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Case Report

Central line-related bacteraemia due to *Tsukamurella tyrosinosolvens* in a haematology patient

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CASE REPORT A 48-year-old female patient with a six-year history of non-secretory multiple myeloma presented to hospital in September 2001 having felt generally unwell for the preceding two weeks. She lived in an urban area and had limited mobility. She described fevers with rigors on a number of occasions particularly associated with flushing of a Hickman line, which had been *in situ* since July 1999. Her white cell count was 2.7 x 10^9/l and she was mildly pancytopaenic following a course of doxombicin and methylprednisolone, which she had received four weeks previously. Blood culture taken from the central line had grown Gram-positive bacilli and coagulase-negative staphylococci in the first 48 hours from both aerobic and anaerobic bottles drawn from the white and red lumens of the Hickman line. This episode was managed in the community as a central venous catheter infection with oral ciprofloxacin and intravenous teicoplanin as empirical therapy. On review two weeks later, following completion of the 10-day course of antibiotics, the patient continued to be unwell complaining of general malaise and further rigors. At this time, in the absence of any other source of infection, a decision was made to remove the catheter. She dramatically improved thereafter without further antimicrobial therapy.

Phenotypic identification of the Gram-positive organism, (isolate identifier: NIPHL100602/ MK2667), isolated from blood culture was performed employing the API Corynebacterium system (Biomérieux, Las Halles, France) and gave the profile 2150004, which gave an identification of *Rhodococcus* sp. (82.9%), followed by *Corynebacterium* sp. (12.2%). The isolate grew aerobically on blood agar at 37°C forming small, rough, dry yellowish colonies, 2-5 mm in diameter. The isolate was noted to have an unusual colonial morphology, as shown (*Figure*), resembling non-aerial hyphae, similar to fungal growth, which was a useful phenotypic characteristic. The organism was sensitive to erythromycin, clindamycin, fusidic acid, gentamicin, netilmicin, rifampicin, teicoplanin and vancomycin, but was resistant to penicillin, oxacillin, tetracycline, chloramphenicol and trimethoprim, employing a standard *in vitro* antibiotic disk diffusion susceptibility assay. Given the relatively poor phenotypic identification

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obtained, the isolate was subsequently forwarded for molecular identification through PCR amplification and direct sequencing of a large but partial region of the 16S rRNA gene, corresponding to base position of approximately 811-1374 of *E. coli* ATCC 25922 16S rRNA [GenBank Accession number X80724]. All DNA isolation procedures were carried out in accordance with the DNA contamination management guidelines of Millar *et al.*[1] and in a Class II Biological Safety Cabinet (MicroFlow, England) in a room physically separated from that used to set up nucleic acid amplification reaction mixes and also from the "post-PCR" room in order to minimise contamination and hence the possibility of false positive results. Bacterial DNA was extracted from the isolate employing the Roche High Purity PCR Template Preparation Kit (Roche Ltd., England) in accordance with the manufacturer's instructions. Extracted DNA was transferred to a clean tube and stored at -80°C prior to PCR. For each batch of extractions, a negative extraction control containing all reagents minus isolate, was performed. All reaction mixes were set up in a PCR hood in a room separate from that used to extract DNA and the amplification and post-PCR room in order to minimise contamination. Fifty microlitre reaction mixes were set up as follows:- 10 mM Tris-HC1, pH 8.3, 50 mM KC1, 2.5mM MgCl2 200μM (each) dATP, dCTP, dGTP and dTTP; 1.25U of Taq DNA polymerase (Amplitaq; Perkin Elmer), 0.2μM of the appropriate "broad-range" primers PSL[2] (forward) 5′ -AGG ATT AGA TAC CCT GGT AGT CCA-3′(1) and P13P[3] (reverse.) 5′ - AGG CCC GGG AAC GTA TTC AC -3′[3] and 4μl of DNA template.[2] Prior to PCR cycling, sealed tubes containing DNA template and all PCR reagents were introduced to the thermal cycler at 96°C to avoid non-specific annealing during the initial ramp stage. The reaction mixtures were subjected to the following thermal cycling parameters in a Perkin Elmer Cetus 2400 thermocycler: 96°C for 3 min followed by 40 cycles of 96°C for 1 min, 55°C for 1 min, 72°C for 1 min, followed by a final extension at 72°C for 10 min. During each run molecular grade water was included randomly as several negative controls and DNA templates from *Staphylococcus aureus* was included as a positive control, as appropriate. For each batch of extractions, an extraction control containing all reagents minus bacterial organism, was performed. Following amplification, aliquots (15 μl) were removed from each reaction mixture and examined by electrophoresis (80V, 45 min) in gels composed of 2% (w/v) agarose (Gibco, UK) in TAE buffer (40mM Tris, 20mM acetic acid, 1mM EDTA, pH 8.3), stained with ethidium bromide (5 μg/100 ml). Gels were visualised under UV illumination using a gel image analysis system (UVP Products, England) and all images archived as digital graphic (*.bmp) files. Amplicons chosen for automated sequencing were purified using a QIAquick PCR purification kit (Qiagen Ltd., UK) eluted in Tris-HC1 (10mM, pH 8.5) prior to sequencing, particularly to remove dNTPS, polymerases, salts and primers. The amplicon was sequenced on the ALF II Express® automated sequencer using the primer PSL which was labelled with Cy-5 fluorescent dye (Clarke Stevenson, Oligosynthesis Unit, The Queen's University of Belfast, UK) and used in conjunction with the Thermo Sequenase Fluorescent Labelled Primer Cycle Sequencing Kit (Amersham, UK). The resulting sequence obtained (999 bp) was compared with those stored in the GenBank Data system using FASTA alignment software (http://www.ebi.ac.uk), as well as being deposited in GenBank (Accession number AY259830). On BLAST analysis in combination with previously reported criteria used for interpretation of partial 16S rRNA gene sequences,[4] the sequence gave a 100% identification for *Tsukamurella tyrosinosolvens* (GenBank Accession numbers AY238514, Y12246, Y12245 & Y12247) followed by *T. columbiensis* AF272835 (99% identity), *T. spumae* AY238513

