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# Increasing Antibiotic Resistance in Shigella Bacteria in the United States

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**Introduction**

*Shigella* are a group of pathogenic bacteria under the Enterobacteriaceae family. They are gram-negative, non-motile, facultatively anaerobic bacteria which include four main subgroups: *Shigella flexneri*, *Shigella boydii*, *Shigella sonnei*, and *Shigella dysenteriae*. Of the four subgroups, *S. dysenteriae* is associated with the most severe outbreaks due to its high infection-fatality rate in low-income areas of central Asia, central America, and southeastern Africa. *S. flexneri* and *S. sonnei* are considered to be the most ubiquitous subgroups, and collectively, they are responsible for the majority of *Shigella* cases globally. *S. boydii* is the least common subgroup, most likely due to its specificity to the India sub-region. When considering the United States and other high-income countries, *S. sonnei* is considered to be the most infectious subgroup (Kotloff *et al*, 2018; Baker and The, 2018). Figure 1 shows that from 1990-2014, the United States had a >3:1 ratio of *S. sonnei* to *S. flexneri* isolates.

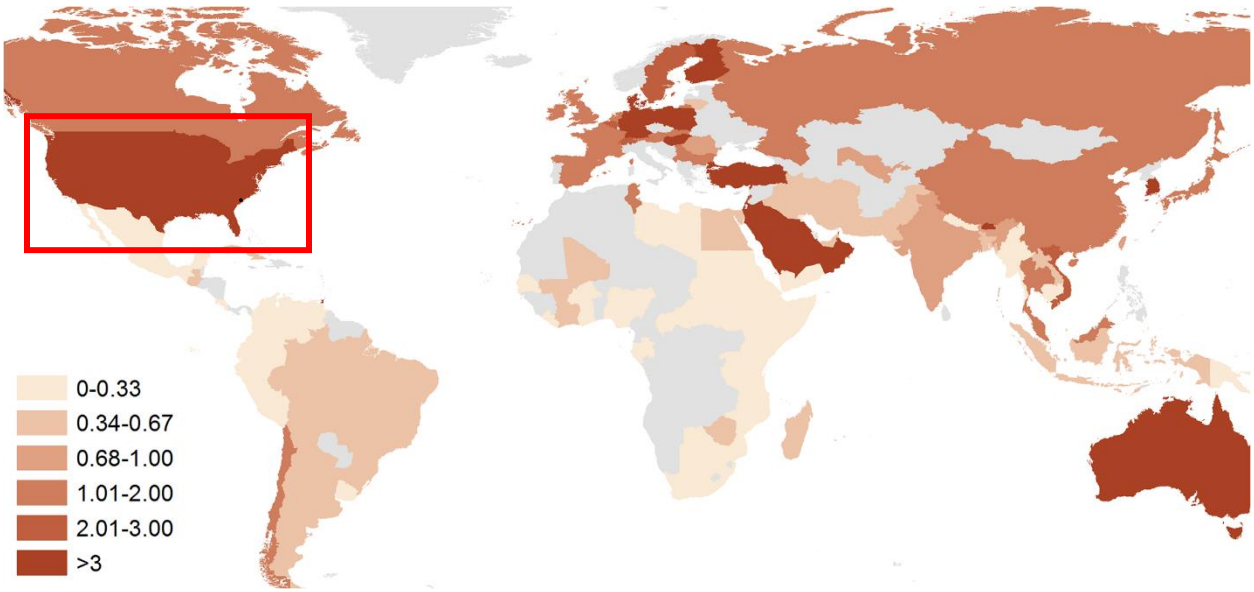


**Fig. 1:** The ratio of *S. sonnei* to *S. flexneri* infections isolated from 100 countries, 1990-2014. The darker the color, the more often *S. sonnei* was isolated compared to *S. flexneri*. The lighter the color, the more proportionally *S. flexneri* was isolated. Grey areas indicate countries where no data was collected (Thompson *et al*, 2015).

