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Endocarditis Caused by *Abiotrophia* and *Granulicatella* Species

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Abstract

Endocarditis caused by *Abiotrophia* and *Granulicatella* species, formerly known as nutritionally variant streptococci (NVS) is rare. It is associated with increased complications such as heart failure, systemic emboli, valve replacement surgery, treatment failures and mortality. The diagnosis of these infections is challenging due to specific nutritional growth requirements although modern techniques such as 16S rRNA sequence analysis and Matrix-assisted laser desorption ionization time-of-flight mass spectrometry (MALDI-TOF MS) are particularly useful. Penicillin resistance among these organisms is a growing problem. Penicillin and gentamicin combination or alternatively Vancomycin alone are the recommended treatment options, however there is increasing data regarding susceptibilities to other antibiotics. Varying susceptibilities to antibiotics among different species of NVS needs to be studied further.

Keywords: *Abiotrophia* and *Granulicatella* endocarditis, aorto-right ventricular fistula, *Abiotrophia* endocarditis

1. Introduction

Nutritionally variant (deficient) streptococci (NVS) were first described by Frankel and Hirsch in 1961 [1]. These gram positive bacteria resembled streptococci but had specific nutritional growth requirements. Since their first identification, over the years nomenclature of NVS changed several times based on DNA-DNA hybridization studies and 16S rRNA sequence analysis. They were first included in the genus *Streptococcus*, then genus *Abiotrophia*, and finally they were recognized as two separate genera; genus *Abiotrophia* and genus *Granulicatella*. Genus

Abiotrophia consists of *Abiotrophia defectiva* and genus *Granulicatella* consists of *Granulicatella adiacens*, *Granulicatella elegans*, *Granulicatella balaenopterae* and *Granulicatella para-adiacens*.

NVS are members of the normal flora of human pharynx, human urogenital and intestinal tracts [2].

Infective endocarditis caused by NVS is rare, causing approximately 2% of all cases of infective endocarditis [3]. Over 125 cases of infective endocarditis caused by *Abiotrophia* and *Granulicatella* spp. have been reported to date. It is estimated that approximately 5–6% of all cases of streptococcal endocarditis are caused by NVS [4]. Due to the fastidious nature of these organisms and difficulties in diagnosis, it is possible that endocarditis caused by NVS may be under-recognized. NVS are among the organisms causing culture negative endocarditis. The main reservoir of infective endocarditis inducing NVS is oral cavity as in the case of other viridans streptococci [5].

Although endocarditis and bacteremia are the most common infections associated by *Abiotrophia* and *Granulicatella* spp., the literature for infections caused by *Abiotrophia* and *Granulicatella* spp. has been growing with new sites of infections being reported as our awareness of these bacteria heightens and our diagnostic capabilities improve.

Ophthalmological infections have been encountered, ranging from keratitis to endophthalmitis [6]. NVS are known to cause corneal ulcers [7], vitreous infections [8] and infectious crystalline keratopathy [9]. Orthopedic infections, including prosthesis infection, septic arthritis, discitis and sacroiliitis have been reported [10–12]. Synovial biopsy sample from a patient with culture negative endocarditis also yielded NVS [13]. NVS are also associated with central nervous system infections; more commonly brain abscesses but rarely meningitis [14], subarachnoid hemorrhage [15] and intracranial aneurysms [3] have been reported. CNS infections have been commonly linked to embolic phenomena, neurosurgical instrumentation and immunosuppression [10, 16]. NVS have been isolated from patients with otitis media [1], otitis externa [2], sinusitis [17], parapneumonic effusion [18], cirrhosis [19], peritonitis [20], pancreatic abscess [21], bacteremia associated with postpartum or postabortal sepsis [19], tubo-ovarian abscess [22], breast implant associated infection [23], wound infections, and vaginal discharge [24]. Endarteritis caused by *A. defectiva* involving the main pulmonary artery in a patient with asymptomatic patent ductus arteriosus has been reported [25].

