Travel Medicine and Infectious Disease (2016) 14, 407–413

Available online at www.sciencedirect.com

ScienceDirect

journal homepage: www.elsevierhealth.com/journals/tmid

Multi drug resistance and Extended Spectrum Beta Lactamases in clinical isolates of *Shigella*: A study from New Delhi, India

Prabhav Aggarwal [a,*], Beena Uppal [a], Roumi Ghosh [a], S. Krishna Prakash [a], Anita Chakravarti [a], Arun Kumar Jha [a], Krishnan Rajeshwari [b]

[a] *Department of Microbiology, Maulana Azad Medical College, Bahadur Shah Zafar Road, New Delhi 110002, India*
[b] *Department of Pediatrics, Maulana Azad Medical College and Associated Lok Nayak Hospital, Bahadur Shah Zafar Road, New Delhi 110002, India*

Received 27 September 2015; received in revised form 15 April 2016; accepted 6 May 2016
Available online 24 May 2016

KEYWORDS
Diarrhea;
Dysentery;
Antimicrobial susceptibility testing;
Minimum inhibitory concentration

Summary *Background: Shigella* is an important cause of gastroenteritis in local Indian population, as well as of traveler's diarrhea in the international visitors to India. These patients often require appropriate antimicrobial therapy; however, rapid development of antimicrobial resistance poses a major hurdle in achieving this goal.
Method: A prospective study was conducted during 2009–12 in New Delhi, India, including 6339 stool samples from gastroenteritis patients. 121 *Shigella* strains were identified on the basis of colony morphology, biochemical reactions, serotyping and *ipaH* gene based PCR. Antimicrobial susceptibility testing by disc diffusion, MIC determination by Vitek® 2 and phenotypic tests for ESBL/AmpC production were done.
Results: Nineteen percent strains (23/121) were found to be resistant to third generation cephalosporins and all were phenotypically confirmed to be ESBL producers; one strain was positive for AmpC. ESBL producing strains were also found to be significantly more resistant ($p < 0.05$) to several other antimicrobials agents in comparison to ESBL non-producers, [ampicillin (100% vs. 62.2%), ampicillin/sulbactam (100% vs. 30.6%), cotrimoxazole (100% vs. 77.6%), ciprofloxacin (87.0% vs. 49.0%), ofloxacin (87.0% vs. 52.0%) and gentamicin (30.4% vs. 7.1%)]. Multidrug resistance was seen in 76% strains.
Conclusions: Inappropriate use of antimicrobial agents puts high selection pressure on the higher-end antibiotics. Multi-drug resistance and high rates of ESBL production by *Shigella* is

* Corresponding author. E-49, Sector-55, Noida, Uttar Pradesh 201301, India. Tel.: +91 9899303626, +91 1204347120.
E-mail address: prabhavaggarwal@yahoo.co.in (P. Aggarwal).

http://dx.doi.org/10.1016/j.tmaid.2016.05.006

a matter of concern for the local population as well as international travelers. Therefore, better national level antimicrobial management programs are the priority needs.

1. Introduction

Diarrhea is the second most common cause of death among young children around the world, following closely behind pneumonia. This is of particular importance in India, which has the highest number of diarrhea associated deaths compared to any other country in the world [1]. *Shigella* is an important cause of diarrhea, particularly in children, senior citizens and other high risk groups such men who have sex with men. Recently, in 2013, the Global Enteric Multicenter Study (GEMS) has recently found rotavirus, *Cryptosporidium*, enterotoxigenic *Escherichia coli* and *Shigella* to be the most common organisms requiring targeted intervention, in children less than 5 years of age in the sub-Saharan African and south Asian regions [2]. Shigellosis is a highly contagious disease due to its low infectious dose and high transmission in areas with crowding and poor sanitary conditions; making it an important agent of traveler's diarrhea. Clinical manifestations may vary from asymptomatic infections, acute diarrheal illness or severe bacillary dysentery, sometimes terminating in severe and life threatening complications.

