Challenge M244-3

February 2025

Stool: *Yersinia enterocolitica*

HISTORY

A simulated stool sample collected from a 44 year old pig farmer with diarrhea was sent to category A laboratories.

Participants were expected to isolate and report *Yersinia enterocolitica.*

CMPT QA/QC/STATISTICS

All simulated stool samples are produced at CMPT according to CMPT internal protocols. The sample contained a culture of *Yersinia enterocolitica* and *Escherichia coli* as background

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 15 days from shipment day. 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

Identification: 12/13 (92%) labs reported *Yersinia enterocolitica* ± group, 1 lab reported the comment: "Due to low incidence of *Yersinia, Aeromonas* and *Vibrio* the laboratory adopted a molecular multi-assay platform for the BDMAX. We currently test for *Salmonella, Shigella, Campylobacter* and Shiga-toxin producing *E.coli* (STEC). If the physician requested testing for *Yersinia, Vibrio* and/or *Aeromonas* the sample would be forwarded to our reference laboratory for full culture."

MAIN EDUCATIONAL POINTS from M244-3

1. Susceptibility testing should always be performed on *Yersinia* isolated from extra-intestinal sites or immunocompromised patients.
2. When performing susceptibility testing on *Yersinia enterocolitica*, reporting cascades should be similar to those cascades used for species that are intrinsic AmpC producers.
3. *Yersinia enterocolitica* is an important food borne pathogen requiring notification to Public Health authorities, when isolated, in all provinces and territories, with the exception of Nova Scotia – primary sources are undercooked and/or contaminated pork and pork products, however epidemics have been linked to other animal and plant products.

Public Health (PH) notification: 10/12 (83%) reporting labs indicated they would notify PH; one lab indicated they would notify infection control but not PH, one lab did not notify either PH or infection control; one lab did not identify *Yersinia.*

Participants

Identification: 28/38 (74%) reporting labs identified *Yersinia enterocolitica* or *Yersinia* species in the sample; 5 participants specifically indicated that they did not isolate *Yersinia*; 3 labs listed organisms not isolated but did not list *Yersinia* among them; 2 labs indicated that they do not investigate *Yersinia* in stool samples (Table 1)

Public Health notification: 27/28 (96%) labs reporting Yersinia indicated they would report to PH. One participant indicated they would NOT report to PH but indicated they would report to infection control (Table 2)

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 and PH notification 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.

Grading

Maximum grade: 8

Reporting *Y. enterocolitica* or *Yersinia* species was graded 4.

Reporting the organism to PH was graded 4.

Table 1. Identification results

Reported	Total	Grade
Yersinia enterocolitica ± biotype 1A ± group ± NSSCAE and other common Shiga-toxigenic E. coli strains (STEC) ± refer	25	4
Yersinia species, refer	1	4
Yersinia enterocolitica/frederiksenii, ± refer	2	4
Comment* ± NSSCE (STEC)	2	ungraded
NSSCYE/no enteropathogens isolated	5	0
NSSCAPE ± (shiga toxin producing)	3	0
no report	1	0
sample not normally processed, refer	12	ungraded
Total	**51**	

**Comment: Due to low incidence of Yersinia, Aeromonas and Vibrio the Laboratory adopted a molecular multi-assay platform for the BDMAX. We currently test for Salmonella, Shigella, Campylobacter and Shiga-toxin producing E.coli (STEC). If the physician requested testing for Yersinia, Vibrio and/or Aeromonas the sample would be forwarded to our reference laboratory for full culture.*

Table 2. Public Health notification results

Notification	Total	Grade
Yes	27	4
No – not required by province	1	Ungraded
No	1	0
n/a, did not report ID	10	ungraded
snnp, refer	12	ungraded
Total	**51**	

COMMENTS ON RESULTS

It was expected that participants would isolate and identify Yersinia enterocolitica, and report this isolate to their provincial Public Health authority.

Reporting *Yersinia enterocolitica* +/- group, or *Yersinia* species refer, was graded 4. Reporting the organism to Public Health authorities, including a Medical Officer of Health, was graded 4 - participants that did not report *Yersinia enterocolitica*, reported *Yersinia* species refer, or are not required by their provincial regulations to report *Yersinia enterocolitica* to Public Health, were ungraded for the PH notification portion of this challenge

ISOLATION AND IDENTIFICATION

Yersinia is a genus of gram negative bacilli, belonging to the newly formed family *Yersiniaceae* in the order *Enterobacterales*.[2] There are three species of indisputable clinical significance: *Yersinia enterocolitica*, *Y. pestis*, and *Y. pseudotuberculosis*.

Y. enterocolitica, as a member of *Enterobacterales*, shares the common characteristics of being glucose-fermenting, oxidase-negative, facultatively anaerobic, and nitrate-reducing. They are able to grow on routine media, including MacConkey, in ambient air or with 5% CO_2; most strains grow optimally at 25-28°C.

Because of their slower growth at 35-37°C compared to other *Enterobacterales,* isolation of *Y. enterocolitica* without overgrowth of other organisms from stool specimens may require additional selective media. The most commonly used one is cefsulodin-Irgasan-novobiocin (CIN) agar, which inhibits most non-*Yersinia* organisms including *E. coli, P. aeruginosa, Proteus* spp., and *Enterococcus* spp. Colonies of *Y. enterocolitica* on CIN agar develop red centres surrounded by a translucent rim, described as 'bull's eye' colonies.

