Differential Antimicrobial Susceptibilities of *Granulicatella adiacens* and *Abiotrophia defectiva*

Ammara Mushtaq,^a Kerryl E. Greenwood-Quaintance,^a Nicolynn C. Cole,^a Peggy C. Kohner,^a Sherry M. Ihde,^a Gregory J. Strand,^a Lance W. Harper,^a Abinash Virk,^b Robin Patel^{a,b}

Division of Clinical Microbiology, Department of Laboratory Medicine and Pathology, Mayo Clinic, Rochester, Minnesota, USA^a; Division of Infectious Diseases, Department of Medicine, Mayo Clinic, Rochester, Minnesota, USA^b

MICs of 25 *Abiotrophia defectiva* and 109 *Granulicatella adiacens* isolates were determined by broth microdilution. Using CLSI breakpoints, the susceptibilities of *A. defectiva* and *G. adiacens* isolates were, respectively, 24% and 34% to penicillin, 92% and 22% to ceftriaxone, 48% and 3% to cefepime, 72% and 87% to meropenem, 92% and 10% to cefotaxime, 100% and 97% to levofloxacin, 92% and 80% to clindamycin, and 24% and 50% to erythromycin. All isolates were susceptible to vancomycin. In the penicillin-susceptible subgroup, all *A. defectiva* isolates were susceptible to ceftriaxone; however, 62% of *G. adiacens* isolates were ceftriaxone nonsusceptible.

Abiotrophia and *Granulicatella* species, historically referred to as nutritionally variant streptococci (NVS), can cause various infections, including endocarditis (1–3). Endocarditis caused by these organisms may be severe and may have a higher relapse rate, longer course of illness, and greater mortality than endocarditis caused by streptococci (2, 4), with complications, including heart failure, embolization, and abscess formation (4, 5).

American Heart Association (AHA) guidelines for treatment of *Abiotrophia* and *Granulicatella* endocarditis (and, by inference, other serious infections caused by these organisms) include administration of penicillin (or ceftriaxone, if susceptible) (3). Ceftriaxone has the advantage of once-daily dosing and thus is often selected. Traditionally, *Abiotrophia* and *Granulicatella* species were reported as NVS; with the advent of matrix-assisted laser desorption ionization–time of flight mass spectrometry (MALDI-TOF MS), the individual species are readily identified. As a result, it is being realized that there may be differences in the antimicrobial susceptibility of the two genera that may inform treatment regimens (6).

We aimed to describe the antimicrobial susceptibility profile of *Abiotrophia* and *Granulicatella* species. We studied a collection of 135 isolates, including 25 *Abiotrophia defectiva* and 109 *Granulicatella adiacens* isolates and 1 *Granulicatella elegans* isolate. Fifteen *A. defectiva* samples were isolated from blood, and the remaining were from heart valves, spinal tissue, peritoneal fluid, and skull tissue; the sources of 3 isolates were unknown. Sixty *G. adiacens* were isolated from blood, with the remaining isolated from long-term intravascular catheters, peripherally inserted central venous catheters, corneal scrapings, body tissues (finger, foot, heart, ankle, abdomen, leg, neck, wrist, spine, eye, and joint), body fluids (synovial, peritoneal, pleural, cerebrospinal, and ventricular fluid), prosthetic joints, and implantable vascular accesses; the sources of 7 were unknown. The single *G. elegans* isolate was from blood. The isolates were received at the Clinical Bacteriology Laboratory of the Mayo Clinic, Rochester, MN, USA, from January 1986 to November 2015 and frozen at -80°C . Isolates were identified to the species level by use of MALDI-TOF MS or 16S rRNA gene sequencing. This study was approved by the Institutional Review Board of the Mayo Clinic.

Isolates were cultured on chocolate agar and incubated in 5%

CO_2 overnight. MICs were determined by broth microdilution, per the manufacturer's guidelines, using the STP6F Sensititre commercial system from Trek Diagnostics Systems (Oakwood Village, OH), which includes a panel of antibiotics. Clinical and Laboratory Standards Institute (CLSI) recommendations for susceptibility testing by broth microdilution were followed (7). *Streptococcus pneumoniae* ATCC 49619 was used as a QC strain. Inoculum density was standardized at 5×10^5 CFU/ml. Cation-adjusted Mueller-Hinton broth with 2.5% lysed horse blood (Trek Diagnostics Systems) was used and supplemented with 0.001% pyridoxal (7). Microdilution plates were incubated at 35°C in ambient air for 20 to 24 h. Each isolate was tested only once, unless the QC strain failed, in which case testing was repeated.

