64 mm Hg; pulse, 72 beats/min with a regular rhythm; SpO_2, 96% in a room air condition; and respiratory rate, 16 breaths/min. Cyanosis, cardiac murmur, and breath sounds were absent. Moreover, her liver, spleen and lymph nodes were not palpable. Her white blood cell count was 7.3 $\times$ 10^3/mL, with a shift to the left (71% neutrophils), and her C-reactive protein value was 5 mg/dL (normal range, 0–0.3 mg/dL). The chest computed tomography (CT) images revealed multiple round lesions located on both lobes, with diameters of 0.5–1 cm (Figures 1 and 2). The radiological findings strongly suggested metastatic tumors; hence, digestive tract endoscopy and positron emission tomography (PET) of the entire body were performed. No other lesions, except the lesions in the lungs, were found to contribute to the findings. Two weeks later (clinical day 21), the multiple pulmonary lesions had grown in size by 3-fold, with partial cavity formation (Figures 1 and 2). Owing to the extreme rapid progression of the lesions, the patient was transferred to our hospital for further examination and treatment. An oral antibiotic, faropenem (FRPM), at 600 mg/day was given from clinical day 21 to 24. On admission (clinical day 25), we suspected acute pulmonary coccidioidomycosis because of her travel history to the US West Coast and

the similarities of her CT images with those of typical pulmonary *Coccidioides* infection,[3] and we administered liposomal amphotericin B (L-AMB) at 150 mg/day intravenously on clinical day 26. We also initiated tazobactam/piperacillin (TAZ/PIPC) therapy at 18 g/day concurrently because the presence of other bacterial infections was not completely excluded. Before the initiation of antibiotic therapy, a transbronchial biopsy (TBB) using endobronchial ultrasonography with a guide sheath was performed 48 h after the cessation of FRPM treatment. The presence of antibodies against *Coccidioides* was also examined before the initiation of antifungal treatment (clinical day 26). After 9 days of treatment (clinical day 35), her fever subsided, but the pulmonary lesions that were observed on radiological examination had not improved.

Primary culturing of bronchoalveolar lavage fluid (BALF) was performed with blood and chocolate agar plates. BALF (inoculum volume, 5 μL) was streaked onto the plate quantitatively and incubated at 37°C in 5% CO_2. Only molar tooth-like colonies were observed in 3 days. Hemolysis around the colonies was not observed, and the quantitative culture yielded 1×10^4 CFU/mL. The organism was a coryneform gram-positive rod that did not

produce catalase. On commercial biochemical testing (ID panel; Phoenix Automated Microbiology System, Becton Dickinson Co., Ltd., Japan), the organism was initially identified as *Erysipelothrix rhusiopathiae*, contrary to its colony characteristics. Upon repeated biochemical testing, the isolate was reidentified as *Arcanobacterium haemolyticum*. The microphotographs and colony characteristics of the organism isolated from the BALF are shown in Figure 3. The MIC values of the tested antibiotics, including penicillin G, ampicillin, piperacillin, cefotiam, cefotaxime, ceftizoxime, ceftazidime, ceftriaxone, cefepime, imipenem, meropenem, erythromycin, and clarithromycin were found to be less than 0.05 μg/mL. Only minocycline had an MIC value of 4 μg/mL. Results of the acid-fast staining and PCR testing of the patient's BALF for *Mycobacterium tuberculosis*, *M. avium*, and *M. intracellulare* were negative.

Although a gram-positive rod bacterium was isolated from the BALF cultures, the histological findings of the TBB specimen could only reveal nonspecific inflammation. Thus, there was a possibility of coinfection with other etiologic pathogens. The *Coccidioides* antibody was not detected on clinical day 32. However, owing to her unique travel history and radiological

findings, we could not completely exclude the possibility of acute pulmonary coccidioidomycosis. Therefore, we performed an additional histological assessment and repetitive examinations for detection of *Coccidioides* antibody to confirm the diagnosis. On clinical day 36, video-assisted thoracoscopic surgery (VATS) was performed to acquire lung tissue. During VATS, the nodule (30 mm) in the right lower lobe was resected and examined. On histological examination, lymphocyte infiltration, fibrous change, and several Masson bodies were found within the resected nodule, but no pathogen, including *Coccidioides*, was identified by pathological or microbiological examination. On clinical day 53, after confirming the result of VATS and obtaining negative results for the repetitive tests for serum antibody against *Coccidioides*, we replaced TAZ/PIPC and L-AMB with amoxicillin (AMPC) therapy at 2 g/day. After 2 months of administration, the pulmonary abscesses were no longer detected on radiological examination.

