Challenge M234-5

February 2024

Joint fluid: 4+ (>10/oif) neutrophils; 4+ (>50/oif) gram positive cocci (group C Streptococcus)

HISTORY

A simulated wound sample collected from 20 year old male with sore elbow was sent to category A laboratories.

Participants were expected to isolate and report beta hemolytic group C Streptococcus

CMPT QA/QC/STATISTICS

All simulated joint fluid samples are produced at CMPT according to CMPT internal protocols. The sample contained a pure culture of group C Streptococcus.

The samples are assessed for homogeneity and stability using in-house quality control methods and random selection of samples before and during production, and post sample delivery. The number of random samples selected is 15% of the total production batch.

The challenge sample lot was confirmed to be homogeneous and stable for 28 days.

Organism identification was confirmed by a reference laboratory.

All challenge components have in-house assigned values based on the most clinically appropriate result; the most clinically appropriate result is determined by expert committee evaluation. No further statistical analysis is performed on the results beyond that described under "Suitability for grading."

MAIN EDUCATIONAL POINTS from M234-5

1. Large colony forms of beta-hemolytic *Streptococcus* that group using the commercial kits for Strep typing as Group C or G are most commonly identified as *Streptococcus dysgalactiae* ± spp equisimilis. The colonies of this organism are differentiated from the *S. anginosus* group isolates that may cross-react with the typing antisera by their colony size (> 0.5mm), as well as by testing positive for β-D-glucuronidase (BGUR), commonly referred to as the MUG test.

2. Vogues-Proskauer is also negative, as opposed to being positive for *S. anginosus* group. If laboratories rely on the Strep grouping kit for identification, one of these two biochemical tests should be used to confirm that the organism is indeed a *S. dysgalactiae*. If MALDI identification is performed and an ID is obtained, Strep grouping can be done to further classify the organism for epidemiological purposes.

3. *Streptococcus dysgalactiae* can cause pharyngitis as well as other more serious and systemic disease similar to those caused by *Streptococcus pyogenes* and being seen more frequently in blood cultures and other serious infections such as bacteremia, osteomyelitis, toxic shock syndrome, and as in this case, septic arthritis in some patient populations.

SURVEY RESULTS

Reference laboratories: 12/12 (100%) labs reported group C Streptococcus (*S. dysgalactiae*) ± spp equisimilis ± beta hemolytic, 1 lab indicated it does not process this type of sample

Grading

Maximum grade: 4

Reporting group C Streptococcus was graded 4

Table 1. Identification results

Reported	Total	Grade
group C Streptococcus (Streptococcus dysgalactiae) ± spp equisimilis ± beta hemolytic	45	4
Streptococcus dysgalactiae (group C/G)	3	4
Streptococcus dysgalactiae/canis	1	3
gram positive cocci, suggestive of Streptococcus, refer for ID	1	3
refer, snnp	4	ungraded
Total	**54**	

Participants: 48/50 (96%) participants reported group C streptococcus/Streptococcus dysgalactiae; one participant reported *Streptococcus dysgalactiae/canis*, and another one reported gram positive cocci, refer (Table 1)

Suitability for Grading

A challenge is considered suitable for grading if agreement is reached by 80 percent of selected reference group and at least 50 percent of the participants.

Organism identification was correctly performed by at least 80 percent of reference laboratories and greater than 50 percent of all laboratories and was thus, determined to be suitable for grading.

COMMENTS ON RESULTS

The majority of laboratories identified the organism as group C *S. dysgalactiae* ± *ssp equisimilis* ± beta hemolytic, which was the expected result. A few labs gave an identification of *Streptococcus dysgalactiae* (group C/G), which was likely due to obtaining an identification by MALDI but not performing Strep grouping due to lab protocol. These participants were given full marks for the identification.

One lab reported a result of *Streptococcus dysgalactiae/canis. Streptococcus canis* types as a Group G Streptococcus and this isolate was a Group C *Streptococcus dysgalactiae*, therefore the identification of *S. dysgalactiae/canis* was downgraded to a grade of 3. The lab that called reported this ID also typed it as a Group C Streptococcus, so should have been able to report the correct ID.

Those labs that would refer for further identification were given a grade of 3 and those that don't process these types of specimens were not graded.

ISOLATION AND IDENTIFICATION

Group C beta hemolytic *Streptococcus dysgalactiae* are catalase negative, gram-positive cocci that exhibit a large zone of beta hemolysis. Beta hemolytic streptococci are grouped according to the presence of the Lancefield antigen.

