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Salmonella, Shigella, and *Yersinia*

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Synopsis

Salmonella, Shigella, and *Yersinia* cause a well-characterized spectrum of disease in humans, ranging from asymptomatic carriage to hemorrhagic colitis and fatal typhoidal fever. These pathogens are responsible for millions of cases of food-borne illness in the U.S. each year, with substantial costs measured in hospitalizations and lost productivity. In the developing world, illness caused by these pathogens is not only more prevalent, but is also associated with a greater case-fatality rate. Classical methods for identification rely on selective media and serology, but newer methods based on mass spectrometry and PCR show great promise for routine clinical testing.

Keywords

Salmonella; *Shigella*; *Yersinia*; Enteric; Gram-negative bacilli; gastroenteritis

Introduction

In this review, we discuss three enteric pathogens: *Salmonella, Shigella*, and *Yersinia*. These important members of *Enterobacteriaceae* are responsible for significant morbidity and mortality, causing diarrhea and a spectrum of associated symptoms from mild to severe in most parts of the world. In this review, we cover infection and epidemiology, taxonomic classification, collection, transport, and storage of specimens, culture techniques, molecular detection methods, susceptibility testing, and treatment. Discussions that pertain to individual organisms are organized into individual sections starting with *Salmonella*, followed by *Shigella*, then *Yersinia*. Topics common to all three (such as collection, transport and storage of specimens, and molecular multiplex methods of detection) are discussed as they first occur, denoted as such in paragraph headings. Throughout, priority of discussion is placed on newer techniques and data, with less emphasis on details of classical methods available in reference textbooks.

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Salmonella Introduction

Members of the genus *Salmonella* cause a well-characterized spectrum of disease in humans, ranging from asymptomatic carriage to fatal typhoidal fever. In the developed world, food-borne acute gastroenteritis and enterocolitis are the most common forms of *Salmonella* infection, with an estimated 1.2 million annual cases of non-typhoidal Salmonellosis occurring in the U.S.[1–3] Though relatively uncommon in the U.S., typhoid, paratyphoid, and enteric fever constitute a very serious global public health problem, with 25 million new infections and >200,000 deaths occurring annually.[4, 5]

Salmonella is a member of the *Enterobacteriaceae*, originally characterized by their ability to metabolize citrate as a sole carbon source and lysine as a nitrogen source, as well as their ability to produce hydrogen sulfide.[6] However, classical biochemical testing alone does not unambiguously distinguish key pathogenic members of this genus and modern classification relies instead on serology and increasingly on molecular methods.

Salmonella Disease Manifestations

Infection with *Salmonella* typically follows two very different disease courses, depending on whether the infecting *Salmonella* strain is a typhoidal or non-typhoidal serovar. Infection with non-typhoidal serovars ordinarily presents as diarrhea associated with fever and abdominal cramping 12–72 hours after infection.[7] In most cases in healthy individuals, this infection runs a self-limited course over 4–7 days but, in susceptible hosts, certain non-typhoidal strains of *Salmonella* may spread systemically to other sites in the body. Though this is more common in those with compromised immune systems or underlying medical conditions (e.g., sickle cell anemia), systemic spread of non-typhoidal *Salmonella* strains may be seen in otherwise healthy individuals as well.

In contrast to infection with non-typhoidal *Salmonella*, infection with typhoidal strains (primarily serovars Typhi and Paratyphi) presents as a systemic, often serious, disease. After invading through intestinal mucosa, typhoidal strains disseminate through a transient primary bacteremia that may occur without diarrhea.[5] Following hematogenous dissemination, some individuals will develop typhoid fever, which involves high temperature (>39° C), vomiting, and headache, sometimes with complications that include neurologic involvement, intestinal perforation and death.[5]

Salmonella Taxonomic classification

The classification of the salmonellae has a complicated history, resulting in part from multiple independent investigators using phenotypic, serologic, and genotypic methods to characterize phylogenetic relationships within the genus, and in part from disagreements on nomenclature. The most recent consensus defines a classification scheme that recognizes two principle species of *Salmonella*: *S. enterica* and *S. bongori* (Figure 1). In this scheme, *S. enterica* is further classified into six subspecies: Subspecies I, or *S. enterica* subsp. *enterica*; Subspecies II, or *S. enterica* subsp. *salamae*; Subspecies IIIa, or *S. enterica* subsp. *arizonae*; Subspecies IIIb, or *S. enterica* subsp. *diarizonae*; Subspecies IV, or *S. enterica* subsp. *houtenae*; and Subspecies VI, or *S. enterica* subsp. *indica*.[8–10] Recent sequence analysis has

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shed light on the genetic relationships within this genus and has largely supported the above classification scheme.[4, 11–20]

The seven principle members of the *Salmonella* genus can be further subtyped by serologic methods, based on three antigens: O, H, and Vi. The serologic typing scheme identifies >2500 serovars.[21] The resolution provided by serologic typing methods has proved valuable to epidemiologic tracking of isolates in outbreaks. Given that *S. enterica* subsp. *enterica* strains constitute the majority (as much as 99.5%) of isolates cultured from humans and other warm-blooded animals, it is perhaps not surprising that the majority of disease causing serovars belong to this subspecies.[6, 21] In contrast, *S. bongori* and the other members of *S. enterica* are more commonly isolated from cold-blooded animals and environmental sources, and Salmonellosis caused by serovars representing these other species is relatively rare, though infections do occur.

