Case Report

In vitro aggregate formation and unusual colony morphology impairing identification of *Roseomonas* sp. from a septic patient

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Introduction: The Gram-stained morphology of organisms from positive blood culture bottles often suggests diagnoses and therapeutic options. *In vitro* aggregate formation by the organism in this case led to initial confusion in the diagnosis.

Case presentation: A 57-year-old woman with a recent stroke returned to the emergency department with acute exacerbation of her neurological deficit. Although afebrile, she had an elevated white blood cell count. Urine analysis revealed 4+ blood, nitrates, leukocyte esterase and bacteria. Blood and urine cultures were collected and intravenous piperacillin/tazobactam treatment was begun. The urine culture grew >100 000 colonies *Escherichia coli* ml⁻¹; the antibiotic was changed to ceftriaxone when susceptibilities became available at 48 h. At 61 h, the aerobic blood culture bottle was flagged as positive. A Gram stain revealed spherical structures, which stained predominantly Gram-negative but were Gram-positive in some areas of the smear. Subcultures grew overnight on blood, chocolate and MacConkey agars and on brain–heart infusion (BHI) broth. Growth on MacConkey agar was sparse. A Gram stain of the snow-white, mucoid colonies revealed Gram-negative rods, spherical aggregates and thick-walled tetrads. Subsequent cultures on solid medium grew only Gram-negative rods, but inoculation of isolated colonies from these cultures into BHI broth or negative blood culture bottles caused the thick-walled spheres and tetrads to reappear.

Conclusion: MicroScan and matrix-assisted laser desorption/ionization time of flight failed to identify the organism, but 16S rRNA gene sequencing identified it as *Roseomonas* genomospecies 5 (100 % match). To the best of our knowledge, there are no reports in the literature of aggregate-forming *Roseomonas*.

Keywords: Aggregated bacteria; bacteraemia; Gram stain; *Roseomonas* genomospecies 5.

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Introduction

Gram staining of positive blood culture bottles often allows a provisional diagnosis of the aetiological agent of infection followed by initiation of more appropriate antibiotics. However, in some cases, the Gram stain may be confusing rather than informative. Whilst some organisms are known to aggregate (e.g. *Sarcina* spp., *Pantoea agglomerans*), such forms are rarely seen. We present here a case of a blood culture isolate that produced thick-walled, Gram-variable aggregates when incubated in the blood culture bottle. This led to a differential diagnosis that included fungi

and *Prototheca* sp., but the organism was ultimately identified as *Roseomonas* genomospecies 5. To the best of our knowledge, there are no reports of *Roseomonas* sp. with white colonies or the rounded, thick-walled aggregates illustrated here in the literature.

Case presentation

A 57-year-old female returned to the emergency department 2 days after hospital discharge, complaining of an acute exacerbation of lower extremity weakness that had begun 1 week earlier after a stroke. She was afebrile and somnolent. Relevant laboratory findings included a white blood cell count of 16×10^9 l⁻¹ and a urine analysis reveal-

Abbreviations: BHI, brain–heart infusion; MALDI-TOF, matrix-assisted laser desorption/ionization time of flight.

ing 4+ blood, nitrates, leukocyte esterase and bacteria. Urine and blood cultures were collected. She was started on intravenous piperacillin/tazobactam 4.5 g every 6 h. The next day, the urine culture grew *Escherichia coli* at $>100\,000$ colonies ml^{-1} . When the susceptibilities became available 48 h later, the antibiotic was changed to ceftriaxone 2 g every 24 h for 6 days. When the blood culture Gram stain was reviewed after it turned positive at 61 h, she also received one dose of amphotericin B, which was changed to fluconazole for 5 days when the blood culture grew on solid medium (Fig. 1b). She was discharged and did well.

