Challenge M242-4

August 2024

Peritoneal fluid – *Escherichia coli* (ESBL) – *Klebsiella pneumoniae*

HISTORY

A simulated peritoneal fluid sample collected from a 53 year old surgical patient admitted with perforation was sent to category A laboratories.

Participants were expected to isolate and report *Escherichia coli* (ESBL) and *Klebsiella pneumoniae* and report susceptibilities for both organisms.

CMPT QA/QC/STATISTICS

All simulated peritoneal fluid samples are produced at CMPT according to CMPT internal protocols. The sample contained a culture of *E. coli* and *K. pneumoniae*.

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 14 days.

Organism identification and susceptibility 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."

SURVEY RESULTS

Reference laboratories

Identification - *E. coli*: 12/12 (100%) labs reported *Escherichia coli* ± ESBL, 1 lab does not normally process this type of sample

Identification – *K. pneumoniae*: 12/12 (100%) labs reported *Klebsiella pneumoniae* ± ssp *pneumoniae*, 1 lab does not normally process this type of sample

MAIN EDUCATIONAL POINTS from M242-4

1. Peritonitis is a serious condition that results from perforations into the peritoneum. It is usually associated with multiple pathogens as in this challenge. The peritonitis can be spontaneous so that empiric antimicrobials are always started before lab results are known. The conditions can also result from leakage into the peritoneum in patients who are on peritoneal dialysis.
2. Empiric antimicrobial therapy is essential and is almost always provided in combinations that are usually successful (based on literature and experience.
3. Multi-drug resistant strains of gram-negative bacilli (in the present case a multi-resistant *E. coli*) can present significant issues for treatment. Providing S/R test results can be helpful for clinicians to tailor therapy.

Susceptibility – *E. coli*: 11/12 (92%) labs reported Ampicillin R, 1 lab did not report; 11/12 (92%) labs reported Cefazolin R, 1 lab did not report; 12/12 (100%) labs reported Ceftriaxone R; 10/12 (83%) labs reported Ciprofloxacin S, 1 lab reported Levofloxacin S, 1 lab did not report; 10/12 (83%) labs reported Gentamicin/Tobramycin R, 2 labs reported Gentamicin I and Tobramycin R; 11/12 (92%) labs reported SXT S, 1 lab did not report. 10/12 (83%)labs reported Carbapenems S (in combination or single antibiotic), 2 labs did not report, 1 lab does not normally process this type of sample

Susceptibility – *K. pneumoniae*: 12/12 (100%) labs reported Ampicillin R; 11/12 (92%) labs reported Cefazolin S, 1 lab did not report; 8/12 labs reported Ceftriaxone R, 4 labs did not report (no consensus); 8/12 labs reported Ciprofloxacin S, 1 lab reported Levofloxacin S, 3 labs did not report (no consensus); 12/12 labs reported Gentamicin/Tobramycin S; 11/12 labs reported SXT S, 1 lab did not report.

Participants

Identification - *E. coli*: 47/48 (98%) labs reported *Escherichia coli* ± ESBL (Table 1A).

Identification – *K. pneumoniae*: 47/48 (98%) labs reported *Klebsiella pneumoniae* ± ssp *pneumoniae* (Table 1B).

Grading

Maximum grade: 56

A grade of 4 was awarded for each correctly identified organism (*E. coli* and *K. pneumoniae*).

Reporting the expected results for the antimicrobial agents for each organism was graded 4 for each agent.

Table 1A-B. Identification results

1A *E. coli* reported	Total	Grade
Escherichia coli ± ESBL ± refer	44	4
Escherichia coli, exhibits a broad spectrum beta lactamase	2	4
Escherichia coli (MDR organism.)	1	4
no report	1	0
sample not normally processed	3	ungraded
Total	**51**	

1B *K. pneumoniae* reported	Total	Grade
K. pneumoniae ± ssp pneumoniae ± complex	46	4
Klebsiella oxytoca	1	3
no report	1	0
sample not normally processed	3	ungraded
Total	**51**	

Susceptibility – *E. coli*: laboratories reported susceptibility testing results for ampicillin, cefazolin, ceftriaxone, ciprofloxacin, gentamicin/tobramycin, SXT and carbapenems. Results and grades are summarized in Table 2A-G.

