CMPT Clinical Bacteriology Program

Innovation, Education, Quality Assessment, Continual Improvement

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Challenge M142-4

August 2014

Blood culture: *Granulicatella adiacens* (Nutritionally variant streptococci)

HISTORY

A simulated blood culture obtained from a 52 year old male in-patient with a heart murmur was sent to category A laboratories.

Participants were expected to isolate and identify *Granulicatella adiacens*.

CMPT QA/QC

CMPT control yielded a pure culture of *Granulicatella adiacens*, viable for 8 days.

SURVEY RESULTS

Reference Laboratories: 14/15 (93%) laboratories reported *G. adiacens*, *Granulicatella* species, or nutritionally variant streptococci (NVS). One laboratory reported results using the incorrect identifier. Consensus was reached, therefore the challenge was considered suitable for grading.

Participants: 49/63 (78%) laboratories processing the sample correctly isolated and identified the organism as *G. adiacens, Granulicatella* sp., or NVS; these laboratories received a grade of 4 (Table 1).

One participant reported *Abiotrophia/ Granulicatella* species and was graded as 4. Three laboratories reported *Abiotrophia* sp. and were graded as 3.

Participants also reported the identification as *Streptococcus alactolyticus*, β hemolytic streptococci, gram positive cocci, gram variable bacilli, and *Neisseria* species, among others. Please refer to Table 1 for grading.

COMMENTS ON RESULTS

The majority of participants were able to identify *G. adiacens*, or some taxonomic variant thereof, as a pathogen in this sample.

Incorrect submissions may have been due to errors in automated identification systems (e.g. "*S. alactolyticus*"), in recognizing the distinctive Gram stain (e.g. "gram variable bacilli"), or in using appropriate media to achieve growth of the organism (e.g. "anaerobic gram positive cocci").

MAIN EDUCATIONAL POINTS from M142-4

1. *Granulicatella adiacens* and other nutritionally variant streptococci (e.g. *Abiotrophia defectiva*, other *Granulicatella* species) are infrequent but important isolates from blood cultures.
2. Media or techniques (e.g. chocolate agar or a streak of *Staphylococcus aureus*) that allow for these organisms to be isolated from blood cultures should be routinely used.
3. The Gram stain may appear quite pleomorphic, but should not be mistaken for bacilli or for gram negative organisms.
4. Correct identification to a genus or species level may be achieved using identification systems in the laboratory. However, even in their absence, it is important for a laboratory to have the ability to cultivate and recognize a nutritionally variant streptococci.

Taxonomically, *Abiotrophia* species is not correct (discussed below), but was considered sufficiently close to merit partial grading.

ISOLATION and IDENTIFICATION

Granulicatella species are catalase-negative gram positive cocci which grow as facultative anaerobes. They were first described as nutritionally variant streptococci (NVS) because of their pyridoxal growth requirement.[1,2] In 1995, all organisms classified as NVS were placed in the genus *Abiotrophia*.[3] Subsequently, 16S rRNA gene sequencing has revealed further phylogenetic divisions within the genus. They have been split into two genera: *Abiotrophia* and *Granulicatella*, to which currently belong one (*A. defectiva*) and three (*G. adiacens*, *G. elegans*, and *G. balaenopterae*) species, respectively.[4]

Their Gram stain appearance varies depending on the culture media. In liquid media containing pyridoxal (e.g. thioglycolate broth), they appear as gram positive cocci in chains, indistinguishable from other streptococci. More commonly, when the Gram stain is performed on colonies from agar plates, especially those poor in pyridoxal, they may appear pleomorphic and even gram variable.[5] Extremely elongated forms may be mistaken for bacilli, and swollen cells may resemble spores, leading to confusion in interpretation of the stain.[4,5]

Grading

Maximum grade: 4

Reporting *Granulicatella, Granulicatella/Abiotrophia*, or nutritionally variant streptococci was graded 4.

Reporting *Abiotrophia* species or α-hemolytic streptococci refer, was downgraded to 3.

Reporting gram positive cocci was graded 1.

Reporting *Streptococcus alactolyticus*, gram variable bacilli, and *Neisseria* species, was graded 0.

Failing to grow the bacteria within the expected time frame was graded 0.

Reporting results using the wrong identifier was graded 0.

