Infection (2013) 41:999–1003
DOI 10.1007/s15010-013-0501-4

BRIEF REPORT

High rates of quinolone-resistant strains of *Shigella sonnei* in HIV-infected MSM

C. Hoffmann · H. Sahly · A. Jessen · P. Ingiliz · H.-J. Stellbrink · S. Neifer · K. Schewe · S. Dupke · A. Baumgarten · A. Kuschel · I. Krznaric

Received: 28 January 2013 / Accepted: 24 June 2013 / Published online: 13 July 2013

Abstract
Purpose There is increasing evidence that shigellosis is a predominantly sexually transmitted disease among men who have sex with men (MSM) and that infection with the human immunodeficiency virus (HIV) is a risk factor for shigellosis.
Methods Retrospective analysis of antibiotic resistance profiles of *Shigella* species isolated from stool specimens of patients presenting with diarrhea from January 2010 to July 2012 in three German outpatient clinics specialized in HIV care.
Results Among 79 cases of *Shigella sonnei*, 56 occurred in HIV-infected MSM, while 23 were observed in HIV-negative MSM. High resistance rates (>90 %) were found for doxycycline, tetracycline, aminoglycosides, all cephalosporins of first and second generations tested, and trimethoprim/sulfamethoxazole. In total, 54 % of cases were resistant to ciprofloxacin. Compared to negative subjects, HIV-infected MSM had a significantly higher rate of quinolone resistance. For ciprofloxacin, the resistance rates were 66 versus 24 %, respectively ($p = 0.0016$). Individual resistance patterns did not indicate that this was due to a limited outbreak. Rates of resistance to other antibiotics than quinolones showed no differences between HIV-infected and HIV-negative cases. No resistance was found for carbapenems or newer cephalosporins such as ceftriaxone.
Conclusions The high rates of *S. sonnei* isolates resistant to quinolones and other traditional antibiotics are of concern. Innovative prevention efforts are urgently needed. The empirical use of quinolones in HIV-infected patients presenting with *S. sonnei* infection is no longer recommended.

Keywords *Shigella sonnei* · HIV · Quinolones · Resistance

This work has been presented, in part, at the 19th Conference on Retroviruses and Opportunistic Infections (CROI), held at the Washington State Convention Center in Seattle, March 5–8, 2012 and at the 112th American Society for Microbiology General Meeting (ASM), held in San Francisco, California, June 16–19, 2012.

C. Hoffmann · H.-J. Stellbrink · K. Schewe
Infektionsmedizinisches Centrum Hamburg, ICH, Glockengiesserwall 1, 20095 Hamburg, Germany

C. Hoffmann · H. Sahly
University Hospital Schleswig-Holstein, Kiel Campus, Kiel, Germany

H. Sahly
Labor Lademannbogen, Hamburg, Germany

A. Jessen
Private Practice Dres. Jessen and Colleagues, Berlin, Germany

P. Ingiliz · S. Neifer · S. Dupke · A. Baumgarten · I. Krznaric (✉)
Medical Center for Infectious Diseases, Berlin, Germany
e-mail: Krznaric@mvz-mib.de

A. Kuschel
Bioscientia Institut für Medizinische Diagnostik GmbH, Berlin, Germany

Introduction

Shigellosis is a major cause of diarrhea-related morbidity and mortality, with an estimated 165 million cases of diarrhea and 1.1 million deaths attributed to *Shigella* infections in the developing countries annually, mainly among young children [1]. In industrialized countries, a travel history to endemic areas was long believed to be the

only significant risk to develop shigellosis. During recent years, there is increasing evidence that, among men who have sex with men (MSM), shigellosis is predominantly a sexually transmitted disease (STD) and that human immunodeficiency virus (HIV) infection is a risk factor for acquiring shigellosis [2–4]. We evaluated the current resistance patterns of *Shigella* species found in MSM in two metropolitan regions of Germany with special regard to their HIV serology status.

Methods

This was a retrospective analysis of the antibiotic resistance profiles of all *Shigella* species which were isolated from the stool specimens of patients presenting with diarrhea from January 2010 to July 2012 in three large outpatient clinics specialized for HIV and infectious diseases. The clinics were located in two metropolitan regions of Germany (Hamburg and Berlin). Cases were identified by computer database queries. For all patients, STD history was extracted by chart review. For syphilis and hepatitis C infection, serologic tests prior to shigellosis were evaluated. For gonorrhea and chlamydial infections, medical charts were screened for microbiologically confirmed diagnosis prior to shigellosis. HIV serology was documented by either positive or negative enzyme-linked immunosorbent assay (ELISA). For HIV-infected subjects, data on HIV infection [CD4 T-cells, HIV RNA, acquired immunodeficiency syndrome (AIDS) events, antiretroviral therapy] were extracted. Charts were also reviewed for the use of antibiotics within a 6-month period prior to shigellosis. Fisher's exact test and Chi-squared tests were used for the comparison of frequencies and to calculate odds ratios, respectively. A p-value of <0.05 was considered to be statistically significant.

