Annals of Global Health
VOL. 82, NO. 5, 2016

ISSN 2214-9996
http://dx.doi.org/10.1016/j.aogh.2016.09.009

ORIGINAL RESEARCH

Molecular Evaluation of High Fluoroquinolone Resistant Genes in Endemic Cases of Shigellosis, Northeast Part of Karnataka, India

Prabhurajeshwar C, MSc, PhD, Pramod Desai, MSc, PhD, Kelmani Chandrakanth R, PhD
Gulbarga, India

Abstract

OBJECTIVES Shigellosis is an acute infection of the intestine caused by bacteria in the genus *Shigella* and also an important cause of diarrhea in developing countries. This study was carried out to find the extent and nature of the emerging resistance in north part of Karnataka, India, and surrounding region with huge population, and also focused on the molecular mechanism of development of resistance against different generations of fluoroquinolones and explored the diversity of restriction endonucleases; we also tried to establish the significance of reduced minimal inhibitory concentrations (MIC) values.

METHODS A total of 32 multidrug-resistant *Shigella* species (isolated from infants' stools) were subjected to MICs of fluoroquinolone-resistant isolates done by both broth dilution and E-test method. The genes implicated in resistance to fluoroquinolone generations ciprofloxacin, ofloxacin, and gatifloxacin (gyrA, gyrB, parC, and parE) were amplified using polymerase chain reaction (PCR) method and restriction digestion analysis of PCR product were performed using PvuI and HaeII enzymes.

FINDINGS Fluoroquinolone-resistant *Shigella* species (n = 32) comprising *S dysenteriae, S flexneri,* and *S sonnei* were selected for MIC; 90.6% (29/32), 93.75% (30/32), and 93.75% (30/32) of isolates were ciprofloxacin, ofloxacin, and gatifloxacin resistant and showed the MIC range from 4-128 μg/mL. The PCR amplification results were positive for all species and asserted the presence of gyrA, gyrB, parC, and pare and sizes of the amplified products. The restriction banding patterns of amplified resistant genes were employed to detect differences among the *Shigella* species.

CONCLUSIONS The present study found that the genetic basis and its characterization of fluoroquinolone resistance in *Shigella* isolates was considered for the common resistant genes, namely, gyrA, gyrB, parC, and pare, and had mutations at position 83 of gyrA and at position 80 of parC of the quinolone-resistant determining regions and associated molecular mechanism. Our study beneficial in identification of the causative agents of the infections, careful control and cautions use of antibiotics must be promoted, particularly to monitor the emergence of isolates that are fully resistant to fluoroquinolones.

KEY WORDS *Shigella* species, minimal inhibitory concentration (MICs), fluoroquinolone genes, QRDRs, gyrA, gyrB, parC, and parE, genomic DNA, PCR amplification, restriction digestion and shigellosis

This Medical Biotechnology and Phage Therapy Laboratory, Department of Biotechnology approved ethical clearance by Institutional Clearance Certificate (IECC) for in vitro and in vivo studies.
The authors declare no conflict of interest.
From the Medical Biotechnology and Phage Therapy Laboratory, Department of Post Graduate Studies and Research in Biotechnology, Gulbarga University, Gulbarga, Karnataka, India. Address correspondence to R.K.C. (ckelmani64@gmail.com).

INTRODUCTION

Acute gastroenteritis is one of the leading causes of illness and death in infants, children, and immune-compromised and aged individuals throughout the world, Like *Campylobacter* spp and *Vibrio cholera*, *Shigella* spp have managed to survive the antibiotic era via ingenious mechanisms of resistance. Unlike diarrhea caused by *Campylobacter jejuni*, shigellosis may occur in epidemic form, causing considerable morbidity and mortality, especially in developing nations such as India. Asia, Africa, and Latin America had an estimated 2.5 million deaths each year in children younger than 5 years of age.[1-4] Shigellosis is primarily a disease of resource-poor, crowded communities that do not have adequate sanitation or safe water and where disease rates may be high. Among the enteric pathogens, *Shigella* species are of particular concern as causes of food poisoning, abdominal tenderness, and gastroenteritis.[5] Although more prevalent in developing countries, shigellosis is a worldwide problem.[6,7] *Shigella sonnei* is predominating in Europe and the United States, and *S dysenteriae* and *S flexneri* are more prevalent in Asian and African countries.[8]

The traditional antishigellosis drugs chloramphenicol, ampicillin, and sulfamethoxazole have become outdated. In recent years, fluoroquinolones, especially ciprofloxacin, have been very successful in combating shigellosis, but unfortunately, resistant strains have emerged. The emergence of high-level ciprofloxacin resistance in *Shigella* spp has also been reported in India.[9,10] Among the *Shigella* species, a major therapeutic challenge required to control this disease. One of the reasons for emergence of multidrug-resistant *Shigella* spp is the unique capability of the pathogen to acquire resistance factors (transmissible genes) from the environment or from other bacteria. Antimicrobial resistance is usually conferred by certain genes. A large number of resistance-related genes have reported for each group of antimicrobials. It is impossible to study all the reported genes, so most commonly isolated predominant isolates and reported genes were selected for this study. Fluoroquinolones, especially ciprofloxacin, are the most commonly used drugs for shigellosis treatment. Reduced susceptibility to the fluoroquinolone group of antibiotics is usually linked with point mutations in the bacterial target genes gyrA, gyrB encoding DNA gyrase and parC, parE encoding DNA topoisomerase IV.

The aim of the present study was to find the extent and nature of the emerging resistance in Gulbarga (north part of Karnataka), India, and the surrounding region, with a huge population; we also focused on molecular mechanisms of development of resistance against different generations of fluoroquinolones—ciprofloxacin, ofloxacin, and gatifloxacin—and explored the diversity of restriction endonucleases to determine if restriction endonuclease production is useful for epidemiological studies and correlates the occurrence of restriction endonucleases with serotype antibiotic resistance in local collected clinical isolates of *Shigella* species, with priority on predictive value of fluoroquinolone's resistance. We also tried to establish the significance of reduced minimal inhibitory concentrations (MIC) values.

MATERIALS AND METHODS

Bacterial Isolation. The clinical isolates used in the present study were isolated from infants' stools. They were identified up to species level on the basis of colonial and Gram stain morphology, carbohydrate fermentation, and indole test, and final confirmation was performed with specific polyvalent antisera (Deben Diagnostics Ltd, Ipswich, Suffolk, UK).

Among 43 previously isolated *Shigella* isolates,[10] 32 multidrug-resistant isolates were considered for assessment in the present study. For each study, an overnight culture was diluted in fresh tryptic soy broth and further incubated to ensure exponential growth conditions.

Antibiotic Susceptibility Test. Resistance level and synergistic activity of previously isolated *Shigella* isolates to various antibiotics (Hi-Media Private Ltd., Mumbai, India) were determined by the microdilution method as described by the Clinical and Laboratory Standards Institute 2010. *Shigella dysenteriae* 13313 and *Shigella flexneri* 12022 obtained from American type culture collection were used as the reference and standard control organism in all the susceptibility procedures.

Minimal Inhibitory Concentration. Minimum inhibitory concentration was determined to evaluate the phenotypic antimicrobial resistance of a strain to a certain antibiotic. MIC was defined as the lowest antibiotic concentration that resulted in no visible growth. The antibiotic (ciprofloxacin, ofloxacin, and gatifloxacin) phenotype was determined for all 32 *Shigella* isolates.

E-strip/Hi comb test. Antibiotic E-strips, having a gradient concentration of 0.001-64 μg/mL, were used in accordance with the protocols from the manufacturer (Himedia, HiComb MIC test,