Commercially available Lancefield antigen grouping sera are primarily used for the differentiation of beta hemolytic streptococci. Group C streptococci can also be differentiated from group A streptococci using other commercially available phenotypic tests including, bacitracin/Taxo A discs and/or PYR (pyrrolidonyl aminopeptidase). Group A streptococci are susceptible to bacitracin and PYR positive while Group C streptococci are resistant to bacitracin and PYR negative.

S. anginosus may react with Lancefield group A, C or G therefore it is important to differentiate beta-hemolytic streptococci of the pyogenic group from those belonging to the *S. anginosus* group, which may be present as normal oropharyngeal flora.

Beta hemolytic *Streptococcus* of the pyogenic group (*S. pyogenes, S. agalactiae*, *S. dysgalactiae* subsp. equisimilis) form large colonies of >0.5 mm after 24 hours of incubation on blood agar, in contrast to the beta hemolytic strains of the *S. anginosus* group, which present with pinpoint colonies of ≤ 0.5 mm after the same incubation time.

The Voges-Proskauer (VP) and β-D-glucuronidase (BGUR) can be used to differentiate small colony *S. anginosus* group, which is VP positive and BGUR negative, from large colony group C streptococci, which is VP negative and BGUR positive.[1]

MALDI-ToF is able to identify large colony beta hemolytic *Streptococcus,* however difficulty in distinguishing between *S. pyogenes, S. dysgalactiae* and *S. canis* has been reported. Different groups report that removal of three reference spectra from the database significantly improved the identification of *S. dysgalactiae* to 94%, without compromising identification *of S. canis*. *Streptococcus canis* can also be differentiated from Group C *Streptococcus dysgalactiae* through the use of the streptococcal typing kits, as *S. canis* types as a Group G. [2,3]

ANTIMICROBIAL SUSCEPTIBILITY

Group C streptococci are uniformly susceptible to penicillin, the treatment of choice. Susceptibility testing for penicillin does not need to be performed routinely because non-susceptible isolates are extremely rare in beta-hemolytic streptococci. However, antimicrobial susceptibilities may need to be performed and reported when a patient is allergic to penicillin and in the event of failure of current antibiotic therapy.

Susceptibility to erythromycin and clindamycin is variable. Isolates need to be screened for inducible clindamycin resistance when isolates demonstrate erythromycin resistance and are susceptible to clindamycin. Inducible clindamycin resistance can be detected by disc diffusion using the D-zone test or broth microdilution.[4]

The same agents used to treat group A streptococcal infections are appropriate to treat group C streptococci.

CLINICAL RELEVANCE

Human strains of Group C streptococci are commensal organisms in the oropharynx, gastrointestinal tract, and can colonize the skin. They have been reported as the etiological agent in a variety of infections including pharyngitis, skin infections, bacteremia, osteomyelitis, endocarditis, meningitis, and toxic shock syndrome.[5,6]

Strains from invasive infections have been shown to have some of the virulence factors of *S. pyogenes* e.g., M protein expression which confers resistance to phagocytosis, also streptolysin O and hyaluronidase production.[7] These strains have also been shown to carry genes for the superantigens that are present in invasive strains of *S. pyogenes.* [8]

Infectious arthritis usually follows hematogenous inoculation of pathogenic organisms in patients predisposed to infectious arthritis. [9]

The most common risk factor for septic arthritis is preexisting joint disease which is present in up to 47% of patients.[10]

Staphylococcus aureus account for the majority of septic arthritis, and account for 52% of cases.[10] After *S. aureus*, *Streptococcus* spp. are the next most commonly isolated bacteria in adult patients with septic arthritis.[11,12] *Streptococcus pyogenes* is usually the most common streptococcal isolate, often associated with autoimmune diseases, chronic skin infections, and trauma.[11,12] Group B streptococci have emerged as invasive pathogens in the elderly, especially those with diabetes, cirrhosis, and neurologic disease causing up to 10% of septic arthritis in some reports. [13]

Groups G, C, and F, in order of decreasing preponderance, are also isolated, especially in patients with immunocompromise, diabetes mellitus, malignancy, and severe genitourinary or gastrointestinal infections.[10]

Blood cultures should be obtained in all patients with suspected septic arthritis. In up to 14% of patients, a bacteriologic diagnosis is made only on the basis of blood cultures. [10]

There have been reports of a delayed or poor response to therapy in patients with septic arthritis, endocarditis, and even pharyngitis caused by *S. dysgalactiae* ssp equisimilis which, while the mechanism is not well understood, may be the result of a combination of microbiological and host factors. The addition of gentamicin for synergistic effect has been proposed for patients with invasive disease. [14]

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