Collection, Transport and Storage of Specimens for Detection of *Salmonella/Shigella/Yersinia*

For laboratory diagnosis in cases of gastrointestinal disease, fecal specimens should be collected at the early stages of illness, ideally before antibiotics have been initiated.[6, 22] Whole stools are the preferred specimen for culture, and examination of multiple specimens may improve the recovery of *Salmonella/Shigella/Yersinia*.[23] Consultation with the laboratory may be required where specimen rejection rules do not allow for serial cultures. Most commonly used pH-buffered stool transport media are compatible with recovery of *Salmonella/Shigella/Yersina*, though Cary-Blair transport medium is preferred by many labs, due to its compatibility with other common stool pathogens.[6, 22]

Fecal specimens should be examined immediately on receipt, or stored at 4° C if plating for culture or inoculation of broth will be delayed for greater than 1–2 hours following collection.[6, 22] However, it should be noted that refrigeration of specimens containing *Shigella* in non-pH-buffered transport media may decrease recovery in culture.[22] In cases of suspected systemic spread, as with typhoidal *Salmonella*, cultures from other sources (blood, bone marrow, lymph node, and bone biopsy) that may be submitted should be collected and transported according to standard procedures appropriate to these specimen types.

Salmonella Culture and Isolation

Stool culture is the most common source from which non-typhoidal serovars of *Salmonella* are recovered. Non-typhoidal strains of *Salmonella* may also be recovered from blood and tissue (lymph node, bone marrow, etc.) in cases with systemic spread. Typhoidal strains may be more easily isolated from cultures of extra-intestinal sites than from fecal cultures.[6]

Salmonella may be cultured on a variety of solid media. Typically, two selective and differential media, one of which is highly selective, are inoculated with the stool specimen. Hektoen and xylose-lysine-deoxycholate (XLD) agars are highly selective and both detect H_2S production, facilitating identification of *Salmonella* species. More highly selective agars, including Salmonella-Shigella, bismuth sulfite, and brilliant green agars may inhibit some strains of *Salmonella* sp., and thus are often used in combination with a less selective

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agar.[22] For this reason, a less selective differential enteric medium, such as MacConkey or eosin methylene blue, and a nonselective medium, such as 5% sheep blood agar may be inoculated in addition as part of the stool culture work up, depending on lab preference.[22] However, the growth of fecal flora on non-selective agars may obscure *Salmonell*a colonies that are present in low numbers.

Stool may be inoculated into enrichment broths in addition to plated cultures. Enrichment broths serve to allow the growth of *Salmonella* while suppressing the growth of normal fecal flora, and thereby can improve recovery yield. Two commonly used enrichment broths are tetrathionate broth and selenite broth.[22] Once isolated, *Salmonella* should be sub-cultured using standard techniques to obtain colonies for identification and susceptibility testing (if indicated), as well as for submission to the local public health laboratory.

Identification Methods for *Salmonella*

Once *Salmonella* has been isolated in culture, there are a variety of methods available for identification and classification.[6] Definitive identification ordinarily relies on a combination of phenotypic and serologic methods. The widely-used Kauffmann-White serologic typing scheme is based on the LPS O antigen, the H1 and H2 flagellar antigens, and the Vi antigen.[21] While the O and H1 antigens are detectable in almost all strains of Salmonella, the H2 antigens are present only in certain strains and the Vi antigen is found predominantly in typhoidal strains.[6] It should be noted that Vi, though useful for detection of serovar Typhi, may also be expressed in *Citrobacter* sp. and therefore the Vi antigen alone cannot be used for definitive identification.[6]

O/H/Vi typing by the Kauffmann-White scheme yields greater than 2500 serovars, which may be designated by antigenic formulae expressed with the following convention: O antigen(s), Vi antigen if present: phase 1 H antigens(s):phase 2 H antigen(s) if present [6, 21]. In the Kauffman-White scheme, the >1500 serovars from *S. enterica* subsp. *enterica* receive names; those from the other subspecies of *S. enterica* and *S. bongeri* are referred to only by formulae.[21] By convention serovar names are capitalized and not italicized, and it is not necessary to include the *enterica* subspecies designation when referring to the serovar, as only subspecies *enterica* serovars are named. Thus both *S. enterica* subsp. *enterica* ser. Enteriditis and *S. enterica* ser. Enteriditis are correct designations [21]. It is important to note that serovars do not have taxonomic status and should not be confused with species or subspecies of *Salmonella*.

As stated above, the majority of identified serovars and the majority of human pathogens belong to *S. enterica* subsp. *enterica*. Some serovars, such as Typhi and Paratyphi are largely restricted to humans. Other serovars are generally restricted to particular zoonotic reservoirs and only occasionally cause human infection. However, infections caused by these serovars may be severe with systemic spread. These species include Derby and Choleraesuis (pig-adapted), Saintpaul (poultry-adapted), and Dublin (bovine-adapted).[6] Yet other serovars, particularly Typhimurium and Enteritidis pass between human and zoonotic reservoirs with ease, perhaps accounting for their high prevalence. CDC Survey data from 2011 suggests that the three most commonly reported serovars in the U.S. during the reporting period were Enteritidis, Typhimurium, and Newport.[24] Non-typhoidal

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Salmonellosis was traditionally associated with meat and poultry products, but more recently outbreaks have been caused increasingly by produce.[1–3]

A number of techniques now exist for screening of primary specimens and enrichment broths before isolated colonies are available for the above methods. Selenite enrichment broth cultures may be screened by the Wellcolex Color *Salmonella* (Remel Inc., Lenexa, KS), a serologic agglutination-based method.[25] There are important limitations in sensitivity and specificity that one must be aware of with the Wellcolex test; in particular, expression of Vi antigen in *Citrobacter* sp. and may lead to false positives with this assay and not all *Salmonella* sp. are identified by this test.[26]