2. Microbiology

Nutritionally variant streptococci were first described by Frenkel and Hirsch in 1961 from blood cultures of cases of subacute bacterial endocarditis and from otitis media. These cell wall deficient, L form 'streptococci' were noted to grow in satellite colonies around other bacteria requiring substances secreted by other bacteria for growth [1]. 'Abiotrophia' means life nutrition deficiency, referring to the need of specific nutrients in media for growth of these bacteria [26]. They are catalase-negative, oxidase-negative, facultative anaerobic gram positive bacteria [27]. They often form white-gray, non-hemolytic colonies. These organisms hardly grow in culture media that streptococci ordinarily grow, such as sheep blood agar. They require supplementation of L-cysteine or pyridoxal HCl. In the absence of these supplements, NVS can also grow forming satellite colonies adjacent to streaks of helper bacteria such as *Staphylococcus aureus* or *Staphylococcus epidermidis*.

Bouvet et al. in 1989 showed that NVS could be divided into two groups, *Streptococcus defectivus* and *Streptococcus adiacens* by DNA–DNA hybridization studies. They noted that there was less than 10% DNA homology with the reference streptococcus species [28].

In 1995, Kawamura et al. proposed that these distinct species be transferred to a new genus, *Abiotrophia*, as *Abiotrophia adiacens* and *Abiotrophia defectiva* by using 16S rRNA gene sequencing. Subsequently two new species were added to this genus; *Abiotrophia elegans* [29] isolated from a patient with endocarditis and *Abiotrophia balaenopterae* [30] from a minke whale (*Balaenoptera acutorostrata*).

Finally, in 2000, Collins et al. proposed the taxonomy of NVS that we use today. They pointed out that genus *Abiotrophia* consisted of two distinct lines. *Abiotrophia defectiva* and a robust group consisting of *A. adiacens*, *A. balaenopterae* and *A. elegans*. They reclassified *A. adiacens*, *A. balaenopterae* and *A. elegans* into genus *Granulicatella* (*small chain of small grains in Latin*) and *Abiotrophia defectiva* into genus *Abiotrophia* [27]. Shortly before this taxonomy revision, Kanamoto et al. proposed a new strain, *Abiotrophia para-adiacens*, related to *Granulicatella adiacens* which is rarely reported but not widely published [31, 32].

3. Pathophysiology

Bacterial attachment to damaged heart valves is the key factor in infective endocarditis. Intact vascular endothelium can resist the development of endocarditis [33]. Experimental animal models showed that when catheter induced endocardial damage is produced; these endocardial lesions can be infected by direct inoculation of bacteria or by intravenous inoculation [34]. Pathophysiology of infective endocarditis typically would start with endothelial cell denudation, followed by exposure of underlying extracellular matrix (ECM) and finally binding of fibrin and platelets [33]. Extracellular matrix proteins are exposed during damage to the cardiac endothelium providing potential sites of attachment for virulent organisms [35] *Granulicatella* and *Abiotrophia* spp. have the ability to bind to fibronectin and other extracellular matrix proteins. The ability to bind to extracellular matrix proteins appears to correlate with the degree of infectivity of NVS [5].

Some groups of NVS are more pathogenic and other groups are less pathogenic. Highly pathogenic *G. adiacens* has high fibronectin binding ability. Highly pathogenic *A. defectiva* strains also have strong ability to bind to fibronectin and other ECM proteins whereas less pathogenic *G. para-adiacens* and *G. elegans* strains show low ability to bind to fibronectin and all other ECM proteins [5]. Similarly, among non-NVS streptococci that are commonly associated with infective endocarditis, *S. mutans*, *S. mitis*, *S. sanguis* and *S. fecalis* also have the ability to bind to the extracellular matrix [35].

By binding to the extracellular matrix proteins, bacteria are able to adhere to the damaged endocardium and subsequently producing colonization and infection. ECM binding ability however is not the sole indicator of pathogenicity. Some strains of NVS have high infectivity without significant binding to the ECM proteins suggesting other mechanisms involved in pathogenesis. Other mechanisms of endocardial infectivity of NVS remains to be discovered [5].

As a group, NVS have heterogenous properties of pathogenicity. *A. defectiva* has higher pathogenicity compared to other species of NVS [5]. About 73% of all NVS isolates from patients with bacterial endocarditis are *Abiotrophia defectiva*. *G. para-adiacens* and *G. elegans* strains are less virulent than *A. defectiva* and *G. adiacens* [5].

Okada et al. noted that [5] NVS isolates from endocarditis patients and from normal oral flora both had the ability to cause infective endocarditis.

