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CASE REPORT

Bacteremia due to the fastidious bacterium *Granulicatella adiacens*: A diagnosis that was almost missed

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ABSTRACT

Bacteremia due to *Granulicatella adiacens* has been rarely reported in the medical literature. A middle-aged gentleman developed necrotizing fasciitis on his left second toe after stepping on a nail. A ray amputation was performed and ceftazidime-susceptible *Pseudomonas aeruginosa* was isolated from his bone culture. However, while receiving ceftazidime for the necrotizing fasciitis, his blood culture vial was positive for gram-positive cocci-shaped bacteria in short chains which grew as tiny non-lytic colonies on sheep blood agar only following extended incubation. There was no culture evidence of *P. aeruginosa* in the same blood specimen. The gram-positive organism was conclusively identified by matrix-assisted laser desorption ionization-time of flight mass spectrometry as *G. adiacens.* The patient was treated with benzylpenicillin (to which the organism tested susceptible) for 14 days before he was discharged home.

Keywords: Bacteremia; penicillin; *Granulicatella adiacens*; nutritionally variant streptococcus.

INTRODUCTION

The fastidious bacterium that is now known as *Granulicatella adiacens* was believed to be a new type of viridans *Streptococcus* when it was first described more than half a century ago. It was even once named *Streptococcus adjacens* (Liao *et al*., 2004). *G. adiacens* is a member of the nutritionally variant streptococci (NVS) group due to its specific growth requirements (Alberti *et al*., 2016). Like the viridans streptococci, *G. adiacens* is a commensal of the human oropharynx and gastrointestinal tract although 16S rRNA sequencing has since rebutted its relatedness to streptococci. *G. adiacens* has only been rarely implicated in bacteremia and infective endocarditis, due to its difficult isolation compared to other streptococci (Gupta *et al*., 2018). On a more sinister note, compared to viridans streptococci, infections caused by NVS are more likely to result in complications such as relapse, treatment failure, embolization (in the case of endocarditis) and even death (Alberti *et al*., 2016). We report a case of *Pseudomonas aeruginosa* necrotizing fasciitis in a middle-aged man. If it was not for our blood culture vials that were already preadded with the necessary compounds, a concomitant *G. adiacens* bacteremia would have been missed, with potentially dire consequences.

CASE REPORT

A 50-year-old man with a background history of diabetes mellitus and ischemic heart disease complained of a four-day history of left foot swelling and pain after stepping on a nail at home. Despite being diabetic, the patient's sensation was intact enough to alert him that a penetrating injury had occurred. A day before he presented to us, his condition deteriorated with the onset of fever and chills. When he arrived at the emergency department of UKM Medical Centre, the patient was alert but lethargic. He had a body temperature of 37°C, a pulse rate of 86 beats per minute, a blood pressure reading of 143/80 mmHg, and his room air oxygen saturation was 98%. Systemic examination was unremarkable. However, the distal half of his left foot was warm and swollen. Slough was visualized at the webspace between the first and second toes. A bluish discoloration was also noted on the second toe, together with crepitation and reduced sensation. Radiological examination revealed the presence of second toe gas shadow until the medial part of the first toe, leading to a diagnosis of left second toe necrotizing fasciitis.

Laboratory tests revealed, among others, a raised total white blood cell count of 21.0 x 10^9/L (with neutrophil predominance) and a high C-reactive protein level of 31.27 mg/L. An urgent wound debridement surgery was scheduled, and a blood specimen was sent for bacteriological culture. Empirical therapy with intravenous (IV) ampicillin-sulbactam 1.5 g q8h was commenced while awaiting surgery. Wound debridement and a ray amputation of the second left toe were successfully performed on day two of admission. Intraoperative bone and soft tissue samples were also sent to the laboratory for bacteriological culture.

