Effect of *Psidium guajava* (guava) L. Leaf Decoction on Antibiotic-resistant Clinical Diarrhoeagenic Isolates of *Shigella* spp.

Poonam Daswani¹, Vigneshwaran Muthuraman¹, Ragini Macaden², Mary Dias², Tannaz Birdi*¹

¹Department of Medicinal Plants, The Foundation for Medical Research, Mumbai, India
²Division of Infectious Diseases, St. John's Research Institute, St. John's Medical College, Bengaluru, India

*Corresponding Author:
Tannaz Birdi,
Department of Medicinal Plants, the Foundation for Medical Research, Dr. Kantilal J. Sheth Memorial Building, 84A, RG Thadani Marg, Worli, Mumbai 400018, Maharashtra, India
Tel: 91-22-24934989
Email: tfmrm@dialink.org

Published Online: December 30, 2020

Keywords: Antibiotic resistance, Infectious diarrhoea, Guava, *Shigella*, India

Abstract
**Background:** Although shigellosis is self-limiting, antibiotics are recommended to minimize the severity of symptoms and reduce mortality rates. However, due to the increasing reports of antibiotic resistance, alternative approaches are needed to combat shigellosis. Interest for research on medicinal plants has increased in recent years, and hence, they can be explored to treat this infectious diarrhoea.

**Objective:** To study the effect of *Psidium guajava* (guava) leaf decoction (GLD) on the antibiotic-resistant clinical isolates of *Shigella* spp.

**Materials and Methods:** A total of 43 isolated *Shigella* spp. from diarrhoeal patients were used in this study. The effect of GLD on the bacterial viability was initially assessed. The isolates were divided into two categories: sensitive and resistant to GLD. For sensitive isolates, antibacterial activity of GLD was evaluated while for resistant strains, the ability of GLD for reducing the bacterial invasion of the HEP-2 cell line underwent an investigation.

**Results:** Among the 43 *Shigella* isolates, GLD affected the growth of 23 strains. The invasion of 9 strains from the 20 remaining resistant isolates was unaffected. Although the number of isolates was less, the data suggested that isolates belonging to *S. flexneri* serogroup were more sensitive to GLD in comparison with other spp (i.e., *sonnei*, *boydii*, and *dyssenteriae*).

**Conclusion:** The results of this study revealed the efficacy of GLD against drug-resistant *Shigella* spp. and thus could be considered for the treatment of diarrhoea. GLD can be a cost-effective alternative to antibiotics.

**Background**
Antibiotics are the primary treatment for infections. However, some studies have documented the rampant and irrational use of antibiotics leading to antibiotic resistant microbes.¹²³ Considering the growing rates of treatment failures in infectious diseases, the emergence of drug-resistant pathogens is a significant threat to public health prompting the World Health Organization (WHO) to declare antibiotic resistance as a subject of global crisis.⁴⁵ Shigellosis, a common gastrointestinal infection, is responsible for about 700,000 deaths per year across the globe.⁶ Although the infection is a self-limiting diarrhoea, it is among the few diarrhoeal diseases wherein antibiotics are recommended by the WHO for reducing the severity of symptoms and minimizing the death rate.⁷ A study by Das et al associated the use of antibiotics for shigellosis to aid faster recovery and the reduction of complications and mortality.⁸ With resistance developing to earlier recommended antibiotics including sulphonamides, chloramphenicol, tetracycline, and fluoroquinolones, third-generation cephalosporins were introduced for treating shigellosis.⁹ However, reports of drug-resistant *Shigella* have emerged from various regions. For example, the resistance of *Shigella* to quinolones (nalidixic acid and ciprofloxacin) has been documented in the regions Europe-America and Asia-Africa.¹⁰ In another prospective study conducted between 2009-2012 in New Delhi (India), involving 6339 stool samples from gastroenteritis patients, 121 strains (19%) were found to be *Shigella* spp. Further, 76% of these strains were multidrug-resistant, including three drugs of ceftriaxone, cefotaxime, and ceftazidime belonging to the third generation of cephalosporins.¹¹ In another study spanning between 2007-12, 67 isolates belonged to *Shigella* spp. These isolates showed high levels of resistance toward trimethoprim-sulfamethoxazole, tetracycline, chloramphenicol, and ampicillin. Multi-drug resistance was observed in 55%.¹² Moreover, these Indian studies,

© 2020 The Author(s); Published by Alborz University of Medical Sciences. This is an open-access article distributed under the terms of the Creative Commons Attribution License (http://creativecommons.org/licenses/by/4.0), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.
in Shiraz, Iran, resistance of Shigella to co-trimoxazole was reported as 90.24%, and high resistance of Shigella isolates to cotrimoxazole, tetracycline, and ampicillin was shown in an Ethiopian investigation.\textsuperscript{15,16} Therefore, multi-drug resistant Shigella including fluoroquinolone-resistant Shigella has been included in the WHO priority list (Priority 3 – medium) for research and development of new antibiotics for the treatment of infections caused by them.\textsuperscript{2}