MIC₅₀ and MIC₉₀ values were calculated and, when available, results interpreted as susceptible, intermediate, or resistant per CLSI breakpoints (7, 8). Intermediate and resistant categories are together referred to here as nonsusceptible.

Susceptibility profiles and MICs at which 50% and 90% of isolates were susceptible are shown in Table 1. Overall, 33% of the isolates were susceptible to penicillin, with *G. adiacens* having somewhat higher susceptibility than *A. defectiva* (34% versus 24%). Of the 6 penicillin-resistant *A. defectiva* isolates, 3 had an MIC of 4 $\mu\text{g}/\text{ml}$ and 3 had an MIC of >4 $\mu\text{g}/\text{ml}$, whereas all 13 penicillin-resistant *G. adiacens* isolates had penicillin MICs of >4 $\mu\text{g}/\text{ml}$. Overall, there were high nonsusceptibility rates to penicillin in both genera. Ceftriaxone, on the other hand, had a similar overall susceptibility rate of 36%, but there was a striking difference in the proportion of susceptible isolates between *A. defectiva* and *G. adiacens* (92% versus 22%, respectively), which was a po-

Received 2 March 2016 Returned for modification 25 March 2016
Accepted 16 May 2016

Accepted manuscript posted online 23 May 2016

Citation Mushtaq A, Greenwood-Quaintance KE, Cole NC, Kohner PC, Ihde SM, Strand GJ, Harper LW, Virk A, Patel R. 2016. Differential antimicrobial susceptibilities of *Granulicatella adiacens* and *Abiotrophia defectiva*. Antimicrob Agents Chemother 60:5036–5039. doi:10.1128/AAC.00485-16.

Address correspondence to Robin Patel, patel.robin@mayo.edu.

Copyright © 2016, American Society for Microbiology. All Rights Reserved.

TABLE 1 Susceptibility percentages and MIC₅₀ and MIC₉₀ values for 13 antibiotics against 25 *Abiotrophia defectiva* and 109 *Granulicatella adiacens* isolates

Antibiotic	MIC (μg/ml)			Susceptibility (% [n])		
	Range	MIC ₅₀	MIC ₉₀	Susceptible	Intermediate	Resistant
β-Lactams						
Penicillin						
<i>A. defectiva</i>	≤0.03 to >4	0.5	>4	24 (6)	52 (13)	24 (6)
<i>G. adiacens</i>	0.06 to >4	0.25	2	34 (37)	54 (59)	12 (13)
Cefepime						
<i>A. defectiva</i>	≤0.5 to 4	1	2	48 (12)	44 (11)	8 (2)
<i>G. adiacens</i>	1 to >8	4	>8	3 (3)	10 (11)	87 (95)
Ceftriaxone						
<i>A. defectiva</i>	≤0.12 to 2	0.5	1	92 (23)	4 (1)	4 (1)
<i>G. adiacens</i>	0.5 to >2	2	>2	22 (24)	33 (36)	45 (49)
Meropenem						
<i>A. defectiva</i>	≤0.25 to 1	0.5	1	72 (18)	28 (7)	0 (0)
<i>G. adiacens</i>	≤0.25 to 2	≤0.25	0.5	87 (95)	6 (7)	6 (7)
Cefotaxime						
<i>A. defectiva</i>	≤0.012 to 2	1	1	92 (23)	4 (1)	4 (1)
<i>G. adiacens</i>	0.5 to >4	4	>4	10 (11)	21 (23)	69 (75)
Ertapenem						
<i>A. defectiva</i>	≤0.5 to 1	≤0.5	1	NA ^a	NA	NA
<i>G. adiacens</i>	≤0.5 to 4	≤0.5	1	NA	NA	NA
Non-β-lactams						
Vancomycin						
<i>A. defectiva</i>	≤0.5 to 1	1	1	100 (25)	0 (0)	0 (0)
<i>G. adiacens</i>	≤0.5 to 1	1	1	100 (109)	0 (0)	0 (0)
Levofloxacin						
<i>A. defectiva</i>	≤0.5	≤0.5	≤0.5	100 (25)	0 (0)	0 (0)
<i>G. adiacens</i>	≤0.5 to >4	1	1	97 (106)	1 (1)	2 (2)
Clindamycin						
<i>A. defectiva</i>	≤0.12 to >1	≤0.12	≤0.12	92 (23)	4 (1)	4 (1)
<i>G. adiacens</i>	≤0.12 to >1	≤0.12	>1	80 (87)	0 (0)	20 (22)
Erythromycin						
<i>A. defectiva</i>	≤0.25 to >2	>2	>2	24 (6)	4 (1)	72 (18)
<i>G. adiacens</i>	≤0.25 to >2	≤0.25	>2	50 (55)	1 (1)	49 (53)
Linezolid						
<i>A. defectiva</i>	≤0.25 to 2	1	2	NA	NA	NA
<i>G. adiacens</i>	≤0.25 to 2	1	2	NA	NA	NA
Daptomycin						
<i>A. defectiva</i>	0.5 to >2	2	2	NA	NA	NA
<i>G. adiacens</i>	0.25 to >2	>2	>2	NA	NA	NA
Tigecycline						
<i>A. defectiva</i>	≤0.015 to 0.03	≤0.015	≤0.015	NA	NA	NA
<i>G. adiacens</i>	≤0.015 to 0.12	≤0.015	≤0.015	NA	NA	NA

