Journal of Biosciences and Medicines, 2018, 6, 22-33
http://www.scirp.org/journal/jbm
ISSN Online: 2327-509X
ISSN Print: 2327-5081

Prevalence and Drug-Resistance Patterns of Enterotoxigenic *Escherichia coli* and *Shigella* Species among Children with Diarrhea in Merida City, Mexico

Claribel Huchin[1], Mario A. Briceño[1], Tahali Mendoza[2], Ana P. Martínez[2], Mario A. Ramírez[3], Julio C. Torres[3]

[1]Laboratorio de Análisis Clínicos de Servicio a la Comunidad, Facultad de Química, Universidad Autónoma de Yucatán, Mérida, México
[2]Laboratorio de Bioquímica y Genética Molecular, Facultad de Química, Universidad Autónoma de Yucatán, Mérida, México
[3]Centro de Información de Medicamentos, Facultad de Química, Universidad Autónoma de Yucatán, Mérida, México
Email: julio.torres@correo.uady.mx

How to cite this paper: Huchin, C., Briceño, M.A., Mendoza, T., Martínez, A.P., Ramírez, M.A. and Torres, J.C. (2018) Prevalence and Drug-Resistance Patterns of Enterotoxigenic *Escherichia coli* and *Shigella* Species among Children with Diarrhea in Merida City, Mexico. *Journal of Biosciences and Medicines*, **6**, 22-33.
https://doi.org/10.4236/jbm.2018.61004

Received: September 14, 2017
Accepted: January 1, 2018
Published: January 4, 2018

Abstract

Enterotoxigenic *Escherichia coli* (ETEC) and *Shigella* are two of the leading causes of diarrhea among children in developing countries. The prevalence of ETEC and *Shigella* species resistant to antimicrobial agents is increasing. The aim of this study was to determine prevalence and antimicrobial resistance patterns of ETEC and *Shigella* species among under-five children with diarrhea in an urban region of southeastern Mexico. A cross-sectional study was conducted among under-five children with acute diarrhea from January 2013 to January 2014 at Merida city. Isolation, identification and antimicrobial susceptibility test of ETEC and *Shigella* species were performed using standard bacteriological protocols. Of 200 stool samples collected, 18 (9.0%) ETEC and 12 (6.0%) *Shigella* strains were isolated. Among 12 *Shigella* species *Shigellaflexneri* founded as 8 (66.7%), followed by *Shigellaboydii* 4 (33.3%). One hundred percent of ETEC and *Shigella* isolates showed resistance to ampicillin, carbenicillin and cephalothin. Also, high frequency of resistance for both ETEC and *Shigella* isolates was observed to nitrofurantoin (100%, 83.3%), respectively. However, when we analyzed the resistance patterns of *Shigella* by species, *S. boydii* showed more resistance (8 of 12 antimicrobials tested) in comparison to *S. flexneri* isolates. Multidrug resistance (MDR) (≥3 drugs) was observed among all ETEC and *Shigella* isolates, being the aminoglycosides the more effective drugs against these pathogens. In conclusion, these findings indicate that ETEC and *Shigella* spp. are important etiological

agents of diarrhea among under-five children and a high rate of drug resistance, including MDR, to the commonly used drugs was observed in our region.

Keywords

ETEC, *Shigella*, Diarrhea, Antibiotic Resistance

1. Introduction

Diarrhea caused by enteric infections is a major factor in morbidity and mortality worldwide. The World Health Organization (WHO) has estimated that billion episodes of infectious diarrhea occur each year and are especially prevalent in infants and children younger than 5 years [1] [2].

Enterotoxigenic *Escherichia coli* (*E. coli*), or ETEC, and *Shigella* species remain major contributors to acute enteric infections, particularly in the developing countries, where they account for about one billion cases of diarrhea annually. They are responsible for almost one-third of child deaths from diarrhea, as well as many deaths in older age groups [3] [4] [5].

The ETEC infection in developing countries is usually frequent in infants younger than 2 years of age, with a decrease after 5 years of age. Repeated ETEC infections among children in these countries are not rare and may be due to both environmental and immunological factors. The diarrhea produced by ETEC is characterized by a rapid onset of watery stool (without blood or inflammatory cells) due to intestinal colonization and production of one or more plasmid-encoded enterotoxins [6].

Infection caused by *Shigella* species (shigellosis) is endemic worldwide and is responsible for an estimated 120 million cases of severe dysentery annually, the majority of which occur in children from developing countries. Shigellosis symptoms include abdominal pain, cramps, fever, vomiting and bloody diarrhea and with mucus in stool [1] [7].

Diarrheal illness rarely requires antimicrobial treatment and often treatment is given empirically depending on the severity of the disease and on the risk of complications. To guide the empirical choice of antibiotics, it is crucial to know both which pathogens are most likely to be infecting the patient in a particular geographic area and the most effective antibiotics for treating them [8].

