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MINIREVIEW

Miscellaneous Catalase-Negative, Gram-Positive Cocci: Emerging Opportunists

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"*In a whimsical mood and quite extemporaneously, Justice Oliver Wendell Holmes once remarked: 'Facts in isolation amount to mere gossip; facts in relation become philosophy.' And this, perhaps, gives the key to one of the chief ailments of bacterial taxonomy. There are too many 'facts in isolation' and too few 'facts in relation'*"

From James M. Sherman's classic 1937 treatise on the genus *Streptococcus* (20)

Clinical microbiologists who contemplate the current bewildering assortment of catalase-negative gram-positive cocci that grow aerobically are likely to feel bombarded by what appear to be facts in isolation. They may find themselves longing for the days when laboratory identification of this group of bacteria was simple and straightforward. They no doubt abhor, in Sherman's (20) words, "...the hair-splitting divisions which have been set up by some taxonomically-minded bacteriologists."

Until the mid-1980s clinical microbiologists viewed catalase-negative gram-positive coccal isolates in essentially the same way Sherman and his colleagues did. All such isolates were streptococci, unless, of course, their cells were arranged in clusters, indicating a probable *Aerococcus viridans* strain (usually considered a contaminant). The streptococcal isolates could be divided into beta-hemolytic strains, enterococcal strains, pneumococci, and the puzzling "*Streptococcus viridans*" group, an unruly collection that defied classification by Lancefield's serological methods. This simplistic view of catalase-negative gram-positive cocci fell by the wayside as molecular taxonomic studies of these bacteria gave rise to an ever-expanding list of new genera and species. Taxonomic hair-splitting was, however, not the only factor contributing to the increasing number of recognized catalase-negative gram-positive cocci.

Concomitant medical progress in the treatment of serious diseases created a growing population of patients with compromised defenses against infection and altered normal flora. We began to see more frequent opportunistic infections in this patient group, and catalase-negative, gram-positive cocci, some familiar and some not previously described in human clinical specimens, emerged as new agents of infection. Today we recognize at least 15 genera (in addition to *Streptococcus* and *Enterococcus*) and numerous new species of catalase-negative gram-positive cocci that have been isolated from human clinical specimens. This review examines their basic characteristics and clinical significance and offers a philosophy (based on facts in relation) on dealing with these bacteria in the clinical microbiology laboratory.

VIRIDANS STREPTOCOCCI

Clinical microbiologists traditionally associated viridans streptococci with subacute bacterial endocarditis and considered these predominantly alpha- or non-hemolytic members of normal oral flora to be contaminants in most other settings. The production of extracellular polysaccharides (dextrans and levans) carried out by some viridans strains by using sucrose as a substrate is thought to contribute to their ability to cause infection by aiding colonization of host surfaces. We now divide viridans streptococci into groups of closely related species whose characteristics are summarized below, in Table 1, and in reference 19. While hemolytic reactions on blood agar may vary, all of the viridans streptococci are pyrrolidonyl arylamidase (PYR)-negative and leucine aminopeptidase (LAP)-positive.

Mutans group. *S. mutans* and *Streptococcus sobrinus* are the two species of the mutans group that are principally isolated from humans. These oral cavity inhabitants are associated with dental caries and endocarditis, and produce extracellular polysaccharides. *S. mutans* may display cellular dimorphism, forming cocci under optimal growth conditions but short rods on agar media and in acidic liquid media. Most strains are alpha-hemolytic on blood agar, but beta-hemolytic strains have also been described. Colonies of some strains are dry and adherent.

Salivarius group. *Streptococcus salivarius* and *Streptococcus vestibularis* are the salivarius group species usually found as part of the normal human oral flora. On blood agar, these strains form non-hemolytic or alpha-hemolytic colonies, and *S. salivarius* produces distinctive mucoid colonies on sucrose agar due to the production of extracellular polysaccharides. *S. vestibularis* is polysaccharide production negative and, along with some *S. salivarius* isolates, urease positive. *S. salivarius* has been noted as an infrequently isolated opportunistic pathogen, producing septicemia in neutropenic patients.