Susceptibility – *K. pneumoniae*: laboratories reported susceptibility testing results for ampicillin, cefazolin, ceftriaxone, ciprofloxacin, gentamicin/tobramycin, and SXT. Results and grades are summarized in Table 3A-F.

Suitability for Grading

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

The 0rganisms identification were 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. Susceptibility testing results that reached consensus both from reference and participant laboratories were considered suitable for grading.

Table 2. Susceptibility results *E. coli*

2A Ampicillin	Total	Grade
R	43	4
no report	3	ungraded
n/a, no ID reported	1	ungraded
sample not normally processed	4	ungraded
Total	**51**	
2B Cefazolin	**Total**	**Grade**
R	37	4
no report	9	ungraded
n/a, no ID reported	1	ungraded
sample not normally processed	4	ungraded
Total	**51**	
2C Ceftriaxone	**Total**	**Grade**
Ceftriaxone R	45	4
Cefotaxime R	1	4
Ceftazidime R	1	4
n/a, no ID reported	1	ungraded
sample not normally processed	3	ungraded
Total	**51**	
2D Ciprofloxacin	**Total**	**Grade**
S	43	4
Levofloxacin S	1	4
no report	3	0
n/a, no ID reported	1	ungraded
sample not normally processed	3	ungraded
Total	**51**	

2E Gentamicin/Tobramycin	Total	Grade
R	28	4
Gen I, Tob R	16	3
Gen I	3	3
n/a, no ID reported	1	ungraded
sample not normally processed	3	ungraded
Total	**51**	
2F SXT	**Total**	**Grade**
S	44	4
no report	3	0
n/a, no ID reported	1	ungraded
sample not normally processed	3	ungraded
Total	**51**	
2G Carbapenems	**Total**	**Grade**
Ert, Imi, Mer S	9	4
Ert, Mer S	19	4
Ert S	1	4
Imi S	2	4
Mer S	9	4
Ert R, Imi and Mer S	1	3
no report	5	0
n/a, no ID reported	1	ungraded
sample not normally processed	4	ungraded
Total	**51**	

Ert: ertapenem; Imi: imipenem; Mer: meropenem

Table 2. Susceptibility results *K. pneumoniae*

3A Ampicillin	Total	Grade
R	45	4
no report	2	Ungraded
n/a, no ID reported	1	ungraded
sample not normally processed	4	ungraded
Total	**51**	
3B Cefazolin	**Total**	**Grade**
S	31	4
I	1	3
no report	15	Ungraded
n/a, no ID reported	1	ungraded
sample not normally processed	4	ungraded
Total	**51**	
3C Ceftriaxone	**Total**	**Grade**
S	32	ungraded
no report	15	ungraded
n/a, no ID reported	1	ungraded
sample not normally processed	3	ungraded
Total	**51**	

3D Ciprofloxacin	Total	Grade
S	40	4
Lev S	1	4
no report	6	0
n/a, no ID reported	1	ungraded
sample not normally processed	3	ungraded
Total	**51**	
3E SXT	**Total**	**Grade**
S	42	4
I	1	3
no report	4	0
n/a, no ID reported	1	ungraded
sample not normally processed	3	ungraded
Total	**51**	
3F Gentamicin	**Total**	**Grade**
S	43	4
Tobramycin S	4	4
no report	1	0
n/a, no ID reported	1	ungraded
sample not normally processed	3	ungraded
Total	**51**	

COMMENTS ON RESULTS

This challenge was performed very well by the participating laboratories. Correct identifications were provided by almost all the laboratories. The one lab that reported *K. oxytoca* instead of *K. pneumoniae* was given a grade of 3 (species difference). Some automated systems may occasionally call a *K. pneumoniae* as *K. oxytoca*, but clinically in this setting it likely makes little difference.