In addition, the overuse and misuse of antibiotics in the treatment of diarrhea could lead to an increase of antibiotic resistance. Several studies from developing countries showing worrying trends in multiple resistance among enteric pathogens such as *Escherichia coli* and *Shigella* spp. [9].

Despite the high prevalence of ETEC and *Shigella* infections, few studies have been conducted to investigate their prevalence and susceptibility pattern in children our country. Therefore, the aim of this work was to determine the current prevalence and antimicrobial susceptibility patterns of ETEC and *Shigella*

isolates from children in Merida city, Mexico, to provide information for the selection of appropriate empirical treatment of these infections in our region.

2. Methods

Specimen collection: This study was conducted in Merida city and was approved by the Ethical Committee of the Regional Research Centre Dr Hideyo Noguchi of the Autonomous University of Yucatán (UADY) (protocol CEI-CIR-UADY-2012-15). This was a descriptive study conducted over period of one year from January 2013 to January 2014.

Microbiological Analysis: A total of 200 stool samples of children with diarrhea referred to the Clinical Analysis Laboratory of Community Services of the Faculty of Chemistry, UADY, and Friendship's hospital of Mérida City from Mexico. There were spread on Mac Conkey agar, xylose lysine deoxycholateagar (XLD), and Salmonella-Shigella agar(S-S) agar and were incubated at 37˚C for 18 - 24 h. The presumptive colonies were fully identified using standard biochemical tests, including Triple Sugar Iron (TSI), Sulphur Indole and Motility (SIM), Urea, Simmons citrate, and Lysine Iron Agar (LIA) according to results described on Table 1.

Identification of Enterotoxigenic *Escherichia coli* by PCR: *E. coli* clinical isolates were processed for isolation of genomic DNA as previously described [10]. In brief, overnight liquid cultures were centrifuged, and the pellet was resuspended in TE buffer (10 m MTris-HCL pH 8.0, 5 m MEDTA), boiled for 10 min, and centrifuged again. The supernatant containing a crude DNA extract was used as a DNA template for PCR assays.

Multiplex PCR reactions with specific ST primers (5'-GCTAAACCAGTAGAGCTCTTCAAAA-3' and 5'-CCCGGTACAAGCAGGATTACAACA-3') to amplify a 147-bp fragment and specific LT primers(5'-GCACACGGAGCTCCTCAGTC-3' and 5'-TCCTTCATCCTTTCAATGGCTTT-3') to amplify a 218-bp fragment were performed as previously reported by Rugeles *et al*. (2010).

Serological identification of *Shigella* species: All *Shigella* clinical isolates were identified by biochemical properties by slide agglutination test, using commercially available antisera (Serobac, BD BBLTM, USA). These antisera were *S. dysenteriae* poly A, *S. flexneri*poly B, *S. boydii* poly C, and *S. sonnei* poly D.Briefly, strains were subcultured on tryptic soyagar (Difco) and tested for agglutination on glass slides. Slides were divided into two sections with a wax pencil. A drop (20 µl) of 0.85% NaCl solution was placed in one section for use as a negative

Table 1. Biochemical identification of *E. coli* and *Shigella* spp.

Bacteria	TSI	Citrate	H_2S	Motility	Indole	Urea	LIA
Escherichia coli	A/A, Gas+	–	–	+	+	–	K/K
Shigella spp.	K/A Gas–	–	–	–	–	–	K/A

control, and a drop of the appropriate antiserum was placed in the other section. By using a sterile inoculation loop, a portion of the culture was emulsified with the NaCl solution and a second independent portion of the culture was mixed in the section of the slide containing the corresponding antiserum. The slide was then gently rocked, and relative agglutination was scored after 60 s. Two reference strains, *Shigella flexneri* ATCC 12022 and *Shigella sonnei* ATCC 9290, were used as control strains for positive agglutination.

Antimicrobial susceptibility testing: ETEC and *Shigella* strains were analyzed for their antimicrobial susceptibility by Kirby-Bauer disc diffusion method according to the Clinical and Laboratory Standards Institute (CLSI) guidelines. The antibiotics tested were: ampicillin (AMP, 10 μg), amikacin (AK, 30 μg), carbenicillin (CB, 100 μg), cephalothin (CF, 30 μg), cefotaxime (CTX, 30 μg), ciprofloxacin (CIP, 5 μg), chloramphenicol (CL, 30 μg), gentamicin (GE, 10 μg), netilmicin (NET, 30 μg), nitrofurantoin (NF, 300 μg), norfloxacin (NX, 10 μg), and trimethoprim/sulfamethoxazole (SXT, 30 μg). *E. coli* ATCC 25922 was used for quality control purposes according to the CLSI standards. After 24 hrs of incubation the diameter of zone of inhibition was measured in millimeter (mm) and the sensitivity pattern of each isolate was recorded accord to the inhibition zone size scale provided by the CLSI standards. For the data analysis, isolates categorized as intermediate were considered as resistant isolates.