4. Diagnosis

Identification of *Abiotrophia* and *Granulicatella* spp. in blood cultures is extremely difficult. NVS do not grow well in subcultures and may be regarded as contaminant bacteria. Their extreme pleomorphism may lead to misidentification of these bacteria as other bacteria, even fungi [36, 37]. Christensen and Facklam [38] studied 101 NVS isolates and reported that isolates were gram variable and pleomorphic, forms varied from bacilli with spore like swellings to cocci predominantly in pairs and chains when gram stain preparations were made from agar plates. NVS can demonstrate bulbous swelling and filament formation and they can form rough colony morphology on chocolate agar that can be suggestive of other microorganisms such as *Streptobacillus moniliformis* or *Erysipelothrix rhusiopathiae* [36].

NVS show morphologic variations depending on the pyridoxal concentrations in the growth medium [39]. Due to the difficulties in identification of these bacteria, it is crucial for microbiology staff to be vigilant and be aware of the pleomorphic nature of the NVS to prevent misidentification.

NVS should be suspected when gram stain shows microbial cells but cultures are negative [2]. Once their nutritional growth requirements are supplemented in media, NVS convert to streptococci-like cells [39] and gram positivity making them easier to identify, although it was also shown that correcting nutritional deficiency may not convert all abnormalities [40]. For *G. elegans*, addition of cysteine to growth media would have the effect of reversal of pleomorphic morphology but addition of pyridoxal HCl does not [29]. The difficulty in identifying these organisms leads to delays in diagnosis and thus timely initiation of appropriate antimicrobial treatment [41].

Contemporary blood culture methods enable *Abiotrophia* and *Granulicatella* spp. to grow routinely, visible colonies of these organisms appear in 48 h on subculture from positive blood culture media supplemented with *Brucella* and chocolate subculture media [3].

Cargill et al. noted that anaerobic blood culture bottles became positive sooner than aerobic blood cultures bottles; (3.56 h, standard deviation 8.49 h) although they noted that this was not significant or reliable [31].

Specific phenotypic characteristics of NVS can be identified by examining their patterns of production of α-galactosidase, β-galactosidase, β-glucosidase, N-acetyl-β-glucosaminidase and β-glucuronidase, and fermentation of trehalose, pullulan, tagatose and sucrose [32, 38].

A. defectiva produces α-galactosidase, β-galactosidase and produces acid from trehalose, sucrose and pullulan. Its acid production from tagatose is variable [38].

G. adiacens produces β-glucuronidase and produces acid from sucrose and tagatose. *G. elegans* hydrolyses arginine. Its hippurate hydrolysis is variable. It produces acid from sucrose. *G. balaenopterae* hydrolyses arginine and produces acid from trehalose and pullulan. [38]

G. para-adiacens produces β-glucosidase, does not produce α- or β-galactosidase or arginine and does not ferment trehalose, pullulan or tagatose [32].

Molecular diagnostic techniques can be used for rapid and accurate diagnosis of NVS in blood or tissue samples. PCR amplification of 16S rRNA and restriction fragment length polymorphism (RFLP) for routine detection of NVS was developed by Ohara-Nemoto et al. in 1997 [42]. For culture negative infective endocarditis, molecular techniques appear to be more sensitive in resected valvular tissue compared to blood samples [43].

Matrix-assisted laser desorption ionization time-of-flight mass spectrometry (MALDI-TOF MS) is a fast, reliable and cost-effective technique used to identify microorganisms by utilizing MALDI-TOF MS devices in the clinical microbiology labs [44]. These devices carry the potential to complement or replace the phenotypic identification of various microorganisms including bacteria [44]. MALDI-TOF MS is a rapid and accurate diagnostic tool that has been used to identify and timely diagnose NVS [45].

In culture negative endocarditis, *Abiotrophia* and *Granulicatella* spp. should be suspected and supplemented media should be performed for the organisms to grow. Once the growth is achieved, PCR amplification of 16S rRNA or MALDI-TOF mass spectrometry can be utilized for rapid and accurate diagnosis [46].

5. Clinical presentation and complications

Endocarditis caused by NVS typically follows a slow and indolent course. Endocarditis develops as a result of bacteremia. *Abiotrophia* and *Granulicatella* spp. are causes of endocarditis with severe complications such as congestive heart failure, valvular destruction, systemic embolization in both immunocompetent and immunocompromised patients.