With respect to susceptibility testing, again the large majority of test results were reported correctly. We'll look at each species separately.

With regard to the *E. coli* isolate, for both ampicillin and cefazolin the small number of laboratories that provided no report were ungraded.

For a multi-resistant strain (indicated as an ESBL but there was no further testing to confirm that by the testing labs) some laboratories prefer not to report the penicillins and first-generation cephalosporins. While that's not wrong, providing a resistant result may prevent use for treatment.

The majority of laboratories reported test results for ciprofloxacin and SXT. The few labs that provided no report were graded as 0. In a intraperitoneal infection these agents if susceptible, can be used in combination with other susceptible agents and should therefore be reported.

The ceftazidime resistant result was also given a grade of 4. Depending on regional formularies, ceftazidime may be incorporated into testing panels and therefore like the other 3rd generation cephalosporins would be reported as resistant.

With regard to the *K. pneumoniae* isolate, like the *E. coli*, some laboratories do not report ampicillin for *Klebsiella* (over 95 % of isolates test resistant, so it is almost intrinsic. Like the earlier comment about *E. coli*, it does not hurt to call the isolate resistant as did all the other testing labs).

Some strains of *Klebsiella* test susceptible to cefazolin. It's thought that inhibition kinetics may be slower (thus an S or I report) but might be acceptable in combination with other more potent agents. Therefore, the "No report" for cefazolin was ungraded for similar reasons to the *E. coli* strain. For the *Klebsiella* results for ciprofloxacin and SXT were both susceptible the the scores for "No Report" (0) were based on similar issues. They can have efficacy if used in combination with other agents in peritonitis, but a "No Report" is less helpful to clinicians.

The reported results for ceftriaxone for this isolate are confusing. Thirty-two laboratories reported the *Klebsiella* as susceptible, but 15 labs indicated "No Report". While the *Klebsiella* was not a multi-resistant strain, treatment with ceftriaxone will not affect the *E. coli*, and it is presumed that's why there were a significant number of labs that indicated "No Report" for *Klebsiella* and ceftriaxone. In practice notification would be helpful to indicate this disconnect on the final report to clinicians.

With respect to the multi-resistant *E. coli* strain, testing for carbapemens in serious infections should be completed. They may be in practice the primary agents of choice for treatment, and both meropenem and imipenem tested as susceptible. One laboratory reported ertapenem as resistant, but meropenem and imipemen as susceptible. This lab was down-graded to 3; ertapenem is rarely reported but can be a marker for carbapenem resistance so such a disconnect in this isolate should be further investigated. An additional 5 labs that indicated no report were graded 0, because of the importance of reporting additional susceptible agents in a serious infection with a multi-resistant strain.

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% CO2, 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]

Intra-abdominal fluid can be contaminated with gastrointestinal microflora in cases of perforating abdominal wounds. Peritoneal fluid should be sent to the laboratory in an an-aerobic transport system for Gram stain and aerobic and anaerobic bacterial cultures. Inoculation of blood culture bottles alone with peritoneal fluid is not appropriate in this setting, as competitive bacterial growth in broth cultures could mask the recovery of clinically important pathogens.[3]

"Because of the polymicrobic nature of secondary peritonitis, clinicians should not expect or request identification and susceptibility testing of all organisms isolated. Rather, the laboratory should provide a general description of the culture results (eg, mixed aerobic and anaerobic intestinal flora) and selective identification of certain organisms such as MRSA, β-hemolytic *Streptococcus* spp, multi-drug-resistant gram negative bacilli, VRE, etc.) to guide empiric anti-microbial therapy." [3]

Patients who do not respond to conventional therapy should have additional specimens collected to examine for resistant organisms or for the presence of intra-abdominal abscesses. [3]

It is important to correlate the colonial morphotypes isolated with the direct Gram stain made from the specimen. Laboratory reports that communicate a presumptive identification of *B. fragilis* group are important to clinicians, because these organisms possess resistance to more antimicrobial agents than most other anaerobic organisms.[3]

CLINICAL RELEVANCE

The incidence or multi-drug resistant *Enterobacterales* has increased significantly in recent years. Some are ESBL- producers but there are other mechanisms that result in similar degrees of resistance. Increased incidence in community acquired infections[4] appear to be contributory to the persistence of these multi -drug resistant species.