3. Results and Discussion

During the 12-month study period a total of 200 stool specimens were examined from children with diarrhea. The subjects comprised 89 males (44.5%) and 111 females (55.5%), with a median age of 2.3 years (SD ± 1.3) and ranging from 0 - 5 years old.

Escherichia coli strains were detected in 124 out of 200 (62.0%) samples analyzed. Of these *E. coli* strains, 18 (14.5%) were positive for ETEC by PCR (Figure 1). The genes *lt* and *st* were successfully amplified in all samples, indicating that all strains correspond to ETEC.

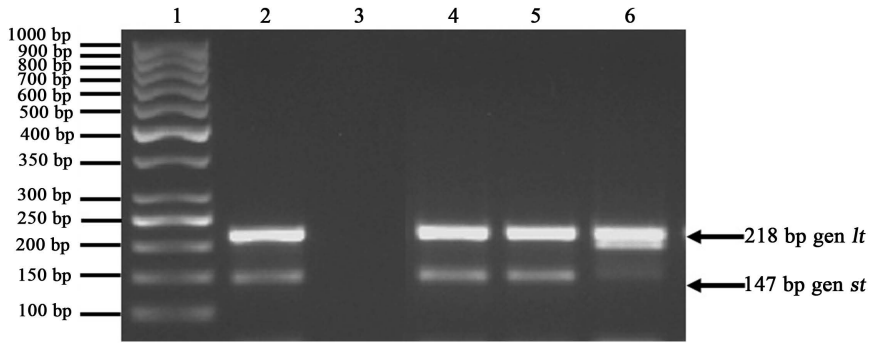

Figure 1. Identification of ETEC by PCR based on the *lt* and *st* genes. Ethidium bromide stained agarose gel showing PCR products of 147 and 218 bp for *st* and *lt* genes, respectively; after amplification of genomic DNA extracted from *E. coli* isolates from diarrheic stools in children. Lane 1: 50 bp molecular weight marker; lane 2: positive control; lane 3: negative control; lanes 4 - 6: positive strains for ETEC.

In our study, the prevalence rate of ETEC infection was of 9.0% among children with diarrhea under 5 years of age. These results agree with the rates determined in Turkey and Iran [11] [12]. However, this rate is lower than the reports from Tunisia and Egypt [13] [14].

The highest prevalence of ETEC infection (55.5%) was found between the 2 and 3-year-old-children, followed by the children under one year of age and between the 1 and 2-year-old-children, with a prevalence of 27.7% and 16.7%, respectively (**Table 2**). These findings reflect that ETEC was an important pathogenic bacteria in the population of children studied.

Some reports indicate that ETEC infection is the first cause of diarrhea illness experienced by infants from endemic areas of developing countries during their first year of life [6] [15]. In a study conducted in our country in 2009, Estrada-García *et al*. [16] determined a 37.5% prevalence rate for ETEC in children (<2 years) with acute diarrhea. In that study for 0 - 1-year-old group; ETEC was detected only in 17% and for our study the prevalence was 28.7% for children in the same age. Nevertheless our study demonstrated that the infection for ETEC was more prevalent in children < 2.5 years of age. Our findings are supported by previous studies of incidence of ETEC infections in developing countries, which revealed that ETEC was detected at a constant rate from cases of acute diarrhea during the first two years of life, with a de-crease of infections after three years [6].

In our study, both toxins coding genes was amplified in all ETEC strains isolated from children with diarrhea. LT expressing ETEC strains are considered to be less pathogenic than those expressing ST [17].

Among the 200 stool samples subjected to analysis, 12 (6.0%) were positive for *Shigella* species infection in children under five years old. The highest prevalence of *Shigella* infection (9 of 12, 75.0%) was found in children under one year of age, and especially those aged 7 - 11 months (**Table 2**). *Shigella flexneri* was identified in 8 out of 12 *Shigella* isolates (66.7%), corresponding the most prevalent specie detected in these children, with a median age of 1.3 years (SD ± 0.8), followed by *Shigella boydii* (4/12; 33.3%), with a median age of 1.2 years (SD ± 0.1). Neither *Shigella sonnei* nor *Shigella dysenteriae* were detected in any of the children studied.

Table 2. Enterotoxigenic *E. coli* and *Shigella* isolates from children with diarrhea in Merida, Mexico.

Age range (years)	ETEC n (%)	*S. flexneri* n (%)	*S. boydii* n (%)
0.0 - 1.0	5 (27.7)	7 (58.33)	2 (16.66)
1.1 - 2.0	3 (16.7)	1 (8.33)	2 (16.66)
2.1 - 3.0	10 (55.5)	NI	NI
3.1 - 4.0	NI	NI	NI
4.1 - 5.0	NI	NI	NI

NI: Not identified.