Bovis group. Formerly set apart from other streptococci as nonenterococcal group D streptococci, bovis group species are now considered as part of the viridans streptococcal division. *S. bovis* is the most well characterized human isolate in this spe-

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TABLE 1. Some characteristics of the viridans streptococcal species groups[a]

Species group and strain type	Result of phenotypic test				Comments
	VP	ARG	MAN	SOR	
Mutans	+	−	+	+	*S. mutans* and *S. sobrinus* (may be sorbitol variable) are the species isolated from humans; associated with dental caries and endocarditis
Salivarius	+	−	−	−	*S. salivarius* and the infrequently isolated *S. vestibularis* (VP-negative) are isolated from humans; infrequent agents of infections in neutropenic patients
Bovis	+	−	V	−	Biotypes of *S. bovis* are the primary isolates of this group from humans; *S. bovis* causes endocarditis, and bacteremia with this organism may be associated with colonic cancer
Anginosus	+	+	V	−	*S. anginosus, S. constellatus*, and *S. intermedius* display variable hemolytic reactions and Lancefield antigens; associated with purulent infections
Mitis					
Arginine hydrolysis positive	−	+	−	V	*S. sanguis* and *S. gordonii* are the most frequently isolated arginine hydrolysis-positive members of the mitis group; associated with endocarditis
Arginine hydrolysis negative	−	−	−	−	*S. mitis* and *S. oralis* are arginine hydrolysis-negative members of the mitis group; *S. mitis* has been isolated as an agent of bacteremia and septic shock in compromised patients

[a] Abbreviations: VP, Voges-Proskauer test; ARG, hydrolysis of arginine; MAN, acid production from mannitol; SOR, acid production from sorbitol; V, variable. See reference 19 for methods for performing tests for the phenotypic traits mentioned in this table and for additional information and differentiating features.

cies group, and can be divided into a number of biotypes based on phenotypic characteristics. A strong association between the presence of the extracellular polysaccharide-forming biotype of *S. bovis* in the bloodstream and endocarditis and colonic cancer has been demonstrated (18).

Mitis group. The mitis group includes species we think of as viridans streptococci along with *Streptococcus pneumoniae*. As suggested in Table 1, the predominantly alpha-hemoytic viridans species in this complex group can be divided phenotypically into two subgroups on the basis of their ability to hydrolyze arginine. Among the arginine hydrolysis-positive strains, *Streptococcus sanguis* and *Streptococcus gordonii* are both common isolates from cases of endocarditis, with the majority of strains producing extracellular polysaccharides. Of the arginine hydrolysis-negative species, *S. mitis* and *Streptococcus oralis, S. oralis* is found more commonly in cases of endocarditis and is variable for extracellular polysaccharide production. The polysaccharide production-negative *S. mitis* has been noted as an agent of infection in neutropenic patient and as a species that displays penicillin resistance (7).

Anginosus group. At one time anginosus group strains were considered to constitute a single species, but they are currently divided into three species named *S. anginosus, Streptococcus constellatus*, and *Streptococcus intermedius*. These organisms have undergone repeated revisions in taxonomy and nomenclature during the past 50 years. Previous epithets for streptococci in this group include "*Streptococcus milleri*," "*Streptococcus* MG-*intermedius*," and "*Streptococcus anginosus-constellatus*." *S. anginosus* group strains may be alpha-, non-, or beta-hemolytic, and may produce Lancefield group A, C, F, or G antigen or no detectable antigen. Beta-hemolytic strains produce small colonies that, along with physiological traits, differentiate them from large colony-forming pyogenic, beta-hemolytic group A, C, or G streptococci. Anginosus group strains are PYR-negative (unlike *Streptococcus pyogenes*) and beta-glucuronidase-negative (unlike large colony-forming human isolates of group C and G streptococci). Anginosus group species do not produce extracellular polysaccharides and are, unlike other viridans streptococci, noted for their association with serious purulent infections.