Most of these gram-negative bacteria remain susceptible to carbapenems however, these organisms often harbor additional genes or mutations in genes that mediate resistance to a broad range of antibiotics.[5] These drug-resistant strains can also be resistant to fluoroquinolones, trimethoprim/sulfamethoxazole (SXT) and aminoglycosides but these agents, if susceptible may be utilized as part of combination therapy, particularly in peritonitis once the pathogens have been isolated, identified and susceptibility test performed.

Although any *Enterobacterales* have the potential to harbor ESBL and other resistance genes; the most prevalent are *Escherichia coli, Klebsiella pneumoniae, Klebsiella oxytoca*, and *Proteus mirabilis*.[7]

Routine testing for ESBLS and other resistance mechanisms (e.g. carbapenemases) is often not performed by most clinical microbiology laboratories.[8] Rather, non-susceptibility to ceftriaxone (i.e., ceftriaxone minimum inhibitory concentrations [MICs] ≥2 μg/mL), is often used as a proxy for ESBL production, although this threshold has limitations with specificity as organisms not susceptible to ceftriaxone for reasons other than ESBL production may be falsely presumed to be ESBL-producers.[5,9]

The *E.coli* strain in this challenge appears to have harboured an ESBL enzyme which conferred resistance to all cephalosporins. Detection of ESBLs in *Enterobacterales* had been recommended until when CLSI (2009) and EUCAST (2010). lowered the breakpoints for the third generation cephalosporins.[4] New recommendations support the notion that it is not necessary to confirm the ESBL result with a clavulanic acid disk test or a combination E-test when these new breakpoints are applied.

Most laboratories that use automated systems for their reporting algorithms should now have implemented the lower breakpoints for cephalosporins and many also incorporate testing for the presence of ESBL enzymes or incorporate algorithms that will flag typical resistance mechanisms, although this is not necessarily accurate.[10]

The initial screen test for ESBL production requires susceptibility testing to cefpodoxime, ceftazidime, aztreonam, cefotaxime, or ceftriaxone. The guidelines recommend the use of more than one antimicrobial agent for improved sensitivity.[4] Growth at or above the screening concentrations suggests the production of ESBLs or other mechanisms, and may warrant the performance of phenotypic confirmation tests.

The application of the new susceptibility breakpoints has eliminated the need for complicated screening and confirmation as-

says by most clinical laboratories, which is seen as additional workload on busy labs and causes a delay in reporting results.[9] If necessary these additional tests can be performed in reference laboratories but may only be necessary for infection control purposes or If outbreaks in a clinical unit warrant such testing. There continues to be controversy about testing, (further reading can be found in CMPT Critique M232-1 – references are supplied here).

CLINICAL RELEVANCE

Peritoneal infections are classified as primary (spontaneous bacterial peritonitis, lacking an intra-abdominal source), secondary (related to a pathologic process in a visceral organ, such as perforation or trauma, including iatrogenic trauma), or tertiary (persistent or recurrent infection after adequate initial therapy).[12,13] Patient's on chronic peritoneal dialysis are at increased risk of peritoneal infections.

Causes of secondary peritonitis can be iatrogenic, or resulting from accidental trauma, perforated appendix or intra-abdominal abscess. Secondary peritonitis tends to be polymicrobial and usually includes anaerobic flora.[13] *B. fragilis* is the most frequently isolated obligate anaerobe after colonic perforation, and *E. coli* is the most frequently isolated facultative anaerobe.[14] Other commonly isolated organisms include *Klebsiella* species and a range of gram positive organisms.[3]

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