Mortality rate associated with endocarditis caused by NVS is 17% which is higher than that of viridans streptococci (0–12%) and enterococci (9%) [47].

Underlying valvular disease is commonly seen as a predisposing factor for development of endocarditis. Over 90% of the cases have preexisting heart disease and 10% of patients have prosthetic heart valves [48]. Newer data however, suggest that there is increased involvement of normal heart valves in the past decade [49].

Embolization is a common complication of Abiotrophia endocarditis affecting one-third of patients. Typical peripheral manifestations of endocarditis such as petechia, digital clubbing, Osler nodes are not frequently found [41].

It has been known that infective endocarditis caused by NVS carries a higher risk of embolization, treatment failure and increased mortality as compared to infective endocarditis caused by viridans streptococci [4].

Stein et al. reviewed 30 published case reports of endocarditis caused by NVS and found that 17% of patients had relapses after antibiotic therapy. Bacteriologic failure rate was 41% (defined

as positive blood cultures after 7 days of appropriate antibiotic therapy, relapse following a course of therapy with appropriate antibiotics, or a positive valve culture). It is notable that bacteriologic failure was seen despite the sensitivity of the organisms to the antibiotics used in two thirds of the cases. About 31% of the patients required surgery. Mortality rate was 17% which was higher than that of endocarditis caused by enterococci or viridans streptococci [41].

Similarly a more recent review of 29 cases of solely *Granulicatella* endocarditis by Adam et al. showed very high rates of complications and adverse outcomes. Incidence of heart failure was 30%, embolism was seen in 30% of patients and perivalvular abscess was seen in 11%. The mortality rate was 17%. The average vegetation size was 16 mm (31). They found that aortic (44%) and the mitral (38%) valves were the most commonly effected and multivalvular involvement was (13%) [50].

Large vegetation sizes are associated with increased risk of systemic embolism in infective endocarditis. Case studies reveal large vegetation sizes with infective endocarditis caused by NVS (greater than 10 mm in 7 out of 8 cases reviewed by Lin et al., and average vegetation size of 16 mm in a case series of 29 patients by Adam et al.) [50]. These findings correlate with the high rates of systemic embolism seen in endocarditis caused by NVS.

Endocarditis caused by NVS is associated with high rates of infectious intracranial cerebral aneurysms although the exact incidence is unknown. Having a low threshold for obtaining imaging of the CNS is reasonable even for patients with vague complaints such as severe localized headaches or mild confusion [3]. Many infectious intracranial cerebral aneurysms resolve by antibiotic treatment with reductions in size in the first 1–2 weeks. The risk of rupture decreases with time on antibiotic therapy [3].

Endocarditis caused by NVS is associated with 13% of aortic valve damage and 11% of mitral valve damage. If not recognized on a timely basis, these patients may present with congestive heart failure as the first presenting manifestation of the infection [51]. Congestive heart failure is a potential complication of valvular destruction which can necessitate heart valve replacement surgery.

Aorto-RV fistula is a rare complication of *A. defectiva* endocarditis that requires early surgical intervention for closure [52]. Development of hemophagocytic lymphohistiocytosis was reported in a previously healthy patient with *A. defectiva* endocarditis [53].

Endocarditis caused by NVS has rarely been reported in children. According to a review of 13 pediatric cases in children, 69% had underlying heart disease [54]. Similar to adult patients, endocarditis caused by NVS in pediatric populations also appears to be associated with high complication rates including severe valvular damage, surgical valve replacement and systemic embolization [54, 55].

6. Treatment

Antimicrobial susceptibility testing is very difficult for *Abiotrophia* and *Granulicatella* due to their fastidious nature. In addition, the results of susceptibility testing may not be accurate or

reliable. Microbiological cure is difficult and infective endocarditis caused by these organisms is associated with high rates of treatment failures. Therefore, AHA (American Heart Association) and British Society for Antimicrobial Chemotherapy (BSAC) Infective Endocarditis treatment guideline for *Abiotrophia defectiva* and *Granulicatella* species is very similar to treatment guidelines for enterococcal endocarditis [33, 56].

Recommended treatment regimen is Ampicillin (12 g/d in divided doses) or penicillin (18–30 million U/D in divided doses or by continuous infusion) plus gentamicin 3 mg/kg/d in 2–3 divided doses).