Isolation, identification, and antibiotic susceptibility testing of *S. sonnei* strains

The strains were isolated from the fecal samples of patients and controls. Samples were cultured overnight in selenite F broth (Oxoid, Wesel, or heipha Dr. Müller GmbH, Eppelheim, Germany) and, in addition, plated onto MacConkey and XLD/OSCM/Hektoen (Oxoid), or XLD/SS (heipha) agars at 37 °C overnight. Suspected colonies were subcultured and identified biochemically using the API 20E system (bioMérieux, Nürtingen, Germany) and the automated VITEK 2 system (bioMérieux, Marcy l'Etoile, France, Version 05.04). The results were interpreted following the guidelines of the Clinical and Laboratory Standards Institute (CLSI, http://www.clsi.org/).

Shigellae were grouped serologically by slide agglutination with polyvalent O-antigen specific antisera and subsequently serotyped with specific monovalent antisera (SIFIN, Berlin, Germany). Alternatively, serogrouping was performed by the Wellcolex Colour Shigella latex agglutination test (Remel, Dartford, UK). Susceptibility profiles of the strains to aminopenicillins, cephalosporins, quinolones, trimethoprim/sulfamethoxazole (TMP/SMX), aminoglycosides, and carbapenems were determined using the VITEK 2 system.

Results

Among a total of 85 cases documented, four cases of *S. flexneri* occurring in MSM and two cases of *S. sonnei* occurring in one woman and in one heterosexual man (both with a documented travel history) were excluded from further analysis. Of the remaining 79 cases of *S. sonnei*, 56 (71 %) were observed in HIV-infected MSM, while 23 (29 %) occurred in HIV-negative MSM. The 79 cases were observed continuously during the observation period. Except for April 2010 and June 2012, at least one case was found each month, with a maximum of seven cases in May 2011 and six cases in July 2010.

The median age at the time of shigellosis did not differ significantly between HIV-infected (41 years, range 23–63) and HIV-negative patients (38 years, range 23–53). In the 56 HIV-infected patients, the median absolute CD4 T-cell count was 466/µl (range 15–1,360/µl). Only three patients had an absolute CD4 T-cell count of less than 200/µl, and three patients had a prior AIDS-defining event. In 41 (73 %) HIV-infected patients, shigellosis occurred during antiretroviral therapy, while 15 (27 %) patients were untreated. Of 55 patients with an available plasma viremia at the time of shigellosis, 34 (62 %) had a viral suppression of <50 HIV-RNA copies/ml, while 21 (38 %) patients were viremic (median 11,600 copies/ml, range 77–200,000).

The majority of the 79 patients with *S. sonnei* infection had at least one other previously documented STD (55 % prior or active syphilis, 38 % gonorrhea, 32 % chlamydial infections, 22 % active or prior hepatitis C). Only 21 % exhibited no evidence for at least one of these four infections. The rates for syphilis (66 vs. 24 %, $p = 0.002$) and hepatitis C (30 vs. 0 %, $p = 0.002$) were significantly higher in HIV-infected patients, while there were no differences with regard to gonorrhea and chlamydial infections.

A total of 43 % of all patients had been treated with at least one antibiotic agent during the last 6 months prior to shigellosis. Only six patients (8 %) had received a quinolone. There were no differences between HIV-infected and

Table 1 Percentage of resistance of *Shigella sonnei* against selected antimicrobial agents for human immunodeficiency virus (HIV)-positive and -negative cases