Because of the rarity of her clinical course, we performed molecular identification by PCR amplification and sequencing analysis of the 16S rRNA gene using DNA extracted from the isolates. The universal primers 8UA (5′-AGAGTTTGATCMTGGCTCAG-3′) and 1485B

(5′-ACGGGCGGTGTGTRC-3′) were used, as described previously.[9] We performed a sequencing analysis using a GenBank BLAST search and BiBi (http://pbil.univ-lyon1.fr/) phylogenetic tools. The sequence of the 16S rRNA gene showed 99.7% identity (1403 base pairs [bp] over the entire 1407-bp fragment) with that of the type strain *Actinomyces graevenitzii* (CCUG27294, Genbank accession no. AJ 540309). On the basis of this result, we identified the isolate as *A. graevenitzii*.

Discussion:

Actinomyces spp. are the most common commensal anaerobic bacterium in the human oral cavity, and 6 species of this genus are considered pathogenic in humans: *A. israeli*, *A. naeslundii*, *A. odontolyticus*, *A. viscosus*, *A. meyeri* and *A. gerencseriae*.[8,13] Pulmonary actinomycosis is well known as a cause of chronic infection, and it constitutes 15% of the total burden of actinomycosis in humans.[8] The clinical features usually include low-grade inflammation with indolent advancement, which is similar to the presentation of fungal infection or lung neoplasms.[8] Several reports[7,8,15] found that 25%–49% of cases of pulmonary actinomycosis were suspected to be lung malignancy upon hospital admission, and the mean duration of illness before a definitive

diagnosis was 2–6 months. A diagnosis of pulmonary actinomycosis is often confirmed by histological findings, which reflect chronic inflammation consisting of granulomatous change with sulfur granules.[6] Bacterial confirmation of a clinicopathological diagnosis is usually obtained in <50% of cases owing to inadequate culturing techniques, previous antibiotic therapy, and bacterial overgrowth.[1] Song et al.[15] found that positive culture results were obtained in only 3 of 40 cases of pulmonary actinomycosis. Thus, the diagnosis of conventional pulmonary actinomycosis requires a combination of several factors, including respiratory specimen culture, correlation with clinical and radiological features, histological findings, and response to antibiotic treatment.

In addition to these traditional actinomycotic forms, some coryneform anaerobic bacteria have also recently been assigned to the genus *Actinomyces* by the US Centers for Disease Control.[4,5] *A. graevenitzii* is a newly recognized *Actinomyces* sp. that was first isolated from 4 clinical human specimens in 1997 by Ramos et al.[11] It is a filamentous gram-positive rod with no catalase production and is facultatively anaerobic with a distinct biochemical profile.[11] Sarkonen et al. isolated *A. graevenitzii* from failed

dental implant surfaces for their study on the distribution of *Actinomyces* spp. in 33 dental implant fixtures. [12] Similar to other *Actinomyces spp.*, *A. graevenitzii* is possibly a component of the oropharyngeal flora. Very little is known about the clinical features and pathogenesis of *A. graevenitzii*,[14] and only 1 case report has described the disseminated infection of *A. graevenitzii*, which showed coinfection with *Mycobacterium tuberculosis*.[16]