BEYOND THE VIRIDANS STREPTOCOCCI

As knowledge of the catalase-negative, gram-positive cocci advanced, it became apparent that some opportunistic agents resembling viridans streptococci in colonial or cellular morphology were taxonomically unrelated to the genus *Streptococcus*. The characterization of these new opportunistic pathogens contributed to the expansion of genera and species in this group of bacteria.

***Leuconostoc* and *Pediococcus*.** *Leuconostoc* and *Pediococcus* were among the first new genera of catalase-negative, gram-positive cocci to be described as clinical isolates in the mid-1980s. These genera display intrinsic high-level vancomycin resistance that is unrelated to the acquired vancomycin resistance observed in enterococci and staphylococci. *Leuconostoc* and *Pediococcus* had previously been well known to food microbiologists, since they occur in various foods and on vegetation, but they were not recognized in human infection until an optimal combination of compromised hosts and antibiotic usage patterns most likely created the conditions for their emergence as opportunists. Members of both genera are PYR-negative and produce alpha- or non-hemolytic viridans streptococcal-like colonies on blood agar. *Leuconostoc* strains form cocci in pairs and chains but, unlike viridans streptococci, produce gas as an end product of glucose metabolism and are LAP negative. *Pediococcus* strains form cocci in clusters, are negative for gas production from glucose, and are LAP positive (Table 2 and references 1, 9, and 17). Members of both genera have been implicated in bloodstream and other types of infections in compromised hosts.

***Abiotrophia* and *Granulicatella* (formerly nutritionally variant streptococci).** Organisms once considered nutritionally deficient mutants of viridans streptococci are now classified in

TABLE 2. Reactions of miscellaneous catalase-negative gram-positive cocci in basic phenotypic tests[a]

Result of phenotypic test			MORPH[b]	Possible identity
PYR	LAP	NaCl		
+	+	+	Chains	*Enterococcus*, *Vagococcus*,[c] *Lactococcus*,[d] *Facklamia* spp. other than *F. languida*, or *Ignavigranum*
+	+	+	Clusters	*Facklamia languida*, or *Dolosigranulum*
+	+	−	Chains	*Abiotrophia, Granulicatella*, or *Gemella* spp. other than *G. haemolysans*
+	+	−	Clusters	*Rothia mucilaginosa* or *Gemella haemolysans*
+	−	+	Chains	*Globicatella*
+	−	+	Clusters	*Aerococcus viridans* or *Helcococcus*
+	−	−	Chains	*Dolosicoccus*
−	+	+	Clusters	*Aerococcus urinae* or *Pediococcus* (vancomycin resistant)
−	+	−	Chains	Viridans streptococci
−	−	+	Chains	*Leuconostoc* (vancomycin resistant)

[a] Abbreviations: PYR, production of pyrrolidonyl arylamidase; LAP, production of leucine aminopeptidase; NaCl, growth in broth containing 6.5% sodium chloride; MORPH, cellular morphology. Organisms listed in the table are, unless otherwise noted, vancomycin susceptible. Results shown are typical of the majority of strains. Reactions of individual strains may vary. See references 8 and 17 for methods for performing the phenotypic tests mentioned in the table and for additional tests and information on differentiation of the organisms mentioned.

[b] Chains refers to cocci in pairs and chains; clusters refers to cocci that form pairs, tetrads, and irregular groups.

[c] *Vagococcus* strains may show variable reactions in the NaCl test.

[d] *Lactococcus* strains may show variable reactions in the PYR and NaCl tests.

two new genera, *Abiotrophia* and *Granulicatella* (4, 10). These organisms form gram-positive cocci in pairs and chains under optimal nutritional conditions (pyridoxal-supplemented media) but may display pleomorphic cellular morphology when growth conditions are suboptimal. *Abiotrophia* and *Granulicatella* strains usually grow as small alpha-hemolytic colonies on chocolate agar but not on sheep blood agar unless the medium is supplemented or other bacteria (e.g., *Staphylococcus aureus*) are present to provide compounds needed for growth. Most strains are both PYR and LAP positive. These members of oral cavity normal flora are well documented as agents of endocarditis.

