Challenge M242-3

August 2024

Stool - *Shigella sonnei*

HISTORY

A simulated stool sample collected from a 62 year old male, returning from vacation was sent to category A laboratories. Participants were expected to isolate and report *Shigella sonnei.*

CMPT QA/QC/STATISTICS

All simulated stool samples are produced at CMPT according to CMPT internal protocols. The sample contained a pure culture of *Shigella sonnei.*

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 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: 11/12 (92%) labs reported *Shigella* species (7 reported *S. sonnei*, 3 reported *Shigella* species, refer, 1 reported *Shigella* species), 1 reported *Shigella*/Enteroinvasive *E.coli,* 1 lab indicated it does not normally process this type of sample

Public health notification: 12/13 (92%) labs indicated they would notify to PH. I lab indicated they would not.

Participants

Identification: 28/42 (67%) participants reported *Shigella sonnei/Shigella* species, refer; 5 participants reported *Shigella*/Enteroinvasive *E coli* as their identification system is unable to differentiate these organisms.

The 5 participants that reported Shigella/ Enteroinvasive *E. coli* used only Multiplex (Biofire) for organism identification.

Most of the laboratories that reported *Shigella sonnei*, used Vitek (11/23) or complemented the identification with biochemical tests.

Public health notification: 35/36 (97%) labs indicated they would notify PH authorities (Table 2).

MAIN EDUCATIONAL POINTS from M242-3

1. *Shigella* spp. continue to be important enteropathogens and potential causes of outbreaks.
2. Specimens positive for *Shigella* spp on molecular testing should be cultured or referred for culture.
3. Culture is useful to get susceptibility information and to allow further characterization for public health follow up.

Grading

Maximum grade: 8

Reporting *Shigella sonnei/ Shigella* species, refer was graded 4.

Reporting to Public health was graded 4.

Table 1. Identification results

Reported	Labs	Grade
Shigella sonnei ± refer ± presumptive/probable	23	4
Shigella species, refer ± presumptive/probable	5	4
Shigella species	1	3
Shigella/Enteroinvasive Escherichia coli, refer (uses Biofire and would refer for culture)	1	4
Shigella + Enteroinvasive E. coli NAAT (multiplex)	4	3
Shigella species detected by NAAT. Isolate failed to grow for further testing. NSaCYEAP.	1	3
E.coli (presumptive STEC), refer	1	0
E. coli	1	0
no enteropathogens isolated/ NSaShCYE. ± Refer for shiga toxin testing/NSaShCAE, *Staphylococcus aureus* and *Bacillus cereus.*	5	0
refer, sample not normally processed	9	ungraded
Total	**51**	

Table 2. Public health notification

Public Health Notification	Total	Grade
Yes	35	4
No	1	0
n/a, incorrect ID submitted	6	ungraded
refer, sample not normally processed	9	ungraded
Total	**51**	

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 was 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.

COMMENTS ON RESULTS

This challenge demonstrates the increasing impact of molecular testing on enteric testing. Laboratories that isolated *Shigella* spp. or detected *Shigella/*EIEC and indicated that they would culture or refer the specimen for culture were graded 4.

Detection of *Shigella* spp. by culture or using molecular testing without referral was graded 3. Laboratories that did not detect *Shigella* were graded 0.

Of the 42 laboratories that processed the sample, 36 did not indicate the use of NAAT, and of these, 29 (81%) cultured *Shigella* and 28 referred it for further testing. Of the 6 laboratories that used NAAT, all detected *Shigella* but only 1 (17%) indicated they would refer the sample for culture.

Culture is important for susceptibility testing and for public health follow up and detection of outbreaks.

ISOLATION AND IDENTIFICATION

For optimal isolation of *Shigella*, two different selective media should be used: a general purpose plating medium of low selectivity (e.g. MacConkey agar) and a more selective agar medium (e.g. xylose lysine deoxycholate –XLD- agar). Salmonella-Shigella (SS) agar should be used with caution because it inhibits the growth of some strains of *S. dysenteriae*.[1] *Shigella* strains appear as colourless colonies (i.e. lactose- or xylose-nonfermenting) on the isolation media described above. Isolates may be screened using biochemical tests, for example triple sugar iron agar to determine no gas production, and motility media to determine lack of motility.

Isolates that react appropriately with the screening biochemicals should then be identified with a complete set of biochemical tests and should be typed with grouping antisera.[1] *Shigella sonnei* may be differentiated from other *Shigella* species as it is ornithine decarboxylase positive.

The genus *Shigella* includes four species: *S. dysenteriae, S. flexneri, S. boydii*, and *S. sonnei*, also designated groups A, B, C, and D, respectively. Each species is more predominant in different areas of the world. In the United States, approximately 86% of *Shigella* infections are caused by *S. sonnei*, whereas in African and Asian countries, *S. flexneri* is more common.[2]

The MALDI-tof MS is unable to distinguish *Shigella* from *E. coli* probably because of their very similar protein profiles. 16S rRNA gene sequencing does not distinguish between them, and molecular tests, many of which are based on the detection of the pINV invasion plasmid, do not distinguish them as the pINV plasmid is found in both *Shigella* and enteroinvasive *E. coli.*[3]

All *Shigella sonnei* strains originated from a common ancestor about 1500, and there is only a single serotype. It has 5 subtypes, referred to as lineages, but most clinical isolates belong to lineage III.[4]