Victims of *Shigella* infection often contract a form of shigellosis, a disease which primarily induces aggressive dysentery. Other symptoms associated with shigellosis include fever, abdominal cramping, vomiting, dehydration, and nutrient deficiency. In rare cases, shigellosis from *S. flexneri* can additionally cause Reiter's syndrome (reactive arthritis) post-infection. *Shigella* are predominantly transferred via fecal-oral contamination, but the bacteria can also be foodborne or waterborne. Recent studies have also concluded that *Shigella* can be transferred via houseflies (Kotloff *et al*, 2018). In the United States alone, *Shigella* infections cause approximately half a million infections, 6,000 hospitalizations, and 70 deaths annually (Kozryeva *et al*, 2016). This manuscript will compare *Shigella* to pathogenic *Escherichia coli* and explain how *Shigella* developed their own virulence mechanisms. Then, groups that are of particular risk to *Shigella* infection will be discussed, followed by an analysis of the increasing antibiotic resistance in *Shigella* found in the United States, and what those increases imply for the future.

**Genotypic comparison of *Shigella* to *E. coli***

Current genomic research indicates that *Shigella* species originated from pathogenic strains of *Escherichia coli*. Both *Shigella* and pathogenic *E. coli* contain the virulence plasmid pINV, which contains coding for a unique virulence mechanism called the type 3 secretion system (T3SS). T3SS allows bacteria to inject effector proteins directly into the cytosol of host cells to illicit an array of responses (Kotloff *et al*, 2018). The pINV plasmid also holds sequences for a number of Shiga toxins shared between *Shigella* and *E.coli*. Despite these fundamental similarities to *E. coli*, however, *Shigella* subgroups have a number of evolutionary divergences that are unique. Primarily, *Shigella* bacteria have developed traits such as proton consumption systems, producing mucinases, and host antimicrobial resistance (Kotloff *et al*, 2018). All of these play an important role in *Shigella* survivability. *Shigella* also diverged from *E. coli* by losing motility, and several metabolic pathways common to *E. coli* are no longer present in *Shigella*. Most of these metabolic pathways are still not fully understood. Table 1 compares key relationships between *Shigella* and pathogenic *E. coli*.

| Trait | *Shigella* | Pathogenic *E. coli* |
|---|---|---|
| Lactose fermentation | -* | + |
| Salicin fermentation | - | + |
| Motility | - | + |
| pINV plasmid | + | + |
| Can cause Haemolytic Uraemic Syndrome | + | + |
| Presence of Shiga toxins | + | + |

*Only in the case of *S. sonnei*

**Table 1:** Comparison of trait presence/absence between *Shigella* and pathogenic *E. coli*. "-" denotes the absence of a trait, whereas "+" represents the presence of a trait. Table synthesized using information from another manuscript (Ud-Din and Wahid, 2014).

Much of *Shigella* genome divergence can be related to the high ratio of insertion sequences (IS) to gene counts within *Shigella* chromosomes (Fig. 2). These IS are transposable elements able to move genetic material on chromosomes, thus causing mutations. Studies have proven that *Shigella* chromosomes can contain approximately 13-42 times more IS than those of pathogenic *E. coli* (Hawkey *et al*, 2019). This means that *Shigella* bacteria are likely to mutate more often than other similar bacteria, thus increasing the chance for genome divergence.

