

More than a Bite.

The Importance of 16S PCR in the Diagnosis of Culture Negative Infective Endocarditis.

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Case History

- A healthy 33 year old postman received a dog bite on his index finger, whilst delivering a letter.
- He developed night sweats and shortness of breath weeks later. Numerous GP tele-consultations prompted SARS-CoV2 testing. He received antimicrobials for presumed LRTI with negative PCR.
- 3 months later, progressive dyspnoea resulted in an emergency admission to hospital, with acute decompensated heart failure. CTPA ruled out pulmonary embolism but highlighted profound pulmonary oedema. Echocardiogram demonstrated severe aortic regurgitation, vegetations on the non-coronary cusp and an aortic root abscess (Figure 1).
- He underwent urgent early aortic valve replacement with a 25mm carbomedics mechanical aortic valve as per International Guidelines (Class 1 Indication)(1).
- Intra-operatively he was noted to have a congenital abnormality: a bicuspid aortic valve, with fusion of right and left coronary cusps, and anomalous origin of his right coronary artery from his left main stem.
- The patient was empirically commenced on an antibiotic regimen for infective endocarditis (IE); ceftriaxone 2g OD, vancomycin and gentamicin.
- Five sets of blood cultures failed to yield an organism after prolonged incubation.
- Valve tissue culture yielded no growth.
- Capnocytophaga canimorsus* was revealed as the causative pathogen following 16S ribosome DNA sequencing of the valve tissue undertaken by Public Health England
- Gentamicin and vancomycin were stopped after 14 days of treatment and targeted treatment with ceftriaxone 2g OD was continued for a total of six weeks treatment.

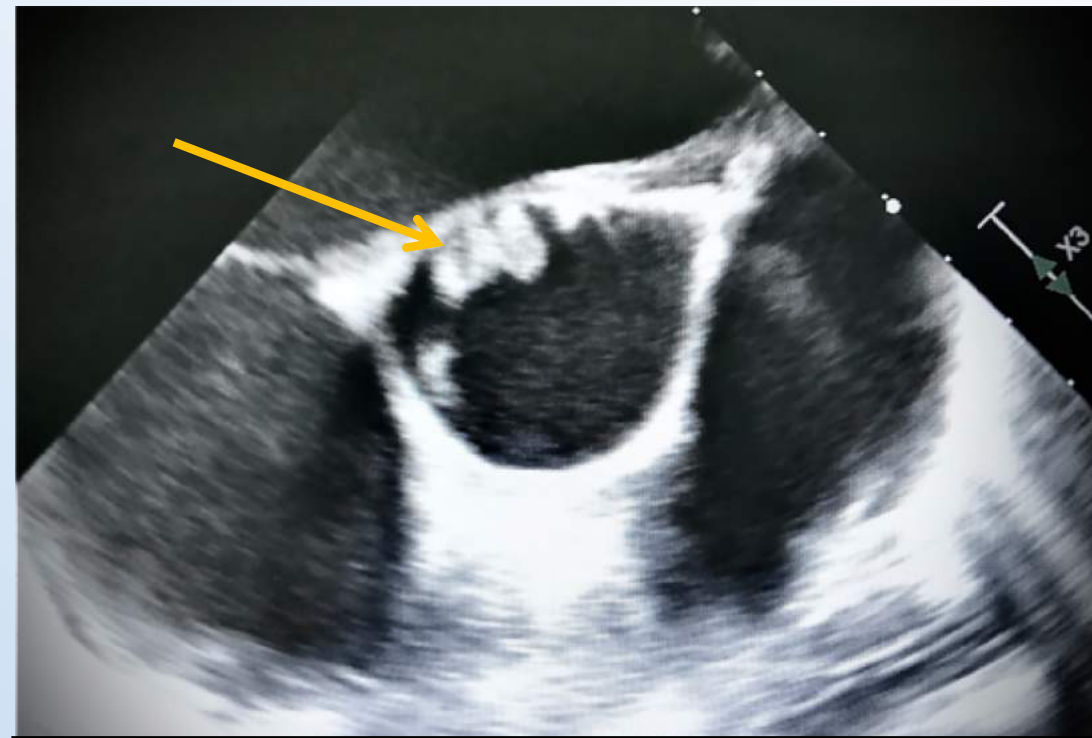


Figure 1: TOE demonstrating large vegetation on non-coronary cusp (yellow arrow)

Table 1: Features of 18 case reports published in the literature from 1977-2019.

Average age	52.8 years (range 24-73 years)
Gender	• 80% male (n=14)
Affected valves	• Aortic (n=11) • Tricuspid (n=6) • Mitral (n=4)
Treatment	• 50% (n=9) received surgical treatment with mostly mechanical valves
Treatment	• Penicillin (n=8) • Cephalosporin (n=4)
Underlying illness	• Underlying cardiac disease (n=4) • alcohol abuse (n=4) • CLL undergoing chemotherapy (n=1)

Capnocytophaga canimorsus

- Is a facultatively anaerobic, fastidious Gram-negative bacillus considered to be a commensal oral flora in dogs. It was originally described by Bobo & Newton in 1976 in a patient with septicaemia and meningitis.
- Patients who are immunocompromised, particularly asplenia and alcoholics are predisposed to severe infections with fulminant sepsis.
- It has been associated with a wide variety of infections including meningitis, septic arthritis and infective endocarditis (2).

Capnocytophaga canimorsus in Infective Endocarditis

- IE accounts for under 2% of *C. canimorsus* blood stream infections and is rare.
- Between 1977 and 2019 there were 18 cases of *C. canimorsus* IE reported in the literature (Table 1). Twelve of these cases had animal contact with a dog, one with a lion and in five cases animal contact was not described (3).

Antimicrobial resistance

- Recent publications report B-lactamase production in between 32 and 70% of isolates.
- A class D beta-lactamase has been described among in 4 strains of *C. canimorsus*. This gene, designated 'blaOXA-347, is phenotypically associated with resistance to penicillin, cephalosporins & imipenem and is classified as a carbapenemase.

16S ribosomal DNA

- Bacterial 16S ribosomal RNA (rRNA) gene sequencing is commonly used for bacterial identification of culture-negative samples.
- The 16S rRNA gene is a highly conserved component of the transcriptional machinery of all bacteria.
- Universal PCR primers can be designed to target the conserved regions of 16S making it possible to amplify the gene in a wide range of different microorganisms from a single sample. Conveniently, the 16S rRNA gene consists of both conserved and variable regions; while the conserved region makes universal amplification possible, sequencing the variable regions allows discrimination between specific different microorganisms.
- In this case, 16SPCR of the infected valve tissue clinched the diagnosis and provided justification for targeted antimicrobial treatment. The patient would otherwise have been treated with a broader regimen increasing the risk of adverse drug reactions and increased healthcare cost.

Bibliography

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