^a NA, not applicable (breakpoints not established).

tentially clinically important observation. The 22% ceftriaxone susceptibility rate of *G. adiacens* was lower than the 43% susceptibility rate reported recently (6). A similar trend was observed for other third-generation cephalosporins. While 92% of *A. defectiva* isolates were susceptible to cefotaxime, only 10% of *G. adiacens* isolates were susceptible to it. Of the 2 ceftriaxone-nonsusceptible *A. defectiva* isolates, 1 was also resistant to cefotaxime. Of the 85 ceftriaxone-nonsusceptible *G. adiacens* isolates, all except 1 were also cefotaxime nonsusceptible. Although there was low susceptibility to cefepime for both species, *A. defectiva* was considerably more susceptible than *G. adiacens* (48% versus 3%) and had 2-fold lower MIC₅₀ and MIC₉₀ values (1 and 2 versus 4 and >8 μg/ml). This is in contrast to the low rate of cefepime nonsusceptibility reported by Liao et al. (9); only 17% of NVS in their

collection of 25 isolates were nonsusceptible to cefepime. The susceptibility profile to cefepime was not previously reported by species. A reported case of *Abiotrophia* species as a cause of neutropenic fever that was empirically treated with cefepime involved an isolate ultimately shown to be resistant to this antibiotic (10).

There was a relatively high susceptibility rate of 84% to meropenem without a marked difference between the two genera, although *G. adiacens* had lower MIC₅₀ and MIC₉₀ values than *A. defectiva* (≤0.25 and 0.5 versus 0.5 and 1 μg/ml, respectively). Of note, all 7 meropenem-resistant isolates had ertapenem MICs of ≥2 μg/ml. Uniform susceptibility to meropenem was reported in a previous study, a finding that was different from that reported here, despite comparable MIC₅₀ and MIC₉₀ values (6). Ertapenem

TABLE 2 Ceftriaxone MICs of penicillin-susceptible versus nonsusceptible bacteria by species

Isolate type	% (n) of isolates with indicated ceftriaxone MIC ($\mu\text{g/ml}$)					
	Susceptible				Intermediate	Resistant
	≤ 0.12	0.25	0.5	1	2	> 2
Penicillin susceptible						
<i>A. defectiva</i> (n = 6)	17 (1)	17 (1)	17 (1)	50 (3)	0 (0)	0 (0)
<i>G. adiacens</i> (n = 37)	0 (0)	0 (0)	19 (7)	19 (7)	38 (14)	24 (9)
Penicillin nonsusceptible						
<i>A. defectiva</i> (n = 19)	5 (1)	5 (1)	47 (9)	32 (6)	5 (1)	5 (1)
<i>G. adiacens</i> (n = 72)	0 (0)	0 (0)	3 (2)	11 (8)	31 (22)	56 (40)

MICs have not been widely reported for these species and were generally low (MIC_{50} , $\leq 0.5 \mu\text{g/ml}$; MIC_{90} , $1 \mu\text{g/ml}$), suggesting that this agent may be active against them. Using the viridans streptococcal breakpoint for ertapenem, 96% (24/25) of *A. defectiva* isolates and 89% (97/109) of *G. adiacens* isolates would have been considered susceptible.