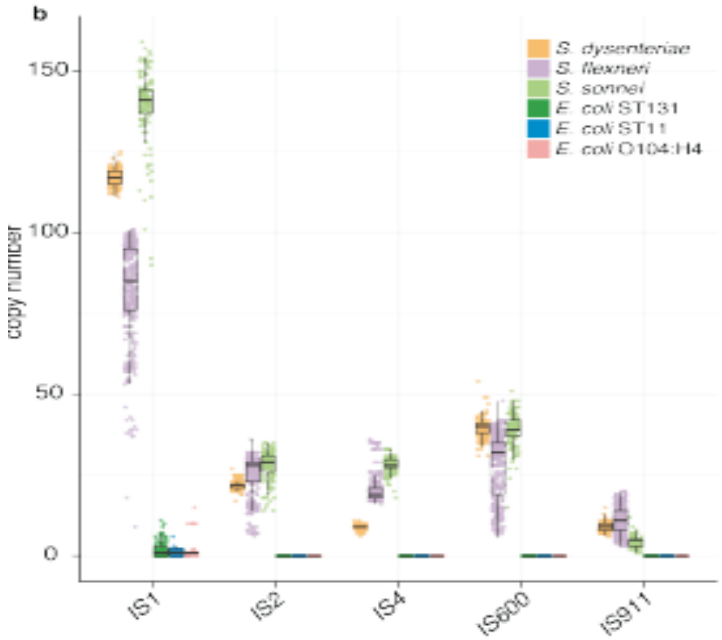

**Figure 2**: Box plots of five IS genes common to shown *Shigella* species and *E. coli* species (Hawkey *et al*, 2019).

**Pathogenicity of *Shigella***

Research has connected *Shigella* pathogenicity to high in-host survivability and low infectious dose. Infections can be contracted after ingestion of less than 10-200 organisms, and symptoms usually show within 12-50 hours. Once within the human body, *Shigella* can survive stomach acid and the numerous competitive intestinal microbiota, ultimately settling on the mucosal layers of the ileum, colon, and rectum. The bacterial cells are phagocytized by Microfold cells (M-cells), allowing *Shigella* to inject effector proteins into the M-cells using T3SS (Kotloff *et al*, 2018).

Although the exact mechanism is not fully understood, research indicates that the injection of effectors like the invasion plasmid antigen H (IpaH) family proteins ultimately induces the reorientation of actin polymerization, allowing *Shigella* to provoke intracellular motility. Therefore, the bacterial cells are able to hijack host cells to move and replicate in neighboring epithelial cells, consequently stirring inflammatory cell death and severe intestinal inflammation (Ashida and Sasakawa, 2016).

**Groups at risk**

Although *Shigella* poses a threat to everyone, there are some groups that are notably more susceptible than others. Transmission in these areas is mostly a result of underdeveloped food/water quality and under-privileged sanitation/hygiene. Therefore, it is not surprising that the majority of shigellosis cases arise from young children (ages 1-4) in low to middle-income countries. In the United States, the age trend is similar. Most shigellosis cases stem from children held in day-care facilities (Kotloff *et al*, 2018). However, a recent *S. sonnei* outbreak within a retirement community in Vermont revealed that elderly and immunocompromised individuals are also likely to be more susceptible (Strysko *et al*, 2019). People traveling from high-income to low/middle-income countries are another group that is more vulnerable to *Shigella* infection. Studies have shown that *Shigella* is one of the leading causes of diarrheal infection for these groups (Khalil *et al*, 2018). It is also worth mentioning that over the past few

years, men who have sex with other men (MSM) have shown an increasing number of *Shigella* infections. Several case studies have identified instances where *Shigella* have been passed sexually between MSM (Kennedy *et al*, 2017; Bowen *et al*, 2016; Heiman *et al*, 2014). The spread of *Shigella* infection via travel and MSM are also proven to be large contributors to the growing antibiotic resistance in *Shigella*, particularly *S. sonnei*.

**Antibiotic resistance in *Shigella***

The use of antibiotics has been the primary solution for *Shigella* infections for many years, but as a result, drug resistance is an ever-increasing issue. Studies on antibiotic resistance in *Shigella* all show trends of increasing resistance to commonly used antibiotic treatments. In the mid-late 1900s, streptomycin, tetracycline, and trimethoprim-sulfamethoxazole were some of the most prescribed antibiotics in the United states to reduce symptoms and pathogen shedding from *Shigella* species. However, by the 21$^{st}$ century, the majority of *Shigella* isolates showed resistance to all these (Howie *et al*, 2010). Afterward, ampicillin and co-trimoxazole became the primary antibiotics used to treat *Shigella* cases. Similarly, *Shigella* quickly developed resistance to these drugs. Nalidixic acid and fluoroquinolones were introduced to replace previous treatments, but *Shigella* species acquired resistance to these antibiotics as well (Puzari *et al*, 2018). In 2006, a study tested 1,604 *Shigella* isolates for antibiotic resistance and concluded that all of them were susceptible to ceftriaxone (Sivapalasingam *et al*, 2006). More recently however, studies have found *Shigella* isolates with resistance to ceftriaxone (Puzari *et al*, 2018).