Psidium guajava (guava) leaves are a traditional remedy for gastrointestinal infections and have been widely reported to have antimicrobial activity.\textsuperscript{15-17} Our previous studies with guava leaves demonstrated that its decoction acts against different diarrhoeal pathogens including Shigella and exhibits a wide spectrum of anti-diarrhoeal activity.\textsuperscript{18,19} In the present study, a decoction prepared from guava leaves was tested \textit{in vitro} for its ability to combat antibiotic-resistant clinical isolates of Shigella spp. Towards this, the decoction was studied for its bactericidal action and effect on the bacterial invasion of epithelial cells.

\section*{Materials and Methods}

\subsection*{Bacterial Strains}
A total of 43 clinical isolates of Shigella spp. were included in the study. Forty of these isolates were provided by the St. John’s Research Institute (SJRI), Bengaluru, and three strains were collected from Hinduja Hospital, Mumbai. The provided strains were those received by the hospitals for diagnostic purposes and were either archival or freshly collected samples. The antibiotic susceptibility profile of the isolates was also provided by the hospitals. All the isolates were frozen in the brain heart infusion broth with 20\% glycerol and stored at -80\°C.

\subsection*{Antibiotic Susceptibility Testing}
This procedure was conducted using the Kirby-Bauer method of disc diffusion as per the Clinical Laboratory Standards Institute (CLSI) guidelines.\textsuperscript{20} Briefly, Mueller-Hinton agar (MHA) Himedia Laboratories, India was inoculated as a lawn using a pure culture of the \textit{Shigella} isolate in peptone water after the inoculum size was adjusted to the 0.5 McFarland standard. Commercially available antibiotic discs (HiMedia Laboratories) impregnated with the recommended concentration of the antibiotics were placed on MHA. The plates were incubated overnight at 37\°C, and the diameter of the zones of inhibition around the discs was measured and recorded as “resistant” or “susceptible” based on the reference table provided by CLSI. Quality control for the antimicrobial disks was performed using reference strain \textit{Escherichia coli} (ATCC 25922). The antibiotic discs and their concentration were ampicillin (10 \(\mu\)g), cefotaxime (30 \(\mu\)g), ceftriaxone (30 \(\mu\)g), chloramphenicol (30 \(\mu\)g), ciprofloxacin (5 \(\mu\)g), cotrimoxazole (23.75 \(\mu\)g/1.25 \(\mu\)g), furazolidone (50 \(\mu\)g), nalidixic acid (30 \(\mu\)g), neomycin (30 \(\mu\)g), norfloxacin (30 \(\mu\)g), and tetracycline (30 \(\mu\)g).

\subsection*{Plant Material}
Mature guava leaves belonging to the Sardar variety were collected from Shirwal, Satara district, Maharashtra in June 2018. The leaves were authenticated by an ethnobotanist (Dr. P. Tetali), and a voucher specimen was deposited at the Naojoi Godrej Centre for Plant Research (NGPCR, Shirwal) under herbarium number NGCPR 1099.

Leaves were sent to the National Agriculture and Food Analysis and Research Institute (NAFARI, Pune) for the analysis of the guava batch obtained for the presence of aflatoxins (B1, B2, G1, & G2), heavy metals viz., tin, copper, lead, zinc, and cadmium, and the determination of the microbial load.

\subsection*{Preparation of Decoction}
GLD was prepared by boiling 1 g of crushed leaves in 16 mL of distilled water until the volume was reduced to 4 mL. The resultant decoction was centrifuged and filtered through a 0.22 \(\mu\) membrane before use. A fresh decoction was prepared for each bioassay. A few decoctions were also air-dried, and the dry weight was recorded to know the yield of the decoction.

\subsection*{Bio-assays}
Each isolate was initially tested for growth in MHA containing 1\% GLD (4 mL of freshly prepared decoction was considered 100\%). Accordingly, the isolates were divided into two categories, including those - sensitive and resistant to GLD. For sensitive isolates, the antibacterial activity of GLD was undertaken, and for resistant strains, and the ability of GLD to reduce bacterial invasion was studied.

\subsection*{Antibacterial Activity}
The antibacterial activity of GLD was assessed using the agar dilution method.\textsuperscript{21} Briefly, bacteria grown to logarithmic phase in Luria broth were plated on MHA alone (as control) and MHA containing different concentrations of GLD (test). Bacteria showing no growth at 1\% GLD were considered as sensitive, and for these isolates, the percentage decrease in bacterial growth was undertaken at 0.5\% and 0.3\% GLD. The colony formation units (CFUs) were enumerated following 48 hours of incubation without and with GLD. Data were expressed as percentage viability, and CFU from the control was taken as 100\%. Each assay was performed in triplicates and repeated thrice.