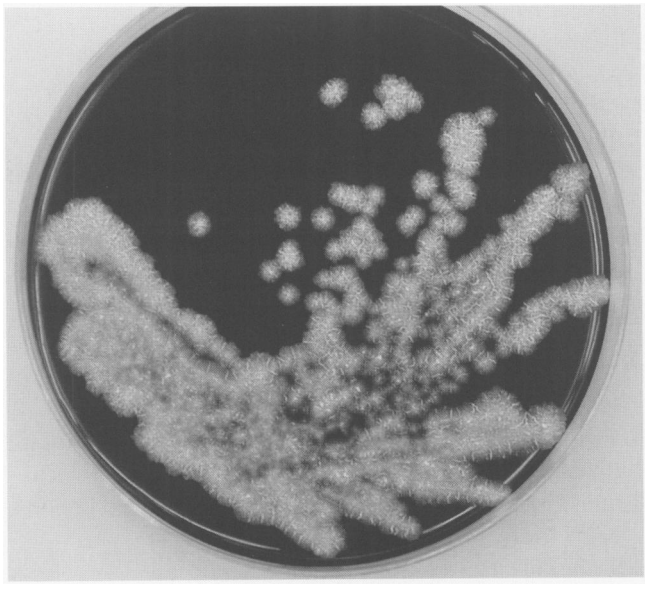

Figure Colonial morphology of *Tsukamurella tyrosinosolvens* NIPHL100602/MK2667 cultured at 37°C Columbia blood agar supplemented with 5% [v/v] defibrinated horse blood. Note the fungal-like hypael appearance of the colonies, which is characteristic of the genus *Tsukamurella*.

(99% identity), *T pulmonis* X92981 (99% identity) and *T. strandjordae* AF283283 (99% identity). The closest *Rhodococcus* neighbour was *Rhodococcus opacus* (AY027586) which was 95% (958/999 bases) similar.

In this case, the patient did not respond to intravenous teicoplanin to which the organism was sensitive for 10 days, thus necessitating the removal of the central line, as this was believed to be the focus of infection. After removal of the line, the patient showed a dramatic response without the use of further antibiotics and continued to remain well (within the limitations of her disease) at 18 months following the *Tsukamurella* infection. We were unable to isolate the coagulase-negative staphylococci or the *Tsukamurella* from the central line tip and this could be explained by the use of teicoplanin therapy, to which the organism was sensitive.

The organism was not identified as *Tsukamurella* until the patient improved. It is therefore important that *Tsukamurella* should be suspected as a possible pathogen in an immunocompromised patient with a foreign implant such as a central line. Previous reports demonstrated that there is a good prognosis by combining the administration of appropriate antibiotic therapy with removal of the catheter, which was the case with our patient.[5]

The genus *Tsukamurella*, was first described by Collins *et al*[6] in 1988 following the reclassification and further molecular and phenotypic charactization of *Gordana aurantiaca, Rhodococcus aurantiaca* and other related organisms, including *Corynebacterium paurometabola* which were distinct from the other mycolic acid-containing actinomycetes. The genus is phylogenetically related to the genera *Nocardia, Gordonia, Streptomyces, Rhodococcus, Corynebacterium* and *Mycobacterium* and taxonomically comprises of at least six described species, including *Tsukamurella inchonensis, T. paurometabola, T. pulmonis, T strandjordii, T. tyrosinosolvens* and *T. wratislaviensis. Tsukamurella* infections have emerged over the last decade as a rare but significant cause of serious infection in immunocompromised individuals. For a comprehensive review of these cases, see Schwartz *et al*.[5] Further to this review, an additional case of line-related sepsis has been shown,[7] as well as a three case of *Tsukamurella* conjunctivitis, which were treated successfully after 10 days with polymyxin B-neomycin or chloramphenicol eyedrops.[8] Consensus opinion from a synthesis of published reports indicates that underlying serious disease, including haematological malignancies, where the patient is immunocompromised combined with indwelling catheters are important risk factors for infection with this genus.[5] Therefore patients with indwelling catheters and haematological malignancies are susceptible populations for *Tsukamurella* infection.

Intravascular catheters have become indispensable in modern medical care. Their use is expected to be increased in the near future with improvement in sophisticated medical care leading to increased numbers of immunocompromised patients, including those with a haematological malignancy, needing indwelling intravascular catheters. The range of catheter-related infection varies from local insertion site infection through to metastatic deepseated infections.

This report highlights the benefits of the integration of a sequence-based typing approach in the identification of difficult-to-identify bacterial isolates employing partial regions of the 16S rRNA gene. Continued routine adoption of such techniques by clinical diagnostic laboratories may prove beneficial for the correct identification of bloodborne infections, as well as for the correct epidemiological characterization of unusual causal agents of bacteraemia in patients with haematological malignancies.

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