As of now, ciprofloxacin is recommended as the primary antibiotic used to treat *Shigella* in adults and children. Unfortunately, studies have shown an increasing resistance to this antibiotic as well (Fig. 3). In 2017, the CDC issued a health advisory urging that the use of antibiotics for *Shigellosis* be reserved for only the most serious cases due to the noticeable increase in ciprofloxacin resistance. As *Shigella* continue to develop resistance to our best treatment methods, it is imperative that the mechanisms and origins of resistance in *Shigella* be further identified and understood.

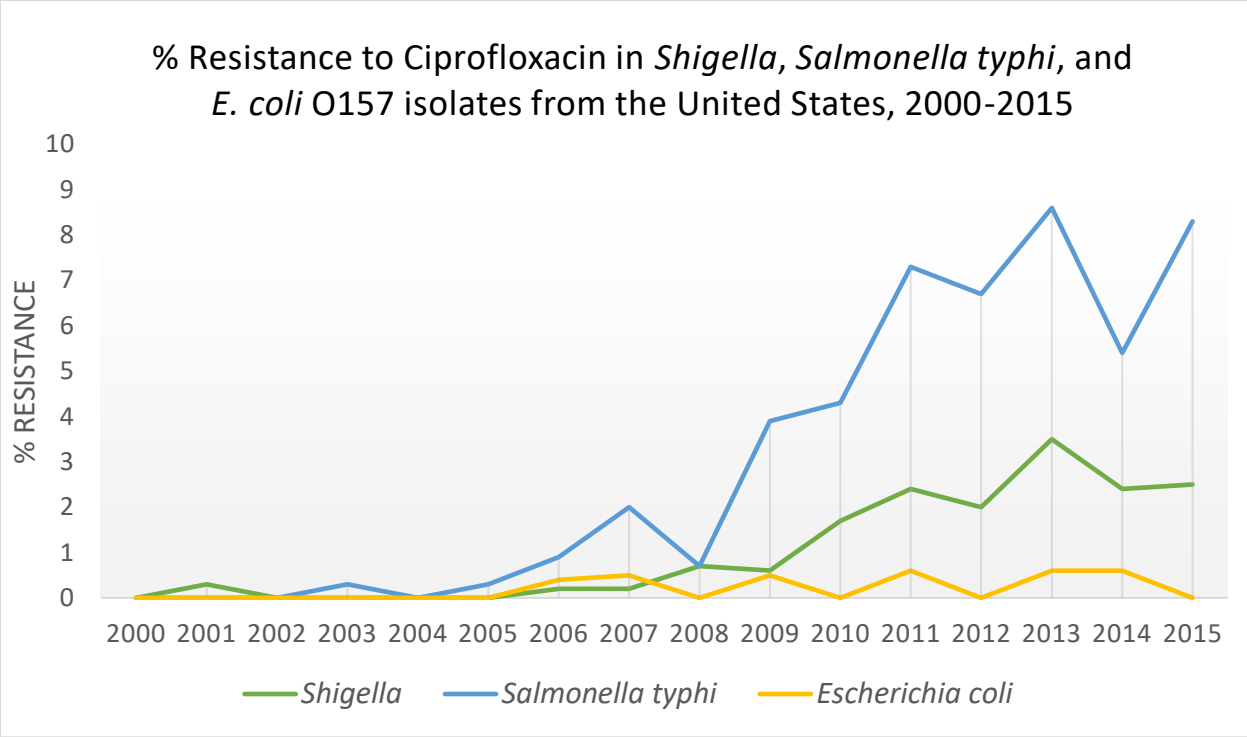


**Figure 3:** Data was collected and synthesized from the National Antimicrobial Resistance Monitoring System (NARMS)

As mentioned previously, much of the growing antibiotic resistance in *Shigella* correllates with the high amount of IS present on *Shigella* chromosomes, the transmission of bacterial strains from travel, and the transmission via MSM. Antibiotic resistance in *Shigella* and other pathogenic bacteria resides within specific genes. An antibiotic resistance gene codes for mechanisms that ultimately give resistance against a specific antibiotic, and these genes are able to be passed between different strains of *Shigella*. As people travel between high to low/middle-income countries, antibiotic resistance genes are able to be passed amongst *Shigella* and spread around the world. Table 1 lists antibiotic resistance in *S. sonnei* isolates collected from the United States and how resistance relates with travel (Abelman *et al*, 2019). Studies also have concluded that MSM can not only transfer *Shigella* to one another sexually, but MSM also may be more susceptible to multi-drug resistant strains of *Shigella*. It is known that HIV-positive men have a higher potential for co-infections with *Shigella* as well as increased potential for relapsed infection.

This raised risk of infection combined with sexual interaction increases the transmission of antibiotic resistance genes among strains of *S. sonnei* and *S. flexneri* (Baker *et al,* 2015).