\subsection*{Effect on Bacterial Invasion}
The bacterial invasion of GLD resistant to 1\% GLD to HEp-2 cells was assessed by the previously described method.\textsuperscript{22} Briefly, a 48-hour culture of HEp-2 cells grown in a 24-well tissue culture plate was infected with 10\(^5\)
mL of bacteria without (control) and with 0.25% GLD (a pre-determined non-cytotoxic dose) and incubated for 2 hours. The extracellular bacteria were washed off and the culture was further incubated with a medium containing gentamycin (100 µg/mL) for another 2 hours. Following incubation, the medium containing gentamycin was washed off, and then the epithelial cells lysed using chilled distilled water and the released bacteria were plated on nutrient agar. The CFUs were then recorded. Data were expressed as percentage viability and CFU from the control was taken as 100%.

Higher doses of the antibiotic were used in clinical isolates resistant to 100 µg/mL gentamicin. However, kanamycin (150 µg/mL) was used for isolates that were highly resistant to gentamicin. The invasion rate was considered unaffected if the percentage of invasion was ≥ 50%. Each assay was carried out in duplicate wells and repeated thrice.

**Results**

**Presence of Alfa Toxins/Heavy Metals**

The report by NAFARI showed that all the tested parameters were within permissible limits or below detectable levels (data not shown).

**Dry Weight of the Decoction and Percentage Yield**

The average dry weight of GLD per mL and the yield were 0.013 ± 0.004 g and 5.2%, respectively.

**Bacterial Isolates**

Among the 43 isolates, 31 (72.09%), 8 (18.6%), and 2 (4.65%) belonged to the flexneri serogroup (*S. flexneri*), *S. sonnei* serogroup, and *S. dysenteriae*, respectively, one isolate belonged to the *S. boydii* serogroup (2.33%) while one isolate could not be characterized. The antibiotics included antibiotics in the antibiotic susceptibility testing profile were ampicillin, quinolones (ciprofloxacin, norfloxacin, and nalidixic acid), cephalosporins (ceftriaxone and cefotaxime), sulfonamide (cotrimoxazole), nitrofuran (furazolidone), aminoglycoside (neomycin), tetracycline, and chloramphenicol. Thirty-seven of the 43 (86%) isolates were resistant to ≥ 6 antibiotics. Among all the isolates, a high degree of resistance was observed for quinolones (88%) and tetracycline (83%).

**Antibacterial Activity**

GLD at 1% inhibited the growth of 23 isolates including 22 *S. flexneri* isolates and only one isolate belonging to *S. sonnei*. The growth of the majority of these isolates was also inhibited at 0.5% GLD. At 0.3% GLD, the bacterial viability was in the range of 0-136% (Table 1).

**Effect on Bacterial Invasion**

Bacterial invasion of HEp-2 cells undertaken at 0.25% GLD was in the range of 0-322% (Table 2).

*S. flexneri*: Among the nine strains resistant to GLD, the invasion of six strains reduced in the presence of GLD while it was unaffected in three strains. The assay could not be performed for one of the isolates as it was highly resistant to gentamicin and kanamycin. However, based on the extracellular count following infection, it was seen although the bacterial invasion decreased, the reduction was >50%.

*S. sonnei*: Invasion assay was performed for eight resistant strains. In three strains, the invasion was unaffected while it reduced in the remaining four strains. Amongst the three strains for which the invasion was unaffected, gentamycin was substituted by kanamycin in one strain (150 µg/mL).

*S. boydii*: Invasion of the single strain of *S. boydii* was reduced.

*S. dysenteriae*: Among the two isolates of *S. dysenteriae*, one strain being resistant to gentamicin and kanamycin, the extracellular count was estimated, and based on the

---

**Table 1. Antibacterial Activity of GLD Against Sensitive Clinical Isolates of Shigella**

| Isolate No. | % Bacterial Viability | Mean ± SD * |
|-------------|-----------------------|-------------|
|             | 0.5% GLD              | 0.3% GLD    |
| *Shigella flexneri* |                     |             |
| 1           | 78 ± 4                | 92 ± 7      |
| 2           | 0                     | 38 ± 9      |
| 3           | 0                     | 48 ± 7      |
| 4           | 11 ± 3                | 56 ± 8      |
| 5           | 0                     | 20 ± 5      |
| 6           | 0                     | 51 ± 8      |
| 7           | 0                     | 61 ± 6      |
| 8           | 0                     | 84 ± 8      |
| 9           | 0                     | 59 ± 6      |
| 10          | 0                     | 42 ± 7      |
| 11          | 0                     | 57 ± 10     |
| 12          | 0                     | 29 ± 9      |
| 13          | 0                     | 59 ± 8      |
| 14          | 0                     | 78 ± 6      |
| 15          | 0                     | 71 ± 7      |
| 16          | 0                     | 86 ± 9      |
| 17          | 0                     | 64 ± 3      |
| 18          | 0                     | 79 ± 6      |
| 19          | 0                     | 77 ± 10     |
| 20          | 0                     | 78 ± 6      |
| 21          | 0                     | 136 ± 7     |
| 22          | 0                     | 73 ± 8      |

*Note*: SD: Standard deviation; GLD: Guava leaf decoction; CFUs: Colony formation units.