Our case presented different clinical features of conventional pulmonary actinomycosis, such as the rapid progression of lung lesions and the lack of specific histological features, including granulomatous change or presence of sulfur granules. Acute pulmonary coccidioidomycosis was first suspected because of the patient's travel history to an endemic area of coccidioidomycosis, and the radiological features of multiple round shadows predominantly located in the lower lobes. A histological examination of lung specimens by VATS was necessary for definite differentiation. Although the pathogen could not be identified by histological examination, the quantitative culture of BALF yielding 1×10^4 CFU/mL organisms supports the diagnosis of infection by *A. graevenitzii* as the etiological pathogen present in the patient's lesions. Quantitative culturing of BALF is one of

the most reliable methods for differentiating respiratory tract pathogens from colonization related to pneumonia, particular for organisms that can colonize the respiratory tract.[2,10] Because the progression of the lung lesions in our case was more rapid than that of conventional pulmonary actinomycosis, the histological findings revealed acute inflammatory change, which is different from the features of typical pulmonary actinomycosis. As our patient was a 32-year-old previously healthy woman with no known predisposing conditions, the rapid growth of pulmonary actinomycosis in our case was assumed to be due to pathogenic factors rather than host factors. Interestingly, the strain of *A. graevenitzii* isolated in our case formed molar tooth-like colonies within 48–96 h of incubation. Its growth rate is faster than that of other *Actinomyces spp.* that can be cultured anaerobically for up to 3 weeks. [17] We presume that the rapid growth of *A. graevenitzii* in aerobic conditions may contribute to rapidly progressive pneumonia. However, the reason for the rapid growth of the pulmonary lesion in this patient is unknown.

To our knowledge, this is the first report describing multiple lung abscesses caused by *A. graevenitzii*, which was diagnosed using a quantitative culture

of BALF.

In our patient, a PET examination before treatment revealed that the lesions were located only in the lungs. Combined with the onset of clinical manifestation after inhalation of dust on a US beach, it could be considered that the pulmonary multiple abscesses were caused by the entry of the pathogens to the lungs via the respiratory tract as opposed to hematogenous infection. As the habitat of *A. graevenitzii* is unknown, further caution is necessary for this organism, in particular when differentiation of such cases is required from those of acute pulmonary abscesses developing after the inhalation of soil, such as in cases of acute pulmonary coccidioidomycosis.

In conclusion, we report a unique case of lung abscesses caused by *A. graevenitzii* that resembled pulmonary coccidioidomycosis in its clinical features and CT findings. Because of its rarity, the documentation of more cases is required to define the pathogenesis of *A. graevenitzii*.

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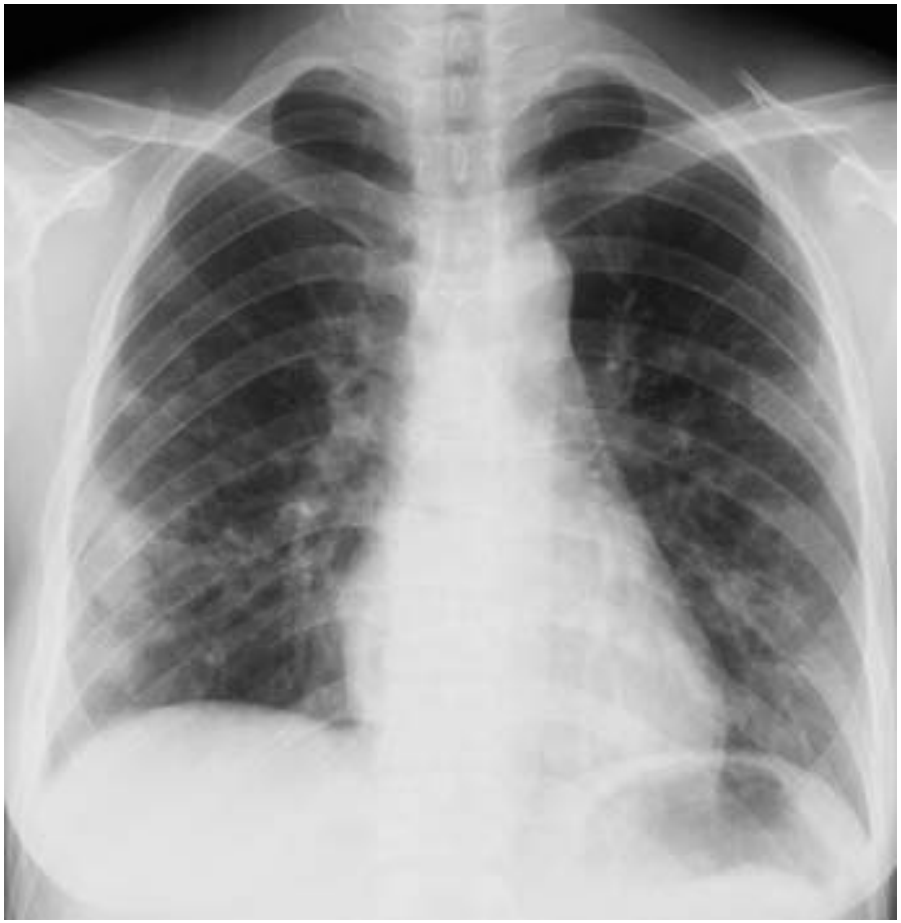
Figure Legends:

Figure 1. Chest radiography film images: (A) 2 weeks before admission (clinical day 8), showing several nodules in the bilateral lower lung field; (B) on admission (clinical day 25), showing enlargement of the lung nodules, as the bilateral lower lung field is almost entirely covered by the lesions.

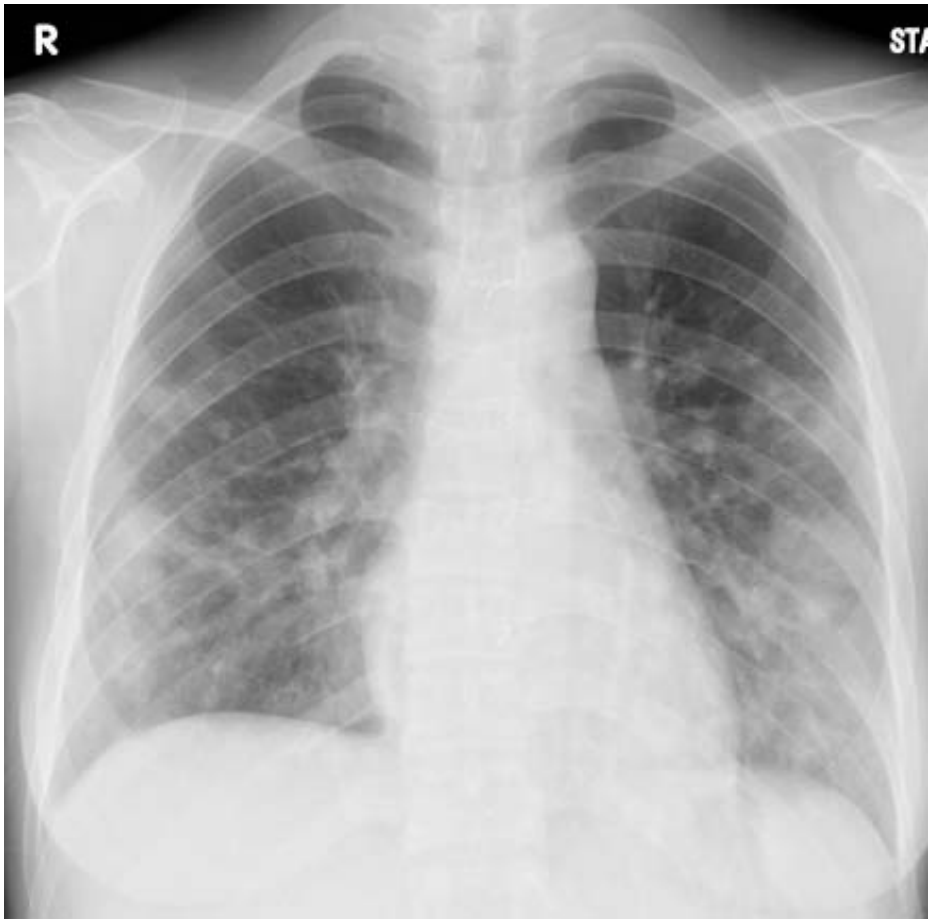
Figure 2. Chest CT image of the thorax (A) 2 weeks before admission (clinical day 8), showing multiple round lesions located in both lobes with diameters of 1–2 cm; (B) on admission (clinical day 25), showing increases in the number and size (diameters of 2–4 cm) of the lung nodules.