Shigella species are predominantly isolated from the stools of children with bloody diarrhea causing 50% or more of all episodes in endemic regions of the developing countries. In this study, *Shigella* infection prevalence was 6.0% (12 of 200). This prevalence was similar to other studies performed across the world with children in the same age group, particularly African and Asian countries [18] [19] [20] [21] [22]. However, it is lower than the rate reported in a study conducted in our region by Zaidi *et al*. [23] where determined a 12.1% prevalence rate for *Shigella* infection in children (0 - 10 years) with diarrhea in 2011. In that study, 54.0% of children belong to an age group of 1 - 4 years. If the rate for 1 - 4-year-old group had been calculated, the *Shigella* prevalence rate would probably have been around the same percentage as in our study, since ten of the twelve isolates correspond to children with a median age of 1.5 years (SD ± 0.5). Evaluation of the results of the study by Zaidi *et al*. and our study shows that the prevalence rate of *Shigella* in our region is predominately in children less than 5 years.

The geographical repartition and the pathogenicity of the four *Shigella* species are different by country [24]. In this study, we found *S. flexneri* was the most common species, followed by *S. boydii*. These results agree with previous reports that indicate that *S. flexneri* is the most commonly isolated species in the developing world and the most frequent cause of bacterial dysentery [25].

There were no significant sex-related differences in the incidence of both ETEC and *Shigella* infections in the children studied. The antimicrobial susceptibility testing results of all 18 ETEC and 12 *Shigella* strains isolated from the stool samples of children of 0 - 5 years of age are shown in **Table 3**.

Several of the ETEC strains were resistant to many antimicrobial drugs. The highest level of resistance was detected for ampicillin, carbenicillin, cephalotin, and nitrofurantoin in which all (100%) ETEC isolates were found to be resistant. The resistance rates of ETEC isolates to the other antibiotics were 66.6% for cefotaxime, 61.1% for chloramphenicol, followed by ciprofloxacin and gentamicin

Table 3. Antimicrobial drug resistance of ETEC and *Shigella* species isolated from children with diarrhea in Merida, Mexico.

Strains (n)	% Resistant (n)											
	AMP	AK	CB	CF	CTX	CIP	CL	GE	NET	NF	NX	SXT
ETEC (18)	100 (18)	44.4 (8)	100 (18)	100 (18)	66.6 (12)	50.0 (9)	61.1 (11)	50.0 (9)	33.3 (6)	100 (18)	27.7 (5)	33.3 (6)
Shigella (a)	100 (12)	0 (0)	100 (12)	100 (12)	33.3 (4)	16.2 (2)	33.3 (4)	16.2 (2)	16.2 (2)	83.3 (10)	33.3 (4)	33.3 (4)
S. flexneri (8)	100 (8)	0 (0)	100 (8)	100 (8)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	75.5 (6)	0 (0)	0 (0)
S. boydii (4)	100 (4)	0 (0)	100 (4)	100 (4)	100 (4)	50.0 (2)	100 (4)	50.0 (2)	50.0 (2)	100 (4)	100 (4)	100 (4)

AMP, ampicillin; AK, amikacin; CB, carbenicillin; CF, cephalothin; CTX, cefotaxime; CIP, ciprofloxacin; CL, chloramphenicol; GE, gentamicin; NET, netilmicin; NF, nitrofurantoin; NX, norfloxacin; STX, trimethoprim/sulfamethoxazole. (a)-*Includes S. flexneri and S. boydii strains.*

with a 50.0% of resistance each, and 44.4% ETEC resistance for amikacin. ETEC isolates showed 33.3% resistance to netilmicin and trimethroprim-sulfamethoxazole. The lowest level of resistance was detected for norfloxacin (27.7%).

Resistance patterns of *Shigella* spp. against the applied antimicrobials is given in Table 2. All of the 12 strains of *Shigella* (100%) were resistant to ampicillin, carbenicillin, and cephalothin, followed by nitrofurantoin (83.3%). Amikacin was the only antimicrobial to which all *Shigella* isolates were susceptible.

When we analyzed the results considering resistance patterns in different species, we identified that *S. flexneri* isolates displayed 100% resistance to ampicillin, carbenicillin, and cephalotin, and 75.5% for nitrofurantoin; while resistance for the other antibiotics were not detected. On the other hand, all *S. boydii* strains showed resistance to almost all antimicrobials tested except ciprofloxacin, gentamicin and netilmicin, to which 50% showed resistance.

All ETEC and *Shigella* isolates showed resistance against at least three antibiotics. These isolates were defined as multidrug resistant (MDR) strains. Six MDR patterns were dominated by ETEC isolates (Table 4). Of the 18 ETEC isolates, 8 (44.4%) showed multidrug resistance to nine antibiotics with two different MDR patterns, followed by resistance to seven antibiotics (33.3%), a MDR patterns to six antibiotics with a prevalence of 11.1%, and MDR patterns for eight and four antibiotics, with a prevalence of 5.6% each.