*Values are represented as the Mean ± SD of CFUs from three independent experiments each carried out in triplicates.*
counts, it was observed that the invasion was unaffected similar to the other isolate.

Uncharacterized *Shigella* spp.: The bacterial invasion remained unaffected in the single uncharacterized strain of *Shigella* spp.

In summary (Table 3), GLD has the potential to arrest the growth and/or reduce the invasion of a significant proportion of multidrug-resistant (MDR) *Shigella* isolates. Among the 43 isolates, GLD could arrest the growth of 23 strains (53%). The invasion of 11 strains (26%) resistant to GLD was reduced. Overall, nine strains (21%) were resistant to GLD.

**Discussion**

Diarrheal diseases continue to be a cause of global concern. Among the *Shigella* spp, *S. flexneri* is known to be the leading and common cause of diarrhoea, especially in low- and middle-income countries.\(^2\)\(^,\)\(^3\) This is also observed in our study wherein the predominant strain was *S. flexneri*. This organism is highly infectious and can survive in low acidic conditions in the stomach.\(^2\)\(^,\)\(^3\) *S. sonnei* infections are the second leading *Shigella* spp. causing diarrhoea and more commonly found in regions where there is industrialization.\(^2\)\(^,\)\(^3\) Further, this has been observed in Vietnam and China where rapid industrialization has taken place, leading to a contaminated water supply.\(^2\)\(^,\)\(^3\)

Similar to the case with other diarrhoeal pathogens, the reports of antibiotic resistance with *Shigella* spp are on the rise. The *Shigella* isolates in the current study showed high levels of resistance toward quinolones, which was also observed in studies conducted in Bangladesh and Assam indicating resistance to ciprofloxacin and nalidixic acid.\(^2\)\(^,\)\(^3\) Increased resistance to tetracycline among the applied isolates in the present study was also reported in a study conducted in Ethiopia.\(^2\)

Due to the increasing incidences of antibiotic resistance in *Shigella* spp., there is a need for novel and efficient curative agents. Plants have been known to cater to a number of ailments, and it is estimated that 60% of the current antimicrobials are obtained from plants.\(^3\)\(^,\)\(^2\)\(^,\)\(^3\) Hence, plant extracts have been proposed as an alternative in this regard.\(^3\)

*Psidium guajava* (guava) is an important food crop that is commonly found in tropical and subtropical countries with several medicinal properties.\(^3\)\(^,\)\(^2\)\(^,\)\(^3\) All parts of this plant are known to possess several therapeutic properties. Particularly, the leaves have been widely attributed to have varied pharmacological properties probably due to the presence of flavonoids, especially quercetin.\(^3\)\(^,\)\(^5\)\(^,\)\(^3\)\(^,\)\(^4\) Our previous studies also demonstrated the anti-diarrhoeal activity of guava leaves.\(^3\)\(^,\)\(^5\)\(^,\)\(^3\)\(^,\)\(^4\)\(^,\)\(^2\)\(^,\)\(^3\) These included the *in vitro* effect of GLD on the viability of *S. flexneri* and a reduction in the invading ability of the bacterium to HEp-2 cells.\(^3\) Based on the promising results with the reference strain used, the current study was undertaken to elucidate the effect of guava leaves against drug-resistant clinical *Shigella* isolates.

The novelty of the current study lies in the fact that it explored the antibacterial activity of GLD against antibiotic resistant clinical isolates of *Shigella* spp. from India, including *S. flexneri*, *S. sonnei*, *S. boydii*, and *S. dysenteriae*. Additionally, the study focused on the effect of GLD on the invasion (an important virulent feature) of *Shigella* strains whose growth was not affected by GLD in order to understand if GLD has an alternate mechanism to combat antibiotic-resistant isolates. To the best of our knowledge, this parameter has not been reported for antibiotic-resistant strains with GLD.

