Shigella spp. are the etiologic agents of acute invasive intestinal infections clinically manifested by watery or bloody diarrhea. Shigellosis represents a major burden of disease, especially in developing countries, and is estimated to affect at least 80 million persons, predominantly children, each year (1). Disease may be caused by any of the 4 Shigella species: S. dysenteriae, S. flexneri, S. boydii, and S. sonnei. In industrialized countries, the most common species is S. sonnei, but this species is spreading intercontinentally to developing countries as a single, rapidly evolving lineage (2). By contrast, in developing countries, the predominant species is S. flexneri, which is characterized by long-term persistence of sublineages in shigellosis-endemic regions with inadequate hygiene conditions and unsafe water supplies (3). More rarely isolated are S. dysenteriae, responsible for large epidemics in the past, and S. boydii (4). Although shigellosis is principally a self-limiting disease, the World Health Organization guidelines recommend antimicrobial drug treatment as a means of reducing deaths, disease symptoms, and organism-excretion time; the current drug of choice is ciprofloxacin (1). Of growing concern is multidrug resistance, and in particular the increasing rate of resistance to ciprofloxacin reported for Shigella isolates from Asian and African regions (5). Furthermore, resistance to recommended second-line antimicrobial drugs, such as the third-generation cephalosporin ceftriaxone and the macrolide azithromycin, is emerging (1).

The Study
To determine antimicrobial drug resistance mechanisms, we analyzed 344 isolates representing 344 Shigella spp. collected during 2004–2014. We focused on molecular resistance mechanisms that promote resistance to currently recommended antimicrobial drugs.

We performed susceptibility testing by using the Kirby–Bauer disk-diffusion method. Results were interpreted according to Clinical and Laboratory Standards Institute performance standards (6). All 344 isolates were screened for plasmid-mediated quinolone resistance (PMQR) genes (7). A subset of 34 isolates eliciting reduced susceptibility to nalidixic acid, ciprofloxacin, or both, and representing different years of isolation was subjected to PCR-based detection of mutations in the quinolone resistance-determining regions (QRDRs) of the gyrA and parC genes (7). Isolates showing an extended-spectrum β-lactamase (ESBL) phenotype were screened by PCR for the presence of genes belonging to the blaTEM, blaSHV, and blaCTX-M families, by using primers described previously (8). All 344 isolates were analyzed for mph(A) by PCR by using previously published primers (9). Resulting amplicons were purified and sequenced. For database searches, we used blastn (http://www.ncbi.nlm.nih.gov/blast/).

Multidrug resistance was defined as resistance to ≥3 classes of antimicrobial agents. Multidrug resistance was detected in 150 (83.8%) of the S. sonnei, 84 (78.5%) of the S. flexneri, 20 (60.6%) of the S. dysenteriae, and 16 (64%) of the S. boydii isolates (Table 1).

Resistance to nalidixic acid was detected in all species, but none of the S. dysenteriae and S. boydii isolates were resistant to ciprofloxacin (Table 1). The time distribution and the frequency of ciprofloxacin-resistant S. sonnei isolates showed a rising tendency (Figure). A similar tendency was noted for ciprofloxacin-resistant S. flexneri isolates, which, however, revealed higher variability throughout the study period (Figure). No ciprofloxacin-resistant isolates were found before 2008. In total, 27 (15%) S. sonnei and 9 (8.4%) S. flexneri isolates were resistant to ciprofloxacin.

The qnrS1 gene was found in 13 (3.8%) of the strains: 4 S. dysenteriae, 4 S. flexneri, 4 S. boydii, and 1 S. sonnei. Other PMQR genes included qnrB19, detected in S. sonnei (n = 1), and qnrB4, detected in combination with qepA in S. sonnei (n = 1). Of the 15 PMQR-positive isolates, only 2 were resistant to nalidixic acid and ciprofloxacin, illustrating the potential for development of resistance in susceptible strains (Table 2, http://wwwnc.cdc.gov/EID/article/22/6/15-2088-T2.htm).

Most of the 34 isolates analyzed for mutations in their QRDR carried mutations in the gyrA and parC genes (Table 2). Most frequently observed was the first-step amino acid substitution within GyrA at Ser83Leu (n = 14), which
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was associated with resistance to nalidixic acid. The double substitutions within GyrA at Ser83Leu/Asp87Gly (n = 11) and Ser83Leu/Asp87Asn (n = 2) occurred invariably in combination with the substitution in ParC (Ser80Ile) and occurred in ciprofloxacin-resistant isolates. In addition, some unusual genotypes were detected; strains containing only second-step mutations within GyrA were observed for Asp87Tyr (n = 4) and Asp87Asn (n = 1) and were associated with resistance to nalidixic acid. The substitution ParC(Ala85Ser) was observed in nalidixic acid–resistant S. boydii isolates with Gly(Ser80Leu) (n = 2) (Table 2).