| PSU-ID | Travel history | Year of isolation | AMR profile |
|---|---|---|---|
| SS-27 | None | 2010 | STR, AMP, SXT, SMX, TET |
| SS-2 | None | 2011 | STR, AMP, AMC, FOX, TET |
| SS-28 | None | 2011 | STR, AMP, AMC, FOX |
| SS-29 | None | 2011 | STR, AMP, AMX, FOX |
| SS-32 | None | 2012 | AMP, AMC, SXT, CHL, SMX, TET |
| SS-36 | None | 2012 | STR, SXT, CIP, NAL, SMX, TET |
| SS-39 | None | 2013 | STR, SXT, CIP, NAL, SMX, TET |
| SS-40 | None | 2013 | STR, AMP, SXT, SMX, TET |
| SS-3 | None | 2013 | GEN, STR, SXT, SMX, TET, AZM |
| SS-4 | None | 2013 | STR, SXT, SMX, TET |
| SS-5 | None | 2014 | STR, AMP, SXT, SMX, TET, AZM |
| SS-43 | Mexico | 2014 | STR, SXT, NAL, SMX, TET |
| SS-38 | Dominican Republic | 2013 | STR, AMP, SXT, SMX, TET |
| SS-42 | Dominican Republic | 2014 | STR, SXT, NAL, SMX, TET |
| SS-37 | Haiti | 2013 | STR, AMP, SXT, SMX, TET |
| SS-26 | Jamaica | 2010 | STR, SXT, NAL, SMX, TET |
| SS-30 | Peru | 2012 | AMP, AMC, SXT, CHL, SMX, TET |
| SS-31 | Peru | 2012 | AMP, AMC, SXT, CHL, SMX, TET |
| SS-21 | India | 2009 | STR, SXT, NAL, SMX, TET |
| SS-24 | India | 2010 | STR, SXT, CIP, NAL, SMX, TET |
| SS-35 | India | 2012 | STR, SXT, CIP, NAL, SMX, TET |
| SS-23 | Nepal | 2009 | STR, SXT, CIP, NAL, SMX, TET |

**Table 2**: All isolates were taken from the Pennsylvania Department of Health. Travel History highlights where the *Shigella* isolate originated from. “None” values in this column imply that the person from which this isolate was collected from did not report any travel outside of the U.S. Isolates that showed resistance to ciprofloxacin or azithromycin are highlighted. Antimicrobial Resistance (AMR) abbreviations represent as follows: streptomycin (STR), gentamicin (GEN), ampicillin (AMP), amoxicillin (AMC), cefoxitin (FOX), trimethoprim-sulfamethoxazole (SXT), sulfamethoxazole (SMX), tetracycline (TET), chloramphenicol (CHL),

ciproflaxin (CIP), nalidixic acid (NAL), and azithromycin (AZM). Table modified from (Abelman *et al*, 2019).