All the isolates of *S. flexneri* showed two MDR patterns. The most prevalent MDR patterns in *S. flexneri* strains were AMP-CB-CF-NF with a prevalence of

Table 4. Multidrug resistance patterns of ETEC and *Shigella* isolates from children with diarrhea in Merida, Mexico.

Organism and MDR patterns	Number of isolates (%)
ETEC	
AMP-CB-CF-NF-CTX-CL-SXT$^{(7)}$	6 (33.3%)
AMP-CB-CF-NF-CTX-CIP-GE-NET-AK$^{(9)}$	5 (27.8%)
AMP-CB-CF-NF-CIP-CL-GE-NX-AK$^{(9)}$	3 (16.6%)
AMP-CB-CF-NF-CL-NX$^{(6)}$	2 (11.1%)
AMP-CB-CF-NF-CTX-CIP-GE-NET$^{(8)}$	1 (5.6%)
AMP-CB-CF-NF$^{(4)}$	1 (5.6%)
S. flexneri	
AMP-CB-CF-NF$^{(4)}$	6 (75.0%)
AMP-CB-CF$^{(3)}$	2 (25.0%)
S. boydii	
AMP-CB-CF-CTX-CL-NF-NX-SXT-CIP-GE-NET$^{(11)}$	2 (50.0%)
AMP-CB-CF-CTX-CL-NF-NX-SXT$^{(8)}$	2 (50.0%)

AMP, ampicillin; AK, amikacin; CB, carbenicillin; CF, cephalothin; CTX, cefotaxime; CIP, ciprofloxacin; CL, chloramphenicol; GE, gentamicin; NET, netilmicin; NF, nitrofurantoin; NX, norfloxacin; STX, trimethoprim/sulfamethoxazole. $^{(n)}$–number of antibiotics in the MDR pattern.

75.0%, and the AMP-CB-CF pattern was the second most prevalent multiresistance pattern with a prevalence of 25.0%. Likewise, all *S. boydii* isolates were shown to be MDR strains. Two *S. boydii* isolates showed a MDR pattern to 11 out of 12 antibiotics tested and the other two isolates showed a resistance for 8 antibiotics (**Table 4**). The aminoglycosides were the most active antibiotics against both *Shigella* species.

The increase in antibiotic resistance among ETEC and *Shigella* isolates are becoming a serious problem, particularly in developing countries. The incidence varies with the area of isolation of these strains. In our study, a total of 18 ETEC and 12 *Shigella* strains isolated from children were tested against 12 different antibiotics. We report a high prevalence of antimicrobial resistance among ETEC and *Shigella* isolates. All ETEC and *Shigella* isolates were 100% resistant to nitrofurantoin and to two penicillins: ampicillin and carbenicillin. Previous studies reported a prevalence of ampicillin resistance more than 75% for ETEC isolates from children in developing countries, including other regions of Mexico, although resistance levels does not raise the obtained in the present study [26] [27] [28]. On other hand, penicillins are not recommended for *Shigella*empiric treatment because of the high resistance in many regions of the world [9] [29].

Cephalosporins and fluoroquinolones constitute another two major groups. We used two drugs representing the cephalosporins: cephalotin and cefotaxime. All ETEC isolates showed a total resistance to both antibiotics. *Shigella* isolates were 100% resistant to cephalotin. Interestingly, *S. boydii*, but not *S. flexneri*, showed a total resistance to cefotaxime. With the fluoroquinolones: ciprofloxacin and norfloxacin, *S. boydii* also showed the higher resistance pattern in comparison with *S. flexneri*. These data indicates that the appropriate antibiotic treatment of shigellosis should depend on identifying resistance patterns to species-level. However, norfloxacin was the most effective drug against ETEC, as 72.3% isolates were sensitive to it.

Aminoglycosides are an important group of drugs highly effective against Gram-negative bacteria. We used amikacin, gentamicin and netilmicin as representatives of this group. Sensitivity pattern was identified in more than 50% of all ETEC isolates for the three aminoglycosides. Netilmicin was more effective showing resistance 33.3% by ETEC strains. Regarding *Shigella* isolates, no resistance was detected to amikacin. These results are in accordance with some earlier reports of resistance pattern for both enteric pathogens [30] [31]. Nevertheless, these drugs have generally not been the first choice of treatment for diarrheic illness and the use in clinical practice is limited, and therefore, no higher prevalence of resistant bacteria are expected.

The growing problem of multidrug resistant enteric pathogens is especially common in developing countries from Latin America, Africa and Asia [32] [33] [34]. All our isolates showed resistance to at least three drugs in different combinations, therefore all the isolates could be classified as MDR. Also, we found a high frequency of MDR to drugs belonging to structurally different antimicrobial groups. Until six different MDR patterns with a minimum of four drugs

(AMP, CB, CF, and NF) were present in all ETEC strains. It is unclear why ETEC isolates from this area manifests such high rates of resistance; could be because of differences in antimicrobial usage which is not effectively controlled.