ANTIMICROBIAL SUSCEPTIBILITY

The CLSI guidelines recommend that when isolates of *Shigella* species are tested, only ampicillin, a fluoroquinolone, and trimethoprim-sulfamethoxazole should be reported routinely.[5]

Resistance has become widespread in *Shigella* isolates because of their ability to disseminate resistance genes through horizontal gene transfer. Resistance plasmids encoding for resistance to a wide range of antimicrobials including ampicillin, sulfonamides, trimethoprim, tetracyclines, ciprofloxacin, macrolides and ceftriaxone have been described resulting in the development of multidrug resistant (MDR) strains. *Shigella* species are intrinsically resistant to first and second generation cephalosporins and aminoglycosides. Rates of resistance to ampicillin and trimethoprim-sulfamethoxazole have been rising over the last decades, making these agents unsuitable for empiric treatment. Resistance to ceftriaxone, ciprofloxacin, and azithromycin have been reported depending on the geographic source of the infection.

In a 2020 study, of 2781 *S. sonnei* tested of isolates from 2011-2018 in the US, 2.9% had decreased susceptibility to azithromycin (MIC ≥32mg/L) and resistance to ampicillin, ciprofloxacin, and cotrimoxazole, defined as MDR, and a further isolate was resistant to ceftriaxone (XDR). MDR isolates increased from 0% in 2011 to 15.3% in 2018. Five more XDR isolates were cultured from passengers on a cruise ship in 2020.[6] Many outbreaks have occurred in the MSM population. In one that involved patients in Australia, the UK, France Belgium and the US, it was hypothesized that resistance may be driven by treatment of sexually transmitted infections using azithromycin and ceftriaxone.[7]

CLINICAL RELEVANCE

Shigellosis is endemic in most developing countries and is the most important cause of bloody diarrhea worldwide. Humans are the only reservoir and Shigella is estimated to have caused around 188 million cases of diarrhea in 2010 of which 62.3 million were in children below the age of 5. It is the second most common cause of death from diarrhea after rotavirus, accounting for 164,300 deaths annually, of which 54,900 were in children below the age of 5 in south Asia and Sub Saharan Africa, an improvement of the rates seen in previous times.[8]

In developed countries, such as the US, person to person transmission predominates, and is manifested by institutional outbreaks. Transmission is by the fecal-oral route and is facilitated by the very low infectious dose (10 to 100 organisms).[9] There have been a number of outbreaks in daycare centres in the US.[10,11] Contamination of food or water occurs where there is poor sanitation, with poor sewage and overcrowding. This route is important in much of the developing world, in addition to person to person spread. Flies may act as mechanical vectors. Transmission during sexual activity is important in men that have sex with men (MSM), and may result in localized outbreaks.[12]

After an incubation period of up to 8 days (most commonly 1-4 days), patients typically present with fever, headache, malaise, anorexia and vomiting followed by initially watery diarrhea, which may progress to the frequent passage of small liquid stools that may contain visible blood, with or without mucus with abdominal cramps and tenesmus.[13]

Although most patients recover uneventfully within seven to ten days, serious complications may occur, including: metabolic abnormalities, sepsis, convulsions, rectal prolapse, toxic megacolon, intestinal perforation, and, with *S. dysenteriae* infection, haemolytic-uremic syndrome.[8]

Shigella is able to survive stomach acidity as they are relatively acid resistant. They multiply in the small bowel as they pass through. Once in the large bowel, they cause infection by inducing uptake by M cells in the bowel, and after causing the death of macrophages, invade the tissues and induce an inflammatory response causing further tissue damage and further invasion by disruption of the epithelium. Epithelial invasion occurs from the tissue aspect, rather than the bowel lumen aspect.

The inflammatory response with disruption of absorption is likely to be responsible for the initial watery diarrhea experienced by many patients. As the inflammation continues, blood and pus are produced, leading to a dysentery.

TREATMENT

Supportive treatment of shigellosis with rehydration is important. The use of anti-motility agents should be avoided. Although shigellosis is a self-limiting condition in most cases, effective antimicrobial therapy is important for reducing the duration of symptoms and the transmission to close contacts.

Susceptibilities should always be done on isolates of Shigella, and most authorities would recommend treating all Shigella-positive patients. Even patients whose disease is mild or resolving may benefit from treatment by eliminating shedding and the consequent risk of transmission. Empiric treatment may be required until the results of susceptibility testing are available to guide therapy.

Recommendations for the treatment of shigellosis in adults (>17 years of age) differ from those for children.[13,14] In adults ciprofloxacin is the first line of treatment, with azithromycin as an alternative. In children, azithromycin is first line of treatment, while cefixime is an alternative. Ciprofloxacin is effective and may be used as empiric treatment, especially for severe disease or treatment failure. Although quinolones have been reported to cause arthropathy in immature animals, the risk of joint damage in children appears to be minimal and is clearly outweighed by the value of these drugs for treatment of this potentially life-threatening disease.[8]

If oral treatment is not possible, or there is bacteremia, ceftriaxone or ciprofloxacin may be used parenterally.

Traa et al. (2010) reviewed the scientific evidence supporting the WHO-recommended antibiotics ciprofloxacin or ceftriaxone for the effective treatment of dysentery. Extrapolating clinical failure to mortality, their meta-analyses indicated that >99% of dysentery deaths can be prevented with ciprofloxacin or ceftriaxone.[15]

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