Challenge M171-5

May 2017

Peritoneal fluid: *Klebsiella pneumoniae* + *Streptococcus constellatus* (c G171)

HISTORY

A simulated peritoneal fluid sample collected from a 50 year old patient with intra-abdominal sepsis was sent to category A laboratories.

Participants were expected to identify *Klebsiella pneumoniae* and *Streptococcus constellatus/ anginosus* group. Participants were also expected to report susceptibility results for *K. pneumoniae*.

CMPT QA/QC/STATISTICS

All simulated peritoneal fluid samples are produced at CMPT according to CMPT internal protocols. The sample contained a mixed culture of *Klebsiella pneumoniae* and *Streptococcus anginosus* group.

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 at least **20** days.

The identification of both organisms and antibiotic susceptibilities were 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."

SURVEY RESULTS

Reference laboratories

Identification: 13/13 (100%) laboratories reported *Klebsiella pneumoniae* ± subspecies *pneumoniae*; 13/13 (100%) laboratories reported *Streptococcus anginosus* group (9 laboratories reported *Streptococcus constellatus*)

Susceptibility: 13/13 (100%) laboratories reported ampicillin resistant (R); 13/13 (100%) laboratories reported gentamicin/tobramycin susceptible (S).

Participants

Identification (Tables 1 and 2): 61/61 (100%) reporting laboratories reported *Klebsiella pneumoniae* ± subspecies *pneumoniae*; 48/61 (77%) reported *Streptococcus anginosus* group / *Streptococcus constellatus.* See tables 1 and 2 for grading

Susceptibility: ampicillin: 59/61 (97%) laboratories reported R; aminoglycosides: 60/61 (98%) participants reported gentamicin/tobramycin S; SXT: 59/61 (97%) laboratories reported S; ciprofloxacin: 55/61 (90%) participants reported S. See tables 3A-D for grading.

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.

MAIN EDUCATIONAL POINTS from M171-5

1. The isolation of more than one pathogen in a sample from an intra-abdominal infection is commonplace.
2. Depending on the degree of infection identification and susceptibility testing of multiple species may be warranted. In this sample only the results for the *Klebsiella* were graded.
3. The large majority of *S. anginosus* group Isolates have predictable susceptibilities and thus were not graded.
4. Many laboratories add a treatment comment when the isolate is considered part of the pathogenic component, indicating that they are predictably susceptible to beta-lactam agents. It is rarely necessary to sub-speciate members of the *S. anginosus* group.

Grading

Maximum grade: 4

Reporting *Klebsiella pneumoniae* was graded 4.

Reporting *Streptococcus constellatus/S. anginosus* group was graded 4.

Reporting streptococcus group F, *Streptococcus* species or gram positive cocci and referring the sample was downgraded to 3.

Reporting *K. pneumoniae* resistant to ampicillin was graded 4.

Reporting *K. pneumoniae* susceptible to aminoglycosides, SXT, and ciprofloxacin was graded 4 for each component.

Table 1. Identification results - *Klebsiella pneumoniae*

Reported results	Total	Grade
Klebsiella pneumoniae	48	4
Klebsiella pneumoniae subspecies *pneumoniae*	13	4
snnp	3	ungraded
Total	**64**	

Organism identification and *K. pneumoniae*'s susceptibility to ampicillin, aminoglycosides, SXT, and ciprofloxacin were correctly performed by at least 80 percent of reference laboratories and greater than 50 percent of all laboratories and were thus, determined to be suitable for grading.

COMMENTS ON RESULTS

All laboratories correctly identified the gram-negative bacillus as *K. pneumoniae*, and the large majority identified the gram positive coccus in the sample within the *Streptococcus anginosus* group.

A small number of laboratories still include the *S. anginosus* group isolates as Group F streptococci, but this has now been supplanted. As noted below these beta-haemolytic species may have Group A antigens but they form small colonies and should not be confused with large colony beta-haemolytic streptococci. These are micro-aerophilic strains that have a butterscotch odor, and are often associated with soft tissue infections. Those laboratories that suggested these isolates were Group A strains should review their quality control for this procedure.

There were no anaerobes in the sample. These errors likely result from problems with Gram-staining of the sample.

Table 2. Identification results *Streptococcus anginosus* group

***Streptococcus constellatus* Identification**	**Total**	**Grade**
Streptococcus constellatus ± subspecies *constellatus* ± (*Streptococcus anginosus/milleri* group) ± refer	34	4
Streptococcus anginosus group, ± refer	14	4
group F Streptococcus, refer	2	3
Streptococcus Group F	1	3
Streptococcus species, refer	1	3
gram positive cocci, refer	1	3
unable to ID: Beta strep sent to reference lab for grouping	1	1
anaerobic gram positive cocci, refer	1	0
group A Streptococcus, refer	1	0
beta hemolytic strep group A/*Streptococcus pyogenes*	2	0
Peptostreptococcus sp., refer	1	0
Clostridium perfringens, refer	1	0
snnp	3	ungraded
Total	**64**	

Table 3. Susceptibility results *K. pneumoniae*

3A. Ampicillin	**Total**	**Grade**
Resistant	59	4
No Report	2	0
snnp	3	ungraded
Total	**64**	
3B. Aminoglycosides	**Total**	**Grade**
Gentamicin S	57	4
Tobramycin S	3	4
High-Level Genta S	1	0
snnp	3	ungraded
Total	**64**	
3C. SXT	**Total**	**Grade**
S	59	4
no report	2	0
snnp	3	ungraded
Total	**64**	
3D. Ciprofloxacin	**Total**	**Grade**
S	54	4
Levofloxacin S	1	4
no report	6	0
snnp	3	ungraded
Total	**64**	

