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Monoclonal outbreak of catheter-related bacteraemia by *Ralstonia mannitolilytica* on two haemato-oncology wards

Sabine Gröbner*, Peter Heeg, Ingo B. Autenrieth, Berit Schulte

Institute of Medical Microbiology and Hygiene, Eberhard-Karls-University of Tübingen, Elfriede-Aulhorn-Str. 6, D-72076 Tübingen, Germany

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Summary *Background: Ralstonia mannitolilytica* is a non-fermentative, Gram-negative bacterium isolated infrequently from clinical samples. However, within a period of 11 weeks five inpatients of the tertiary care hospital of the University of Tübingen developed clinical signs of infection and *R. mannitolilytica* was cultivated from blood samples of all patients suggesting an outbreak.
Methods: Blood cultures and one catheter tip were analysed by standard microbiological procedures. Genetic relatedness of the isolates was investigated by pulsed-field gel electrophoresis. To ascertain the possible source of the outbreak, environmental sampling and challenge-recovery experiments to test filters used for multi-dose solution bottles were performed.
Results: In the present study a monoclonal outbreak with *R. mannitolilytica* causing catheter-related infection of five haematological patients is reported. Underlying severe diseases with consecutive immunosuppression, permanent indwelling intravenous devices, multiple intravenous applications, and chemotherapy were possible risk factors promoting the infection.
Challenge-recovery experiments revealed that *R. mannitolilytica* to a high extent even passed through Mini-spike Plus® filters of pore size 0.2 μm.
Conclusion: Although the source of the outbreak could not be identified, it is possible that solutions given intravenously were contaminated. Since *R. mannitolilytica* had never been isolated in our laboratory before and environmental testings performed were negative, it cannot be excluded that commercial products like drugs, saline solutions or infusion systems (filters) were contaminated.

* Corresponding author. Tel.: +49 7071 298 1528; fax: +49 7071 295 440.
E-mail address: sabine.groebner@med.uni-tuebingen.de (S. Gröbner).

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Introduction

The genus *Ralstonia* (formerly *Burkholderia*) is a group of non-fermentative, Gram-negative bacteria isolated from environmental sources and infrequently from clinical samples.[1] The main pathogenic species of this genus is *R. pickettii*, of which several nosocomial catheter-related outbreaks have been described.[2–7] *R. pickettii* are non-fastidious microorganisms which have the ability to survive in 0.05% chlorhexidine solutions[3,8] and can penetrate 0.2 μm filters.[9] Therefore, many of the cases of infection with *R. pickettii* were due to contaminated water or aqueous solutions given intravenously.[5,6,10,11] Interestingly, even ''sterile'' medical products like ranitidine[12] and saline solution[13,14] were reported to be contaminated with *R. pickettii*. Several infections of patients with permanent indwelling intravenous devices like central venous catheters or Port-A-Caths have been described.[15–18]

R. mannitolilytica (formerly *R. pickettii* biovar 3/'*thomasii*') is distinguished from all described *Ralstonia* species by its acidification of D-arabitol and mannitol and by its lack of nitrate reduction.[19] Due to misidentification as ''*P. fluorescens*'' the clinical importance of *R. mannitolilytica* may have been overlooked, as only a limited number of infections with *R. pickettii* biovar 3/'*thomasii*' (*R. mannitolilytica*), partly due to contaminated fluids or plastic drains, have been reported.[20–24]

Although Pasticci et al.[7] have described three clusters of catheter-related infections with *R. picketti*, which represents another *Ralstonia* species, we demonstrate the first outbreak with *R. mannitolilytica* clinically suspected to be an intravenous catheter infection.

In January 2006 from two inpatients on the same haemato-oncology ward *R. mannitolilytica* was cultivated from blood samples. Within a period of 11 weeks *R. mannitolilytica* with the same antibiotic resistance profile was isolated from blood cultures of three further haemato-oncological patients suggesting an outbreak.

To unravel the outbreak with a pathogen we never isolated from any patients' material, neither before nor after the outbreak, we analysed the patients' data, performed pulsed-field gel electrophoresis, environmental sampling, and challenge-recovery experiments to test filters used for multi-dose solution bottles.

Methods

Hospital setting

The university hospital of Tübingen in the south-western part of Germany is a tertiary care hospital with 1500 beds and about 68,000 inpatients and 220,000 outpatients treated per year. The five haemato-oncology wards altogether have 83 beds. The outbreak was focused on two of these wards. Additionally, there are two day hospitals associated with altogether 23 beds.

Microbiological procedures

For cultivation of *R. mannitolilytica* an aliquot of signal-positive blood culture bottles (BACTEC 9240, Becton Dickinson, Heidelberg, Germany) was plated on brain heart agar and then incubated for 18—48 h at 37 °C in 5% carbon dioxide.

R. mannitolilytica was identified by Gram-staining, colony morphologic features, oxidase, API 20 NE and Vitek II (bioMérieux, Nürtingen, Germany). Identification was confirmed by amplification of the 16s rDNA gene followed by single-strand sequencing. Susceptibility testing of the isolates and meropenem-E-test (AB Biodisk, Solna, Sweden) were performed by agar diffusion following the Clinical and Laboratory Standards Institute (CLSI) guidelines.

Pulsed-field gel electrophoresis (PFGE)

To investigate the relatedness of the *R. mannitolilytica* isolates, SpeI-digested (Roche Biochemicals, Mannheim, Germany) chromosomal DNA from isolates collected from patients' blood cultures were analysed by PFGE according to the modified protocol of Pasticci et al.[7] Gels were interpreted according to the criteria of Tenover et al.[25]

Challenge-recovery tests

Commercial available Mini-spike Plus® filters are used in our hospital to protect from potentially contaminated multi-dose i.v. solutions. The related species *R. pickettii* is known to pass through 0.2 μm rated filters. To investigate the ability of several filters with different pore size to retain *R. mannitolilytica*, challenge-recovery tests were performed. A total of 5 mL of a challenge PBS solution spiked with 9×10^2 cfu (colony forming units) of *R. mannitolilytica*/mL were passed across the test filters using a 10 mL syringe. Filters used for challenge-recovery-experiments were Minisart® 0.45 μm (Sartorius, Goettingen, Germany), Acrodisc® Syringe Filter with 0.2 μm *Supor*® Membrane (Pall, Cornwall, UK) and Mini-spike Plus® filters of pore size 0.2 μm and 0.45 μm (Braun, Melsungen, Germany).

Log_{10} titer reduction (LTR*) factor was calculated by log_{10} (Challenge [cfu/mL]/Recovery [cfu/mL]). Cases in which the number of organisms in the filter effluent was zero, the LTR value was calculated using one in the denominator.

Environmental sampling

Environmental sampling started immediately after *R. mannitolilytica* had been isolated from the blood culture of the second patient. Materials considered to be the most relevant sources for bloodstream infections were subjected to microbiological testing. These included: (1) swabs from medical devices (minispikes for drawing fluids from vials; $N = 5$); and (2) sterility testing of opened infusates ($N = 5$), filled syringes ($N = 2$) and aqueous octenidine-based disinfectant solution in use (Octenisept®; $N = 4$). Swabs and solutions for environmental sampling were cultured in tryptic soy broth for 48 h.

Routine environmental sampling is performed on the paedriatric BMT (bone marrow transplant) ward once monthly including swabs and contact plates from the patients' rooms as well as from functional areas (drug preparation, kitchen, decontamination area). In addition,