Antimicrobial Susceptibility Testing for *Salmonella*

Current CLSI guidelines [27] state that routine susceptibility testing is indicated for typhoidal *Salmonella* serovars (Typhi and Paratyphi A-C) from all sites, and for non-typhoidal serovars from extra-intestinal sites. Fecal isolates should be tested for susceptibility to ampicillin, a fluoroquinolone, and trimethoprim-sulfamethoxazole. For extra-intestinal isolates of *Salmonella*, a third-generation cephalosporin should be tested and reported additionally, and chloramphenicol may be tested and reported if requested. It should be noted that 1st and 2nd generation cephalosporins, cephamycins and aminoglycosides may appear active *in vitro* against *Salmonella*, but are not effective clinically and should not be reported as susceptible.[27]

Current CLSI breakpoints for ciprofloxacin and levofloxacin for *Salmonella* are lower than for other *Enterobacteriaceae*.[27] CLSI recommends that laboratories unable to implement the current (lowered) MIC breakpoints for fluoroquinolone testing should use nalidixic acid to test for reduced fluoroquinolone susceptibility. Strainsresistant to nalidixic acid may be associated with clinical fluoroquinolone failures. *Salmonella* strains producing ESBL beta-lactamases and NDM-1 type carbapenemases have been reported, as well as *S. enterica* ser. Typhi isolates with chromosomally-integrated multi-drug resistance islands.[28–30] It is therefore important for the laboratory and clinicians to be aware of the possibility of multi-drug- and carbapenem-resistant isolates.

Identification of *Salmonella/Shigella/Yersinia* by Mass Spectrometry

Molecular methods, including mass spectrometry and PCR-based multiplex panels have been developed for the detection of enteric bacteria, and some laboratories are beginning to incorporate these techniques. These newer methods have the potential to improve our ability to provide rapid and accurate bacterial identification, but they have limitations that are important to understand. Commercial matrix assisted laser desorption ionization – time of flight mass spectrometry (MALDI-TOF MS) instruments can provide rapid identifications of *Salmonella* and *Yersinia*, and limited identification of *Shigella*.[31, 32] Both the Bruker Biotyper and Biomerieux Vitek MS MALDI-TOF MS systems are FDA approved for *in vitro* diagnostic use to identify cultured isolates of *Y. enterocolitica* and *Y. pseudotuberculosis* (species level identification) and *Salmonella* (genus level identification), but not *Shigella*. The Biomerieux Vitex MS system carries a manufacturer's note that confirmatory testing is recommended when identifications of *Salmonella* are made. Neither

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system is FDA approved for species-level or serovar-level classifications of *Salmonella*. However, recent literature suggests that MALDI-TOF mass spectrometry-based systems may have the power to distinguish among subspecies and some *Salmonella* serovars.[33] As this technology is new and experience is still relatively limited, caution is appropriate, as illustrated by a recent report of a patient with both bacteremia and gastrointestinal symptoms for whom an isolate of *Y. pseudotuberculosis* was misidentified as *Y. pestis* using a MALDI-TOF MS method. This highlights the need for comprehensive MALDI-TOF MS spectral databases in combination with careful validations and appropriate additional confirmatory testing for this group of less commonly seen bacteria.[34]

Pulsed field gel electrophoresis (PFGE) has been used extensively for epidemiologic typing of strains of *Salmonella*. Recently, a variety of molecular methods have been developed for distinguishing among strains of *Salmonella/Shigella/Yersina*, including multi-locus sequence typing approaches, and PCR-based approaches.[35–42] Whole genome sequencing-based approaches have also been used, facilitated by published reference genomes.[13, 14, 43–45]

PCR-Based Molecular Methods to Detect *Salmonella/Shigella/Yersinia*

Recently, a number of PCR-based multiplex GI pathogen identification panels have been marketed for use with primary stool specimens. These include the bioMerieux Biofire Filmarray system,[46] BD MAX system,[47] Luminex xTAG system,[48, 49] Savyon Diagnostics system,[48] and Genetic Signatures system.[50] These panels allow rapid identification of *Salmonella, Shigella*, and *Yersinia* from primary stool specimens, and offer substantially improved turnaround time on primary laboratory diagnosis compared with culture-based methods. Recovery of isolates from culture is still required for taxonomic classification and susceptibility testing. It should be noted that none of the commercially available multiplex PCR panels include *Y. pseudotuberculosis* in the target list.

Though experience with these new panels is still relatively limited, a number of recent publications have begun to shed light on their performance. Wessels et al. found that in comparison with conventional diagnostic methods, the Luminex xTAG panel reported almost twice as many pathogen identifications.[51] However, not all of these positive results were confirmed by independent PCR assays, including one *Shigella* sp. and four *Salmonella* sp. out of a total of 83 positives reported by the instrument. The authors therefore recommended that confirmatory testing be performed before reporting *Salmonella* with this method. A study of Luminex xTAG ASR reagents demonstrated sensitivity for *Salmonella* of 92% and for *Shigella* of 93%.[52] Conclusions regarding the sensitivity of this assay for detecting *Yersinia* sp. were limited by the inclusion of only 3 isolates.[52] A study comparing the Biofire FilmArray and Luminex xTAG systems demonstrated higher positivity rate with both assays compared to routine methods; however, presumed false positives were observed, consistent with other studies.[46] The Genetics Signatures EasyScreen system tested in Australia showed very good correlation of results between conventional and molecular methods for *Salmonella*, but evaluation of performance for the detection of *Shigella* and *Yersinia* was limited by the small numbers of isolates of these genera included in the study.[50] Adequate validation of these multiplexed panels can be quite difficult due to the

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number of pathogens that must be tested, and the need to verify all presumed false positives with another equivalently-sensitive method. Significant testing and experience should be accumulated before a lab considers replacing traditional methods with a single molecular method.

