Infected endocarditis caused by *Kingella denitrificans*

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**SUMMARY** The clinical and bacteriological findings in a case of infective endocarditis caused by *Kingella denitrificans* are presented. This appears to be only the second report providing clear evidence for a pathogenic role for this species.

Several species of fastidious Gram negative bacteria have been identified as causes of infective endocarditis. Such organisms, including *Actinobacillus actinomycetemcomitans, Cardiobacterium hominis, Eikenella corrodens,* and *Haemophilus spp* are all members of the upper respiratory commensal flora. This group also includes *Kingella kingae* (previously known as *Moraxella kingae*), the first named species of a new genus proposed in 1976 by Henriksen and Bøvre. In the same year Snell and Lapage proposed the name *Kingella denitrificans* for a group of organisms described by Hollis et al under the designation TM-1. This new species is regarded as an upper respiratory tract commensal without pathogenic potential. There is a single report, however, of this organism acting as a pathogen in a case of endocarditis. We describe here a second case of endocarditis caused by this species.

**Case report**

A 49 year old woman was admitted to hospital on the 13 January 1984 with a two week history of malaise and weakness. A month before admission her general practitioner had treated a respiratory complaint with erythromycin followed by cephalaxin. Subsequently, she had complained of poor appetite, disturbed sleep, and inability to cope, which were attributed to depression. Antidepressant therapy was complicated by confusion and memory loss and the patient was referred for psychiatric assessment. The psychiatrist noted a deterioration in the patient's mental state but was concerned with a two week history of breathlessness on exertion and ankle swelling. On examination the patient was feverish with a tachycardia. A recent blood count showed anaemia and polymorphonuclear leucocytosis. Accordingly, the patient was referred to a cardiologist for further investigation.

On further direct questioning in hospital a one month history of night sweats and shivering episodes was elicited. There was no history of rheumatic fever or recent dental extractions. Examination showed an ill looking woman with anaemia, a temperature of 40·0°C, and sub-conjunctival haemorrhages. A pansystolic murmur was heard at the apex radiating to the axilla and an ejection systolic murmur maximal at the left sternal edge was noted. The jugular venous pressure was raised to 6 cm and the patient had bilateral basal crepitations and ankle oedema. Urinalysis showed haematuria. Haemoglobin concentration was 8·3 g/dl and the peripheral white cell count 18·2 × 10⁹/l. A diagnosis of infective endocarditis was made, and echocardiography showed a massive vegetation on the anterior leaflet of the mitral valve. Treatment was started with benzyl penicillin 12 megaunits/day and gentamicin 360 mg/day.

Within 24 h the patient's fever had reduced and her clinical condition improved. Overnight incubation of the initial blood culture set yielded a Gram negative rod in the aerobic broth only. Treatment was changed to piperacillin 16 g/day with continuation of the gentamicin. Two days after admission there was evidence of increasing cardiac failure and the patient was given frusemide and packed blood cell transfusion. When complete antimicrobial sensitivities were available, the antibiotic regimen was changed to ampicillin 12 g/day with the gentamicin continued as before. A Hickman intravenous catheter was inserted for administration of antibiotics. Nine days after admission the patient complained of numbness and discomfort in the right foot. Clinical examination and Doppler studies showed occlusion of the right superficial femoral artery. In view of the large mitral valve vegetation and evidence of major
embolisation the patient was referred for mitral valve replacement.

Surgery confirmed the presence of a large mitral valve vegetation and the valve was replaced by a Starr-Edwards prosthesis. The patient made a good postoperative recovery and the intravenous antibiotic regimen was continued for a total of four weeks with a further two weeks of ampicillin alone. Three weeks after operation she was found to have an aneurysm of the right superficial femoral artery which also involved the common femoral bifurcation. The aneurysm was removed and a saphenous vein graft inserted. Subsequent follow up has showed her to be well with no stigmata of infective endocarditis.

**Bacteriology**

On the day of admission four blood cultures were taken into aerobic and anaerobic bottles. Growth was detected by the radiometric system (Bectec, Johnston Laboratories, Becton Dickinson and Co, Towson, Maryland, USA). Broths were incubated at 37°C, the aerobic bottles being shaken for the first 24 h. The first set gave a positive reading in the aerobic broth on the following day. Gram staining of the broth showed Gram negative rods with some cocccoid forms. The remaining three sets all produced a similar organism in the aerobic broth after 48 h incubation.