For those patients who are intolerant to penicillin, Vancomycin alone without the use of gentamicin can be given for therapy. This is in contrast to enterococcal endocarditis treatment where Vancomycin is combined with gentamicin [33].

The duration of treatment for *Abiotrophia* or *Granulicatella* endocarditis needs to be determined by consultation with an infectious disease expert. As a general guidance, AHA recommendations for treatment durations for enterococcal endocarditis are as follows:

The treatment duration is 4 weeks for native valve endocarditis with symptoms or illness ≤3 months. 6 week therapy is recommended for patients with symptoms >3 months. For prosthetic valve or other prosthetic cardiac material infections, minimum 6 weeks of antibiotic therapy is recommended [33].

Historically, in animal models it was shown that Penicillin alone was inferior to Penicillin plus aminoglycoside or Vancomycin alone for the treatment of infective endocarditis caused by NVS [57, 58]. it was shown that penicillin plus low dose (0.32 mg/kg) vs. high dose (1.05 mg/kg) gentamicin treatment results were virtually identical [57].

There is encouraging data to suggest that shortened courses of aminoglycosides in the treatment regimens (median 15 days) may result is similar clinical outcomes in treatment of enterococcal endocarditis. However this particular issue requires further study and it is not yet known how this would apply to treatment of infective endocarditis caused by *Abiotrophia defectiva* or *Granulicatella* species. [49].

Given the growing concerns over antibiotic resistance among NVS, poor treatment outcomes and high rates of treatment failures it is important to look into data for susceptibilities of a broad range of antibiotics. There is however limited data available regarding the antibiotic susceptibilities of *Granulicatella* and *Abiotrophias* spp. due to the rare nature of the infections, the specific nutritional growth requirements and difficulties in standardization of testing methodologies.

7. Penicillin

NVS have the highest in vitro penicillin resistance compared to any other streptococci. The rate of penicillin resistance among NVS appears to be rising over the years. While an earlier study by Cooksey and Swenson in 1979 [59] and Gephart and Washington in 1982 [60] showed no isolates had a penicillin MIC >1 μg/ml, subsequent studies showed significantly increasing penicillin resistance; Bosley and Facklam in 1990 [61] noted 9% rate of resistance

to penicillin and Alberti in 2016 [62] reported 14% rate of penicillin resistance among NVS. It is also notable that the method of penicillin susceptibility testing has changed over the years. Douglas et al. (1994) [63] found that while historical method of penicillin susceptibility testing by reference dilution method did not find penicillin resistance, when same NVS isolates were tested with E test, 7% penicillin resistance was detected. The high rate of penicillin resistance among VNS appears also to be consistent among NVS isolates from pediatric infections [64].

According to antibiotic susceptibility testing of 132 isolates by Albierti et al. in 2016, only 33% of the 132 isolates were susceptible to penicillin and 14% were resistant with an MIC ≥4 μg/ml. The remaining 53% of the isolates had penicillin MICs in the intermediate category (0.25–2 μg/ml) [62]. Liao et al. reported 50% of their isolates (14 out of 28 isolates) had intermediate susceptibility to penicillin [65].

There appears to be differences in penicillin susceptibilities among different species of NVS. Albierti et al. showed that penicillin susceptibility is much less among *A. defectiva* compared to *G. adiacens* (10.8% vs. 38.9%). *G. elegans* isolates are highly susceptible to penicillin with MIC of 0.03 μg/ml (n = 5) [62].

In an earlier study by Touhy et al. in a review of 39 isolates from 1995 to 1999, similar to Albierti et al.'s findings, *G. adiacens* penicillin sensitivity was higher than that of *A. defectiva*; 55 vs. 8% respectively. [66].

7.1. Penicillin tolerance

It is notable that clinical failures of treatment have frequently been described even for penicillin susceptible strains when appropriate antibiotics are given. Holloway et al. described a phenomenon of penicillin tolerance among NVS which minimum bactericidal concentration (MBC) significantly exceeded (greater than 32) the minimum inhibitory concentration (MIC) that would lead to a slower antibiotic effect and potentially a worse clinical response. In addition to the usual nutritional supplements of vitamin B6 and cysteine to the plates, by adding penicillinase to the subculture medium and a staphylococcal streak across the plates they showed that even though all tested isolates were susceptible to penicillin (MICs of the strains ranged from 0.05 to 0.4 U of penicillin per ml), 100% of the isolates were penicillin tolerant. The isolates did not show any penicillin tolerance if the subculture was supplemented only with pyridoxal and cysteine [67]. Therefore, in order to identify penicillin tolerance and not misidentify the strains as penicillin sensitive, it is necessary to add penicillinase to the medium in addition to the usual growth supplements, pyridoxal HCl, cysteine and staphylococcal streak.