Antibiotics always are used to treat *Shigella* infection in children, because it may help to reduce the clinical course and complications of illness, and also to reduce the duration of the symptoms. However, several studies have been reported multidrug resistance to the antibiotics most commonly used to treat shigellosis [18] [35] [36]. In the present study we detected two MDR pattern for *S. flexneri* isolates, however all isolates were susceptible to 8 of 12 antibiotics tested, including the fluoroquinolones which are recommended as the drugs of choice for shigellosis by WHO. In the case of *S. boydii*, although the number of isolates were low, can give us a perspective of the multiresistance present in our region. All *S. boydii* isolates showed two different MDR pattern with a minimum of eight of the 12 antibiotics tested. This finding was in line with some earlier reports conducted in developing countries [37] [38].

4. Conclusion

This study reflects a significant prevalence of ETEC and *Shigella* strains as responsible of diarrhea episodes in children from southeast Mexico. The majority of the isolated strains showed high rates of resistance to antibiotics which may lead to problems in the cases requiring antibiotic treatment. We conclude that MDR is very frequent in ETEC and *Shigella* species in our region, therefore the prescription and use of antimicrobial agents to treat diarrhea infections should be considered several factors including specific identification of bacteria and their antimicrobial resistance profile.

Acknowledgements

This work was supported by the Universidad Autónoma de Yucatán through the Programa Integral de Fortalecimiento Institucional (PIFI).

References

[1] Hodges, K. and Gill, R. (2010) Infectious Diarrhea: Cellular and Molecular Mechanisms. *Gut Microbes*, **1**, 4-21. https://doi.org/10.4161/gmic.1.1.11036

[2] UNICEF, WHO (2009) Diarrhoea: Why Children Are Still Dying and What Can Be Done. Geneva, WHO.

[3] Kotloff, K.L., Nataro, J.P., Blackwelder, W.C., *et al.* (2013) Burden and Aetiology of Diarrhoeal Disease in Infants and Young Children in Developing Countries (the Global Enteric Multicenter Study, GEMS): A Prospective, Case-Control Study. *The Lancet*, **382**, 209-222. https://doi.org/10.1016/S0140-6736(13)60844-2

[4] Lamberti, L.M., Bourgeois, A.L., Fischer, C.L., Black, R.E. and Sack, D. (2014) Estimating Diarrheal Illness and Deaths Attributable to *Shigella* and Enterotoxigenic *Escherichia coli* among Older Children, Adolescents, and Adults in South Asia and Africa. *PLOS Neglected Tropical Diseases*, **8**, e2705.

[5] Walker, R.I. (2015) An Assessment of Enterotoxigenic *Escherichia coli* and *Shigella vaccine* Candidates for Infants and Children. *Vaccine*, **33**, 954-965.

https://doi.org/10.1016/j.vaccine.2014.11.049

[6] Qadri, F., Svennerholm, A.M., Faruque, A.S. and Sack, R.B. (2005) Enterotoxigenic *Escherichia coli* in Developing Countries: Epidemiology, Microbiology, Clinical Features, Treatment, and Prevention. *Clinical Microbiology Reviews*, **18**, 465-483. https://doi.org/10.1128/CMR.18.3.465-483.2005

[7] Emch, M., Ali, M. and Mohammad, Y. (2008) Risk Areas and Neighborhood-Level Risk Factors for *Shigelladysenteriae* 1 and *Shigellaflexneri*. *Health & Place*, **14**, 96-105. https://doi.org/10.1016/j.healthplace.2007.05.004

[8] Diniz-Santos, D.R., Santana, J.S., Barretto, J.R., Andrade, M.G.M. and Silva, L.R. (2005) Epidemiological and Microbiological Aspects of Acute Bacterial Diarrhea in Children from Salvador, Bahia, Brazil. *Brazilian Journal of Infectious*, **9**, 77-83. https://doi.org/10.1590/S1413-86702005000100013

[9] Kariuki, S. (2010) Antimicrobial Resistance in Enteric Pathogens in Developing Countries. In: Sosa, A.J., Byarugaba, D.K., Amabile-Cuevas, C.F., Hsueh, P.R., Kariuki, S. and Okeke, I.N., Eds., *Antimicrobial Resistance in Developing Countries*, Springer, New York. https://doi.org/10.1007/978-0-387-89370-9_11

[10] Rúgeles, L.C., Bai, J., Martínez, A.J., Vanegas, M.C. and Gómez-Duarte, O.G. (2010) Molecular Characterization of Diarrheagenic *Escherichia coli* Strains from Stools Samples and Food Products in Colombia. *International Journal of Food Microbiology*, **138**, 282-286. https://doi.org/10.1016/j.ijfoodmicro.2010.01.034

[11] Ozerol, I.H., Bayraktar, M.R., Iseri, L., Otlu, B. and Durmaz, R. (2005) The Prevalence and Molecular Typing of Enterotoxigenic *Escherichia coli* Strains Isolated from Diarrheic Stools in Malatya, Turkey. *New Microbiologica*, **28**, 237-243.