	All cases ($n = 79$)		HIV-positive ($n = 56$)		HIV-negative ($n = 23$)		p Value
	%	MIC[a]	%	MIC[a]	%	MIC[a]	
Amoxicillin	26	n.t.	29	n.t.	19	n.t.	n.s.
Amoxicillin + clavulanic acid	12	3.8 ± 6.4	15	4.1 ± 7.3	5	2.9 ± 2.5	n.s.
Ampicillin	25	9.1 ± 10.3	29	8.8 ± 10.1	17	10 ± 11.2	n.s.
Piperacillin	17	15.7 ± 32	18	18 ± 36	14	7.6 ± 9	n.s.
Cefaclor	95	n.t.	93	n.t.	100	n.t.	n.s.
Cephalexin, cefotiam	96	n.t.	94	n.t.	100	n.t.	n.s.
Cefazolin	92	n.t.	92	n.t.	93	n.t.	n.s.
Cefuroxime	94	8.23 ± 12.7	93	8.3 ± 14.5	95	8 ± 4.3	n.s.
Cefixime	4		6		0		n.s.
Ciprofloxacin	54	2.4 ± 1.9	66	2.75	24	1.3 ± 1.8	0.0016
Levofloxacin	55	4.6 ± 3.8	67	5 ± 3.7	27	3 ± 4	0.0018
Moxifloxacin[a]	61	n.t.	73	n.t.	31	n.t.	0.018
Ofloxacin[a]	59	n.t.	71	n.t.	29	n.t.	0.01
Gentamicin	91	n.t.	91	n.t.	91	n.t.	n.s.
TMP/SMX	95	279 ± 96	92	290 ± 83	100	245 ± 128	n.s.
Tobramycin	94	n.t.	95	n.t.	93	n.t.	n.s.
Doxycycline	96	n.t.	94	n.t.	100	n.t.	n.s.
Tetracycline	94	n.t.	91	n.t.	100	n.t.	n.s.

No resistance was found for piperacillin + tazobactam, cefepime, cefotaxime, ceftazidime, ceftriaxone, imipenem, meropenem, or tigecycline

n.s. not statistically significant ($p > 0.05$). Not all isolates were tested for all agents

[a] Only 41 isolates were tested for resistance. *MIC*, minimum inhibitory concentration, μg/ml, mean ± SD; range; *n.t.* MIC not available, resistance deduced automatically via the VITEK 2 system

HIV-negative patients with respect to the use of quinolones (9 and 5 %, respectively), cephalosporins (13 and 15 %, respectively), or any antibiotic agents (44 and 40 %, respectively).

The resistance rates of *S. sonnei* are depicted in Table 1. High resistance rates were found for doxycycline, tetracycline, aminoglycosides, all cephalosporins of the first and second generations tested, and TMP/SMX. All isolates were resistant to at least one agent. Two isolates (isolated from one HIV-infected patient and one HIV-negative patient) were resistant to TMP/SMX only. In total, 54 % of isolates were resistant to ciprofloxacin and 12 % were resistant to both ciprofloxacin and ampicillin. The isolates from two HIV-infected patients showed an extended resistance spectrum including cefixime.

No resistance was found for cefepime, cefotaxime, ceftazidime, and ceftriaxone. In addition, no resistance to penems or tigecycline was detected, indicating the absence of carbapenemases and efflux-mediated resistance patterns in this cohort. The resistance rates of amoxicillin–clavulanate, ampicillin–sulbactam, and piperacillin–tazobactam were significantly lower than those of amoxicillin, ampicillin, and piperacillin, indicating that inhibitors of plasmid-mediated beta-lactamases remained active in a substantial proportion of isolates.

Compared to HIV-negative patients, HIV-infected patients had a significantly higher rate of quinolone resistance. For ciprofloxacin, the resistance rates were 66 versus 24 %, respectively ($p = 0.0016$). The risk ratio was 2.77 [95 % confidence interval (CI) 1.26–6.11]. Significant differences were also found for other quinolones, such as levofloxacin, moxifloxacin, and ofloxacin. All patients with isolates resistant to both ciprofloxacin and ampicillin were HIV-infected (16 vs. 0 %, $p = 0.05$). Patients with isolates resistant to quinolones had been treated more frequently with antibiotics within 6 months prior to shigellosis than patients without resistant isolates. However, this difference was not significant (51 vs. 32 %, $p = 0.15$).

As shown in Table 1, the rates of resistance to antibiotics other than quinolones showed no differences between HIV-infected and HIV-negative cases. Moreover, the frequency and distribution of minimum inhibitory concentrations (MICs) of selected antibiotics did not differ among strains of HIV-positive and -negative patients (Table 2).