On the fourth day of admission, the laboratory reported that his bone sample yielded ceftazidime-susceptible *Pseudomonas aeruginosa*. Accordingly, the clinician commenced IV ceftazidime 2 g q12h and discontinued IV ampicillin-sulbactam. A day later, his

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anaerobic blood culture vial (BD BACTEC™ Lytic/10 Anaerobic/F) was positive for gram-positive cocci-shaped bacteria arranged primarily in short chains. The gram-positive cocci grew as tiny non-hemolytic colonies on sheep blood agar only after 48 hours of incubation (Figure 1). They were also oxidase- and catalase-negative. Despite the absence of the same bacteria in the aerobic blood culture vial (BD BACTEC™ Plus Aerobic/F), they were facultative anaerobes because of their metronidazole-resistant nature when sub-cultured anaerobically (Figure 1). Importantly, there was no culture evidence of *P. aeruginosa* in the aerobic blood culture vial. The VITEK 2 system (bioMerieux, France) identified the gram-positive organism biochemically as *Granulicatella adiacens* (bionumber: 000032300001010; probability: 99%). Our isolate's identity was further confirmed by matrix-assisted laser desorption ionization-time of flight mass spectrometry (MALDI Biotyper, Bruker-Daltonics, Germany), which matched its mass spectral pattern with that of *Granulicatella adiacens* 20_A875 MHH (score value: 1.81).

Using the gradient diffusion method to obtain minimal inhibitory concentration (MIC) values, we concluded that our *G. adiacens* isolate was susceptible to penicillin (MIC: 0.125 ug/mL), ceftriaxone (MIC: 0.25 ug/mL), cefotaxime (MIC: 0.38 ug/mL) and vancomycin (MIC: 1.0 ug/mL). Owing to this latest culture development, the clinician switched the patient's antibiotic from IV ceftazidime to IV benzylpenicillin 4 mega units q4h. An echocardiogram was also performed. Fortunately, there was no evidence of cardiac vegetations or valvular abnormalities. A repeat blood culture after 72 hours of benzylpenicillin therapy was negative. The patient's septic parameters improved significantly after completing 14 days of benzylpenicillin therapy (as evidenced by a reduction in the total white cell count from 21.0×10^9/L to 8.4×10^9/L and a decrease in the C-reactive protein level from 31.27 mg/L to 0.8 mg/L) and he was discharged home.

DISCUSSION

NVS are fastidious organisms because they require thiol compounds such as pyridoxal (a form of vitamin B6) and L-cysteine to be incorporated into standard culture media to facilitate their successful isolation in diagnostic laboratories (Alberti *et al*., 2016). For this reason, NVS are also sometimes referred to as 'nutritionally deficient' streptococci and this elucidates the occurrence of NVS satellite colonies (i.e. satellitism) surrounding a *Staphylococcus aureus* streak when subcultured (because *S. aureus* releases thiol compounds) (Gupta *et al*., 2018). Our centre uses the commercially available BD BACTEC™ Lytic/10 Anaerobic/F blood culture vials to isolate anaerobic bacteria. While these vials contain thiol compounds, the same cannot be said of our BD BACTEC™ Plus Aerobic/F blood culture vials used to isolate aerobic organisms. The supplementation of thiol compounds in only one type of vial explains the recovery of *G. adiacens* only from the inoculated anaerobic vial, even though *G. adiacens* is not an obligate anaerobe.

Also, in our centre, we utilize sheep blood agar (which is naturally deficient in pyridoxal) as one of our primary isolation media when sub-culturing from positive blood culture vials – this explains why the growth of *G. adiacens* was poor (as evidenced by tiny colonies) and required at least 48 hours of incubation. Although we managed to isolate *P. aeruginosa* from our patient's bone and tissue samples, due to the lack of thiol supplementation in the agar media used for our pus and tissue cultures (i.e. sheep blood agar and MacConkey agar), we are unable to confidently exclude a co-infection by *G. adiacens.* Thus, although it is not a routine practice in our centre, some centres practice inoculating non-blood specimens (e.g. synovial fluid) into commercial blood culture vials to increase the likelihood of isolating NVS (Hepburn *et al*., 2003).

