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CMPT Clinical Bacteriology Program

Innovation, Education, Quality Assessment, Continual Improvement

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Challenge M233-3

November 2023

Stool: *Salmonella* Typhi

HISTORY

A simulated stool sample collected from a 43-year-old returning traveler with diarrhea and fever was sent to category A laboratories.

Participants were expected to isolate and report *Salmonella enterica* serotype Typhi (or *Salmonella* Typhi). *Salmonella* species was considered acceptable.

CMPT QA/QC/STATISTICS

All simulated stool samples are produced at CMPT according to CMPT internal protocols. The sample contained a mixed culture of *Salmonella* Typhi and *Escherichia 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 20 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

Identification: 10/13 (77%) of reference laboratories reported an acceptable response: 4 reported *Salmonella* Typhi; 6 reported *Salmonella* species. One reference laboratory reported *Salmonella* Paratyphi and 2 reported *Salmonella* species not Typhi, which were considered unacceptable responses.

Susceptibility: 11/11 (100%) reference labs reported ampicillin as susceptible, with 2 labs indicated they would refer. 12/12 (100%) reported ciprofloxacin as susceptible; one lab indicated they would refer. 11/11 (100%) labs reported trimethoprim-sulfamethoxazole (SXT) as susceptible; 2 labs indicated they would refer.

Public health notification: 13/13 (100%) labs indicated they would notify Public Health authorities.

Participants

Identification: 42/46 (91%) of reporting laboratories reported an acceptable response, with 8 reporting *Salmonella* Typhi and 33 reporting *Salmonella* species. See Table 1 for detailed reporting.

MAIN EDUCATIONAL POINTS from M233-3

1. Despite few domestic cases in Canada, *Salmonella* Typhi remains an important pathogen globally and is a key cause of fever in returning travellers. Laboratories should be equipped to isolate and identify *Salmonella* Typhi.
2. Definitive serotyping of *Salmonella* isolates is usually performed in specialized reference laboratories. *Salmonella* Typhi should optimally be identified more rapidly in order to facilitate appropriate clinical and public health management.
3. Resistance in *Salmonella* Typhi is on the rise globally; laboratories must be vigilant in testing and detecting isolates with resistance to one or more antimicrobial agents.

Grading

Maximum grade: 16

The identification component of this challenge was ungraded due to lack of consensus amongst reference laboratories.

Reporting the isolate susceptible to ampicillin, ciprofloxacin, and SXT was graded 4 for each component.

Reporting the isolate to PH authorities was graded 4.

Table 1. Identification results

Reported	Total
Salmonella Typhi (± presumptive, ± refer)	8
Salmonella sp. (± presumptive, ± refer)	33
Salmonella sp., group D	1
Salmonella Paratyphi (presumptive, refer)	1
Salmonella species, NOT Typhi (± refer)	2
negative (no *Salmonella* or any other stool pathogen isolated)	1
no report	1
shipping delay, no report	1
refer, or sample not normally processed	6
Total	**54**

Susceptibility: 34/36 (94%) labs reporting *Salmonella* reported the isolate susceptible to ampicillin. 33/35 (94%) reported susceptibility to ciprofloxacin. 34/36 (94%) reported susceptibility to SXT (Table 2).

Public health notification: 44/45 (98%) labs indicated they would notify Public Health (Table 3)

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.

An acceptable response to organism identification was provided by fewer than 80 percent of reference laboratories; therefore, this component was NOT suitable for grading.

Susceptibility testing to ampicillin, ciprofloxacin, and trimethoprim-sulfamethoxazole, and public health notification were correctly performed by at least 80 percent of reference laboratories and greater than 50 percent of all laboratories and were determined to be suitable for grading.

COMMENTS ON RESULTS

Most participants performed well on this challenge. However, the threshold for grading was not met on the identification component due to unacceptable responses from reference laboratories. Two laboratories reported that the isolate was *Salmonella* sp., not Typhi, and another reported that it was *Salmonella* Paratyphi, when the isolate was *S.* Typhi. As serotyping of *Salmonella* species is complex and often performed in specialized laboratories, laboratories may consider stopping the practice of reporting "not Typhi", especially if the isolate Is still pending reference serotyping at a specialized laboratory.

The susceptibility portion of the challenge was performed well, with all reports correctly stating that the isolate was susceptible to the three first-line agents that were graded (ampicillin, ciprofloxacin, trimethoprim-sulfamethoxazole). Laboratories that submitted a report that did not include these agents were graded 0, and should review their antibiotic reporting cascades.

ISOLATION AND IDENTIFICATION

Salmonella taxonomy remains complex; reviews elsewhere discuss this topic in more depth.[1] Almost all *Salmonella* seen clinically are the species *Salmonella enterica*. There are a multitude of serotypes within this species, with different epidemiological and in some cases clinical characteristics. *S.* Typhi and *S.* Paratyphi A are two such serotypes that cause enteric fever.