Our data document an ongoing trend toward dominance of S. sonnei, which is reflective of a current global shift in the epidemiologic distribution of this species (10). Of the 18 patients for whom travel to India was documented, isolates from 55.6% were resistant to ciprofloxacin, a finding that supports previous reports of importation of ciprofloxacin-resistant Shigella from India to Europe and the United States (11,12) and emphasizes the need to obtain travel information from patients receiving treatment for shigellosis. Furthermore, therapeutic efficiency of fluoroquinolones may be decreased because of the presence of PMQR determinants in phenotypically susceptible strains. PMQR genes are of concern because they not only promote mutations within the QRDR, resulting in resistance to fluoroquinolones, but they may disseminate among other species of Enterobacteriaceae.

Besides ciprofloxacin, the third-generation cephalosporin ceftriaxone is recommended as an alternative for the treatment of shigellosis (1). Resistance to the broad-spectrum β-lactam ampicillin was observed in all Shigella species (Table 1); however, the ESBL phenotype (resistance to cefotaxime; Table 1) was restricted to S. sonnei and was found in 8 strains (4.5% of S. sonnei isolates). PCR

| Agent                        | S. sonnei, n = 179 | S. flexneri, n = 107 | S. dysenteriae, n = 33 | S. boydii, n = 25 |
|------------------------------|-------------------|----------------------|------------------------|------------------|
| Ampicillin                   | 31 (17.3)         | 73 (68.2)            | 19 (57.6)              | 12 (48)          |
| Amoxicillin/clavulanic acid  | 2 (1.1)           | 1 (0.9)              | 0                      | 0 (0)            |
| Cephalothin                  | 12 (6.7)          | 0                    | 0                      | 0                |
| Cefotaxime                   | 8 (4.5)           | 0                    | 0                      | 0                |
| Nalidixic acid               | 49 (27.4)         | 15 (14)              | 2 (6)                  | 2 (8)            |
| Ciprofloxacin                | 27 (15)           | 9 (8.4)              | 0                      | 0                |
| Azithromycin*                | 2 (1.1)           | 5 (4.7)              | 0                      | 0                |
| Trimethoprim                 | 172 (96)          | 70 (65.4)            | 20 (60.6)              | 15 (60)          |
| Sulfamethoxazole             | 151 (84.4)        | 71 (66.4)            | 19 (57.6)              | 16 (64)          |
| Kanamycin                    | 1 (0.5)           | 1 (0.9)              | 0                      | 0                |
| Gentamicin                   | 4 (2.2)           | 0                    | 0                      | 0                |
| Streptomycin                 | 163 (91)          | 81 (75.7)            | 24 (72.7)              | 18 (72)          |
| Tetracycline                 | 145 (81)          | 83 (77.6)            | 22 (66.6)              | 13 (52)          |
| Chloramphenicol              | 6 (3.4)           | 56 (52.3)            | 9 (27.3)               | 2 (8)            |

*For azithromycin, no Clinical and Laboratory Standards Institute breakpoints for Enterobacteriaceae exist. Isolates harboring mph(A) were regarded as resistant.

Figure. Shigella spp. isolated in Switzerland, 2004–2014, and percentages of ciprofloxacin-resistant S. sonnei and S. flexneri.
analysis confirmed the presence of blaCTX-M genes in all 8 isolates: blaCTX-M-3 (n = 1), blaCTX-M-14 (n = 2), and blaCTX-M-15 (n = 5) (Table 2). The establishment of blaCTX-M-harboring Shigella as an additional reservoir of these widely disseminated resistance determinants poses a threat to the treatment of shigellosis, especially because all ESBLs detected in this study were CTX-M enzymes, which are also potent ceftriaxone hydrolyzers (13).

Screening of the 344 Shigella isolates for the presence of mph(A) revealed 7 (2%) positive strains: 2 S. sonnei and 5 S. flexneri (Table 2). Shigella species exhibiting reduced susceptibility to azithromycin are of great concern because azithromycin, in combination with colistin, has recently been found to represent a potentially invaluable option for the treatment of gram-negative rods expressing MDR, including carbapenem-resistant isolates of Pseudomonas aeruginosa, Klebsiella pneumoniae, and Acinetobacter baumannii (14). Hence, judicious use of this particular drug and susceptibility monitoring are warranted. Furthermore, our data show that mph(A) may be present in isolates displaying MICs as low as 12 µg/mL, highlighting the urgency with which azithromycin susceptibility breakpoints and interpretive criteria for Enterobacteriaceae are needed.

Conclusions

Treatment of shigellosis with currently recommended antimicrobial drugs is increasingly threatened by the emergence of ciprofloxacin resistance, ESBLs, or plasmid-mediated azithromycin resistance in multidrug-resistant Shigella isolates. Because azithromycin is a last-resort antimicrobial agent used to treat shigellosis, the emergence of mph(A) among Shigella spp. is cause for concern.

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Dr. Nüesch-Inderbinen is a research associate at the National Centre for Enteropathogenic Bacteria and Listeria and the Institute for Food Safety and Hygiene, University of Zurich, Switzerland. Her research interest is the dissemination of antimicrobial drug resistance genes among Enterobacteriaceae in humans and food animals.

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Address for correspondence: Roger Stephan, Institute for Food Safety and Hygiene, Vetsuisse Faculty, University of Zurich, Winterthurerstr 272, CH-8057 Zurich, Switzerland; email stephanr@fsafety.uzh.ch