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CMPT Clinical Bacteriology Program

Innovation, Education, Quality Assessment, Continual Improvement

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Challenge M241-1

May 2024

Urine: >100 x 10^6 CFU/L *Proteus mirabilis* and >100 x 10^6 CFU/L *Escherichia coli*

HISTORY

A simulated midstream urine sample collected from an 80-year-old female with dysuria was sent to category A laboratories. Participants were expected to isolate and report colony counts for bacteria identified as *P. mirabilis* and *E. coli*. Susceptibility testing was not required.

CMPT QA/QC/STATISTICS

All simulated urine samples are produced at CMPT according to CMPT internal protocols. The sample contained a mixed culture of *P. mirabilis* and *E. coli*.

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 15 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."

SURVEY RESULTS

Reference laboratories

Colony count: 11/12 (92%) of reference laboratories reported ≥10 to ≥ 100 × 10^6 CFU/L for both organisms. One lab reported <100 × 10^6 CFU/L of mixed insignificant growth.

E. coli identification: 11/12 (92%) labs reported *E. coli*. One lab did not work it up as it reported insignificant mixed growth.

P. mirabilis identification: 11/12 (92%) labs reported *P. mirabilis*. One lab did not work up as it reported insignificant mixed growth.

MAIN EDUCATIONAL POINTS from M241-1

1. Gram-negative *Enterobacterales*, including *E. coli* and *P. mirabilis*, are among the most important uropathogens, both from an epidemiologic and clinical standpoint.
2. Urinary tract infections can involve more than a single pathogen. When three or more organisms are isolated in similar counts, however, this is typically considered mixed growth.

Participants

Colony count: 47/49 (96%) labs reported ≥10 to ≥ 100 × 10^6 CFU/L for both organisms. One lab reported <100 × 10^6 CFU/L of mixed insignificant growth. One did not report a colony count.

E. coli identification: 48/49 (98%) labs identified and reported *E.coli*. One lab did not work it up as it reported insignificant mixed growth (Table 2A).

P. mirabilis identification: 42/49 (86%) labs identified and reported *P. mirabilis*. 6 laboratories did not report a second organism; one lab did not work up as it reported insignificant mixed growth (Table 2B).

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.

Colony count and organism 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.

Grading

Maximum grade: 12

Reporting colony count of >10—>100x 106cfu/L was graded 4

Reporting *E. coli* was graded 4

Reporting *P. mirabilis* was graded 4

Table 1. Colony count results

Count reported (× 10^6 CFU/L)	Total	Grade
≥10 - ≥100	47	4
<100 × 10^6 CFU/L; mixed insignificant growth	1	0
no report	1	0
sample not normally processed	2	ungraded
Total	51	

Table 2A. Identification results—*E. coli*

Reported	Total	Grade
Escherichia coli	48	4
mixed insignificant growth	1	ungraded
sample not normally processed	2	ungraded
Total	51	

Table 2B. Identification results—*P. mirabilis*

Reported	Total	Grade
Proteus mirabilis	42	4
mixed insignificant growth	1	ungraded
no report	6	0
sample not normally processed	2	ungraded
Total	51	

COMMENTS ON RESULTS

Laboratories generally performed well on this challenge. One laboratory reported insignificant mixed growth on this challenge.

This urine specimen contained two uropathogens at high and comparable counts. Guidelines typically recommend that laboratories work-up two pathogens when present, and report mixed growth when ≥3 organisms are isolated. As such, this response was considered unacceptable.

Identification of the pathogens was well done. All laboratories reporting significant growth were able to identify *E. coli*. However, several laboratories missed or were unable to identify the *P. mirabilis*. Laboratories should review their protocols to ensure that they are able to isolate and detect *Proteus* spp. on their primary urine culture media.

ISOLATION AND IDENTIFICATION

Both *E. coli* and *P. mirabilis* belong to the order *Enterobacterales*, notable as facultatively anaerobic Gram-negative bacilli that test negative for oxidase and positive for nitrate reduction. They are not fastidious, growing on all routine media including blood, chocolate, and MacConkey agar. In addition, many laboratories employ specially designed chromogenic agars to select for and differentiate uropathogens, potentially presumptively identifying *E. coli* by colour alone. However, each agar has its own nuances and limitations, including cross-colour reactivity of *E. coli* and *Citrobacter* spp.; protocols used by the laboratory should be accordingly mindful of these.[1]

Identification of these organisms may be done from simple biochemicals, including a combination of TSI, indole, PYR, Voges-Proskauer, citrate, urease, etc. *Proteus mirabilis* has a distinctive swarming colony morphology, and tests positive for urea, phenylalanine deaminase, and H_2S production.

Outside of manual biochemical methods, most common identification platforms, including biochemical (e.g., API, Vitek 2) and mass spectrometry (e.g., MALDI-TOF) can reliably identify these organisms. Note is made of the close genomic relationships of *E. coli* and *Shigella*, though the latter is rarely isolated from urinary specimens.

ANTIMICROBIAL SUSCEPTIBILITY

Interpretive breakpoints to a wide variety of antimicrobial agents are available for *Enterobacterales* in the CLSI M100 guidelines, among others.[2] As of 2023, the categories of recommended antimicrobials for testing and reporting changed to Tiers. For urinary isolates of *Enterobacterales*, Tier 1 agents (appropriate for routine primary testing and reporting) include ampicillin, cefazolin (as a surrogate for 1st generation cephalosporins in uncomplicated UTI), nitrofurantoin, trimethoprim-sulfamethoxazole, as well as amoxicillin-clavulanate, piperacillin-tazobactam, ceftriaxone, gentamicin, and ciprofloxacin. Some of these agents are more clinically used than others, depending on the clinical practices of the site; cascading may still be warranted or needed even among these agents to encourage appropriate antimicrobial use. It may be helpful to involve local antimicrobial stewardship resources in the design of such cascades.

With respect to *P. mirabilis*, laboratories should keep in mind the intrinsic resistance to nitrofurantoin and tetracyclines (and colistin/polymyxins, though these are seldom tested or needed for this organism).

CLINICAL RELEVANCE

Urinary tract infections (UTI's) are very common, including in elderly patients as portrayed in this challenge. However, note should be made that asymptomatic bacteriuria is likewise common in the elderly population. The differentiation of these entities is primarily done by the presence of consistent genitourinary or sepsis symptoms, and must be made by the treating clinician.[3] While laboratories cannot be expected to make this differentiation, laboratories may choose to use a variety of methods to use clinical history in triaging testing and reporting, with the hope of improving both diagnostic and antimicrobial utilization.[4] Laboratories, by way of education, test directory or other means, may discourage collection of urine specimens in asymptomatic patients, with the exception of pregnant patients and those undergoing invasive genitourinary procedures.[5]

In actual UTIs, *E. coli* remains by far the most common etiologic organism. *P. mirabilis* remains a major cause, along with other common *Enterobacterales* such as *Klebsiella* and *Enterobacter* spp.

REFERENCES

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