ISOLATION and IDENTIFICATION

Intra-abdominal fluid can be contaminated with numerous mixed gastrointestinal microbiota in cases of ruptured intestine, so inoculating fluid into blood culture bottles is not recommended. However, in patients with chronic ambulatory peritoneal dialysis (CAPD) or spontaneous bacterial peritonitis (SBP) the pathogen numbers may be low and recovery can be enhanced by inoculating blood culture bottles in addition to submitting fluid for Gram stain and direct plating. [1]

Incubate Blood and Chocolate agar plates at 35 -37°C in 5% CO_2, MacConkey agar at 35-37°C in ambient air, and anaerobic medium at 35-37°C in anaerobic conditions. Other selective media may also be added based on Gram stain showing multiple morphologies of microorganisms. [2]

Species of the *S. anginosus* group (SAG) grow readily on BAP incubated in CO_2 and are small-colony-forming bacteria that can display varia-

ble patterns of hemolysis. Beta hemolytic strains need to be distinguished from large-colony-forming (>0.5 mm) beta-hemolytic streptococci since they can also harbor the Lancefield group antigens A, C, F, or G (or none at all).[3-5] All members of SAG are arginine, esculin and Voges-Proskauer (VP) positive and mannitol, sorbitol and urea negative. [5] The strain sent out in this survey produced beta-hemolytic colonies on 5% sheep's blood agar and possessed Lancefield group F antigen.

Members of *Streptococcus anginosus* group have been described as part of the pyogenic (beta-hemolytic) category and the viridians (non-beta-hemolytic) category of streptococci, which causes confusion when determining the clinical significance of the organism. This group of organisms should be identified to the *S. anginosus* group level and not reported as viridans streptococci (see Clinical Relevance).

Streptococcus constellatus is often alpha-hemolytic, but occasionally can be beta-hemolytic or non-hemolytic. A positive VP reaction separates it from the pyogenic beta-hemolytic streptococci and from the *S. mitis* group. A positive arginine hydrolysis reaction differentiates it from all the other viridans groups. A negative neuraminidase reaction differentiates *S. constellatus* from *S. intermedius* (positive) while a positive hyaluronidase reaction differentiates it from *S. anginosus* (negative). [3, 5]

ANTIMICROBIAL SUSCEPTIBILITY

Antimicrobial susceptibility testing and interpretive standards for *Streptococcus* species viridans group can be found in the CLSI document M100-S27 [6] : "Performance Standards for Antimicrobial Susceptibility Testing, Twentiyseventh Informational Supplement".

CLSI lists penicillin for primary testing and reporting, and ceftriaxone, cefotaxime, cefepime, and vancomycin for primary testing but selective reporting.

CLINICAL RELEVANCE

Peritoneal infections are classified as primary (ie, from hematogenous dissemination, usually in the setting of an immunocompromised state), secondary (ie, related to a pathologic process in a visceral organ, such as perforation or trauma, including iatrogenic trauma), or tertiary (ie, persistent or recurrent infection after adequate initial therapy).

Primary peritonitis is most often spontaneous bacterial peritonitis (SBP) seen mostly in patients with chronic liver disease. Secondary peritonitis is by far the most common form of peritonitis encountered in clinical practice. Tertiary peritonitis often develops in the absence of the original visceral organ pathology.

This intra-abdominal infection case is an example of secondary peritonitis which is normally caused by organisms from the gastrointestinal flora and is typically polymicrobial in nature. [7]

The *Streptococcus anginosus* group (SAG) is composed of the species *S. anginosus*, *Streptococcus constellatus* and *Streptococcus intermedius*. [3] Although SAG isolates are part of the normal flora of the human respiratory, gastrointestinal, and genitourinary tracts, [3, 8] the organisms can also cause serious invasive infections, including infections of the liver and lung, brain abscesses, bacteremia, endocarditis, and intra-abdominal infections. [9-11] Members of the SAG were identified as the most frequent cause of invasive pyogenic streptococcal infections in Canada. [12]

One of the most striking features of species in the SAG is their tendency to cause abscesses, however, many authors have suggested that the members of this group do not cause abscesses with an equal frequency. [9, 13]

Although observed by various authors, the reports on which species is more prevalent varies. Bantar et. al. [13] described that *S. anginosus* was the most prevalent species (64.4%) among the three species belonging to the *S. anginosus* group, and it was more frequently associated

with abdominal sites than either *S. constellatus* or *S. intermedius*. Claridge e.t al. [9] however, reported that *S. intermedius* and *S. constellatus* were more likely to cause deep abscess (86% and 73% of isolates of each species, respectively) than was *S. anginosus* (19%). Abscesses caused by *S. intermedius* tended to be associated with hematogenous spread or were deep-seated, whereas those due to *S. constellatus* were more often superficial. *S. constellatus* also seemed to cause a broader range of infections, including odontogenic and intra-abdominal disease.

In any case, because of their association with the formation of abscesses, the isolation of SAG from blood should alert physicians to the possible presence of parenchymatous abscesses and initiate appropriate investigations for their detection. [14, 15]

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