Another approach under investigation is PCR electrospray ionization mass spectrometry (PCR-ESI-MS). Pierce et al. evaluated a PCR-ESI-MS-based assay for testing of enteric pathogens in food samples that employed broad-range oligonucleotide primers targeted to highly conserved regions in the genomes of target organisms [53]. *Salmonella* was detected in samples tested by this method with high sensitivity, but *Shigella* was detected in only 81% of cases. As with MALDI-TOF MS-based methods, PCR-ESI-MS-based assays are very dependent on the completeness of the database. In this study, identification of bacteria from food samples required the use of selective enrichment media and additional incubation time. Further work is needed to incorporate this method into routine use by food safety investigators.[53]

Shigella Epidemiology, Disease Manifestations & Treatment

Symptoms of *Shigella* infection include fever, malaise, watery diarrhea, cramping abdominal pain, and myalgia. The incubation period is 1–4 days, and the illness often resolves in 5–7 days. After 2–3 days, the volume and frequency of diarrhea may decrease to be replaced with blood and mucus in feces (dysentery), along with straining. Some individuals may not have symptoms, but can still transmit the bacteria to others.[54] Although not as frequent as gastrointestinal disease, there are multiple reports of bacteremia and other extraintestinal infections.[55–57] Complications of *Shigella* infection include hemolytic uremic syndrome, and reactive arthritis.[58]

The worldwide incidence of shigellosis has been reported to be approximately 165 million cases, but the mortality has decreased substantially over the past three decades.[59–61] Though the causes of this decrease in shigellosis-associated mortality are likely to be multifactorial, it has occurred along with a decrease in prevalence of *S. dysenteriae* type 1.[61] Infection with *S. dysenteriae* type 1 carries relatively high mortality in the developing world, as demonstrated by the case fatality rate of 5–15% in Africa and Central America. There are an estimated 500,000 cases of shigellosis per year in the U.S. with 38 deaths.[62]

The only known natural reservoirs of *Shigella* are humans and large primates. Shigellosis is highly infectious, with a minimum inoculum of only 10–100 organisms required to cause disease.[63] Outbreaks often occur in the summer months. Transmission is through the fecal-oral route, with spread occurring person-to-person, and through consumption of water or food contaminated with feces from infected individuals. Not surprisingly, shigellosis seen more commonly under conditions that facilitate spread of bacteria through the fecal-oral route, such as in daycare centers or in areas without indoor plumbing. Outbreaks have additionally been described among people in custodial institutions, orthodox Jews, international travelers, and men who have sex with men.[64–68] Asymptomatic infection is associated with the spread of disease and prolongation of outbreaks due to silent transmission. Severe intestinal and extra-intestinal manifestations can occur with all four species of *Shigella*, but are most common with *S. dysenteriae* type 1, due in part to the

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production of Shiga toxin.[69] Infections due to *S. dysenteriae* are often acquired by international travel and are often multidrug resistant.[70] Shigellosis due to *S. boydii* is uncommon, and limited to the Indian subcontinent.[71] *S. sonnei* is endemic in the United States and other developed countries. Infections associated with *S. sonnei* tend to be mild or asymptomatic.

Antimicrobial therapy has been demonstrated to decrease the duration, transmission, and severity of symptoms. A decision to treat is based on severity of symptoms, and on the desire to reduce spread, balanced against the goal to reduce the development of more resistant isolates. In the U.S., shigellosis is reportable to state-level public health labs, and *Shigella* resistance is tracked by the National Antimicrobial Resistance Monitoring System for Enteric Bacteria, a collaboration of multiple U.S. government agencies.

Shigella Laboratory Identification

Shigella, a member of the *Enterobacteriaceae* family, is classified into four serologic subgroups: *S. dysenteriae* (Group A), S. *flexneri* (Group B), *S. boydii* (Group C), and *S. sonnei* (Group D). Though many authors have treated these subgroups of *Shigella* as distinct taxonomic species, *Escherichia coli* and *Shigella* are very similar genetically (80–90%), and the argument has been made that almost all *Shigella* strains could be considered a biotype of *E. coli*.[72] *S. boydii* 13 is the exception and has been reclassified as *Escherichia albertii*.[73, 74] Because of their substantial genetic similarity, distinguishing *Shigella* from *E. coli* often presents a challenge for the clinical microbiology laboratory. However, though they are genetically similar, *Shigella* and non-Shiga toxin-producing strains of *E. coli* demonstrate different clinical behavior, and their distinction in the microbiology laboratory facilitates their continued epidemiological tracking as distinct entities.

To optimize detection of *Shigella* in stool, samples should be plated on MacConkey and either xylose-lysine-deoxycholate, Hektoen enteric, or deoxycholate citrate agar. Colonies are bluish-green on Hektoen agar and do not have the black center seen with *Salmonella*, as *Shigella* do not produce H2S. *Shigella* do not ferment lactose and xylose and are relatively inert biochemically. Some strains of *S. sonnei* are exceptions and may ferment lactose. Most isolates do not produce gas, except some *S. flexneri*.[6, 75] Appropriate colonies can be characterized further using Kligler iron or triple sugar iron agar. Lysine decarboxylase tests are typically negative for *Shigella*. Groups A, B, and C have similar biochemical characteristics, but *S. sonnei* has ornithine decarboxylase activity and beta-galactosidase activity.[75]

Serologic typing should be performed on *Shigella* isolates. Subgroup A (*S. dysenteriae*) has 17 serotypes, subgroup B (*S. flexneri*) has 14 serotypes further divided into subserotypes, subgroup C (*S. boydii*) has 19 serotypes, and subgroup D (*S. sonnei*) is a single serotype.[76, 77] Confirmation of identification and serotype should be performed at a reference lab or public health lab for isolates from extraintestinal infections, or when the primary lab does not have full serotyping capabilities. Enteroinvasive *E. coli* are evolutionary intermediates between *E. coli* and *Shigella*.[78] Some of the enteroinvasive *E. coli* (EIEC) may cross-react with *Shigella* anti-sera. It should be noted that serological testing of the serum of an infected individual is generally not useful for diagnosis.