Figure 3. Microphotographs and colony morphological features of the organism isolated from the bronchoalveolar lavage. Panel A shows numerous coryneform gram-positive rods (Gram stain). Panel B shows the molar tooth-like appearance of the colonies on blood agar (72 h after incubation).

Figure 1

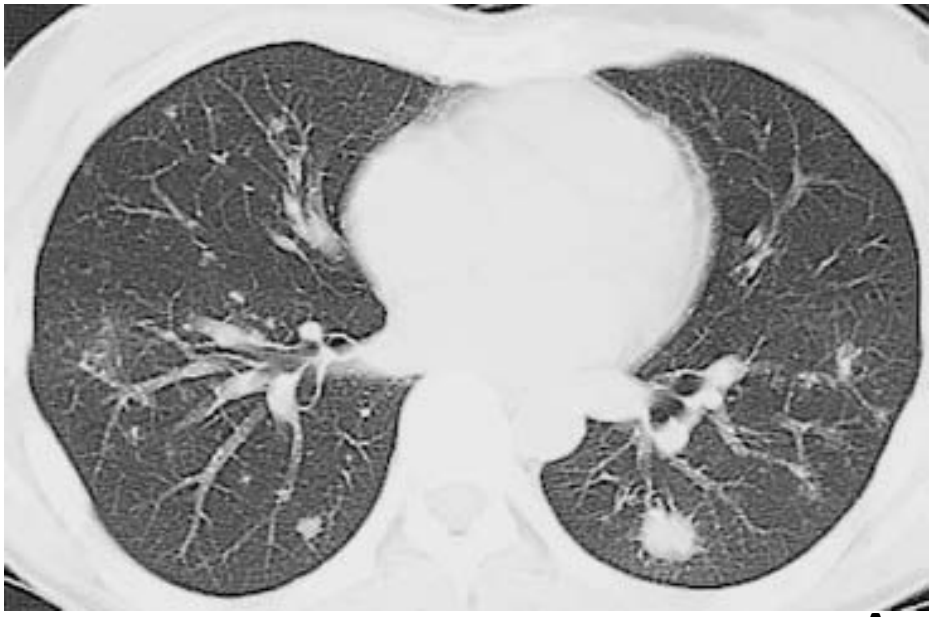


A



B

Figure 2



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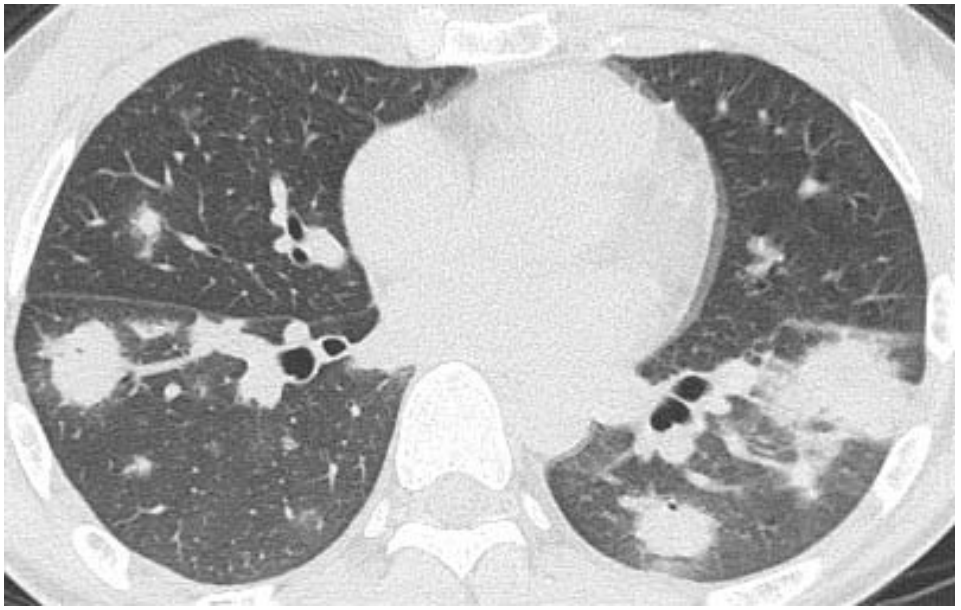

