Case Report

Rhodococcus erythropolis septicaemia in a patient with acute lymphocytic leukaemia

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Rhodococcus erythropolis rarely causes infection in humans. We report the second case of *R. erythropolis* septicaemia in a 7-year-old child. However, to our knowledge it is the first case in a patient with acute lymphocytic leukaemia who had been undergoing chemotherapy. The identification was performed using 16S rRNA gene sequencing. Even though *R. erythropolis* is rarely associated with human infections, it should be considered as a potential causative agent of bacteraemia, rather than overlooked as a contaminant.

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Introduction

Rhodococcus belongs to the order *Actinomycetales* and the family *Nocardiaceae*. *Rhodococcus* is a genus of obligately aerobic, non-sporulating, non-motile, Gram-positive bacteria phylogenetically more closely related to the genera *Corynebacterium* and *Mycobacterium* (Conville & Witebsky, 2007). *Rhodococcus* species have been isolated from a large variety of sources, including soils, rocks, groundwater, seawater, plants, animals and the guts of insects (Gürtler *et al.*, 2004). For the past few decades, much biotechnological interest, especially in relation to bioremediation and industrial wastewater treatment, has been directed at *Rhodococcus* species because of their ability to degrade persistent chemical pollutants (Teramoto *et al.*, 2009). Furthermore, they have been predicted to include a number of potential antibiotic producers (Teramoto *et al.*, 2009).

Although *Rhodococcus* species are generally considered to have low pathogenicity, they cause diseases in animals, plants and humans (Bell *et al.*, 1998). There has been increased interest in infections caused by *Rhodococcus* species in humans since the first clinical case caused by *Rhodococcus equi* was reported in 1967 (Weinstock & Brown, 2002). The vast majority of patients infected with *R. equi* are immunocompromised individuals such as recipients of solid organ transplants and those with AIDS, although infection in apparently immunocompetent persons has also been reported (Weinstock & Brown, 2002; Roy *et al.*, 2009). Infections due to *R. equi* are generally acquired through inhalation, direct inoculation and oral ingestion (Conville & Witebsky, 2007). Bacteraemia caused by *R. equi* has been reported in >80 % of immunocompromised patients and approximately 30 % of immunocompetent patients (Weinstock & Brown, 2002). However,

in contrast to *R. equi*, the pathogenic potential of *Rhodococcus erythropolis* as an invasive pathogen is not well known because *R. erythropolis* has rarely been isolated from human infections despite its widespread environmental existence. Until the present time, only six cases of human infection with *R. erythropolis* have been reported. (Brown & Hendler, 1989; Osoagbaka, 1989; Vernazza *et al.*, 1991; von Below *et al.*, 1991; Roy *et al.*, 2009; Baba *et al.*, 2009). We report what is to the best of our knowledge the first case of bacteraemia due to *R. erythropolis* in a child with acute lymphocytic leukaemia.

Case report

A 6-year-old child presented to the emergency room with a pale appearance, petechiae on both legs and a palpable abdominal mass. The patient had been diagnosed with early precursor B cell type acute lymphoblastic leukaemia. On the fourth day of admission, a Hickman catheter was inserted and chemotherapeutic treatment according to the Children's Cancer Group 1891 protocol was initiated. One month after the chemotherapy, the patient's bone marrow showed a complete remission state. Since that time, the child had been receiving maintenance chemotherapy for leukaemia.

Seven months after acute leukaemia diagnosis, the child was readmitted to the hospital with fever and complaint of intermittent pain in both knees, but no complaints of any other symptoms. On readmission, the patient's temperature was 39 °C, pulse rate was 92 beats min⁻¹ and the respiratory rate was 29 breaths min⁻¹. The white blood cell count was 10.66 × 10⁹ l⁻¹ with 89 % neutrophils (reference range 35–60 %), 5 % lymphocytes (reference range 30–45 %) and 5 % monocytes (reference range 2–8 %).