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Active or typical *E. coli* strains can be distinguished from *Shigella* by several tests, including lysine decarboxylase, motility, gas production, acetate utilization, and mucate and lactose fermentation. However, inactive strains of *E. coli* can have overlapping reactions with *Shigella*. Inactive strains of *E. coli* that are nonmotile, anerogenic biotypes are called Alkalescens-Dispar bioserotypes. In order to address this issue, different laboratories have developed different algorithms for the work-up of *E coli* and *Shigella* in stool specimens; however, many labs do not employ procedures to distinguish *E. coli* from *Shigella* when isolated from other body sites.

Given that *E. coli* is a much more common isolate to recover from most sterile site cultures than *Shigella*, and given that many labs restrict procedures to distinguish *Shigella* from *E. coli* to stool specimens, a *Shigella* occurring in a sterile site specimen may be misidentified as an *E. coli*. This is especially likely if identification is dependent on an automated system, as commercial systems such as the Vitek 2 have a high rate of misidentification.[79] There are a number of reports examining API20E strips for the identification of *Shigella*, with varying reports of accuracy.[80–83] If a *Shigella* isolate from an extra-intestinal site were misidentified as an *E. coli*, the reflex susceptibility performed and reported would be that appropriate for an *E. coli* isolate. However, the CLSI guidelines differ for the two organisms, with first and second generation cephalosporins, cephamycins, and aminoglycosides excluded from testing for *Shigella* isolates, as false *in vitro* susceptibility my occur.[27] As a consequence, a *Shigella* misidentified from a site such as blood, could mistakenly be treated with an ineffective agent (e.g. aminoglycoside), with possible clinical failure. Furthermore, such a case could be missed in the tracking of *Shigella* incidence, unless *Shigella* was isolated from a concurrent stool specimen. An algorithm to distinguish *E. coli* and *Shigella* is presented in Figure 2.[72]

Given the long-standing difficulty in distinguishing *Shigella* from *E. coli*, some newer methods have been evaluated. Khot and Fisher applied ClinProTools software from Bruker Daltonics to develop and algorithm to distinguish *Shigella* from *E. coli* using MALDI-TOF mass spectrometry. The 3% rate of misidentification these authors achieved is better than that seen with automated biochemical instruments. However, given the still significant error rates, such an approach does not obviate the need for traditional biochemical approaches.[84]

Guidelines have been written to test all stool of all patients with acute, community-acquired diarrhea for Shiga-toxin producing *E. coli*, because selective testing strategies such as testing only bloody stool, or stool from children, or during summer months, fail to detect all positive samples.[85] Due to low prevalence, not all hospitals have chosen to test all such stool samples. The Shiga toxin produced by S. *dysenteriae* type 1 is very similar to the Shiga toxin 1 produced by some enterohemorrhagic *E. coli*, so some rapid assays may detect the toxin from either *E. coli* or this specific *Shigella* species.[86–88] *Shigella*s that do not produce the toxin should not be detected with those assays. It should be noted that Shiga toxin-encoding genes can be detected in healthy volunteers by PCR of fecal samples, so correlation with clinical symptoms and culture of an isolate are essential for diagnosis when using this type of molecular assay.[89]

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Shigella Susceptibility Testing

Shigella solates should be tested against ampicillin, a fluoroquinolone, and trimethoprim-sulfamethoxazole.[27] There is significant resistance to ampicillin and trimethoprim-sulfamethoxazole.[68, 90] In Asia, resistance has been reported to ciprofloxacin, pivmecillinam, and azithromycin, with some emerging resistance to third-generation cephalosporins and additional drugs.[91–96] A recent report from studies in Bangladesh show an increase in resistance to ciprofloxacin from 0% in 2004 to 44% in 2010.[97]

Shigella Virulence Mechanism

Shigella pathogenesis involves translocation through ileal and colonic M cells, uptake by macrophages, basolateral invasion of epithelial cells, and dissemination within the mucosa.[98, 99] *Shigella* has a large virulence plasmid, encoding a Type III-secretion system and a set of secreted proteins. The system results in the injection of virulence factors into host colonic epithelial cells, leading to damage to the epithelial lining, as well as proteins that antagonize the adaptive and innate immune response.[98, 100] *Shigella* virulence is enhanced by the presence of enterotoxins, which is separate from the Shiga toxin produced in limited strains.[101] The genes encoding the Shiga toxin genes are located in the genome of heterogeneous lambdoid prophages. These are highly mobile genetic elements that play a role in horizontal gene transfer and genome diversification.[102] Shiga toxin is made by the *Shigella dysenteriae* type I strains and *E. coli* O157:H7 among other *E. coli* shiga toxin-producing strains. Shiga-like toxin II, also seen in some *E. coli* strains is 56% homologous with Shiga toxin (or Shiga-like toxin I).[103]

Shigella Vaccine

The declining incidence of shigellosis with increasing age suggests that natural immunity develops, implying that vaccines eliciting this natural response may be effective. There is currently no licensed *Shigella* vaccine, but there are multiple vaccines are in preclinical stages or clinical trials, including live attenuated, killed whole-cell, conjugate, and subunit vaccines.[104] Vaccines have been designed against the O antigens, and also conjugate vaccines have been developed using several different conserved antigenic molecules.[101] Data are limited regarding the immune parameter that correlates with protective immunity.[77] More studies are needed before we will know if a *Shigella* vaccine will prove effective.

