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Infective 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.[1] In the same year Snell and Lapage[2] proposed the name *Kingella denitrificans* for a group of organisms described by Hollis *et al* under the designation TM-1.[3] 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.[4] 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 cephalexin. 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 × 10^9/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

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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 (Bactec, 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 coccoid 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% CO_2 aerobically and under anaerobic conditions (80% N_2, 10% H_2, 10% CO_2). A limited range of biochemical characteristics was determined using the methods and media described by Cowan.[5] 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[6] 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.[6] 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.[6]

Growth appeared on horse blood agar after 24 h incubation under 5% CO_2 but was better characterised after 48 h incubation. Colonies were roughly 1 mm in diameter, grey, circular, low convex, semitranslucent, 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% CO_2 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*[7]). These results correspond perfectly to those of the four reference strains of *Kingella denitrificans*, on which the original description of the species was based.[2]

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.[3] The organism was identified from throat swabs taken in a survey for carriers of *Neisseria lactamica* and *N meningitidis*. In 1976 Snell and Lapage[2] 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.[8]

The strain described here conforms to the descriptions of *K denitrificans* given by Snell and Lapage[2] and Snell[9] 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[2] but as negative by Snell[9]; 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.[8]) Even the reference strains of the species did not give positive results in these

Characteristics of the isolate of Kingella denitrificans herein reported and of the four reference strains (including the type NCTC 10995) on which the original description of the species[2] was based

All five isolates positive for:		
Cytochrome oxidase production	Growth at 37°C	Nitrate reduction
All five isolates negative for:		
Acid from ASS* glucose		
Acid from ASS adonitol	Gluconate oxidation	
Acid from ASS arabinose	Growth at 5°C	
Acid from ASS cellobiose	Growth at room temperature	
Acid from ASS dulcitol	Growth at 42°C	
Acid from ASS ethanol	Growth on cetrimide	
Acid from ASS fructose	Growth on β-hydroxybutyrate	
Acid from ASS glycerol	Growth on MacConkey agar	
Acid from ASS inositol	Growth on Simmons' citrate	
Acid from ASS lactose	Hydrogen sulphide-production (lead acetate paper)	
Acid from ASS maltose	Hydrogen sulphide production (triple sugar iron agar)	
Acid from ASS mannitol	Indole production (Kovacs')	
Acid from ASS raffinose	KCN tolerance	
Acid from ASS rhamnose	Lysine decarboxylation	
Acid from ASS salicin	Malonate utilisation	
Acid from ASS sorbitol	Motility at room temperature	
Acid from ASS sucrose	Motility at 37°C	
Acid from ASS trehalose	Opalescence on lecithovitellin agar	
Acid from ASS xylose	Ornithine decarboxylase	
Acid from 10% (wt/vol) glucose	Phenylalanine deamination	
Acid from 10% (wt/vol) lactose	Pigment on nutrient agar	
Acid from peptone-water glucose	Pigment on tyrosine agar	
Aesculin hydrolysis	Poly-β-hydroxybutyrate inclusion granules	
Alkali production on Christensen's citrate	Reaction in Hugh and Leifson oxidation fermentation test	
Arginine desimidase	Reduction of 0·4% (wt/vol) selenite	
Arginine dihydrolase	Starch hydrolysis	
Casein digestion	Tween 20 hydrolysis	
Catalase production	Tween 80 hydrolysis	
Deoxyribonuclease production	Tyrosine hydrolysis	
Fluorescence on King's B medium	Urease production	
Gas from peptone-water glucose	β-D-Galactosidase production (ONPG test)	
Gelatin hydrolysis (stab liquefaction)	3-ketolactose production	
Gelatin hydrolysis (plate method)		
Isolates differed in:		
Nitrite reduction†		

*Tested in ammonium salt medium.
†Positive result given only by reference strain A360/72.

tests, however, and the differences may be attributed to differences in test methods. For example, Snell and Lapage[2] and Snell[9] detected acid from carbohydrates using the "sugars for neisserias" medium described by Cowan,[5] and Snell and Lapage[2] 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.[8]

To our knowledge this organism has only once been reported as a pathogen. Goldman *et al*[4] 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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