***Gemella*.** Although the *Gemella* genus has recently grown to include a total of four species (*Gemella haemolysans, Gemella morbillorum, Gemella bergeriae*, and *Gemella sanguinis*), *G. haemolysans* and *G. morbillorum* are the two most well described species of the genus. Most strains of these species are PYR and LAP positive and produce colonies on blood agar that resemble those of viridans streptococci. While the cellular morphology of *G. morbillorum* resembles that of streptococci, *G. haemolysans* forms *Neisseria*-like diplococci that may also be arranged in tetrads and clusters and may appear to be gram variable. Part of the normal flora of the oral cavity (*G. haemolysans*) and the gastrointestinal tract (*G. morbillorum*), *Gemella* strains have been isolated from cases of endocarditis, meningitis, and other infections (8).

***Rothia mucilaginosa* (formerly *Stomatococcus mucilaginosus*).** *R. mucilaginosa* strains, members of normal oral flora, form cocci in clusters and display variable catalase reactions ranging from negative to weakly positive to strongly positive. Their white to grayish non-hemolytic colonies may be mucoid, rubbery, or sticky in consistency and adherent to agar. The inability to grow in the presence of 5% NaCl distinguishes *R. mucilaginosa* from members of the genera *Staphylococcus* and *Micrococcus*. Stomatococci have been described as opportunistic agents of infection in cases of endocarditis, meningitis, peritonitis, and other infections (15, 17).

***Aerococcus*.** *Aerococcus viridans*, the most well known species of this genus of cluster-forming cocci has been noted as an infrequent cause of infection in compromised hosts. A number of newer species have been described more recently, and of these, *Aerococcus urinae* has been well documented as an agent of urinary tract infection and endocarditis in compromised patients (2, 11). Aerococci form alpha-hemolytic colonies on blood agar, and *A. viridans* colonies are larger than those of *A. urinae*. The two species also differ in other phenotypic traits (*A. viridans* is PYR positive and LAP negative, while *A. urinae* is PYR negative and LAP positive).

***Lactococcus* and *Vagococcus*.** The genus *Lactococcus* was created to accommodate non-beta-hemolytic Lancefield group N streptococci normally isolated from dairy products. Eventually, organisms identified as motile strains of lactococci were reclassified in a new genus, *Vagococcus*. Members of these genera resemble either streptococci or enterococci in terms of phenotypic traits and are infrequently isolated opportunistic pathogens. Lactococci have been isolated from cases of endocarditis and a variety of other infections, and while vagococci have been isolated from clinical specimens, little is currently known about the extent of their role in human disease (8).

***Helcococcus*.** *Helcococcus kunzii* is currently the only species in this genus of PYR-positive, LAP-negative cluster-forming cocci that has been isolated from human clinical specimens. *H. kunzii* forms small, non-hemolytic, slowly growing colonies on blood agar and has been described as an agent of wound infections (3, 17).

***Globicatella* and related genera (*Facklamia, Ignavigranum*, and *Dolosicoccus*).** The description of the alpha-hemolytic, PYR-positive, LAP-negative, salt-tolerant species *Globicatella sanguinis* (originally named *Globicatella sanguis*) in the early 1990s was followed by descriptions of the additional related genera, *Facklamia, Ignavigranum*, and *Dolosicoccus* (5, 6, 13, 14). *Dolosicoccus* is, like *G. sanguinis*, alpha-hemolytic, PYR positive, and LAP negative, while *Facklamia* and *Ignavigranum* isolates are positive for both of these enzyme activities and non-hemolytic. All organisms in this group form cocci in chains, except for the cluster-forming *Facklamia languida* (13). Isolates of *Ignavigranum* may, like *Abiotrophia* and *Granulicatella*, display satelliting behavior. Strains of these genera have been isolated from blood cultures and cultures of other specimen types, but more information is needed concerning their role in infection.