**Conclusion**

*Shigella* is a widespread, highly pathogenic bacteria that causes many infections and deaths worldwide. In the United States and other high-income countries, *S. sonnei* is the most concerning subgroup, due to its high drug resistance. Over several years, studies have indicated that *Shigella* is highly adaptable. *Shigella* continues to quickly develop antibiotic resistance to commonly used antibiotics in the United States. Some of the bacteria's fast response to new antibiotics can be connected back to the high amounts of IS sequences present within *Shigella* chromosomes. These sequences allow the bacteria to undergo rapid mutations to develop antibiotic resistant genes. As multi-drug resistance continues to gain more attention, researchers are beginning to develop new solutions to reduce the need for so many antibiotics. Using genome sequencing, scientists are now able to observe the entire genome of a particular strain of *Shigella* to identify the antibiotic resistance genes present. Identification of these genomes will allow for more specific treatment of *Shigella* infections, ultimately increasing the number of successful treatments while also slowing the rate of antibiotic resistance development. However, the exact mechanism of these antibiotic resistance genes needs to be better understood before this form of treatment could be viable. These sequences contain unique, often complex mechanisms for a particular resistance gene, and it will take time to fully understand them at a level which treatment could be effectively prescribed. Additionally, new mechanisms will inevitably continue to develop in *Shigella* species, so researching these mechanisms would essentially be a never-ceasing process. Another treatment option highlights taking preventative measures via vaccination. Tests have already indicated that O-polysaccharide conjugate vaccines reduce the severity of *Shigella* infections (Kotloff *et al*, 2018). However, it is important to note that these vaccinations were serotype specific. Until a vaccine providing cross-species immunity is established, it will be difficult for vaccination to keep up with the rate of change in strains of *S. sonnei* and *S.* flexneri. Until further treatment methods can be bolstered, it is important to moderate *Shigella* infections by practicing good hygiene.

**References:**


Abelman, R. L., N. M. M'ikanatha, H. M. Figler, and E. G. Dudley. (2019). Use of whole-genome sequencing in surveillance for antimicrobial-resistant *Shigella sonnei* infections acquired from domestic and international sources. Microbial Genomics 5:e000270.

Ashida, H. and C. Sasakawa. (2016). *Shigella* IpaH family effectors as a versatile model for studying pathogenic bacteria. Frontiers in Cellular and Infection Microbiology.

Baker, S. and H. C. The. (2018). Recent insights into *Shigella*: a major contributor to the global diarrheal disease burden. Current Opinion in Infectious Diseases 31:449-454.

Baker, K. S., T. J. Dallman, P. M. Ashton, M. Day, G. Hughes, P. D. Crook, V. L. Gilbart, S. Zittermann, V. G. Allen, B. P. Howden, T. Tomita, M. Valcanis, S. R. Harris, T. R. Connor, V. Sitchenko, P. Howard, J. D. Brown, N. K. Petty, M. Gouali, D. P. Thanh, K. H. Keddy, A. M. Smith, K. A. Talukder, S. M. Faruque, J. Parkhill, S. Baker, F. X. Weill, C. Jenkins, and N. R. Thompson. (2015). Intercontinental dissemination of azithromycin-resistant shigellosis through sexual transmission: A cross-sectional study. The Lancet Infectious Diseases 15:913-931.

Bowen, A., J. Grass, A. Bicknese, D. Campbell, J. Hurd, and R. D. Kirkcaldy. (2016). Elevated risk for antimicrobial drug resistant *Shigella* infection among men who have sex with men, United States, 2011-2015. Emerging Infectious Diseases 22:1613-1616.

Bowen, A., J. Hurd, C. Hoover, Y. Khachadourian, E. Traphagen, E. Harvey, T. Libby, S. Ehlers, M. Ongpin, J. C. Norton, A. Bicknese, and A. Kimura. (2015). Importation and domestic transmission of *Shigella sonnei* resistant to ciprofloxacin–United States, May 2014-February 2015. Morbidity and Mortality Weekly Report 64:318-320.

cdc.gov/narms/reports/index.html

Hawkey, J., J. M. Monk, H. Billman-Jacobe, B. Palsson, and K. E. Holt. (2019). Impact of insertion sequences on convergent evolution of *Shigella* species. bioRxiv 680777.