[12] Jafari, F., Garcia-Gil, L.J., Salmanzadeh-Ahrabi S., Shorkrzadeh, L., Aslani, M.M., Pourhoseingholi, M.A., Derakhshan, F. and Zali, M.R. (2009) Diagnosis and Prevalence of Enteropathogenic Bacteria in Children Less than 5 Years of Age with Acute Diarrhea in Tehran Children's Hospitals. *Journal of Infection*, **58**, 21-27. https://doi.org/10.1016/j.jinf.2008.10.013

[13] Al-Gallas, N., Bahri, O., Bouratbeen, A., Haasen, A.B. and Aissa, R.B. (2007) Etiology of Acute Diarrhea in Children and Adults in Tunis, Tunisia, with Emphasis on Diarrheagenic *Escherichia coli*: Prevalence, Phenotyping, and Molecular Epidemiology. *The American Journal of Tropical Medicine and Hygiene*, **77**, 571-582.

[14] Shaheen, H.I., Messih, I.A., Klena, J.D., Mansour, A.E., I-Wakkeel, Z., Wierzba, T.F., *et al.* (2009) Phenotypic and Genotypic Analysis of Enterotoxigenic *Escherichia coli* in Samples Obtained from Egyptian Children Presenting to Referral Hospitals. *Journal of Clinical Microbiology*, **47**, 189-197. https://doi.org/10.1128/JCM.01282-08

[15] Walker, C.F. and Black, R.E. (2010) Diarrhoea Morbidity and Mortality in Older Children, Adolescents, and Adults. *Epidemiology & Infection*, **13**, 1215-1226. https://doi.org/10.1017/S0950268810000592

[16] Estrada, T., Lopez, C., Thompson, R., *et al.* (2009) Association of Diarrheagenic *Escherichia coli* Pathotypes with Infection and Diarrhea among Mexican Children and Association of Atypical Enteropathogenic E. coli with Acute Diarrhea. *Journal of Clinical Microbiology*, **47**, 93-98. https://doi.org/10.1128/JCM.01166-08

[17] Vilchez, S., Reyes, D., Paniagua, M., Bucardo, F., Möllby, R. and Weintraub, A. (2009) Prevalence of Diarrhoeagenic *Escherichia coli* in Children from Leon, Nicaragua. *Journal of Medical Microbiology*, **58**, 630-637. https://doi.org/10.1099/jmm.0.007369-0

[18] Opintan, J.A. and Newman, M.J. (2007) Distribution of Serogroups and Serotypes of Multiple Drug Resistant *Shigella* Isolates. *Ghana Medical Journal*, **41**, 8-29.

[19] Nath, R., Saikia, L., Choudhury, G. and Sharma, D. (2013) Drug Resistant *Shigellaflexneri* in & around Dibrugarh, North-East India. *Indian Journal of Medical Research*, **137**, 183-186.

[20] Njunda, A.L., Assob, J.C., Nsagha, D.S., Kamga, H.L., Awafong, M.P. and Weledji, E.P. (2012) Epidemiological, Clinical Features and Susceptibility Pattern of Shigellosis in the Buea Health District, Cameroon. *BMC Research Notes*, **5**, 54. https://doi.org/10.1186/1756-0500-5-54

[21] Jomezadeh, N., Babamoradi, S., Kalantar, E. and Javaherizadeh, H. (2014) Isolation and Antibiotic Susceptibility of *Shigella* Species from Stool Samples among Hospitalized Children in Abadan, Iran. *Gastroenterology and Hepatology from Bed to Bench*, **7**, 218-223.

[22] Khan, S.A., Hussain, A., Ali, M.A. and Abbas, M. (2014) Clinico-Epidemiology of Shigellosis in Children Suffering from Diarrhea in District Lahore, Pakistan. *International Journal of Current Microbiology and Applied Sciences*, **3**, 950-957.

[23] Zaidi, M.B., Estrada-García, T., Campos, F.D., *et al*. (2013) Incidence, Clinical Presentation, and Antimicrobial Resistance Trends in *Salmonella* and *Shigella* Infections from Children in Yucatan, Mexico. *Frontiers in Microbiology*, **4**, 1-10. https://doi.org/10.3389/fmicb.2013.00288

[24] Ergönül, O., Imre, A., Celikbaş, A. and Dokuzoğuz, B. (2004) Drug Resistance of *Shigella* Species: Changes over 20 Years in Turkey. *International Journal of Antimicrobial Agents*, **23**, 527-528. https://doi.org/10.1016/j.ijantimicag.2004.01.005

[25] Lima, I.F., Havt, A. and Lima, A.A. (2015) Update on Molecular Epidemiology of *Shigella* Infection. *Current Opinion in Gastroenterology*, **31**, 30-37. https://doi.org/10.1097/MOG.0000000000000136

[26] Vila, J., Vargas, M., Casals, C., Urassa, H., Mshinda, H., Schellemberg, D. and Gascon, J. (1999) Antimicrobial Resistance of Diarrheagenic *Escherichia coli* Isolated from Children under the Age of 5 Years from Ifakara, Tanzania. *Antimicrobial Agents and Chemotherapy*, **43**, 3022-3024.