All *Salmonella* spp. (and serotypes) grow well on routine media, including blood, chocolate, and MacConkey agars. The standard laboratory incubation conditions of 35°C in ambient air are likewise able to support growth of these organisms, with visible colonies by 16-18 hours. To distinguish *Salmonella* (and other stool pathogens) from stool flora, different media have been developed and used: selenite and GN broths, Hektoen media, xylose-lysine-deoxycholate (XLD) media, and *Salmonella*-specific chromogenic media.[2] Notably, for the isolation of *S.* Typhi, selenite broth is less inhibitory than GN broth.[3]

As *Salmonella* spp. are relatively biochemically distinct within *Enterobacterales*, identification of these organisms to the genus level can be done reliably using biochemical tests. *Salmonella* spp. are H_2S producers, motile, negative for urease, and positive for lysine and ornithine decarboxylase. On triple-sugar iron (TSI) slants, they test K/A with gas and H_2S production, though *S.* Typhi and Paratyphi A produce less H_2S than other *Salmonella* serotypes.[4] Automated and semi-automated platforms, including MALDI-TOF, Vitek, and api 20E, can routinely identify *Salmonella* to genus level.[5] The biochemical platforms may identify *S.* Typhi and Paratyphi A due to their phenotypic patterns, and certain MALDI-TOF platforms can differentiate typhoidal from non-typhoidal serotypes as well.[6]

Confirmation of isolates as *Salmonella* should be done by serotyping: genus-specific or group-specific somatic (O) antigens can be used, often with a latex agglutination assay. Determination of *S.* Typhi may be done in clinical laboratories using Vi antigen

Table 2. Susceptibility testing reported results

2A – Ampicillin	Total	Grade
S	34	4
no report	2	0
N/A: did not report ID	3	ungraded
refer, or snnp	15	ungraded
Total	**54**	
2B – Ciprofloxacin	**Total**	**Grade**
S	33	4
no report	2	0
N/A: did not report ID	3	ungraded
refer, or snnp	16	ungraded
Total	**54**	
2C – SXT	**Total**	**Grade**
S	34	4
no report	2	0
N/A: did not report ID	3	ungraded
refer, or snnp	15	ungraded
Total	**54**	

Snnp: sample not normally processed

Table 3. Report to Public Health - Results reported

Public Health Notification	Labs	Grade
Yes	44	4
No	1	0
N/A: did not report Salmonella, or did not process sample	9	ungraded
Total	**54**	

(with boiling pre-testing necessary for some isolates). Nevertheless, *Salmonella* spp. should be referred to public health laboratories for typing and surveillance to identify outbreaks and to have specialized serotyping testing performed.

Nucleic acid detection of *Salmonella*, usually as part of gastrointestinal pathogen panels, is available in many laboratories. The performance of each panel (and different analytes on each panel) varies.[7–9] Virtually all such panels include *Salmonella* and *Shigella* spp., but other targets may differ. Because of the importance of serotyping, culture should be performed on all stools positive by nucleic acid testing in order to obtain isolates for further testing.

Reporting

A preliminary report should generally be issued as soon as *Salmonella* sp. is presumptively identified. Serologic testing can be time consuming and a preliminary report should not wait until this is complete. Salmonellosis is a nationally notifiable disease and all isolates should be notified to public health authorities as soon as possible. Surveillance occurs on a provincial as well as a federal basis.

ANTIMICROBIAL SUSCEPTIBILITY TESTING

Unlike other serotypes of *Salmonella* which may not require susceptibility testing when cultured from stool, all *Salmonella* Typhi isolates should have susceptibility testing performed.

As a reminder, *Salmonella* spp. are intracellular pathogens, and therefore are clinically resistant to all first- and second-generation cephalosporins as well as aminoglycosides, as these agents do not concentrate well in host cells. *Salmonella* isolates should never be reported as susceptible to these agents regardless of any in vitro laboratory testing that is performed.

Guidelines for susceptibility testing are laid out by CLSI, and are the same of those recommended for other *Enterobacterales*.[10] Tier 1 agents (routine primary testing and reporting) for *Salmonella* include ampicillin, trimethoprim-sulfamethoxazole (SXT), ciprofloxacin/levofloxacin, and cefotaxime/ceftriaxone.

Multi-drug resistant (MDR) *Salmonella* Typhi isolates, with resistance to ampicillin, SXT, and chloramphenicol, have been found in many countries especially in South and Southeast Asia. In 2016, extensively drug-resistant (XDR) *S.* Typhi was described in Pakistan, and has since become the dominant strain causing enteric fever.[11] In addition to resistance to the three MDR agents, XDR strains are also resistant to third-generation cephalosporins and fluoroquinolones. Laboratories should be vigilant in resistance testing.

CLINICAL RELEVANCE

S. Typhi and Paratyphi A cause enteric fever, historically called typhoid fever.[12] The cardinal symptom is fever, which may be accompanied by diarrhea, nausea and vomiting, and abdominal pain.[13] Severe disease may result in sequelae including hepatitis, nephritis, GI bleeding, encephalopathy, intestinal perforation, and death.

Humans are the primary reservoir of *S.* Typhi, and infection is from fecal-oral transmission, either directly or through contaminated food or water.[13]

Carriage and shedding of *Salmonella* spp. normally lasts a few weeks to months after resolution of symptoms. Chronic carriage, where patients continue to shed the bacterium a year after the acute illness, is rare (<1%), but is important for disease transmission and is a major public health issue, particularly when it occurs in food handlers.

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