Heiman, E. K., M. Karlsson, J. Grass, B. Howie, R. D. Kirkcaldy, B. Mahon, J. T. Brooks, and A. Bowen. (2014). *Shigella* with decreased susceptibility to azithromycin among men who have sex with men—United States, 2002-2013. Morbidity and Mortality Weekly Report 63:132-133.

Howie, R. L., J. P. Folster, A. Bowen, E. J. Barzilay, and J. M. Whichard. (2010). Reduced azithromycin susceptibility in *Shigella sonnei*, United States. Microbial Drug Resistance 16:245-248.

Kennedy, S. L., J. Murira, and C. Y. Wenham. (2017). A case of reactive arthritis secondary to sexually acquired *Shigella flexneri*. Oxford Medical Case Reports 2017:omx070.

Khalil, A. I., C. Traeger, B. F. Blacker, P. C. Rao, A. Brown, D. E. Atherly, T. G. Brewer, C. M. Engmann, E. R. Houpt, G. Kang, K. L. Kotloff, M. M. Levine, S. P. Luby, C. A. MacLennan, W. K. Pan, P. B. Pavlinac, J. A. Platts-Mills, F. Qadri, M. S. Riddle, E. T. Ryan, D. A. Shoultz, A. D. Steele, J. L. Walson, J. W. Sanders, A. H. Mokdad, C. J. L. Murray, S. I. Hay, and R. C. Reiner Jr. (2018). Morbidity and mortality due to shigella and enterotoxigenic *Escherichia coli* diarrhea: The global burden of disease study 1990-2016. The Lancet Infectious Diseases 18:1229-1240.

Kotloff, K. L., M. S. Riddle, J. A. Platts-Mills, P. Pavlinac, and A. K. M. Zaidi. (2018). Shigellosis. The Lancet Infectious Diseases 391:801-812.

Kozryeva, V. K., G. Jospin, A. L. Greninger, J. P. Watt, J. A. Eisen, and V. Chaturvedi. (2016). Recent outbreaks of shigellosis in California caused by two distinct populations of *Shigella sonnei* with either increased virulence or fluoroquinolone resistance. Clinical Science and Epidemiology doi: 10.1128/mSphere.00344-16.

msdsonline.com/resources/sds-resources/free-safety-data-sheet-index/shigella-spp/

Puzari, M., M. Sharma, and P. Chetia. (2018). Emergence of antibiotic resistant *Shigella* species: A matter of concern. Journal of Infection and Public Health 11:451-454.

Sivapalasingam, S., J. M. Nelson, K. Joyce, M. Hoekstra, F. J. Angulo, and E. D. Mintz. (2006). High prevalence of antimicrobial resistance among *Shigella* isolates in the United States tested by the National Antimicrobial Resistance Monitoring System from 1999 to 2002. Antimicrobial Agents and Chemotherapy 50:49-54.

Strysko, J., V. Fialkowski, Z. Marsh, A. Wadhwa, J. Collins, R. Gharpure, P. Kelso, C. R. Friedman, and K. E. Fullerton. (2019). *Notes from the field*: Outbreak of multi-drug resistant *Shigella sonnei* infections in a retirement community—Vermont, October-November 2018. Morbidity and Mortality Weekly Report 68:405-406.

Thompson, C. N., P. T. Duy, and S. Baker. (2015). The rising dominance of *Shigella sonnei*: An intercontinental shift in the etiology of bacillary dysentery. Public Library of Science: Neglected Tropical Diseases doi: 10.1371/0003708.

Ud-Din, A., and S. Wahid. (2014). Relationship among *Shigella* spp. and enteroinvasive *Escherichia coli* (EIEC) and their differentiation. Brazilian Journal of Microbiology 45:1131-1138.

Webb, H. E., K. A. Tagg, J. C. Chen, J. Kim, R. Lindsey, L. K. F. Watkins, B. E. Karp, Y. Sugawara, and J. P. Folster. (2019). Novel quinolone resistance determinant, *qepA8*, in *Shigella flexneri* isolated in the United States, 2016. Antimicrobial Agents and Chemotherapy doi: 10.1128/AAC.01458-19.