[27] Estrada-García, T., Cerna, J.F., Paheco-Gil, L., Velázquez, R.F., Ochoa, T.J., Torres, J. and DuPont, H.L. (2005) Drug-Resistant Diarrheogenic Escherichia coli, Mexico. *Emerging Infectious Diseases*, **11**, 1306-1308. https://doi.org/10.3201/eid1108.050192

[28] Ochoa, T.J., Ruiz, J., Molina, M., *et al*. (2009) High Frequency of Antimicrobial Drug Resistance of Diarrheagenic *Escherichia coli* in Infants in Peru. *The American Journal of Tropical Medicine and Hygiene*, **81**, 296-301.

[29] Pickering, L.K. (2008) Antimicrobial Resistance among Enteric Pathogens. In: *Hot Topics in Infection and Immunity in Children IV*, Springer, New York, 154-163. https://doi.org/10.1007/978-0-387-73960-1_12

[30] Roy, S., Shamsuzzaman, S.M. and Mamun, K.Z. (2013) Antimicrobial Resistance Pattern of Diarrheagenic *Escherichia coli* Isolated from Acute Diarrhea Patients. *International Journal of Pharmaceutical Science Invention*, **2**, 43-46.

[31] Usein, C.R., Tatu-Chitoiu, D., Ciontea, S., Condei, M. and Damian, M. (2009) *Escherichia coli* Pathotypes Associated with Diarrhea in Romanian Children Younger than 5 Years of Age. *Japanese Journal of Infectious Diseases*, **62**, 289-293.

[32] Nys, S., Okeke, I.N., Kariuki, S., Dinant, G.J., Driessen, C. and Stobberingh, E.E. (2004) Antibiotic Resistance of Faecal *Escherichia coli* from Healthy Volunteers from Eight Developing Countries. *Journal of Antimicrobial Chemotherapy*, **54**, 952-955. https://doi.org/10.1093/jac/dkh448

[33] Bii, C.C., Taguchi, H., Ouko, T.T., Muita, L.W., Wamae, N. and Kamiya, S. (2005) Detection of Virulence-Related Genes by Multiplex PCR in Multidrug-Resistant Diarrhoeagenic *Escherichia coli* Isolates from Kenya and Japan. *Epidemiology & Infection*, **133**, 627-633. https://doi.org/10.1017/S0950268805003870

[34] Okeke, I.N., Laxminarayan, R., Bhutta, Z.A., Duse, A.G., Jenkins, P., O'Brien, T.F., Pablos-Mendez, A. and Klugman, K.P. (2005) Antimicrobial Resistance in Developing Countries. Part I: Recent Trends and Current Status. *The Lancet Infectious Diseases*, **5**, 481-493. https://doi.org/10.1016/S1473-3099(05)70189-4

[35] Taneja, N., Lyngdoh, V., Vermani, A. and Mohan, B. (2005) Re-Emergence of Multi-Drug Resistant *Shigelladysenteriae* with Added Resistance to Ciprofloxacin in North India & Their Plasmid Profiles. *Indian Journal of Medical Research*, **122**, 348-354.

[36] Toro, C.S., Farfán, M., Contreras, I., Flores, O., Navarro, N., Mora, G.C. and Prado, V. (2005) Genetic Analysis of Antibiotic-Resistance Determinants in Multidrug-Resistant *Shigella* Strains Isolated from Chilean Children. *Epidemiology & Infection*, **133**, 81-86. https://doi.org/10.1017/S0950268804003048

[37] Nguyen, T.V., Le, P.V., Le, C.H. and Weintraub, A. (2005) Antibiotic Resistance in Diarrheagenic *Escherichia coli* and *Shigella* Strains Isolated from Children in Hanoi, Vietnam. *Antimicrobial Agents and Chemotherapy*, **49**, 816-819. https://doi.org/10.1128/AAC.49.2.816-819.2005

[38] Khan, S., Singh, P., Ansari, M. and Asthana, A. (2014) Isolation of *Shigella* Species and Their Resistance Patterns to a Panel of Fifteen Antibiotics in Mid and Far Western Region of Nepal. *Asian Pacific Journal of Tropical Disease*, **4**, 30-34. https://doi.org/10.1016/S2222-1808(14)60309-1