The slow growth rate of NVS is also thought to be responsible from poor response to antibiotic treatment. NVS generation time is 2–3 h while viridans streptococci generation time is 40–50 min [2, 41, 68].

7.2. Susceptibility testing

According to the latest consensus guidelines from the Clinical and Laboratory Standards Institute (CLSI) for antimicrobial susceptibility testing for infrequently isolated or fastidious bacteria, disk diffusion test for *Abiotrophia* and *Granulicatella* species is not recommended.

Instead, broth microdilution MIC testing by laboratories experienced in such testing is recommended. CLSI suggests broth microdilution MIC testing in Cation adjusted Mueller-Hinton broth with 2.5–5% lysed horse blood and 0.001% pyridoxal HCl [69].

E test is proven to be a rapid and simple method for MIC estimation for NVS, comparable to broth microdilution MIC testing [63].

CLSI consensus guidelines also emphasize that cases of *Abiotrophia* or *Granulicatella* infections can be managed by following the treatment recommendations in the medical literature without antimicrobial susceptibility testing. The antimicrobial susceptibility testing can be reserved for those cases where there is persistent infection, clinical failure, allergy or intolerance to the drugs of choice and possible resistance to the drugs that might be prescribed. Infectious disease specialists or other expert clinicians should make all susceptibility testing decision and test interpretation [69].

7.3. Cephalosporins

Penicillin resistance is often associated with resistance or decreased susceptibility to other beta-lactam antibiotics including ceftriaxone [66].

However, overall cephalosporin susceptibility among NVS appears to be higher compared to penicillin. In addition, A. *defectiva* appears to have higher susceptibility to 3rd generation cephalosporins compared to *G. adiacens*. According to a large review of susceptibilities of antibiotic susceptibility testing for 132 clinical NVS isolates from blood cultures that were isolated from 2008 to 2014 at Los Angeles hospitals by Albierti et al., Ceftriaxone susceptibility was 61.4% and Cefotaxime susceptibility was 43.2% among all isolates. *A. defectiva* was more susceptible than *G. adiacens* to the third generation cephalosporins (94.6% vs. 18.9% for Cefotaxime and 100% vs. 43.3% for ceftriaxone). Ceftriaxone susceptibility breakpoint was MIC ≤1 µg/ml as per CLSI M45 [62, 69]. Touhy et al. had found a similar susceptibility pattern of susceptibility for ceftriaxone; they had observed that 83% of the *A. defectiva* isolates and 63% of *G. adiacens* isolates were susceptible to Ceftriaxone by using MIC ≤0.5 susceptibility breakpoint [66]. Zheng et al. reported that out of 15 isolates of *Abiotrophia* and *Granulicatella*, 9 were resistant to Ceftriaxone (MIC of >2 µg/ml), 13 were resistant to Cefuroxime (MIC of >2 µg/ml) [64]. All six isolates of *G. adiacens* in their review were resistant to Ceftriaxone [64].

Albierti et al. noted that some of the isolates that were resistant to ceftriaxone still remained susceptible to Ceftaroline. (51.6% of *G. adiacens* isolates resistant to ceftriaxone with an MIC ≥4 µg/ml had Ceftaroline MICs of ≤1 µg/ml). On the other hand, 32% of *G. adiacens* isolates that were resistant to ceftriaxone (MIC ≥4) had Ceftaroline MICs ≥4 µg/ml. These isolates were noted to be resistant to penicillin (MIC ≥4 µg/ml). The Ceftaroline MIC90 for all isolates were lower compared to Cefotaxime or Ceftriaxone (2 versus >4 µg/ml) [62].

Resistance to higher generations of cephalosporins have been reported such as Cefepime (2 out of 21 isolates) [70] and Cefotaxime (7 out of 28 isolates were resistant) [65].