The results of the present study showed that GLD can arrest the growth of a significant number of clinical isolates of *Shigella*, especially *S. flexneri*. These observations are similar to available reports on the inhibitory potential of guava against *Shigella* and other drug-resistant

**Table 2. Effect of GLD on the Invasion of Resistant Clinical Isolates of Shigella to HEp-2 Cell Line**

| No. | % Bacterial Invasion (0.25% GLD) | Mean ± SD* |
|-----|---------------------------------|------------|
| 1   | *Shigella flexneri*             | 23 ± 10    |
| 2   |                                 | 3 ± 2      |
| 3   |                                 | 0          |
| 4   |                                 | 14 ± 9     |
| 5   |                                 | 26 ± 14    |
| 6   |                                 | 91 ± 13    |
| 7   |                                 | 2 ± 2      |
| 8   |                                 | 56 ± 9     |
| 9   |                                 | 72 ± 7     |
| 10  | *Shigella sonnei*               | 7 ± 3      |
| 11  |                                 | 33 ± 5     |
| 12  |                                 | 16 ± 9     |
| 13  |                                 | 15 ± 4     |
| 14  |                                 | 118 ± 11   |
| 15  |                                 | 126 ± 24   |
| 16  |                                 | 274 ± 75   |
| 17  | *Shigella boydii*               | 27 ± 13    |
| 18  | *Shigella dysenteriae*          | 157 ± 56   |
| 19  |                                 | 76 ± 7     |
| 20  | Uncharacterized                 | 322 ± 74   |

Note: SD: Standard deviation; GLD: Guava leaf decoction; CFUs: Colony formation units.

* Values are indicated as the Mean ± SD of CFUs from three independent experiments each carried out in triplicates.
* Values ≥ 50 (invasion unaffected).

*Based on extracellular counts.
Several studies have indicated that the enhancement of virulence and increased antibiotic resistance could occur simultaneously. The fundamental event in the pathogenesis of Shigella is its ability to invade, and subsequently, colonize the human intestinal epithelium. The presence of several virulence factors and toxins is associated with the pathogenicity of Shigella spp. Bloody diarrhoea, which is a critical condition observed in shigellosis, is induced by the sen gene and has been reported across all species of Shigella. It has been reported that dehydration in shigellosis leading to watery diarrhoea is potentially caused by the set1B gene predominantly found only in S. flexneri. Recent studies have shown that S. flexneri possesses more virulent factors compared to other Shigella spp. Several studies also indicated that Sat and ShET1 toxins which may be major contributors to the virulence of S. flexneri strains were predominantly found in S. flexneri but absent in other Shigella spp. Although it cannot be stated conclusively, based on the above-mentioned discussion and the results of this study, it is likely that GLD selectively targets virulence factors predominantly found in S. flexneri leading to a decrease in their virulence.

The findings of the current study also revealed that GLD reduced the invasion capabilities of eight (90%) out of nine tested S. flexneri strains, followed by S. sonnei wherein four (58%) out of seven tested strains represented a reduction in the invasion. A plausible reason might be that GLD targets the Type III secretion system (T3SS) used by Shigella spp. to invade human mucosal cells. This hypothesis is supported by Honma et al. and Michel-Briand et al. reporting that erythromycin selectively reduced the invasion rate of S. flexneri and S. sonnei by passing through the T3SS, as well as a recent study which suggested that T3SS in MDR S. flexneri harbours more virulence factors in comparison to their sensitive counterparts. Thus, virulence factors in MDR S. flexneri targeted by GLD would result in a reduction in the invasion rate.

Therefore, it is proposed that multiple components of GLD probably act at multiple targets including virulence genes such as set1B, Sat, and ShET1 harbored in antibiotic-resistant S. flexneri, along with the Type III secretion system, eventually affecting the bacterial survival and the ability to invade epithelial cells. Subsequently, it would also be worth exploring the differential expression of genes associated with bacterial growth pathways and/or bacterial virulence in the presence of GLD using transcriptomics. This would not only contribute to understanding the molecular basis of the anti-diarrhoeal activity of GLD against Shigella species but also provide enlarged insights into the mechanisms underlying its efficacy against drug-resistant clinical isolates of this bacterial species.

**Financial Support**
This study was financially supported by Zoetis Pharmaceuticals Research Private Limited, India

**Authors’ Contributions**
P. D. and V. M. undertook the bioassays, carried out the literature survey, and drafted the manuscript. R. M. and M. D. provided the clinical Shigella isolates and were responsible for the antibiotic susceptibility testing of the isolates. T. B. was responsible for the study, contributed to the study design, and was involved in the preparation of the manuscript.

**Ethical Approval**
This study was approved by the Ethics Committee of Foundation for Medical Research (Reference: FMR/IREC/MP/01/2019) and the St. John’s Research Institute (Reference: IEC/1/354/2019).

**Conflict of Interest Disclosures**
The authors declare that they have no conflict of interests.

**Acknowledgements**
The authors are grateful to Dr. P. Tetali for providing the guava
leaves in the study and obtaining the herbarium number. In addition, we acknowledge the help of Dr. RN Sivanandan (Infectious Diseases Unit, St John’s Research Institute) for rechecking, compiling patient data, and sending the isolates to Mumbai, as well as Miss D. Kirobabai (Department of Microbiology, St. John’s Medical College) for isolating, identifying, screening for antibiotic resistance, and stocking the isolates. The authors are also thankful to Dr. Camilla Rodrigues (Hinduja Hospital, Mumbai) for providing three clinical isolates that were included in the study.