Investigations

After 61 h of incubation in a continuously monitoring blood culture instrument (Bactec FX; BD Diagnostics), the aerobic blood culture bottle (Bactec Plus/F) was flagged as positive. The Gram stain (Fig. 1a) showed thick-walled structures, including large spheres and occasional tetrads. They were predominantly Gram-negative but appeared Gram-positive in certain areas, raising the possibility of a fungus. No bacterial cocci or rods were seen in the initial blood culture bottle. Based on Gram-stain morphology, the differential diagnosis included *Coccidioides immitis*, an ascomycetous fungus, *Prototheca* sp. and *Sarcina* sp. The bottle was subcultured in brain–heart infusion (BHI) broth and on blood, chocolate and MacConkey agars, and, because the

morphology of the organism raised the possibility of fungus, on fungal medium as well. No growth was detected on fungal medium after 4 weeks. The organism grew overnight in BHI broth and on the solid media, although the growth on MacConkey agar was sparse. The colonies were white and mucoid. Gram staining of several isolated colonies from each agar plate revealed that all colonies contained Gram-negative cocci/coccobacilli, the thick-walled spherical structures and tetrads. The spherical structures and tetrads disappeared on further subculture, but reappeared when colonies from sphere-free cultures were inoculated into BHI broth or into inoculated blood culture bottles that had been read as negative and were ready for discard. The organism was inoculated into an NBC44 Gram-negative combination panel for the MicroScan WalkAway FX (Siemens) but was not identified. However, it was susceptible to antibiotics considered appropriate for Gram-negative organisms, including the two that had already been used to treat the patient for 4 days. After the blood culture bottle was reported as positive, four additional blood cultures were drawn over the next 24 h, but all remained negative.

Diagnosis

The organism was sent to two reference laboratories for matrix-assisted laser desorption/ionization time of flight (MALDI-TOF) analysis, which did not identify the organism. No *Roseomonas* spp. are in either the Brucker

Fig. 1. Photographs of the causative organism. (a) Gram stain of fluid from the blood culture bottle. Original magnification $\times 1000$. (b) Subculture directly from the blood culture bottle shown after 48 h of growth on Mueller–Hinton agar. Although this is not a standard medium utilized in our laboratory for direct subcultures from blood culture bottles, it was chosen here to illustrate the lack of pink coloration of the colonies.

BioTyper (Bruker Daltonics) or Vitek MS (bioMérieux) FDA-approved US databases. 16S rRNA gene sequencing followed by BLAST analysis (<http://blast.ncbi.nlm.nih.gov/Blast.cgi>) identified the organism as *Roseomonas* genomospecies 5 (100 % match with GenBank accession no. AF533356).

Discussion

Gilardi and Faur (1984) described the isolation of an unnamed taxon consisting of seven strains of oxidative, pink-pigmented, Gram-negative coccobacilli that characteristically grew as mucoid colonies. Five were isolated from blood. A number of similar organisms were isolated over the years. They were distinguished from *Methylobacterium* spp. by their inability to utilize methanol as a carbon source and their inability to adsorb long-wave (365 nm) UV light. The genus was named *Roseomonas* by Rihs *et al.* (1993).

The mucoid colonies of the organism from our patient were pure white, even on clear, uncoloured medium such as Mueller–Hinton agar (Fig. 1b). Touching the colonies with white cotton swabs revealed no pink coloration. Cultures of colonies containing both the Gram-negative coccobacilli and the ball- and tetrad-shaped structures were sent to two reference laboratories for identification. Neither laboratory identified the organism by MALDI-TOF, but both laboratories performed 16S rRNA gene sequencing and reported the organism to be *Roseomonas* genomospecies 5 (100 % match). Two MALDI-TOF instruments have been approved by the US Food and Drug Administration: Bruker Biotyper (Bruker Daltonics) and Vitek MS (bioMérieux). *Roseomonas* is not in the FDA-approved database of either instrument.

Roseomonas is an unusual cause of bloodstream infections, most of which are found in cancer patients with catheter-related infections (Dé *et al.*, 2004; Rihs *et al.*, 1993; Wang *et al.*, 2012). It is often difficult, as in this case, to determine whether the isolates represent contaminants or

significant infections. However, Wang, *et al.* (2012) reported 20 infections, of which 17 were judged to be significant. Of their four patients with primary bacteraemias, three were not obviously immunocompromised, similar to our patient.

To the best of our knowledge, there are no reports in the literature of *Roseomonas* sp. isolates growing as snow-white colonies or as bacterial aggregates and tetrads *in vitro*. The unusual aggregates disappeared on repeated subculture on solid medium. They recurred after the third and sixth subcultures when colonies containing only coccobacilli were inoculated into BHI broth or into previously inoculated blood culture bottles that had exhibited no growth and were ready for discard. *Roseomonas* should be added to the list of rare genera that can form aggregates under certain culture conditions.

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