Species related differences of penicillin or cephalosporin sensitivies in determining antibiotic choices remains to be investigated. The high rates of ceftriaxone resistance among *G. adiacens* isolates appear to be fairly consistent across various studies.

European Society of Cardiology (ESC) Clinical Practice Guidelines include Ceftriaxone in their recommendations for treatment of endocarditis caused by *A. defectiva* or *Granulicatella* slightly differing from AHA recommendations. ESC recommendation for treatment of IE caused by *A. defectiva or Granulicatella* is Penicillin G, Ceftriaxone or Vancomycin for 6 weeks, combined with an aminoglycoside at least for the first 2 weeks [71].

8. Vancomycin

Iv Vancomycin is recommended as an alternative regimen to iv penicillin for those patients who are not able to tolerate penicillin or ampicillin [33].

Bouvet et al. by using an experimental animal model found that Vancomycin alone was as good as combination of Vancomycin and Gentamicin for treatment of endocarditis caused by NVS [58].

Vancomycin susceptibility breakpoint is typically MIC <1 μg/ml and no resistance to Vancomycin among NVS has been reported thus far [62, 64, 66]. It is notable however that MIC90 for Vancomycin is 2 times higher for *G. adiacens* compared to *A. defectiva* or *G. elegans* [62, 66].

9. Aminoglycosides

NVS remains susceptible to aminoglycosides (MICs for Gentamicin and streptomycin ≤4 μg/ml), high level aminoglycoside resistance has not been reported. As per AHA and BSAC guidelines for treatment of infective endocarditis caused by *Abiotrophia* or *Granulicatella* species, iv gentamicin is combined with iv penicillin (first line treatment) [33, 56].

10. Macrolides

Macrolide resistance is common among *Abiotrophia* and *Granulicatella species* (49.2% of all isolates sensitive to erythromycin vs. 87% of all isolates sensitive to Clindamycin) [62]. Resistance mechanisms include efflux among *mef*(A) positive isolates and *erm*(B) gene causing resistance to both Erythomycin and Clindamycin [64]. It was shown that *erm*(B) gene is located on Tn916-related transposon in *A. defectiva* similar to the pneumococcal transposon Tn3872, enabling Abiotrophia to act as a donor and recipient of antibiotic resistance [72]. Macrolide resistance pattern of NVS is suggestive of constitutive macrolide-lincosamide-streptogramin B (cMLSB) phenotype. Zheng et al. noted that all three isolates of NVS that carried erm(B) (*G. adiacens* and *G. elegans*) also carried *tet*(M), tetracycline resistance gene which is carried on the same transposon as ermB gene [62, 64].

11. Carbapenems

Resistance to Meropenem or Imipenem among *Abiotrophia* and *Granulicatella species* is rare. Review of 132 isolates by Albierti showed 100% sensitivity to Meropenem and Imipenem

(MIC ≤0.06 μg/ml for both antibiotics) [62]. Touhy et al. [66] found that 3 isolates of *A. adiacens* (out of 27 total isolates) and 7 isolates of *G. defectiva* (out of 12 total isolates) had increased MICs of Meropenem (0.5 μg/ml). These isolates also had penicillin MICs ≥0.5 μg/ml. One of their isolates of *A. adiacens* had a high MIC for Meropenem (MIC of 1 μg/ml) which was isolated from a patient with suspected intervertebral disc space infection who had received a prolonged course of various antibiotics including beta-lactams. This particular isolate was also resistant to penicillin and ceftriaxone (MICs ≥8 μg/ml).

12. Quinolones

Resistance to quinolones among NVS is rare. 8 *G. adiacens* [62] and one *G. elegans* [70] isolates have been reported to be resistant to Levafloxacin. The case of *G. elegans* resistant to Levafloxacin was isolated from a patient with neutropenic fever with bacteremia who had previously received Levafloxacin therapy. Mechanism of NVS resistance to quinolones is yet to be determined [62].