References

1. Rathaur VK, Pathania M, Jayara A, Yadav N. Clinical study of acute childhood diarrhoea caused by bacterial enteropathogens. J Clin Diagn Res. 2014;8(5):PC01-05. doi:10.7860/jcdr/2014/6677.4319

2. Tacconelli E, Carrara E, Savoldi A, et al. Discovery, research, and development of new antibiotics: the WHO priority list of antibiotic-resistant bacteria and tuberculosis. Lancet Infect Dis. 2018;18(3):318-327. doi:10.1016/s1473-3099(17)30753-3

3. Chellapandi K, Dutta TK, Sharma I, De Mandal S, Kumar NS, Ralte L. Prevalence of multi drug resistant enteropathogenic and enteroinvasive Escherichia coli isolated from children with and without diarrhea in Northeast Indian population. Ann Clin Microbiol Antimicrob. 2017;16(1):49. doi:10.1186/s12941-017-0225-x

4. Boucher HW, Talbot GH, Bradley JS, et al. Bad bugs, no drugs: no ESKAPE! An update from the Infectious Diseases Society of America. Clin Infect Dis. 2009;48(1):1-12. doi:10.1086/595011

5. World Health Organization. Antibiotic resistance—a threat to global health security May 2013. Available at: http://www.who.int/drugresistance/activities/what66_side_event/en/. Accessed March 31, 2020.

6. Tai AY, Easton M, Encena J, et al. A review of the public health management of shigellosis in Australia in the era of culture-independent diagnostic testing. Aust N Z J Public Health. 2016;40(6):585-591. doi:10.1111/1753-6405.12590

7. World Health Organization. Guidelines for the control of Shigellosis, including epidemics due to Shigella dysenteriae type 1. 2005. http://www.who.int/cholera/publications/shigellosis/en/. Accessed March 31, 2020.

8. Das JK, Salam RA, Bhutta ZA. Global burden of childhood diarrhea and interventions. Curr Opin Infect Dis. 2014;27(5):451-458. doi:10.1097/qco.0000000000000166

9. Puzari M, Sharma M, Chetia P. Emergence of antibiotic resistant Shigella species: a matter of concern. J Infect Public Health. 2018;11(4):451-454. doi:10.1016/j.jiph.2017.09.025

10. Gu B, Cao Y, Pan S, et al. Comparison of the prevalence and changing resistance to nalidixic acid and ciprofloxacin of Shigella between Europe-America and Asia-Africa from 1998 to 2009. Int J Antimicrob Agents. 2012;40(1):9-17. doi:10.1016/j.ijantimicag.2012.02.005

11. Aggarwal P, Uppal B, Ghosh R, et al. Multi drug resistance and extended spectrum beta lactamases in clinical isolates of Shigella: a study from New Delhi, India. Travel Med Infect Dis. 2016;14(4):407-413. doi:10.1016/j.tmaid.2016.05.006

12. Vubil D, Ballest-Delpierre C, Mabunda R, et al. Antibiotic resistance and molecular characterization of Shigella isolates recovered from children aged less than 5 years in Manhiça, Southern Mozambique. Int J Antimicrob Agents. 2018;51(6):881-887. doi:10.1016/j.ijantimicag.2018.02.005

13. Farshad S, Sheikh R, Japoni A, Basiri E, Alborzi A. Characterization of Shigella strains in Iran by plasmid profile analysis and PCR amplification of ipa genes. J Clin Microbiol. 2006;44(8):2879-2883. doi:10.1128/jcm.00310-06

14. Yismaw G, Negeri C, Kassu A. A five-year antimicrobial resistance pattern of Shigella isolated from stools in the Gondar University hospital, northwest Ethiopia. Trop Doct. 2008;38(1):43-45. doi:10.1258/tid.2007.060215

15. Lin J, Puckree T, Mvelase TF. Anti-diarrhoecal evaluation of some medicinal plants used by Zulu traditional healers. J Ethnopharmacol. 2002;79(1):53-56. doi:10.1016/s0378-8741(01)00353-1

16. Adebolu TT, Adeboye PT, Adebola NB. Evaluation of a traditional decoction made from Psidium guajava and Zingiber officinale for anti bacterial activity. Res J Microbiol. 2007;2(12):954-959. doi:10.3923/rjm.2007.954.959

17. Morais-Braga MF, Carneiro JN, Machado AJ, et al. Psidium guajava L., from ethnobiology to scientific evaluation: elucidating bioactivity against pathogenic microorganisms. J Ethnopharmacol. 2016;194:1140-1152. doi:10.1016/j.jep.2016.11.017

18. Birdi T, Daswani PG, Brijesh S, Tetali P, Natu A, Antia N. Newer insights into the mechanism of action of Psidium guajava L. leaves in infectious diarrhea. BMC Complement Altern Med. 2010;10:33. doi:10.1186/1472-6882-10-33

19. Birdi TJ, Daswani PG, Brijesh S, Tetali P. In vitro antiadigarial and antirotaviral activity of Psidium guajava L. leaves. Indian J Pharmacol. 2011;43(5):616-617. doi:10.4103/0253-7613.84990

20. Clinical and Laboratory Standards Institute (CLSI). Performance Standards for Antimicrobial Disk Susceptibility Tests. 13th ed. Wayne, PA: CLSI; 2018.