C-reactive protein was elevated at 62.0 mg l^{-1} (reference range $<5 \text{ mg l}^{-1}$); prothrombin time (reference range 9.5–14 s) and activated partial thromboplastin time (reference range 27.9–37.8 s) were 13.2 and 40.9 s, respectively. Aspartate aminotransferase (reference range $<40 \text{ U l}^{-1}$) and alanine aminotransferase (reference range $<40 \text{ U l}^{-1}$) were elevated at 92 and 52 U l^{-1} , respectively. Blood, urine and stool samples were submitted for culture. History, physical examination and radiographs did not reveal an obvious source of infection. After sampling for culture, cefotaxime, isepamicin and amoxicillin/clavulanate were empirically administered. However, the patient's fever persisted despite being treated with antimicrobial agents. The initial two sets of blood cultures were incubated at 37°C in the BacT/Alert 3D system (bioMérieux) and one bottle became positive for a Gram-negative bacillus on the second day of incubation. The Gram-negative bacillus was identified as *Acinetobacter lwoffii* by using the Vitek 2 GN identification system (bioMérieux). *A. lwoffii* was susceptible to piperacillin, piperacillin/tazobactam, ceftazidime, cefepime, imipenem, meropenem, colistin, gentamicin, tobramycin, amikacin and levofloxacin, but resistant to trimethoprim/sulfamethoxazole. Another bottle of initial blood culture showed a positive signal on the third day of incubation, and was then subcultured to 5% sheep blood agar and MacConkey agar. Smooth white colonies of a Gram-positive rod grew on 5% sheep blood agar but no colonies formed on MacConkey agar after 72 h of aerobic incubation at 35°C . The Vitek 2 ANC identification system (bioMérieux) and BBL Crystal GP ID system (Becton Dickinson Microbiology Systems) were used for the initial identification according to the manufacturers' instructions. The Vitek 2 ANC identification system identified the isolate as *Corynebacterium pseudodiphtheriticum* (probability: 91.22%; confidence level: good identification). However, the BBL Crystal GP ID system could not identify the isolate at the genus level (profile number: 3665000545; confidence level: unacceptable identification). Blood cultures collected 2 days after the initial two sets were collected again grew simultaneously a Gram-negative bacillus and Gram-positive rod in two sets of blood culture bottles, which appeared phenotypically identical to the earlier isolates. On the third day of readmission, six sets of blood cultures were submitted. The Hickman catheter was then removed since it was presumed to be the cause of the persisting fever, and the catheter tip was submitted for semiquantitative culture. Blood culture through the intravascular Hickman catheter line was not performed. The third follow-up blood cultures grew a Gram-positive rod in two bottles and *A. lwoffii* in one bottle. The chest and knee joint roentgenograms were unremarkable. No organism was isolated from urine, stool and catheter tip cultures. On the fourth day of readmission, antimicrobial agents were changed to meropenem and gentamicin according to the results of the antimicrobial susceptibility tests against *A. lwoffii*. After removal of the Hickman catheter and administering antibiotics, the patient's temperature showed a decreasing trend and his

symptoms also improved. In addition, the total lymphocyte count showed an increasing trend, and recovered to $3.11 \times 10^9 \text{ l}^{-1}$ on the seventh day of readmission. The child was discharged on the seventh day of readmission in good health with no detectable sequelae. After that time, cefpodoxime and trimethoprim/sulfamethoxazole were orally medicated for 1 week by outpatient-based follow-up.

Microbiology

All isolates grew in the aerobic bottle of the BacT/Alert 3D system. Because the Gram-positive rod was repeatedly isolated from separate blood culture sets, confirmation of its identity was carried out by a more stringent identification method, i.e. by sequencing of the 16S rRNA gene. After PCR amplification of the 16S rRNA gene with the primers 518F (5'-CCA GCA GCC GCG GTA ATA CG-3') and 800R (5'-TAC CAG GGT ATC TAA TCC-3'), sequencing was conducted using the Big Dye Terminator Cycle Sequencing kit (Applied Biosystems) and ABI PRISM 3730 genetic analyser (Applied Biosystems). All sequences were analysed by using BLAST (a genome database of the National Center for Biotechnology Information) and the Ribosomal Database Project. The sequences showed 99% identity with the 16S rRNA gene sequences of *R. erythropolis*, e.g. *R. erythropolis* strain MM30 (GenBank accession no. EU729738) and *R. erythropolis* strain HS18 (GenBank accession no. AY168596). The MICs of the following antimicrobial agents were determined by the broth microdilution method: penicillin, 2 mg l^{-1} ; cefotiam, 2 mg l^{-1} ; cefoperazone/sulbactam, 2 mg l^{-1} ; vancomycin, 0.5 mg l^{-1} ; clindamycin, 2 mg l^{-1} ; tetracycline, 2 mg l^{-1} ; gentamicin, 2 mg l^{-1} ; and rifampicin, 2 mg l^{-1} .

Discussion

In 1891, Zopf first used the genus name *Rhodococcus* for two species of red-pigmented bacteria (Gürtler *et al.*, 2004). The genus is composed of over 40 species that have a broad range of phenotypic features such as Gram stain and colony morphology, growth characteristics and biochemical reactions (Goodfellow, 1989; Bell *et al.*, 1998; Goodfellow *et al.*, 1998; Gürtler *et al.*, 2004).