CMPT requires the full identifier in the results report form. Please note that the M is part of the identifier and the sample should be identified as M142-4 and not as 142-4

Table 1. Identification results

Reported results	Total	Grade
Granulicatella adiacens or *Granulicatella* species, ± refer	40	4
nutritionally variant/deficient streptococci, ± refer	9	4
Abiotrophia/Granulicatella species, refer	1	4
Gram-positive cocci in chains, sent to reference lab to rule out *Granulicatell*a and *Abiotrophia* spp. (NVS)	1	4
Abiotrophia species, refer ± probable	3	3
α-hemolytic streptococcus, refer	1	3
Streptococcus alactolyticus	1	0
anaerobic Gram-positive cocci sent to reference lab for identification	1	1
Gram-variable bacilli ± with spores, refer	2	0
Neisseria species, refer	1	0
Reported results with incorrect identifier	1	0
no growth in 5 days (received August 13 - CMPT QA/QC 1+-4+ growth)	1	0
no growth after 5 days incubation (sample lost received 2nd sample on 08/15 - CMPT QA/QC: no growth)	1	ungraded
sample not normally processed	5	ungraded
refer	2	ungraded
Total	**70**	

A defining characteristic of NVS is their inability to grow on pyridoxal-deficient media such as sheep blood agar, though this may vary depending on the specific composition.[2,6] These bacteria grow on Brucella blood agar and chocolate agar (both contain pyridoxal), as well as in the broths used in current blood culture systems.[7]

Colonies of gram positive cocci that grow in blood culture bottles or on chocolate agar but not on blood agar plates should trigger suspicion for NVS. Demonstration of satellitism around a streak of *Staphylococcus aureus* or a pyridoxal disk can be useful in identification.

Accurate determination of the different genera/ species in this group may pose a challenge for laboratories. Colonies test catalase negative. These isolates can be identified on automated identification systems, including the API Strep,[8] Vitek 2, and MALDI-TOF platforms.[9] However, the accuracy of these tests is not perfect. Sequencing of the 16S rRNA gene is the most accurate way to distinguish between the species, though this may not be a practical approach for many clinical laboratories.[6,8] From a clinical standpoint, recognition that a bacteria belongs to the group of *Abiotrophia/ Granulicatella* spp. is more important than identification to the species level, as there may be ramifications in pathogenicity and antibiotic susceptibilities.

ANTIMICROBIAL SUSCEPTIBILITY

Susceptibility testing methods and interpretation guidelines for *Abiotrophia* and *Granulicatella* species have been published by the CLSI in the document M45-A2.[10] The prescribed test is a broth microdilution assay, using pyridoxal supplementation of cation-adjusted Mueller Hinton broth with laked horse blood.

Many laboratories may find it more practical to perform testing using E-test (or another antibiotic gradient strip) on a Mueller Hinton agar with blood, supplemented with pyridoxal.[9] Reports indicate, however, that E-tests may produce MICs higher than those with broth microdilution when testing penicillin G, potentially overcalling beta-lactam resistance.[11] There are no Kirby-Bauer disk breakpoints.

There is limited antimicrobial susceptibility data available for NVS, as clinical isolates are relatively rare. It does appear that antimicrobial resistance in *Abiotrophia* and *Granulicatella* is more common than in viridans group streptococci.[12] Penicillin resistance is relatively uncommon but still is seen in up to 20% of isolates, but isolates appear to more likely be resistant to ceftriaxone (13 - 60%).[13] Macrolide resistance, mediated by mefA and ermB,[13] is common: one study reported an azithromycin resistance rate of over 90%.[14] Clindamycin resistance has also been observed; however, no

CMPT reminds participants that they should send a report for every sample received with the survey even if they do not normally process it.

If the laboratory does not normally process the sample, a report should still be completed indicating that is the case or that the sample would be referred out.

Failure to send a report will be considered "no report" and graded 0 as CMPT does not keep records of what kind of samples each laboratory process or not.

vancomycin resistant isolates have been reported.[12-14]

In summary, resistance testing of *Abiotrophia* and *Granulicatella* is logistically challenging, but critical to guide therapy, as resistance to major groups of therapeutic agents is common.

CLINICAL RELEVANCE

Abiotrophia and *Granulicatella* species are normal components of the oral flora; [2] these organisms are not frequently recovered from clinical specimens; most reported isolates associated with disease are recovered from blood. They are important causes of endocarditis, comprising 5-6% of all cases of streptococcal endocarditis.[15]

Once considered an etiology of culture-negative endocarditis, [7] improvements to blood culture broths to contain cysteine have facilitated their recovery from automated blood culture platforms. Thus, NVS are scarcely ever implicated in culture-negative endocarditis in recent studies.[16] In NVS endocarditis, embolization of vegetations is frequent, and infections are difficult to treat and prone to relapse.[17]

Aside from endocarditis, *Granulicatella* infections are rare, but have been implicated in cases of neonatal sepsis,[18] brain abscesses,[19] and meningitis.[20]

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