21. Cruickshank R, Duguid JP, Marmion BP, Swain RH. Test for sensitivity to antimicrobial agents. Med Microbiol. 1975;2:196-197.

22. Vesikari T, Bromirska J, Mäki M. Enhancement of invasiveness of Yersinia enterocolitica and Escherichia coli in HEp-2 cells by centrifugation. Infect Immun. 1982;36(2):834-836. doi:10.1128/iai.36.2.834-836.1982

23. Schroeder GN, Hilbi H. Molecular pathogenesis of Shigella spp.: controlling host cell signaling, invasion, and death by type III secretion. Clin Microbiol Rev. 2008;21(1):134-156. doi:10.1128/cmr.00032-07

24. Anderson MC, Vonaesch P, Saffarian A, Marteyn BS, Sansonetti PJ. Shigella somaii encodes a functional T6SS used for interbacterial competition and niche occupancy. Cell Host Microbe. 2017;21(6):769-776.e3. doi:10.1016/j.chom.2017.05.004

25. Vinh H, Nhu NT, Nga TV, et al. A changing picture of shigellosis in southern Vietnam: shifting species dominance, antimicrobial susceptibility and clinical presentation. BMC Infect Dis. 2009;9:204. doi:10.1186/1471-2334-9-204

26. Qiu S, Xu X, Yang C, et al. Shift in serotype distribution of Shigella species in China, 2003-2013. Clin Microbiol Infect.
international colonization of 

Shigella 

species isolated from stool specimens 

in India: its association with clinical manifestation and antimicrobial resistance. Pathog Glob Health. 2019;113(4):173-179. doi:10.1080/20477724.2019.1632062

Cristea D, Oprea M, Ciontea AS, Antohe F, Usein C-R. Prevalence of virulence markers and HPS-2 like plasmids among Shigella sonnei and Shigella flexneri isolates originating from shigellosis cases in Romania. Rev Rom Med Lab. 2016;22(4):103-110. doi:10.1515/rrml-2016-0003

Medeiros P, Lima AA M, Guedes MM, et al. Molecular characterization of virulence and antimicrobial resistance profile of Shigella species isolated from children with moderate to severe diarrhea in northeastern Brazil. Diagn Microbiol Infect Dis. 2018;90(3):198-205. doi:10.1016/j.diagmicrobio.2017.11.002

da Cruz CB, de Souza MC, Serra PT, et al. Virulence factors associated with pediatric shigellosis in Brazilian Amazon. Biomed Res Int. 2014;2014:539697. 10.1155/2014/539697

Hosseini Nave H, Mansouri S, Emaneini M, Moradi M. Profiling of virulence-associated factors in Shigella species isolated from acute pediatric diarrheal samples in Tehran, Iran. Osong Public Health Res Perspect. 2017;8(3):220-226. doi:10.24171/j.phrp.2017.8.3.09

Torres AG. Current aspects of Shigella pathogenesis. Rev Latinoam Microbiol. 2004;46(3-4):89-97.

Yaghoubi S, Ranjbar R, Soltan Dallal MM, Yaslani Fard S, Shirazi MH, Mahmoudi M. Profiling of virulence-associated factors in Shigella species isolated from acute pediatric diarrheal samples in Tehran, Iran. Osong Public Health Res Perspect. 2017;8(3):220-226. doi:10.24171/j.phrp.2017.8.3.09

sethuvul DPM, Anandan S, Michael JS, et al. Virulence gene profiles of Shigella species isolated from stool specimens in India: its association with clinical manifestation and antimicrobial resistance. Pathog Glob Health. 2019;113(4):173-179. doi:10.1080/20477724.2019.1632062

Cristea D, Oprea M, Ciontea AS, Antohe F, Usein C-R. Prevalence of virulence markers and HPS-2 like plasmids among Shigella sonnei and Shigella flexneri isolates originating from shigellosis cases in Romania. Rev Rom Med Lab. 2016;22(4):103-110. doi:10.1515/rrml-2016-0003

Medeiros P, Lima AA M, Guedes MM, et al. Molecular characterization of virulence and antimicrobial resistance profile of Shigella species isolated from children with moderate to severe diarrhea in northeastern Brazil. Diagn Microbiol Infect Dis. 2018;90(3):198-205. doi:10.1016/j.diagmicrobio.2017.11.002

da Cruz CB, de Souza MC, Serra PT, et al. Virulence factors associated with pediatric shigellosis in Brazilian Amazon. Biomed Res Int. 2014;2014:539697. 10.1155/2014/539697