All of the isolates were susceptible to vancomycin. Notably higher MICs to daptomycin than expected for Gram-positive cocci were observed (MIC_{50} , $2 \mu\text{g/ml}$; MIC_{90} , $> 2 \mu\text{g/ml}$). Using the daptomycin breakpoint for viridans streptococci, 72% of *A. defectiva* and 96% of *G. adiacens* isolates would have been considered nonsusceptible (8). In the context of inconsistent susceptibility to β -lactams and the daptomycin results found here, vancomycin may be a more suitable option than daptomycin for empirical treatment of serious infections caused by *Abiotrophia* and *Granulicatella* species. Viridans streptococci of the *mitis* group exhibit high daptomycin MICs after being exposed to its inhibitory or subinhibitory concentrations (11); whether the same is true for *Abiotrophia* and *Granulicatella* species is unknown.

Of note, all penicillin-susceptible *A. defectiva* isolates were susceptible to ceftriaxone, but 21% (23/109) of *G. adiacens* isolates were susceptible to penicillin but not to ceftriaxone (Table 2). Alternatively described, 62% (23/37) of penicillin-susceptible *G. adiacens* isolates were nonsusceptible to ceftriaxone, a pattern that was not observed for *A. defectiva* isolates. Similarly, 50% (3/6) of penicillin-susceptible *A. defectiva* isolates were nonsusceptible to cefepime, whereas 95% (35/37) of penicillin-susceptible *G. adiacens* isolates were nonsusceptible to cefepime (Table 3). Furthermore, all penicillin-susceptible *A. defectiva* isolates were susceptible to cefotaxime, but 81% (30/37) of penicillin-susceptible *G. adiacens* isolates were nonsusceptible to cefotaxime (Table 4).

Our findings have implications for patient care if ceftriaxone (or cefotaxime) treatment is selected based on penicillin susceptibility. The results emphasize the need to specifically test these agents if they are to be used for treatment.

The single *G. elegans* isolate studied was susceptible to all agents tested, including penicillin, vancomycin, levofloxacin, cefepime, ceftriaxone, clindamycin, erythromycin, meropenem, and cefotaxime. For agents without established breakpoints, this isolate had low MICs (linezolid, $1 \mu\text{g/ml}$; daptomycin, $0.25 \mu\text{g/ml}$; tigecycline, $\leq 0.015 \mu\text{g/ml}$; and ertapenem, $\leq 0.5 \mu\text{g/ml}$).

The British Society of Antimicrobial Chemotherapy recommends benzylpenicillin and gentamicin for 4 to 6 weeks for the treatment of *Granulicatella* or *Abiotrophia* endocarditis (12). The European Society of Cardiology recommends penicillin G, ceftriaxone, or vancomycin for 6 weeks, combined with an aminoglycoside for at least the first 2 weeks (13). Accordingly, a 4- to 6-week course of combination antimicrobial therapy with penicillin or ceftriaxone and an aminoglycoside is often prescribed (3–5). The AHA also recommends combination therapy with ampicillin, penicillin, or ceftriaxone (if susceptible) plus gentamicin (with infectious diseases consultation to determine the length of therapy), with vancomycin being an alternative for patients intolerant of β -lactams (3).

The AHA states that susceptibility testing of *Abiotrophia* and *Granulicatella* species “is often technically difficult, and the results may not be accurate” (3). Susceptibility testing on some of our isolates was previously performed for select antibiotics as part of routine clinical care; this testing was done at an outside laboratory using the same methodology described here. Twenty-eight isolates that had both penicillin and ceftriaxone susceptibility based on this historical testing were reviewed; all results matched those

TABLE 3 Cefepime MICs of penicillin-susceptible versus nonsusceptible bacteria by species