R. erythropolis was originally named *Mycobacterium erythropolis* by Gray and Thornton in 1928 during an investigation of soil bacteria (Baba *et al.*, 2009). After a period of being known as *Nocardia erythropolis*, the organism is now known as *R. erythropolis*. The typical colony of *R. erythropolis* has been described to be rough and orange-to-red coloured on glucose yeast extract agar and Sauton's agar (Goodfellow, 1989). However, these colonial features are not always observed. On the basis of its morphological characteristics, *Rhodococcus* can easily be mistaken for a diphtheroid contaminant such as *Corynebacterium* sp.; on the basis of acid-fast stain results, it can also be mistaken for *Mycobacterium* or *Nocardia* (Funke *et al.*, 1997).

R. erythropolis is widely distributed in nature and is found particularly in soil and faeces of herbivores. As with many other bacteria of environmental origin, *R. erythropolis* likely has limited pathogenicity, but occasional reports have noted clinically significant roles for this organism. As far as we know, six cases of human infection with *R. erythropolis* have been previously described, although molecular confirmation of species identification was not done in all. Brown & Hendler (1989) reported peritonitis caused by *R. erythropolis* during continuous ambulatory peritoneal dialysis. Osoagbaka (1989) described the isolation of *R. erythropolis* from sputum in a patient with pulmonary illness. von Below *et al.* (1991) identified *R. erythropolis* in chronic endophthalmitis after lens implantation. In this case, it was necessary to remove the implant and perform anterior vitrectomy plus coadminister cefotiam and amikacin. Vernazza *et al.* (1991) reported a disseminated skin infection in a human immunodeficiency virus-infected patient. Roy *et al.* (2009) reported osteomyelitis in the toe caused by *R. erythropolis* after first metatarsophalangeal joint fusion. The patient's symptoms and signs subsided and the arthrodesis united after removal of the cerclage wires and oral ciprofloxacin treatment. Moreover, Baba *et al.* (2009) reported the first case of bacteraemia caused by *R. erythropolis* in a 79-year-old man with oesophageal cancer. The patient underwent radiotherapy and chemotherapy, and parenteral nutrition was administered by means of a peripheral venous catheter. The fever resolved after catheter removal with antibiotic treatment.

It has been suggested that non-*equi* *Rhodococcus* species may be more important in human disease than previously thought (Bell *et al.*, 1998). However, information on the clinical significance of *R. erythropolis* is very limited so far due to the paucity of reported cases, especially when the strain is isolated from blood. Graham *et al.* (2007) reported that *R. erythropolis* was found in ocular surface specimens from both normal healthy humans and those with dry eye signs and symptoms, and that it was only detected by PCR. There are no other reports of its presence as normal flora at other human body sites such as the skin or in the faecal flora. Although the exact cause of the rarity of human infections caused by *R. erythropolis* is uncertain, there is a risk of the dismissal of these Gram-positive rods as contaminating 'diphtheroids' because many aerobic Gram-positive rods have been considered to be contaminants and identification of Gram-positive rods is difficult due to the enormous diversity of these organisms and the small number of readily available commercial identification systems in clinical laboratories (Conville & Witebsky, 2007). In our case, the two commercial identification kits initially used not only showed discrepancies in identification results, but also misidentified the organism at the genus level. Additionally, the non-thermophilic growth characteristic of *R. erythropolis* is another possible reason for the rarity of human infections (Vernazza *et al.*, 1991).

The pathogenicity and association with human disease of *R. erythropolis* have yet been determined. In our case, even

though *A. lwoffii* was co-isolated, the fact that *R. erythropolis* was isolated from a number of blood cultures makes it likely that it contributed to the worsening of the patient's clinical status. For *R. equi*, concurrent infection with other opportunistic organisms is common in immunocompromised patients (Weinstock & Brown, 2002).

Our patient had been undergoing maintenance chemotherapy and so was regarded as immunocompromised. Additionally, the total lymphocyte count was decreased at $0.50 \times 10^9 \text{ l}^{-1}$ (reference range $1.5\text{--}6.5 \times 10^9 \text{ l}^{-1}$) when the patient was readmitted. The existence of long-term indwelling catheter placement may allow the colonization and infiltration to the bloodstream, and immunosuppression by maintenance chemotherapy will facilitate bacterial growth. Although the infection source could not be identified as in the previous reports (Baba *et al.*, 2009), it can be hypothesized that immunocompromised conditions and indwelling catheterization may play a crucial role as underlying risk factors for the development of *R. erythropolis* bacteraemia.

A combination of antimicrobials is generally used for the treatment of rhodococci infections. Agents used include aminoglycosides, erythromycin, imipenem, quinolones, rifampicin and vancomycin; linezolid may also be efficacious (Conville & Witebsky, 2007). Use of penicillins should be avoided despite initial demonstration of *in vitro* susceptibility because resistance has been shown to develop rapidly during therapy (Tang *et al.*, 1997).