Table 2 Frequency (%) and cumulative minimum inhibitory concentration (MIC) for selected antibiotics among strains of HIV-positive and -negative patients

MIC (μg/ml)												
Antibiotic	Patients %	0.125	0.25	1	2	4	8	16	32	64	128	>320
Ampicillin	All	–	–	–	23[b]	32	20	5	20[a]	–	–	–
	HIV-pos.	–	–	–	24[b]	33	14	4	25[a]	–	–	–
	HIV-neg.	–	–	–	19[b]	30	34	–	17[a]	–	–	–
Amoxicillin/clavulanic acid	All	–	–	–	84[b]	2	2	7	5[a]	–	–	–
	HIV-pos.	–	–	–	82[b]	–	3	6	9[a]	–	–	–
	HIV-neg.	–	–	–	82[b]	–	13	2	3	–	–	–
Piperacillin	All	–	–	–	–	77	5	2	8	–	7	–
	HIV-pos.	–	–	–	–	75	6	–	9	–	9	–
	HIV-neg.	–	–	–	82	–	–	4	9	–	1	–
Ciprofloxacin	All	–	46[b]	–	–	54[a]	–	–	–	–	–	–
	HIV-pos.	–	34[b]	–	–	66[a]	–	–	–	–	–	–
	HIV-neg.	–	76[b]	–	–	24[a]	–	–	–	–	–	–
Levofloxacin	All	37[b]	–	2	–	4	49[a]	–	–	–	–	–
	HIV-pos.	33[b]	–	–	–	7	60[a]	–	–	–	–	–
	HIV-neg.	64[b]	–	9	–	–	27[a]	–	–	–	–	–
TMP/SMX	All	–	–	–	–	–	–	–	5[b]	–	95[a]	–
	HIV-pos.	–	–	–	–	–	–	–	7[b]	–	92[a]	–
	HIV-neg.	–	–	–	–	–	–	–	–	–	100[a]	–

[a] ≥ to corresponding MIC

[b] ≤ to corresponding MIC

Discussion

During recent years, a relatively large number of *S. sonnei* infections among MSM has been observed in the three participating centers. In Germany, shigellosis is usually a rare event. Mandatory notifications reported anonymously to the Robert Koch Institute yielded a total of 617 cases in 2009, among which 68 % were of *S. sonnei* [5]. Except for a few cases of *S. flexneri* (which were excluded from further analysis), our cases were restricted to *S. sonnei*, suggesting that, in German Metropolitan areas, *S. sonnei* is currently the predominantly transmitted strain among MSM.

Population-based studies have revealed that, among MSM, shigellosis is predominantly an STD, with direct oral–anal contact conferring the highest risk and HIV infection likely contributing to increased host susceptibility [4, 6]. At least 79 % of the patients in this cohort had a history of other STDs or of hepatitis C. Thus, innovative prevention efforts are urgently needed in this high-risk group. Patients should also be informed to avoid practices that might result in the fecal–oral transmission of enteric infections.

Whereas the rates of resistance to most antibiotics showed no differences between HIV-infected and -negative cases, the resistance rates against quinolones were significantly higher in HIV-infected patients. Until recently, in Western countries, resistance to quinolones of *S. sonnei* has been rarely seen [7, 8]. A recent systematic review of resistance rates of shigellosis in the period 1998–2009 revealed that the rates for *S. sonnei* resistant to ciprofloxacin remained at 0.1 % (95 % CI 0.0–0.3 %) in Europe–America [9]. In recent years, however, few cases of quinolone resistance in *S. sonnei* have been described in Canada, USA, or Europe [6, 10, 11]. These early investigations suggested that transmission among MSM was associated with sex venues [6, 10].

The particularly high ciprofloxacin resistance rate of 66 % observed in our cohort of HIV-infected MSM with *S. sonnei* infection suggests that the increase observed during recent years was mainly driven by this subgroup. Due to the retrospective design of the study, it was not possible to perform molecular typing and phylogenetic analysis. Individual resistance patterns (data not shown) and the longitudinal distribution during the 2.5-year observation period did not indicate that our cases represent a limited outbreak, given the short incubation period of shigellosis. However, given the lack of phylogenetic analysis, small outbreaks could not be ruled out definitely.

One explanation for the high resistance rates could be the transfer of quinolone resistance mediated by transferrable plasmids from other Gram-negative bacilli or species

outside the *Enterobacteriaceae* family [12, 13]. For example, ciprofloxacin resistance rates of gonorrhea have reached 50 % in Germany [14]. In our cohort, a total of 44 % of all patients had received any antibiotic treatment within 6 months prior to shigellosis. Moreover, there was a trend towards a more frequent antibiotic use in patients with *S. sonnei* isolates resistant to quinolones. Although we did not find a higher rate of quinolone or other antibiotic use (including third-generation cephalosporins) in HIV-infected patients in the 6-month period prior to shigellosis, we could not rule out a higher usage in the past. The high resistance rates in the HIV population may also be due to other factors, such as sexual or behavioral practices. In retrospect, it was not possible to evaluate these factors, which presents another limitation of this study. However, prior hepatitis C and prior syphilis were found more frequently in HIV-infected patients.