Isolate type	% (n) of isolates with indicated cefepime MIC ($\mu\text{g/ml}$)					
	Susceptible		Intermediate	Resistant		
	≤ 0.5	1	2	4	8	> 8
Penicillin susceptible						
<i>A. defectiva</i> (n = 6)	50 (3)	0 (0)	50 (3)	0 (0)	0 (0)	0 (0)
<i>G. adiacens</i> (n = 37)	0 (0)	5 (2)	24 (9)	41 (15)	27 (10)	3 (1)
Penicillin nonsusceptible						
<i>A. defectiva</i> (n = 19)	16 (3)	32 (6)	42 (8)	5 (1)	0 (0)	5 (1)
<i>G. adiacens</i> (n = 72)	0 (0)	1 (1)	3 (2)	35 (25)	21 (15)	40 (29)

TABLE 4 Cefotaxime MICs of penicillin-susceptible versus nonsusceptible bacteria by species

Isolate type	% (n) of isolates with indicated cefotaxime MIC (μg/ml)						
	Susceptible				Intermediate	Resistant	
	≤0.12	0.25	0.5	1	2	4	>4
Penicillin susceptible							
<i>A. defectiva</i> (n = 6)	17 (1)	0 (0)	33 (2)	50 (3)	0 (0)	0 (0)	0 (0)
<i>G. adiacens</i> (n = 37)	0 (0)	0 (0)	5 (2)	14 (5)	27 (10)	30 (11)	24 (9)
Penicillin nonsusceptible							
<i>A. defectiva</i> (n = 19)	5 (1)	5 (1)	16 (3)	63 (12)	5 (1)	0 (0)	5 (1)
<i>G. adiacens</i> (n = 72)	0 (0)	0 (0)	1.4 (1)	4 (3)	18 (13)	28 (20)	49 (35)

reported here, suggesting that MICs can be reproducibly determined. Furthermore, our overall results are consistent with those of Alberti et al. (6).

A potential limitation of our study was the inability to include the entire QC range of *S. pneumoniae* ATCC 49619 for all antimicrobial agents tested; the Sensititre plates used included the entire QC range for only 5 of 20 antimicrobial agents tested. Also, the Sensititre system used in this study has not been specifically validated for ertapenem and clindamycin.

Further research is needed to determine the mechanism of resistance in the penicillin-susceptible ceftriaxone-nonsusceptible strains, which may relate to altered penicillin-binding proteins, as in enterococci. Furthermore, treatment guidelines may need to be updated to reflect differences in the susceptibility profiles of the two genera, with overall high nonsusceptibility rates to penicillin and ceftriaxone nonsusceptibility in penicillin-susceptible *G. adiacens* isolates.

In conclusion, our results highlight the need to routinely perform susceptibility testing on clinically significant *A. defectiva* and *G. adiacens* isolates. We demonstrate important differences in the susceptibility profiles of the two genera and confirm overall high rates of nonsusceptibility to penicillin. Finally, we show that a significant proportion of *G. adiacens* isolates that are susceptible to penicillin are nonsusceptible to ceftriaxone and cefotaxime.

FUNDING INFORMATION

This study was supported by the Department of Laboratory Medicine and Pathology, Mayo Clinic, Rochester, MN.