Dolosigranulum. *Dolosigranulum pigrum*, the sole species in the genus, has been isolated from blood, ocular fluid, and other body site cultures. The organism forms cocci arranged in pairs, tetrads, and clusters, and produces colonies on blood agar that resemble those of viridans streptococci. *D. pigrum* is PYR and LAP positive (12).

DEALING WITH MISCELLANEOUS CATALASE-NEGATIVE, GRAM-POSITIVE COCCI IN THE CLINICAL LABORATORY

Philosophy for dealing with the miscellaneous catalase-negative, gram-positive cocci. The clinical significance and impact on patient care of characterization of a clinical isolate should guide formulation of a strategy for dealing with the miscellaneous catalase-negative, gram-positive cocci. When isolated from normally sterile body sites or fluids in significant amounts and in pure culture or as the predominating organism or when isolated repeatedly from the same site, these bacteria may be functioning as opportunistic pathogens and should therefore be characterized. Communication with physicians caring for the patient will also aid in establishing clinical significance and in making a decision on how extensively an isolate should be identified.

Many of the miscellaneous catalase-negative, gram-positive cocci can only be accurately identified by reference laboratories. Clinical microbiologists should be aware of the plethora of genera and species of catalase-negative, gram-positive cocci and realize that they may encounter strains that do not fit into the categories of routinely isolated organisms. On the other hand, some of the miscellaneous catalase-negative, gram-positive cocci may be misidentified as more frequently isolated organisms. Clinical microbiologists should be aware of the possibilities when examining isolates in this group of bacteria. Armed with knowledge of the basic traits of the infrequently encountered catalase-negative, gram-positive cocci, the clinical microbiologist should be able to make an educated guess as to the nature of the isolate and send the strain to a reference lab if complete identification is desired. A somewhat vague preliminary report (e.g., gram-positive coccus, probable *Gemella*, sent to reference lab for confirmation) is preferable to an authoritative but incorrect identification.

Identification methods. Although a number of commercially available products are capable of identifying some species of viridans streptococci and a number of the other organisms mentioned here, the accuracy of these products with newer genera and species is not established. Rapidly changing taxonomy within this group has made it difficult to keep the databases of identification products up-to-date. Tests for some of the phenotypic characteristics used to differentiate new genera and species may not be included in some of the commercially available products.

A few basic phenotypic tests (Table 2 and references 8, 17, and 19) can aid in the preliminary characterization or presumptive identification of the miscellaneous catalase-negative gram-positive cocci. Table 2 displays the possible reactions of the genera discussed in this review in tests for PYR and LAP production and growth in 6.5% salt broth. Additional phenotypic characteristics of these organisms and methods for performing the tests can be found in references 8, 17, and 19. If, as mentioned above, a complete identification seems warranted, a reference laboratory should be consulted.

Susceptibility studies. Standardized susceptibility testing methods have been established for the viridans streptococci (16), but no methods have been validated for other miscellaneous catalase-negative, gram-positive cocci. Results of susceptibility testing performed by various methods can be found in the literature, and these studies suggest that, with the exception of *Leuconostoc* and *Pediococcus*, these organisms are uniformly susceptible to vancomycin. Many of the miscellaneous catalase-negative, gram-positive cocci appear to be susceptible to beta-lactam agents and a variety of other antimicrobial agents. Organisms in which elevated MICs for beta-lactams have been observed include some strains of *R. mucilaginosa*, *Abiotrophia* species, and *Granulicatella* species (17). Most viridans streptococci remain susceptible to penicillin, but a survey of U.S. isolates revealed penicillin resistance among strains belonging to the mitis species group (7).

CONCLUSION

The numbers of recognized genera and species of catalase-negative, gram-positive cocci will no doubt continue to grow. While few clinical microbiologists will have the luxury or perhaps even the inclination to become experts in their knowledge of these organisms, we should at least be aware of the existence of this group of bacteria. Basic knowledge of these infrequently encountered organisms and the clinical settings in which to expect their isolation can contribute not only to more accurate microbiology laboratory results but also to our increased understanding of the miscellaneous catalase-negative, gram-positive cocci and their role in infection.

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