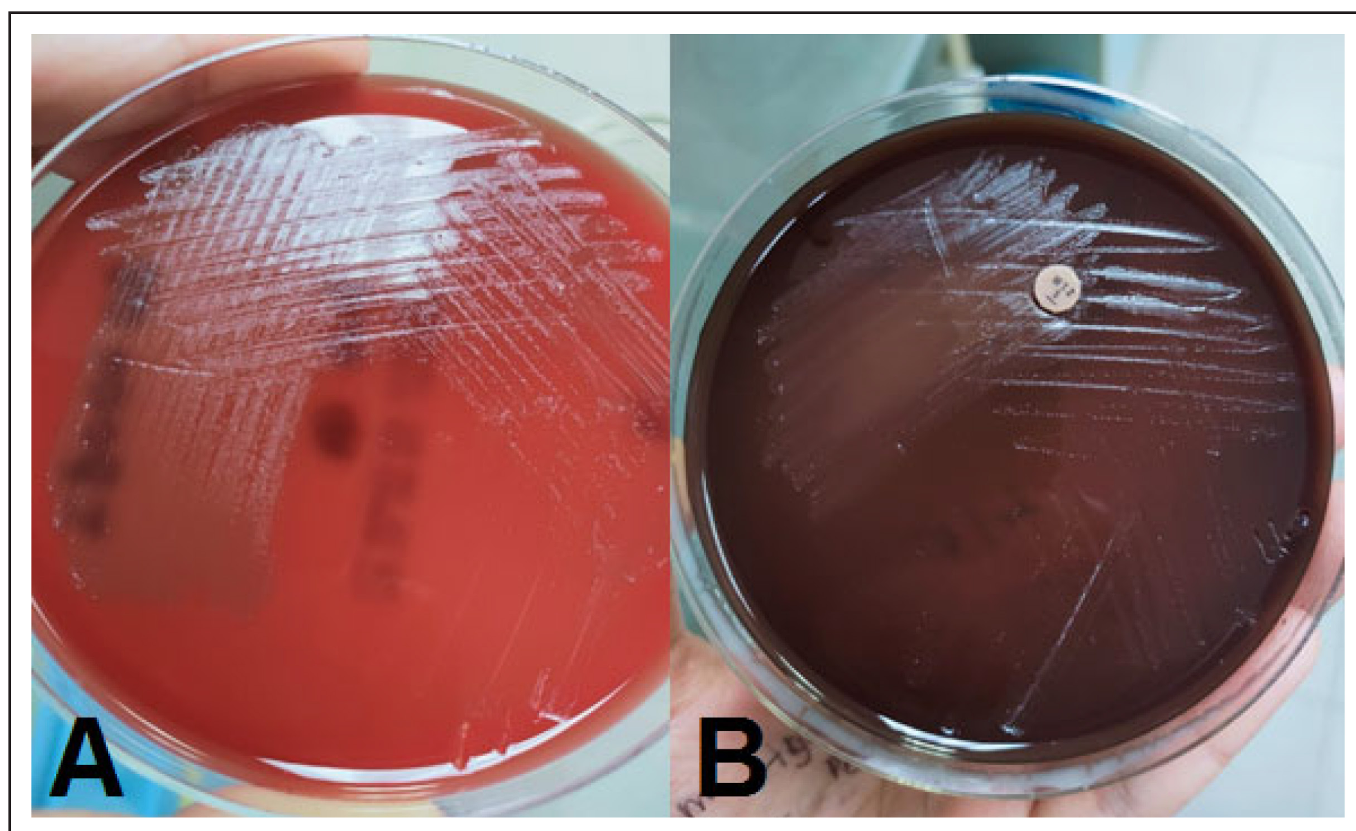

Figure 1. The left image (A) shows tiny non-hemolytic *G. adiacens* colonies on blood agar after 48 hours of incubation. The right image (B) shows metronidazole-resistant *G. adiacens* colonies following 48 hours of anaerobic culture on blood agar.