Other notable biochemical reactions include the absence of gas production in a triple-sugar iron tube when fermenting glucose, positive urease, and positive motility which is typically enhanced at temperatures below 30°C (testing at 35-37°C may yield negative results).

Identification panels and platforms normally include *Y. enterocolitica* in their databases. The API20E reportedly identifies *Y. enterocolitica* well, but struggles with other related species, like *Y. fredriksenii* and especially *Y. intermedia*.[3] The Vitek GNI is able to reliably identify the isolates to the genus level, but accurate species-level identification may be challenging.[4] MALDI-TOF systems, such as the Vitek MS, are able to identify *Y. enterocolitica*, but may still have variable success in differentiating *Y. enterocolitica* from other closely related species.[1]

There have been several biotypes of *Yersinia enterocolitica* described, with varying pathogenicity. (Biotype 1A, in particular, is considered non-pathogenic.) However, aside from a panel of manual biochemical tests, automated platforms cannot reliably identify these biotypes,[1] and it is a task best left to specialized reference laboratories.

Finally, PCR-based gastrointestinal pathogen identification panels have changed the way many laboratories identify stool pathogens, including *Yersinia*. *Y. enterocolitica* is variably included on these panels, which typically have good sensitivity/specificity, and offer substantially improved turnaround time on primary laboratory diagnosis compared with culture-based methods.[5]

ANTIMICROBIAL SUSCEPTIBILITY

Yersinia spp. are tested for antimicrobial susceptibility with the same methods and interpretations as other *Enterobacterales*.[6] Laboratories should be clear that Yersinia enterocolitica does contain an AmpC enzyme with a regulatory system that potentially allows for derepression;[7] thus, reporting cascades for this organism should be similar to reporting cascades for other intrinsic AmpC producers, such as *Enterobacter* spp.

Because most *Yersinia enterocolitica* diarrheal disease is self-limited, routine susceptibility testing of these isolates may not be necessary. However, susceptibility testing should be performed in extraintestinal infection, and in immunocompromised patients.

CLINICAL RELEVANCE

Y. enterocolitica is an important cause of acute gastrointestinal disease. The most important reservoirs are pigs, and hence consumption of undercooked raw pork is the highest risk factor for acquisition of this organism.[8] In addition to pork, epidemics of Yersiniosis have also been traced to dairy products, poultry, fruits and vegetables, and even seafoods.[10]

In young children, acute infection is characterized by diarrhea and fever, along with abdominal pain. Stools may contain mucus or blood. In older children and adults, infection manifests more commonly as a mesenteric adenitis or terminal ileitis, resulting in fever and often right lower quadrant pain, which may be confused with appendicitis.[9]

Extraintestinal infections are rare but may be serious, and occur more commonly in immunocompromised patients. Post-infection sequelae are also frequent, the most common being reactive arthritis.[9]

REFERENCES

1. Morka K, Bystroń J, Bania J, et al. Identification of *Yersinia enterocolitica* isolates from humans, pigs and wild boars by MALDI TOF MS. BMC Microbiol 2018;18.

2. Adeolu M, Alnajar S, Naushad S, Gupta RS. Genome-based phylogeny and taxonomy of the '*Enterobacteriales*': proposal for *Enterobacterales* ord. nov. divided into the families *Enterobacteriaceae, Erwiniaceae* fam. nov., *Pectobacteriaceae* fam. nov., *Yersiniaceae* fam. nov., *Hafniaceae* fam. nov., *Morganellaceae* fam. nov., and *Budviciaceae* fam. nov. Int J Syst Evol Microbiol 2016;66(12):5575–99.

3. Sharma NK, Doyle PW, Gerbasi SA, Jessop JH. Identification of *Yersinia* species by the API 20E. J Clin Microbiol 1990;28 (6):1443–4.

4. Linde H-J, Neubauer H, Meyer H, Aleksic S, Lehn N. Identification of *Yersinia* Species by the Vitek GNI Card. Journal of Clinical Microbiology 1999;37(1):211–4.

5. Simner PJ, Oethinger M, Stellrecht KA, et al. Multisite evaluation of the BD Max Extended Enteric Bacterial Panel for detection of *Yersinia enterocolitica*, enterotoxigenic *Escherichia coli*, Vibrio, and *Plesiomonas shigelloides* from stool specimens. J Clin Microbiol 2017;55(11):3258–66.

6. Clinical and Laboratory Standards Institute. M100:S30 - Performance standards for antimicrobial susceptibility testing: thirtieth informational supplement. 2020;

7. Jacoby GA. AmpC β-lactamases. Clin Microbiol Rev 2009;22 (1):161–82.

8. Tauxe RV, Vandepitte J, Wauters G, et al. *Yersinia enterocolitica* infections and pork: the missing link. Lancet 1987;1 (8542):1129–32.

9. Bottone EJ. *Yersinia enterocolitica*: the charisma continues. Clin Microbiol Rev 1997;10(2):257.

10. Shoaib M, Shehzad A, Rzaz H, Niazi S, Khan IM, Akhtar W, Safdar W, Wang Z, A comprehensive review on the prevalence, pathogenesis and detection of *Yersinia enterocolitica*. RSC Adv., 2019, 41040-41021.