13. Daptomycin and linezolid

There are no CLSI defined sensitivity breakpoints for Daptomycin and Linezolid for NVS. Albierti et al. found that Daptomycin MICs appear to be relatively high for NVS. Daptomycin MIC90 was >4 μg/ml for *A. defectiva*, 4 μg/ml for *G adiacens* and 0.5 μg/ml for *G. elegans*. According to the CLSI breakpoint of susceptibility for viridans group Streptococci is MIC ≤1 μg/ml, majority of the tested isolates (89.4%) would be considered resistant to Daptomycin [62, 69]. The reason for relatively high Daptomycin MICs for *A. defectiva* and *G. adiacens* is not clear. This may be due to an inherent resistance of these bacteria to Daptomycin potentially due to differences in cell wall composition [62]. In a prior study a smaller number of NVS isolates (n = 10) were found to have MICs ≤0.125–2 [73]. The reason for the discrepancy in the findings of these two studies is not known and merits further investigation.

When the breakpoint of Linezolid susceptibility for viridans group streptococci (MIC ≤2 μg/ml) is applied to NVS, all NVS would be considered susceptible to Linezolid according to one study of 132 isolates [62, 69]. It was noted that *G. adiacens* MIC90 for Linezolid is higher (2 μg/ml) than that of *A. defectiva* and *G. elegans* (1 μg/ml) [62].

14. Rifampin

Rifampin appears to be one of the most effective antibiotics against NVS although the data is limited. It was shown that Rifampin had a minimal bactericidal concentration of 2 μg/ml while that of penicillin was 1 μg/ml [60]. Combination of Vancomycin and Rifampin showed synergy

in in vitro studies [74]. According to one review of 15 isolates of *Abiotrophia* and *Granulicatella* species, all isolates were found to be susceptible to Rifampin with MICs ≤0.012 µg/ml [64].

15. Role of surgery

Endocarditis caused by NVS is associated with high rates of complications including heart failure, embolization and valvular damage. The need for surgery and time of surgery remains to be determined. However based on outcomes of several cases published in the literature, the rate of surgical treatment is very high especially due to development of heart failure.

he rate of valve surgery is high; 51% (in a review of 29 cases of *Granulicatella* endocarditis by Adam et al.) [50], 48% (review of 23 cases of endocarditis caused by NVS by Guiliano et al.) [49], 38% (review of 30 cases of endocarditis caused by NVS by Stein et al.) [41], 44% (review of 9 cases of *A. defectiva* endocarditis by Hashimoto et al.) [75].

A vegetation size of 10 mm or more is associated with increased mortality and increased risk of embolic events [76]. EASE Trial showed that early surgery in infective endocarditis in patients with large vegetations significantly reduced the mortality, risk of systemic embolism or recurrence of infective endocarditis (3% in the early surgery group vs. 28% in the conventional treatment group) [77]. Lin et al. [68] reported 7 out of 8 cases of endocarditis caused by NVS had large vegetation sizes (10 mm). In the same review, 7 out of 8 cases required surgery (4 out of 8 cases required early valve replacement due to severe heart failure, while 3 cases underwent mitral valve repair 2,4, and 7 months after the diagnosis of endocarditis).

Combined approach with antibiotic treatment and surgery provides the best outcomes in endocarditis caused by NVS. Specifically, early surgical intervention should be considered for those patients with heart failure due to valvular destruction [68], hemodynamic compromise [49] or large vegetation sizes [68, 77].

16. Conclusion

Infective endocarditis caused by NVS has posed tremendous diagnostic and therapeutic challenges and continue to do so even in the era of modern medicine. Delays in diagnosis due to difficulties in identification frequently cause delays in treatment and poor treatment outcomes. Treatment failure and high complication rates associated with *Abiotrophia* and *Granulicatella* endocarditis is at least partially attributable to the pleomorphic nature of the organisms, lack of growth in subcultures and specific nutritional requirements in media along with the need for the microbiology lab staff to have heightened awareness of these microorganisms. Their fastidious nature of NVS makes the antibiotic susceptibility testing fairly difficult, causing delays in initiation of timely and effective antibiotic treatment.

Interpretation of the medical literature of *Abiotrophia* and *Granulicatella* spp. and its application to current clinical practice is challenging as the names of these organisms have been changed several times and not uncommonly the two genera were addressed together. There is

lack of large clinical studies and our knowledge about these organisms is based on relatively small number of reported cases.

Differences in pathogenicity and susceptibility to antimicrobials have been demonstrated among these heterogeneous group of bacteria. More studies are needed to determine if there are further species specific differences of these fascinating microorganisms which would help us improve our understanding, diagnosis and the treatment outcomes of infections caused by NVS.

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