The Clinical and Laboratory Standards Institute (CLSI), through its M45 document, recommends that antibiotic susceptibility testing (AST) for *Granulicatella* should only be undertaken using the broth microdilution method by utilizing cation-adjusted Mueller Hinton broth supplemented with lysed horse blood and pyridoxal hydrochloride (CLSI, 2015). However, due to the unavailability of these specific reagents and the technically demanding nature of broth microdilution, we attempted an alternative AST method (known as the gradient diffusion method), in which Etest strips impregnated with antibiotics known to have activity against NVS were placed on various media. Specifically, the Etest strips were placed on sheep blood agar plates and on Mueller Hinton agar plates supplemented with sheep blood. All plates were then incubated at 37°C in a 5% CO_2 atmosphere.

Only the sheep blood agar plates could provide us with MIC values following an extended incubation period of 48 hours. The plates with Mueller Hinton agar supplemented with sheep blood failed to produce any bacterial lawn, although it is a CLSI-sanctioned AST medium for viridans streptococci (CLSI, 2022). While we are cognizant that our MIC values were controversially obtained, the lack of published disk diffusion breakpoints for *Granulicatella*, the absence of any FDA-approved automated AST system and the restricted availability of broth microdilution capabilities in many diagnostic laboratories (particularly in developing countries such as Malaysia), make it difficult to report AST results accurately (Gupta *et al*., 2018). Like us, other investigators had also attempted AST with Etest strips on agar media other than that recommended by the CLSI (Alberti *et al*., 2016).

Notwithstanding the challenges faced in obtaining antibiotic MIC values, forsaking AST completely could jeopardize patient management because of the higher risk of drug resistance in *G. adiacens.* A Taiwanese study found that half of the NVS isolated were not susceptible to penicillin and one-third were not susceptible to cefotaxime, although full susceptibility was recorded for drugs customarily reserved to manage infections caused by multidrug-resistant gram-positive pathogens (i.e., vancomycin, quinupristin-dalfopristin and linezolid) (Liao *et al*., 2004). Ceftazidime was administered to our patient when *P. aeruginosa* was cultured from his bone specimen. While ceftazidime has excellent activity against gram-negative bacilli (earning it the 'anti-pseudomonal cephalosporin' moniker), the same cannot be said about its activity against the gram-positive streptococci. Specifically, when pitted against other cephalosporins (e.g., ceftriaxone and cefepime) or even the humble penicillin, the in-vitro activity of ceftazidime against viridans streptococci was dismal, with a susceptibility of only 13% (Pfaller *et al*., 1997). For practical purposes, this poor susceptibility to ceftazidime can also be extrapolated to *G. adiacens*. Even the CLSI does not recommend testing NVS against ceftazidime (CLSI, 2015). Thus, if it was not for his positive blood culture, we would have continued to administer ceftazidime to our patient and may have had to deal with the dire consequences of *G. adiacens* bacteremia (including infective endocarditis).

CONCLUSION

Since not all bacterial pathogens can be cultured with equal ease, blood and other sterile clinical specimens should be inoculated into commercially sourced culture vials that contain thiol compounds to facilitate the isolation of NVS such as *G. adiacens.* A vital clue to the presence of *G. adiacens* in a culture is the poor/sluggish growth of gram-positive cocci colonies on standard blood agar. Organism identification is generally not an issue because commercial biochemical identification systems (e.g., VITEK 2) can readily identify it. The true challenge lies in patient management, where the microbiology laboratory may face hurdles obtaining antibiotic MIC values. While penicillin may be utilized for treatment at the outset, sans any MIC values, the threshold to escalate to a cephalosporin (e.g., ceftriaxone) or even a glycopeptide (e.g., vancomycin) should be low.

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Conflict of Interest

The authors declare no conflict of interest.

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