Subcultures were made on to 5% (vol/vol) horse blood agar and incubated under 5% CO₂ aerobically and under anaerobic conditions (80% N₂, 10% H₂, 10% CO₂). A limited range of biochemical characteristics was determined using the methods and media described by Cowan. For more complete characterisation the isolate was referred to the Computer Identification Laboratory at the National Collection of Type Cultures, Central Public Health Laboratory, Colindale.

Disc diffusion antimicrobial sensitivities were determined by the comparative method using Isosensitest agar with 5% (vol/vol) lysed blood. In addition, the minimum inhibitory concentration and minimum bactericidal concentration of ampicillin were determined in Isosensitest broth with 5% (vol/vol) lysed blood. The minimum bactericidal concentration was defined as that concentration which killed 99.9% of the original inoculum. Bactericidal activity of the patient's serum against the infecting strain was determined in Isosensitest broth containing 50% (vol/vol) horse serum.

Growth appeared on horse blood agar after 24 h incubation under 5% CO₂ but was better characterised after 48 h incubation. Colonies were roughly 1 mm in diameter, grey, circular, low convex, semi-opaque, and produced pitting of the medium. No haemolysis was observed. Gram stained smears showed Gram negative coccobacilli in pairs and short chains.

Colonies grew equally well with or without 5% CO₂ under aerobic conditions but were smaller anaerobically. No growth occurred on horse blood agar at 22°C. Growth was enhanced by the presence of blood or serum but did not require X or V factors. There was no growth on MacConkey agar. The organisms were non-motile at 37°C and 22°C.

The results of the biochemical tests performed on the strain at the National Collection of Type Cultures are shown in the Table (the methods used for these tests are described by Holmes et al). These results correspond perfectly to those of the four reference strains of *Kingella denitrificans*, on which the original description of the species was based.

On disc sensitivity testing the isolate was sensitive to ampicillin, tetracycline, and gentamicin and moderately sensitive to penicillin (inhibition zone diameter with 1U penicillin disc 12 mm smaller than that of *Staphylococcus aureus* NCTC 6571). The ampicillin minimum inhibitory concentration was 0.06 mg/l and the minimum bactericidal concentration 0.125 mg/l. Serum obtained while the patient was receiving intravenous ampicillin and gentamicin was bactericidal to a dilution of 1/256 before and greater than 1/1024 one hour after both antibiotics had been given.

**Discussion**

In 1972 Hollis et al described a Gram negative rod isolated on Thayer-Martin gonococcal selective agar, which they designated TM-1. The organism was identified from throat swabs taken in a survey for carriers of *Neisseria lactamica* and *N meningitidis*. In 1976 Snell and Lapage proposed the name *Kingella denitrificans* for this organism, stating that it is found in the respiratory tract of man and has unknown pathogenicity. Of the strains referred to the Center for Disease Control, Atlanta, USA, more than 80% were isolated from the respiratory tract with five isolates from rectal and genitourinary sources and two from blood.

The strain described here conforms to the descriptions of *K denitrificans* given by Snell and Lapage and Snell except that it does not reduce nitrite, does not produce acid from glucose, and fails to yield a fermentative reaction in the O-F test. (Maltose is described as positive by Snell and Lapage but as negative by Snell; the latter result is correct as the use of horse serum, rather than rabbit serum, to supplement test media may lead to false positive reactions with maltose.) Even the reference strains of the species did not give positive results in these
tests, however, and the differences may be attributed to differences in test methods. For example, Snell and Lapage detected acid from carbohydrates using the "sugars for niesserias" medium described by Cowan, and Snell and Lapage also used O-F medium and phenol red broth base (Difco), both supplemented with 5% (vol/vol) horse serum. We did not use any of these media except for glucose O-F medium, which we did not supplement with serum. Weaver and Hollis indicate that five of their 60 strains did not ferment glucose and that positive reactions are produced slowly even with the addition of rabbit serum.

To our knowledge this organism has only once been reported as a pathogen. Goldman et al describe a case of endocarditis in a 31 year old man with aortic stenosis who had undergone open commissurotomy at the age of 12. The patient gave a history of dental cleaning without prophylaxis two months before admission. Treatment with ampicillin for six weeks produced an uncomplicated recovery. The patient reported here had an infection complicated by a large vegetation with embolisation necessitating valve replacement. There was no history of previous cardiological abnormalities or recent dental manipulations.

This second case of endocarditis provides confirmation of the pathogenic potential of K denitrificans and indicates that this organism should be added to the growing list of fastidious Gram negative organisms associated with this condition.

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References

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