To the best of our knowledge, our case is the second of *R. erythropolis* septicaemia but it is the first report in an acute lymphoblastic leukaemia patient. Even though *R. erythropolis* is rarely associated with human infections, it should be considered as a potential causative agent of bacteraemia, rather than overlooked as a contaminant. As the pool of immunosuppressed patients increases in the future, future cases of infection with non-*equi* *Rhodococcus* species such as *R. erythropolis* are expected. Therefore, further clinical and laboratory research is needed to better define the routes of acquisition and the mechanisms of pathogenesis of *R. erythropolis* infection and the appropriate treatments for it.

References

- Baba, H., Nada, T., Ohkusu, K., Ezaki, T., Hasegawa, Y. & Paterson, D. L. (2009). First case of bloodstream infection caused by *Rhodococcus erythropolis*. *J Clin Microbiol* **47**, 2667–2669.
- Bell, K. S., Philp, J. C., Aw, D. W. & Christofi, N. (1998). The genus *Rhodococcus*. *J Appl Microbiol* **85**, 195–210.
- Brown, E. & Hendler, E. (1989). *Rhodococcus* peritonitis in a patient treated with peritoneal dialysis. *Am J Kidney Dis* **14**, 417–418.
- Conville, P. S. & Witebsky, F. G. (2007). *Nocardia*, *Rhodococcus*, *Gordonia*, *Actinomyces*, *Streptomyces*, and other aerobic actinomycetes. In *Manual of Clinical Microbiology*, 9th edn, pp. 515–542. Edited by P. R. Murray, E. J. Baron, J. H. Jorgensen, M. L. Landry & M. A. Pfaller. Washington, DC: American Society for Microbiology.

- Funke, G., von Graevenitz, A., Clarridge, J. E., III & Bernard, K. A. (1997). Clinical microbiology of coryneform bacteria. *Clin Microbiol Rev* **10**, 125–159.
- Goodfellow, M. (1989). Genus *Rhodococcus*. In *Bergey's Manual of Systematic Bacteriology*, vol. 4, pp. 2362–2371. Edited by S. T. Williams, M. E. Sharpe & J. G. Holt. Baltimore, MD: Williams & Wilkins.
- Goodfellow, M., Alderson, G. & Chun, J. (1998). Rhodococcal systematics: problems and developments. *Antonie van Leeuwenhoek* **74**, 3–20.
- Graham, J. E., Moore, J. E., Jiru, X., Moore, J. E., Goodall, E. A., Dooley, J. S. G., Hayes, V. E. A., Dartt, D. A., Downes, C. S. & Moore, T. C. B. (2007). Ocular pathogen or commensal: a PCR-based study of surface bacterial flora in normal and dry eyes. *Invest Ophthalmol Vis Sci* **48**, 5616–5623.
- Gürtler, U., Fuchs, P., Stangelmayer, A., Bernhardt, G., Buschauer, A. & Spruss, T. (2004). Construction and validation of a microprocessor controlled extracorporeal circuit in rats for the optimization of isolated limb perfusion. *Arch Pharm (Weinheim)* **337**, 672–681.
- Osoagbaka, O. U. (1989). Evidence for the pathogenic role of *Rhodococcus* species in pulmonary diseases. *J Appl Bacteriol* **66**, 497–506.
- Roy, M., Sidhom, S., Kerr, K. G. & Conroy, J. L. (2009). Case report: *Rhodococcus erythropolis* osteomyelitis in the toe. *Clin Orthop Relat Res* **467**, 3029–3031.
- Tang, S., Lo, C. Y., Lo, W. K. & Chan, T. M. (1997). Optimal treatment regimen for CAPD peritonitis caused by *Rhodococcus* species. *Nephrol Dial Transplant* **12**, 1080–1081.
- Teramoto, K., Kitagawa, W., Sato, H., Torimura, M., Tamura, T. & Tao, H. (2009). Phylogenetic analysis of *Rhodococcus erythropolis* based on the variation of ribosomal proteins as observed by matrix-assisted laser desorption ionization-mass spectrometry without using genome information. *J Biosci Bioeng* **108**, 348–353.
- Vernazza, P. L., Bodmer, T. & Galeazzi, R. L. (1991). *Rhodococcus erythropolis* infection in HIV-associated immunodeficiency. *Schweiz Med Wochenschr* **121**, 1095–1098.
- von Below, H., Wilk, C. M., Schaal, K. P. & Naumann, G. O. (1991). *Rhodococcus luteus* and *Rhodococcus erythropolis* chronic endophthalmitis after lens implantation. *Am J Ophthalmol* **112**, 596–597.
- Weinstock, D. M. & Brown, A. E. (2002). *Rhodococcus equi*: an emerging pathogen. *Clin Infect Dis* **34**, 1379–1385.