The emergence of *Shigella* infections with decreased susceptibility to quinolones is a public health concern, especially since plasmid-mediated quinolone resistance determinants may spread among other members of the *Enterobacteriaceae* [13, 15]. The World Health Organization (WHO) currently still recommends ciprofloxacin or other fluoroquinolones as the drug of choice for the therapy of shigellosis in both adults and children [16]. Despite the limitations of this retrospective analysis, we believe that the empirical use of quinolones in MSM with *S. sonnei* infection is no longer advisable. Third-generation cephalosporins may offer an alternative but should be used with caution, given the potential selection of extended-spectrum β-lactamase (ESBL) producers. Stool culture should become a routine procedure in MSM suffering from diarrheal disease for both etiologic and antibiotic resistance investigation. Because shigellosis is often a self-limited illness in adults, more research is needed to help identify individuals at risk of severe disease and who need antibiotic treatment.

Conflict of interest None.

References

1. Niyogi SK. Shigellosis. J Microbiol. 2005;43:133–43.
2. Niyogi SK. Increasing antimicrobial resistance—an emerging problem in the treatment of shigellosis. Clin Microbiol Infect. 2007;13:1141–3.
3. Baer JT, Vugia DJ, Reingold AL, Aragon T, Angulo FJ, Bradford WZ. HIV infection as a risk factor for shigellosis. Emerg Infect Dis. 1999;5:820–3.
4. Aragón TJ, Vugia DJ, Shallow S, Samuel MC, Reingold A, Angulo FJ, Bradford WZ. Case–control study of shigellosis in San Francisco: the role of sexual transmission and HIV infection. Clin Infect Dis. 2007;44:327–34.
5. Robert Koch Institut. Epidemiologisches Bulletin. 27 September 2010/Nr. 38. 2010.
6. Marcus U, Zucs P, Bremer V, Hamouda O, Prager R, Tschaepe H, Futh U, Kramer M. Shigellosis—a re-emerging sexually transmitted infection: outbreak in men having sex with men in Berlin. Int J STD AIDS. 2004;15:533–7.
7. Sivapalasingam S, Nelson JM, Joyce K, Hoekstra M, Angulo FJ, Mintz ED. High prevalence of antimicrobial resistance among *Shigella* isolates in the United States tested by the National Antimicrobial Resistance Monitoring System from 1999 to 2002. Antimicrob Agents Chemother. 2006;50:49–54.
8. Vrints M, Mairiaux E, Van Meervenne E, Collard JM, Bertrand S. Surveillance of antibiotic susceptibility patterns among *Shigella sonnei* strains isolated in Belgium during the 18-year period 1990 to 2007. J Clin Microbiol. 2009;47:1379–85.
9. Gu B, Cao Y, Pan S, Zhuang L, Yu R, Peng Z, Qian H, Wei Y, Zhao L, Liu G, Tong M. 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;40:9–17.
10. Gaudreau C, Ratnayake R, Pilon PA, Gagnon S, Roger M, Lévesque S. Ciprofloxacin-resistant *Shigella sonnei* among men who have sex with men, Canada, 2010. Emerg Infect Dis. 2011;17:1747–50.
11. Folster JP, Pecic G, Bowen A, Rickert R, Carattoli A, Whichard JM. Decreased susceptibility to ciprofloxacin among *Shigella* isolates in the United States, 2006 to 2009. Antimicrob Agents Chemother. 2011;55:1758–60.
12. Ruiz J, Pons MJ, Gomes C. Transferable mechanisms of quinolone resistance. Int J Antimicrob Agents. 2012;40:196–203.
13. Dalhoff A. Resistance surveillance studies: a multifaceted problem—the fluoroquinolone example. Infection. 2012;40:239–62.
14. Enders M, Turnwald-Maschler A, Regnath T. Antimicrobial resistance of *Neisseria gonorrhoeae* isolates from the Stuttgart and Heidelberg areas of southern Germany. Eur J Clin Microbiol Infect Dis. 2006;25:318–22.
15. Jacoby GA. Mechanisms of resistance to quinolones. Clin Infect Dis. 2005;41:S120–6.
16. World Health Organization (WHO). International note on antibiotics in the management of shigellosis. Wkly Epidemiol Rec. 2004;79:202–3.