Yersinia Epidemiology & Disease Manifestations

Yersinia are zoonotic agents distributed worldwide and there are both pathogenic and nonpathogenic strains.[105] *Yersinia* species associated with human disease include *Y. pseudotuberculosis, Y. enterocolitica*, and *Y. pestis*. Of these clinically significant species, we will discuss only *Y. enterocolitica* and *Y. pseudotuberculosis*, as this review is focused on enteric pathogens. Disease due to *Y. enterocolitica* manifests as terminal ileitis, lymphadenitis, and acute enterocolitis due to ingestion of contaminated food or water. Symptoms typically develop 4–7 days after exposure and may last 1–3 weeks. Complications include skin rash, joint pains, and bacteremia.[106] Right-sided abdominal pain in some cases of *Y. enterocolitica* infection may be confused with appendicitis. Illness may include erythema nodosum, polyarthritis, and less commonly septicemia or

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endocarditis.[107–110] Reactive arthritis is an uncommon complication, but may be seen in especially in immunocompromised patients and those with the HLA-B27 allele. Associated disorders also include inflammatory bowel disease and autoimmune thyroid disorders. Iron overload due to underlying conditions, such as hereditary hemochromatosis or beta thalassemia, or treatment with deferoxamine, leads to an increased susceptibility to septicemia manifestations.[105, 111]

Infections with *Y. enterocolitica* are more common in the winter months and often seen in young children. There is one culture-confirmed case of *Y. enterocolitica* per 100,000 individuals each year in the United States. The CDC tracks foodborne disease with the surveillance network, FoodNet. The source of infection can include contaminated prepackaged deli meats, undercooked pork, unpasteurized milk, or untreated water. Preparation of chitterlings (large intestines of pigs), a dish for holiday meals, has been associated with multiple cases of infection in infants, due to caregivers handling of the contaminated food[112–117] This organism is able to multiply at refrigerated temperatures, contributing to its role in food products and transfusion-related infections.[118]

Y. pseudotuberculosis is endemic in a variety of animals, including fowl. *Y. pseudotuberculosis* usually produces a self-limited disease. The infection can manifest as mesenteric lymphadenitis, and may also be confused with appendicitis.[119] Septicemic illness is rare and if seen, would most likely occur in someone with underlying disorders that increase susceptibility to severe infection.[120–122] *Y. pseudotuberculosis* has been reported as a foodborne pathogen.[123, 124]

Yersinia Laboratory Identification

Yersinia is a gram-negative bacillus in the Enterobacteriacae family. There are 11 species of *Yersina*, but only three are clearly human pathogens.[75] Genome sequencing reveals that *Y. pestis* and *Y. pseudotuberculosis* are closely related, but significantly different than *Y. enterocolitica*.[125] *Yersinia* can appear small and coccobacillary in Gram-stained smears. It exhibits bipolar staining described as a safety pin shape on Giemsa staining. *Yersinia* grow on blood, chocolate, and MacConkey agar, but may be overgrown by other organisms due to slow growth. *Yersinia* can form pinpoint colonies on both blood agar and MacConkey agar in 24 hours, particularly *Y. pseudotuberculosis*. *Yersinia* are catalase positive, oxidase negative and ferment glucose. *Yersinia* species have an alkaline over acid pattern on Kligler Iron Agar and are nonmotile at 36°C, but motile at 22°C. Optimal growth is observed at 25–32°C. Longer incubation reveals grey-white, 1–2 mm, convex colonies. *Y. enterocolitica* appears as small, lactose-negative colonies on MacConkey in 48 hours. Selective media, such as cefsulodin-irgasan-novobiocin (CIN) and incubation at lower temperatures can enhance detection. Colonies appear as a bull's-eye with a red center on CIN agar, although some other bacteria will also give this appearance. Use of eosin methylene blue agar and triple-sugar iron agar may not result in a clear distinction from other coliforms. *Y. enterocolitica* incubated at room temperature will be more biochemically active and better identification at this temperature may be obtained with some systems such as API 20E, because commercial systems such as Vitek may fail to identify these organisms under routine conditions.[126, 127]

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Y. pseudotuberculosis is a pleomorphic gram-negative bacillus that can grow at temperatures ranging from 4°C to 43°C, with optimal temperatures between 25–28°C.[128] Enteric *Yersinia* species can be distinguished biochemically; ornithine decarboxylase, sucrose, and sorbitol are all positive for *Y. enterocolitica* and negative for *Y. pseudotuberculosis*. Enzyme-linked immunosorbent assays and lateral-flow assays exist, but are not commonly used for routine labs, instead being used primarily in research.

Yersinia Serogroups

Of the six biovars of *Y. enterocolitica* (1A, 1B, 2, 3, 4), , five are pathogenic for humans.[129] It has been proposed that biotype 1A strains may represent a potential group of emerging pathogens. They can be identified in clinical specimens, but clear association with human disease has not been established.[130] Serology has been used in investigations of plague infections caused by *Y. pestis*, in cases when an organism was not recovered in culture. However, serology for *Y. enterocolitica* and *Y. pseudotuberculosis* is somewhat limited by cross-reactions due to infections with other bacterial species, and due to human exposure to nonpathogenic *Yersinia* species.