Hosseini Nave H, Mansouri S, Emaneini M, Moradi M. Distribution of genes encoding virulence factors and molecular analysis of Shigella spp. isolated from patients with diarrhea in Kerman, Iran. Microb Pathog. 2016;92:68-71. doi:10.1016/j.micpath.2015.11.015

Lluque A, Mosquito S, Gomes C, et al. Virulence factors and mechanisms of antimicrobial resistance in Shigella strains

Sansonetti PJ. Rupture, invasion and inflammatory destruction of the intestinal barrier by Shigella: the yin and yang of innate immunity. Can J Infect Dis Med Microbiol. 2006;17(2):117-119. doi:10.1155/2006/189784

Torres AG. Current aspects of Shigella pathogenesis. Rev Latinoam Microbiol. 2004;46(3-4):89-97.

Yaghoubi S, Ranjbar R, Soltan Dallal MM, Yaslani Fard S, Shirazi MH, Mahmoudi M. Profiling of virulence-associated factors in Shigella species isolated from acute pediatric diarrheal samples in Tehran, Iran. Osong Public Health Res Perspect. 2017;8(3):220-226. doi:10.24171/j.phrp.2017.8.3.09

Sethuvul DPM, Anandan S, Michael JS, et al. Virulence gene profiles of Shigella species isolated from stool specimens in India: its association with clinical manifestation and antimicrobial resistance. Pathog Glob Health. 2019;113(4):173-179. doi:10.1080/20477724.2019.1632062

Cristea D, Oprea M, Ciontea AS, Antohe F, Usein C-R. Prevalence of virulence markers and HPS-2 like plasmids among Shigella sonnei and Shigella flexneri isolates originating from shigellosis cases in Romania. Rev Rom Med Lab. 2016;22(4):103-110. doi:10.1515/rrml-2016-0003

Medeiros P, Lima AA M, Guedes MM, et al. Molecular characterization of virulence and antimicrobial resistance profile of Shigella species isolated from children with moderate to severe diarrhea in northeastern Brazil. Diagn Microbiol Infect Dis. 2018;90(3):198-205. doi:10.1016/j.diagmicrobio.2017.11.002

da Cruz CB, de Souza MC, Serra PT, et al. Virulence factors associated with pediatric shigellosis in Brazilian Amazon. Biomed Res Int. 2014;2014:539697. 10.1155/2014/539697

Hosseini Nave H, Mansouri S, Emaneini M, Moradi M. Distribution of genes encoding virulence factors and molecular analysis of Shigella spp. isolated from patients with diarrhea in Kerman, Iran. Microb Pathog. 2016;92:68-71. doi:10.1016/j.micpath.2015.11.015

Lluque A, Mosquito S, Gomes C, et al. Virulence factors and mechanisms of antimicrobial resistance in Shigella strains
from Periurban areas of Lima (Peru). Int J Med Microbiol. 2015;305(4-5):480-490. doi:10.1016/j.ijmm.2015.04.005

55. Roy S, Thanasekaran K, Dutta Roy AR, Sehgal SC. Distribution of Shigella enterotoxin genes and secreted autotransporter toxin gene among diverse species and serotypes of shigella isolated from Andaman Islands, India. Trop Med Int Health. 2006;11(11):1694-1698. doi:10.1111/j.1365-3156.2006.01723.x

56. Thong KL, Hoe SL, Puthucheary SD, Yasin R. Detection of virulence genes in Malaysian Shigella species by multiplex PCR assay. BMC Infect Dis. 2005;5:8. doi:10.1186/1471-2334-5-8

57. Silué N, Marcantonio E, Campbell-Valois FX. RNA-Seq analysis of the T3SA regulon in Shigella flexneri reveals two new chromosomal genes upregulated in the on-state. Methods. 2020;176:71-81. doi:10.1016/j.ymeth.2019.03.017

58. Honma Y, Sasakawa C, Tsuji T, Iwanaga M. Effect of erythromycin on Shigella infection of Caco-2 cells. FEMS Immunol Med Microbiol. 2000;27(2):139-145. doi:10.1111/j.1574-695X.2000.tb01424.x

59. Michel-Briand Y, Laporte JM, Couetdic G, Sansonetti PJ. Elimination of a virulence plasmid from Shigella sonnei and Escherichia coli by antibiotics. Ann Inst Pasteur Microbiol. 1986;137B(3):291-295. doi:10.1016/s0769-2609(86)80119-3

60. Wang L, Zhu Z, Qian H, et al. Comparative genome analysis of 15 clinical Shigella flexneri strains regarding virulence and antibiotic resistance. AIMS Microbiol. 2019;5(3):205-222. doi:10.3934/microbiol.2019.3.205