REFERENCES

- Giuliano S, Caccese R, Carfagna P, Vena A, Falcone M, Venditti M. 2012. Endocarditis caused by nutritionally variant streptococci: a case report and literature review. *Infez Med* 20:67–74.
- Yemisen M, Koksall F, Mete B, Yarimcam F, Okcun B, Kucukoglu S, Samasti M, Kocazeybek B, Ozturk R. 2006. *Abiotrophia defectiva*: a rare cause of infective endocarditis. *Scand J Infect Dis* 38:939–941. <http://dx.doi.org/10.1080/00365540600606424>.
- Baddour LM, Wilson WR, Bayer AS, Fowler VG, Tleyjeh IM, Rybak MJ, Barsic B, Lockhart PB, Gewitz MH, Levison ME, Bolger AF, Steckelberg JM, Baltimore RS, Fink AM, O'Gara P, Taubert KA. 2015. Infective endocarditis in adults: diagnosis, antimicrobial therapy, and management of complications: a scientific statement for healthcare professionals from the American Heart Association. *Circulation* 132:1435–1486. <http://dx.doi.org/10.1161/CIR.0000000000000296>.
- Padmaja K, Lakshmi V, Subramanian S, Neeraja M, Krishna SR, Satish OS. 2014. Infective endocarditis due to *Granulicatella adiacens*: a case report and review. *J Infect Dev Ctries* 8:548–550. <http://dx.doi.org/10.3855/jidc.3689>.
- Adam EL, Siciliano RF, Gualandro DM, Calderaro D, Issa VS, Rossi F, Caramelli B, Mansur AJ, Strabelli TM. 2015. Case series of infective endocarditis caused by *Granulicatella* species. *Int J Infect Dis* 31:56–58. <http://dx.doi.org/10.1016/j.ijid.2014.10.023>.
- Alberti MO, Hindler JA, Humphries RM. 2015. Antimicrobial susceptibilities of *Abiotrophia defectiva*, *Granulicatella adiacens*, and *Granulicatella elegans*. *Antimicrob Agents Chemother* 14:1411–1420. <http://dx.doi.org/10.1128/AAC.02645-15>.
- Clinical and Laboratory Standards Institute. 2015. Methods for antimicrobial dilution and disk susceptibility testing of infrequently isolated or fastidious bacteria—3rd ed. CLSI guideline M45. Clinical and Laboratory Standards Institute, Wayne, PA.
- Clinical and Laboratory Standards Institute. 2016. Performance standards for antimicrobial susceptibility testing; 26th informational supplement. CLSI document M100-S26. Clinical and Laboratory Standards Institute, Wayne, PA.
- Liao CH, Teng LJ, Hsueh PR, Chen YC, Huang LM, Chang SC, Ho SW. 2004. Nutritionally variant streptococci infections at a University Hospital in Taiwan: disease emergence and high prevalence of beta-lactam and macrolide resistance. *Clin Infect Dis* 38:452–455. <http://dx.doi.org/10.1086/381098>.
- Murray CK, Walter EA, Crawford S, McElmeel ML, Jorgensen JH. 2001. *Abiotrophia* bacteremia in a patient with neutropenic fever and antimicrobial susceptibility testing of *Abiotrophia* isolates. *Clin Infect Dis* 32:E140–E142. <http://dx.doi.org/10.1086/320150>.
- Humphries RM, Pollett S, Sakoulas G. 2013. A current perspective on daptomycin for the clinical microbiologist. *Clin Microbiol Rev* 26:759–780. <http://dx.doi.org/10.1128/CMR.00030-13>.
- Gould FK, Denning DW, Elliott TS, Foweraker J, Perry JD, Prendergast BD, Sandoe JA, Spry MJ, Watkin RW, Working Party of the British Society for Antimicrobial Chemotherapy. 2012. Guidelines for the diagnosis and antibiotic treatment of endocarditis in adults: a report of the Working Party of the British Society for Antimicrobial Chemotherapy. *J Antimicrob Chemother* 67:269–289. <http://dx.doi.org/10.1093/jac/dkr450>.
- Habib G, Lancellotti P, Antunes MJ, Bongiorno MG, Casalta JP, Del Zotti F, Dulgheru R, El Khoury G, Erba PA, Iung B, Miro JM, Mulder BJ, Plonska-Gosciński E, Price S, Roos-Hesselink J, Snygg-Martin U, Thuny F, Tornos Mas P, Vilacosta I, Zamorano JL, Erol C, Nihoyanopoulos P, Aboyans V, Agewall S, Athanassopoulos G, Aytekin S, Benzer W, Bueno H, Broekhuizen L, Carerj S, Cosyns B, De Backer J, De Bonis M, Dimopoulos K, Donal E, Drexel H, Flachskampf FA, Hall R, Halvorsen S, Hoen B, Kirchhof P, Lainscak M, Leite-Moreira AF, Lip GY, Mestres CA, Piepoli MF, Punjabi PP, Rapezzi C, Rosenhek R, Siebens K, Tamargo J, Walker DM. 2015. 2015 ESC guidelines for the management of infective endocarditis: the Task Force for the Management of Infective Endocarditis of the European Society of Cardiology (ESC). Endorsed by: European Association for Cardio-Thoracic Surgery (EACTS), the European Association of Nuclear Medicine (EANM). *Eur Heart J* 36:3075–3128. <http://dx.doi.org/10.1093/eurheartj/ehv319>.