Yersinia Virulence

One virulence factors of *Yersinia* is yersiniabactin , a siderophore iron uptake system present in virulent *Yersinia*, including *Y. pestis*, *Y. pseudotuberculosis*, and *Y. enterocolitica* biotype 1B. The genes involved in the biosynthesis, transport, and regulation of yeriniabactin are located on a mobile genetic element, the high-pathogenicity island. This mobile element has contributed to the horizontal spread of yersiniabactin genes into other Enterobacteriacae. In addition, *Yersina* species contain alternative iron siderophore scavenging systems, with some strains containing all three systems, yersiniabactin, pseudochelin, and yersiniachelin.[131]

Y. pseudotuberculosis has a virulence plasmid, pYV, that carries a type III secretion system that forms a needle structure on the bacterial surface for the injection of *Yersinia* outer membrane proteins (Yops) into target host cells. This system results in disruption of both the innate and adaptive immune response, inhibiting phagocytosis.[128]

Yersinia Susceptibility Testing

Some *Y. enterocolitica* have chromosomally encoded beta lactamases, conferring resistance to ampicillin, cephalothin, and carbenicillin. Treatment of *Y. enterocolitica* may include an aminoglycoside, doxycycline, trimethoprim-sulfamethoxazole, a third-generation cephalosporin, or a fluoroquinolone.[106] There is significant resistance to fluoroquinolones in some regions, due to mutation of the gyr A gene and efflux mechanisms.[132] *Y. pseudotuberculosis* infections do not usually require treatment, but bacteremia may be treated with ampicillin, tetracycline, or streptomycin. The organism is usually susceptible to extended-specturm cephalosporins, aminoglycosides, chloramphenicol, tetracycline, and trimethoprim-sulfamethoxazole.[133]

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Chronic disease associated with *Salmonella/Shigella/Yersinia* Infections

An examination of cases of gastroenteritis due to *Salmonella*, *Campylobacter*, *Shigella*, or *Yersinia* in US military personnel lead to a report of a higher incidence of chronic health sequelae than some previous reports.[134] Pathogen-specific increases were observed with associated increased risk of irritable bowel disease, dyspepsia, constipation, and gastroesophogeal reflux disease, with a relative risk of 13.1 for inflammatory bowel disease following *Yersinia* infection. Three separate metaanalyses have shown a significantly increased risk of irritable bowel syndrome following gastroenteritis due to *Shigella*.[135–137]

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Key points

- The global disease burden from *Salmonella/Shigella/Yersinia*, organisms reportable to public health departments, is quite high, with significant but fewer infections in the developed world.
- Although there is a broad spectrum of disease associated with these pathogens, infections are self-limiting in the majority of cases. Antimicrobial therapy is ordinarily indicated only for severe gastrointestinal and systemic disease.
- Inactive strains of *E. coli* can be difficult to distinguish from *Shigella* strains, using multiple laboratory methods.
- Molecular methods such as PCR and mass spectrometry are being used more routinely for identification of these organisms, but both methods have limitations.
- There is active investigation in the areas of vaccine development and characterization of virulence mechanisms.

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Box 1

Prevention of *Yersinia* Infections

- Avoid raw or undercooked pork.
- Avoid unpasteurized milk and milk products.
- Wash hands with soap and water before preparing food or eating, and after contact with animals or raw meat.
- Avoid contact with young children while preparing raw chitterlings.
- Clean all kitchen boards and utensils after preparing raw meat.
- Dispose of animal feces in a sanitary manner.

(From Yersinia. National Center for Emerging and Zoonotic Infectious Diseases. Centers for Disease Control and Prevention (CDC). Available at: http://www.cdc.gov/nczved/divisions/dfbmd/diseases/yersinia/)

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Box 2

Barriers to development of a *Shigella* Vaccine

- We have a limited understanding of protective immunity for *Shigella*.
- There are any serotypes of *Shigella*.
- There is a lack of an adequate animal model.
- Concerns of producing reactive arthritis in vaccine trial recipients.
- Concerns of gastrointestinal tract side effects or chronic conditions.
- Challenges in manufacturing process.
- Some promote funding for clean water and sanitation over vaccine studies.

From Barry EM, Pasetti MF, Sztein MB, et al. Progress and pitfalls in Shigella vaccine research. Nature reviews Gastroenterology & hepatology 2013;10(4):245–255; with permission.

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SELF ASSESSMENT

1. Which of the following statements is true regarding *Salmonella*?
   - **a.** S. bongeri is a common cause of Salmonellosis in humans.
   - **b.** Typhus is caused by a serovar of *S. enterica* subsp. *Indica*.
   - **c.** Serovars of Salmonella have official taxonomic status similar to the named *Salmonella* subspecies.
   - **d.** Typhoidal strains of *Salmonella* are more likely to cause systemic disease than non-typhoidal strains.
   - **e.** Unpasteurized milk products are the most common source of human *Salmonella* infection in the U.S.
2. Which of the following statements is true regarding *Shigella*?
   - **a.** Diarrhea from *Shigella* infection is almost always bloody.
   - **b.** All patients with S*higella* infection should receive antibiotics promptly to avoid sequelae.
   - **c.** The black-centered colony on Hektoen agar is characteristic for *Shigella*.
   - **d.** Two of the four groups of *Shigella* species typically carry the shiga toxin gene.
   - **e.** Inactive strains of *E. coli* are very difficult to distinguish from *Shigella* with multiple laboratory identification systems.
3. Which of the following statements is true regarding *Yersinia*?
   - **a.** *Y. pestis*, *Y. enterocolitica*, and *Y. pseudotuberculosis* all have equally similar genomes.
   - **b.** It is common for patients with *Y. enterocolitica* to have bacteremia as a complication.
   - **c.** There is currently no role for MALDI-TOF MS in the identification of *Yersinia* due to the poor performance in published studies.
   - **d.** There are multiple reports of chronic gastrointestinal diseases as sequelae of *Yersinia* infections.
   - **e.** There are 50 serotypes of *Yersinia* known to be pathogenic in humans.