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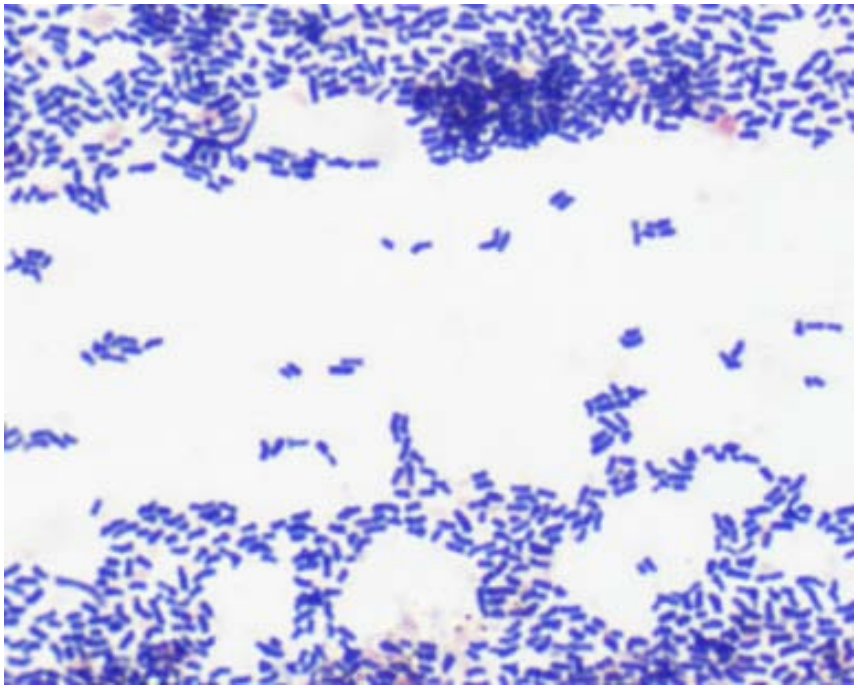
Figure 3



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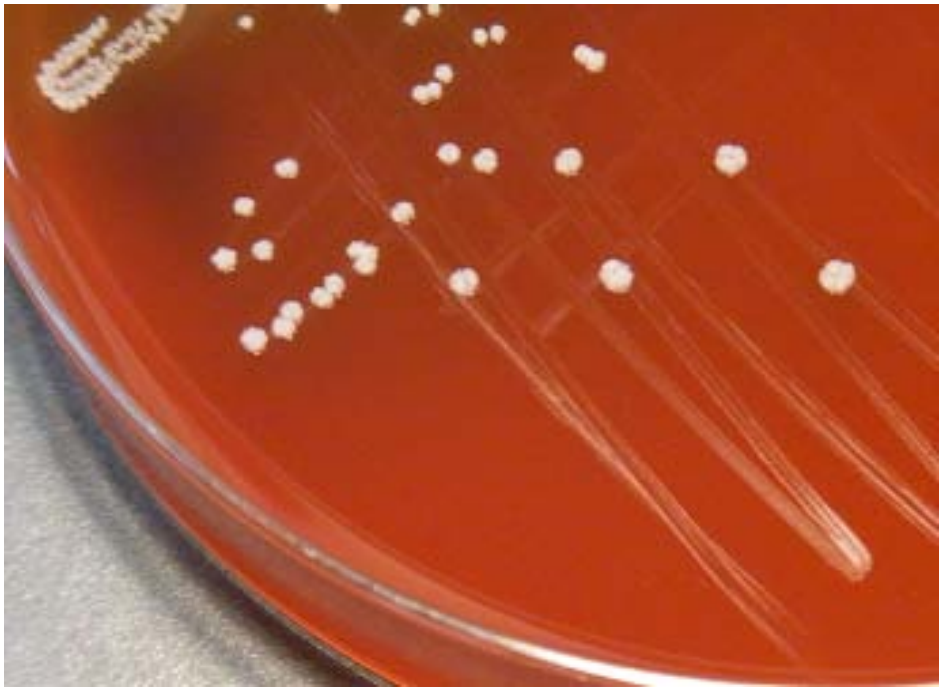

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