Case Report: Shell Vial Cell Culture as a Tool for *Streptobacillus moniliformis* “Resuscitation”

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Abstract. *Streptobacillus moniliformis* is a fastidious growing Gram-negative bacillus responsible of rat-bite fever. We describe here the first report of this disease in la Réunion and the first isolation using shell vial cell culture from a blood culture bottle with a bacterium suspected to be dead.

Streptobacillus moniliformis, a fastidious growing, facultative, non-motile Gram-negative bacillus, is one of the causative agents of rat-bite fever and Haverhill fever in humans.¹ Although the link with rat-bite fever has not been assessed in our case, this is the first report of this disease in la Réunion, France. The major originality of this report is that the bacterium supposed to be lost in subculture from blood culture broth to agar plate could be resuscitated by passage on shell vial cell culture.

An 89-year-old woman, who lived alone in precarious conditions and in contact with rats and a domesticated dog, was admitted into the burn intensive care unit of the Bellepierre hospital, Reunion Island, France, in September 2009 for foot burns linked to a domestic accident. Four days after admission, the patient was somnolent, with a fever of 38.5°C. Two successive blood specimens were taken and inoculated into BACTEC Plus Aerobic/F and Anaerobic/F bottles (Beckton Dickinson Bactec, Shannon, County Clare, Ireland). BACTEC automated systems detected growth in one of the anaerobic bottles. Gram staining revealed the presence of pleomorphic filaments and branching Gram-negative bacilli. Amoxicillin-clavulanate and gentamicin were administered, but fever persisted. At day 9, the treatment was changed to ceftazidime, vancomycin, and metronidazole. At day 11, confronted with an alteration in general status, the treatment was switched to rifampicine and doxycycline. The fever broke, but the patient died 3 days later in a state of renal dysfunction. The positive blood culture broth was subcultured on Columbia blood agar (bioMérieux, Marcy l’Etoile, France), Chocolate-polyViteX agar (bioMérieux), Schaedler broth with Vitamin K1 (AES, Ker Lann, Bruz, France), Heart-Brain infusion broth (Prolab, Saint-Pierre, Réunion, France), and Lowenstein Jensen agar (BioRad, Marnes la Coquette, France) at 35°C and 30°C in aerobic and anaerobic conditions, respectively. None of these media yielded any bacterial growth despite a 3-week incubation time. The blood culture broth was frozen and sent to the Unité de Recherche sur les Maladies Infectieuses et Tropicales Emergentes (URMITE, Marseille, France). DNA extraction and a 16S-rRNA gene amplification polymerase chain reaction (PCR) assay followed by sequencing were performed on the broth as previously described,² and the analyses identified *S. moniliformis* (99.5% sequence similarity to Genbank sequence DQ325537). The 11 closest sequences were all *S. moniliformis*, with the lower similarity at 98%. The second

closest hit was *Leptotrichia* sp., at a similarity of 93%. After this result was obtained, we again inoculated the previously frozen blood culture broth sample on Colombia blood agar (bioMérieux) and ECV 304 human endothelial cell monolayers using the centrifugation shell vial technique.³ Briefly, 1 mL blood culture broth was inoculated onto shell vials (3.7 mL; Sterilin, Feltham, England) containing monolayers of confluent ECV 304 human endothelial cells and centrifuged at 700 × g for 1 hour at 22°C. The cell sheet was rinsed, and 1 mL culture medium was added (RPMI medium with 10% fetal calf serum and 1 mM L-glutamine per liter). No growth was obtained on the agar plate, but after 24 hours, a cytopathic effect was observed on the monolayer cells. Gimenez and Gram staining were performed on the shell vial supernatant, and a Gram-negative bacillus was observed. The infected cell monolayer was scraped and then inoculated onto Colombia blood agar and new shell vials. The strain of *S. moniliformis* was established not only on the shell vial culture but also from cell culture to the agar plate, and the bacterial identity was verified again by 16S rRNA gene sequencing.

S. moniliformis, a fastidious growing, facultative, non-motile Gram-negative bacillus, is one of the causative agents of rat-bite fever and Haverhill fever in humans. Although the link with rat-bite fever has not been assessed in our case, this is the first report of this disease in la Réunion, France. Untreated rat-bite fever has a mortality rate ranging from 7% to 13%.¹ Diagnosis of *S. moniliformis* infections is usually based on bacterial axenic cultures. Its optimal growth requires microaerophilic conditions and medium such as trypticase soy agar or broth enriched with 20% blood, serum, or ascitic fluid, and strain establishment is difficult.¹ Its growth is inhibited by sodium polyanethol sulfonate (SPS), the anticoagulant added to most commercial automatic blood culture systems.⁴ Some anaerobic media for bacterial isolation do not contain SPS and improve isolation of *S. moniliformis*.⁵ Anaerobic BACTEC Plus Anaerobic/F bottles do contain 0.05% SPS (<http://www.bd.com/resource.aspx?IDX=9455>). Our data suggest that carry over of SPS from the blood culture broth to the agar plate prevented growth, whereas the rinsing step during the shell vial procedure removed SPS. Beside *S. moniliformis*, several agents such as *Neisseria meningitidis*, *Peptostreptococcus anaerobius*, *Capnocytophaga* spp., *Gardnerella vaginalis*, and *Mycoplasma hominis* have been shown to be susceptible to SPS at variable degrees. This is the reason that Beckton Dickinson recommends optimal filling of blood culture bottles to reduce the final proportion of SPS in the blood culture vials. The difficulty in making a diagnosis of rat-bite fever by culture is likely responsible for significant underdiagnosis of this disease, especially when history of rat bite is not reported by

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the patient as in the herein reported case. Recently, molecular detection by PCR has been proven efficient for the detection of *S. moniliformis* in humans.^{6–8} However, detection of bacteria is usually more difficult in blood than in tissues because of the low number of copies of the agent, the fact that clearance of dead organisms is quicker after the antibiotic therapy has been initiated, and the inhibitory effect of haemoglobin.⁹ To our best knowledge, this is the first application of a shell vial assay for the isolation of *S. moniliformis*. This technique is routinely used in our laboratory for the culture of strict intracellular bacteria, but it is also highly effective for isolation of some facultative intracellular bacteria, such as *Bartonella* sp.,³ especially from biopsy specimens, *Francisella tularensis* from an inoculation eschar,¹⁰ *Legionella pneumophila* from multiple liver and lung abscesses,¹¹ and *Brucella melitensis* from a liver abscess.¹² For all of these cases, the standard axenic cultures remained negative, and only cell cultures allowed bacterial isolation. They have also been used to isolate *Mycobacterium* sp. from different clinical specimens, such as lymph node biopsies, pericardial fluid samples, cerebrospinal samples, and bronchoalveolar lavages, whereas axenic cultures were negative.¹³ The shell vial culture technique was also helpful in our experience for isolation of unusually fastidious microorganisms, such as a *Staphylococcus aureus* small-colony variant with a suggested intracellular location.^{14,15} In all cases, we hypothesized that cells provided damaged bacteria with some growth factors not present in axenic media and/or that the shell vial procedure with a rinsing step helped to remove potential inhibiting substances. Even if the shell vial culture assay has a very low rate of success, this technique gives valuable results.¹⁴

Received August 19, 2010. Accepted for publication November 2, 2010.

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