Antimicrobial therapy in patients with shigellosis can help to reduce the duration of illness, shedding of the organism in the faeces and the risk of subsequent complications and malnutrition. However, rapid development of antimicrobial resistance among *Shigella* species is a major obstacle. Sulphonamides, tetracyclines, ampicillin, and cotrimoxazole have been useful previously. WHO recommended ciprofloxacin as the drug of choice [3], but high resistance has been reported in several Indian studies [4—6]. Third generation cephalosporins are often used if resistance to the other agents is seen; however, high Minimum Inhibitory Concentrations (MIC) and production of Extended Spectrum Beta Lactamases (ESBL) by *Shigella* species have been reported [7]. Drug resistant *Shigella* infection is rapidly becoming serious threat and a cause of worry for international travelers to India [8]. Thus, this study was undertaken to analyze the multidrug resistance and the current rates of ESBL/AmpC production in *Shigella* strains isolated from the clinical cases of diarrhea and dysentery, that may act as important agents of traveler's diarrhea for international traveler's to India.

2. Methods

A study was conducted at a 1600 bedded tertiary care hospital and medical college in New Delhi, India during 2009—12. Stool samples from all patients presenting with signs and symptoms of gastroenteritis (diarrhea and dysentery) were collected and transported to the Microbiology laboratory within 2 h. In case of delay, samples were transported in Cary Blair medium/buffered glycerol saline and processed within 24 h.

2.1. Culture and identification

The samples were then inoculated directly and after enrichment in selenite F broth onto MacConkey's agar and Xylose Lysine Desoxycholate (XLD) agar (HiMedia, Mumbai, India). The plates were incubated for 18—24 h at 37°c. Non-lactose fermenting colonies from MacConkey's agar and XLD agar were selected and identified on the basis of biochemical reactions and/or Vitek® 2. Biochemically identified *Shigella* strains were serotyped by the slide agglutination test (Denka Seiken Co., Ltd., Tokyo, Japan).

2.2. Polymerase chain reaction

Further confirmation of the *Shigella* strains was carried out by *ipaH* gene based PCR. Bacterial DNA was extracted using the HiPurA kit (HiMedia, Mumbai, India) following the manufacturer's instructions and was used as template DNA. Amplification of a 424 bp region of the *ipaH* gene was done using previously described oligonucleotide primer sequences [9]. *ipaH* gene is also present in Enteroinvasive *E. coli* (EIEC), therefore, exclusion of EIEC was supported by biochemical reactions. Briefly, the reaction mixture (25 μl) consisted of template DNA (6 μl), 10X PCR reaction buffer, 15 mM $MgCl_2$, 50pM of each primer, 0.25 mM concentration of each dNTP (dATP, dCTP, dNTP, dTTP) and 1U of Taq DNA polymerase. Thirty five cycles of amplification were performed, consisting of initial denaturation at 96 °C for 10 min, denaturation at 94 °C for 60s, annealing at 56 °C for 120s, extension at 72 °C for 60s, final extension at 72 °C for 10 min. Positive (*Shigella flexneri* ATCC® 12022) and negative controls (*E. coli* ATCC® 25922) were included in each run of PCR.

2.3. Antimicrobial susceptibility testing/MIC/multidrug resistance

Shigella isolates obtained were subjected to antimicrobial susceptibility testing by the disc diffusion method as per the CLSI guidelines [10]. Susceptibility testing was performed against ampicillin (10 μg), ampicillin/sulbactam (10 μg + 10 μg), gentamicin (10 μg), amikacin (30 μg), doxycycline (30 μg), chloramphenicol (30 μg), cotrimoxazole (1.25 μg + 23.75 μg), naldixic acid (30 μg), ciprofloxacin (5 μg), ofloxacin (5 μg), ceftriaxone (30 μg), ceftazidime (30 μg), cefotaxime (30 μg), piperacillin/tazobactam (100 μg + 10 μg), imipenem (10 μg), meropenem (10 μg) and ertapenem (10 μg) (HiMedia, Mumbai, India).