Answers to multiple choice questions

1. D
2. E
3. D

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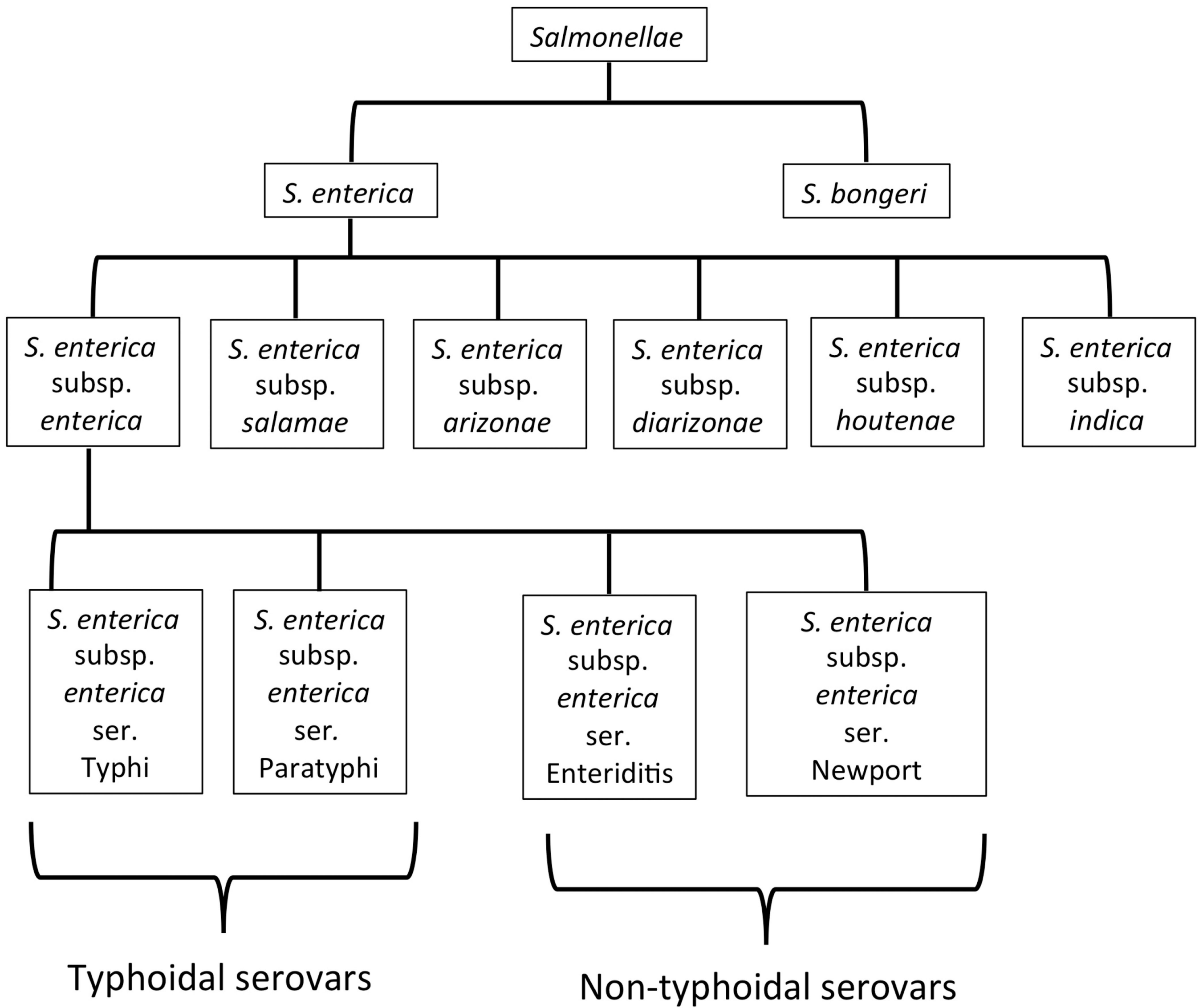

Figure 1.

Relationships within the *Salmonella* genus, including species, subspecies, and serovar designations are illustrated. Note that serovars do not have official taxonomic status. Four representative serovars are shown for *S. enterica* subsp. *enterica*. Only the most common typhyoidal serovars, and representative non-typhoidal serovars are shown. There are greater than 2500 serovars in total, with the most common disease-causing serovars belonging to *S. enterica* subsp. *Enterica*.

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LDC [a]	*ipaH*-gene PCR [b]	Motility [c]	Salicin, acid [a]	Esculin hydrolysis [a]	Gas from D-Glucose and indol [a]	Agglutination with EIEC associated antisera [d]	Agglutination with *Shigella* antisera	
+								*E. coli* non-enteroinvasive
-	-							*E. coli* non-enteroinvasive
	+	+						EIEC
		-	+					EIEC
			-	+				EIEC
				-	+			EIEC
					-	+		EIEC
						-	+	*Shigella*
							-	Provisional *Shigella*

Figure 2.

Key for differentiation of *Shigella*, enteroinvasive *Escherichia coli* (EIEC), and noninvasive *Escherichia coli*. (From van den Beld MJ, Reubsaet FA. Differentiation between Shigella, enteroinvasive Escherichia coli (EIEC) and noninvasive Escherichia coli. European journal of clinical microbiology & infectious diseases : official publication of the European Society of Clinical Microbiology 2012;31(6):899–904; with permission)

- **a.** Based on Edwards and Ewing's identification of *Enterobacteriaceae*, 4th edition, 1986 and/or Cowan and Steel's manual for the identification of medical bacteria, 3rd edition, 1993.
- **b.** Performed with a standard PCR protocol, with primers designed to amplify a part of the conserved region of $ipaH_{7.8}$, as described by Buysse *et al*., Microb. Pathog. 19(5):335–349.
- **c.** Incubated for 24 h in BHI-medium at 37°C.
- **d.** Known O:H serotypes of EIEC according to Bergey's manual of Systematic Bacteriology, 2nd edition, volume 2, The *Proteobacteria*, Part B The *Gammaproteobacteria*.