Genetic characterization of multidrug resistance in *Shigella* spp. from Japan

Ashraf M. Ahmed,^{1,3} Kimi Furuta,² Kei Shimomura,² Yoshio Kasama² and Tadashi Shimamoto¹

Correspondence

Tadashi Shimamoto
tadashis@hiroshima-u.ac.jp

¹Laboratory of Food Microbiology and Hygiene, Graduate School of Biosphere Science, Hiroshima University, Higashi-Hiroshima 739-8528, Japan

²Division of Biological Science, Hiroshima City Institute of Public Health, Hiroshima 733-8650, Japan

³Department of Microbiology, Faculty of Veterinary Medicine, Kafr El-Sheikh University, Kafr El-Sheikh 33516, Egypt

This study characterized the genetic basis of antimicrobial resistance of a number of *Shigella* spp. isolated from humans from 2000 to 2004 in Hiroshima prefecture, Japan. A total of 26 isolates of *Shigella* spp. were included in this study. Antimicrobial susceptibility tests revealed high levels of resistance, especially to ampicillin, streptomycin, trimethoprim, tetracycline, nalidixic acid and ciprofloxacin. PCR and DNA sequencing were used for screening and characterization of antibiotic-resistance determinants. PCR sequencing analysis revealed the presence of only one type of class 1 integron in one isolate of *Shigella sonnei*. This class 1 integron was 1904 bp and contained two gene cassettes: a probable esterase/lipase (*estX*) and *aadA1*, which confers resistance to streptomycin and spectinomycin. Two types of class 2 integron were identified in this study. One was the classic type (2158 bp) and carried the three conserved resistance gene cassettes of the class 2 integron, *dfrA1*, *sat1* and *aadA1*, which confer resistance to trimethoprim, streptomycin and streptomycin/spectinomycin, respectively. This type was detected in both *Shigella sonnei* (14 isolates) and *Shigella flexneri* (five isolates). The other type was shorter (1313 bp) and carried only two gene cassettes, *dfrA1* and *sat1*. This integron was detected in a single isolate of *Shigella sonnei*. PFGE patterns showed limited diversity within clusters of the same species. Furthermore, an extended-spectrum β -lactamase gene, *bla*_{OXA-30}, which confers resistance to ampicillin, was characterized in all isolates of *Shigella flexneri* except the oldest strain, which was isolated in 2000. Southern blot hybridization and conjugation experiments showed that *bla*_{OXA-30} was located in the chromosome.

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INTRODUCTION

Shigella is a major cause of dysentery throughout the world and is responsible for 5–10 % of diarrhoeal illnesses in many areas. There are four species of *Shigella*, *Shigella dysenteriae*, *Shigella flexneri*, *Shigella boydii* and *Shigella sonnei*, also designated groups A, B, C and D, respectively. Each year, 1·1 million people are estimated to die from *Shigella* infection (Kotloff *et al.*, 1999). Recently, it was estimated that 91 million people worldwide contract shigellosis each year; in Asia, 410 000 children, mostly malnourished, die (World Health Organization, 2005). In the USA, shigellosis is the

third leading bacterial gastrointestinal disease, with 25 000 cases reported in 1998 and 18 000 cases reported in 1999 (Cimons, 2000). In Japan, according to the National Epidemiological Surveillance of Infectious Diseases, there were 471 notifications of shigellosis in 2003, 597 in 2004 and 560 in 2005, totalling 1628 cases (as of 6 February 2006) (National Institute of Infectious Diseases, 2006). A multi-prefectural outbreak of *Shigella sonnei* infections associated with eating oysters occurred in Japan at the end of November 2001 (Terajima *et al.*, 2004). A summary report of the Ministry of Health, Labour and Welfare stated that 160 cases of shigellosis had been reported from 30 different prefectures by 30 January 2002.

Problems associated with the development and spread of antibiotic resistance have been increasing since the early 1960s and are currently viewed as a major threat to global public health (World Health Organization, 2001). The ability of bacteria to acquire and disseminate exogenous genes via

Abbreviations: CS, conserved segment; MDR, multidrug resistance.

The GenBank/EMBL/DDBJ accession numbers for the sequences of the *Shigella sonnei* class 1 integron, *S. sonnei* class 2 integron, *Shigella flexneri* class 2 integron, *S. sonnei* small-size class 2 integron and *S. flexneri* *bla*_{OXA-30} genes are AB234884, AB234885, AB234886, AB234887 and AB234888, respectively.

Table 1. Oligonucleotides used in this study

Primer	Nucleotide sequence (5'→3')	Target size	Reference/GenBank accession no.
5'-CS	GGCATCCAAGCAGCAAG	Variable	Lévesque <i>et al.</i> (1995)
3'-CS	AAGCAGACTTGACCTGA		
hep74	CGGGATCCCGGACGGCATGCACGATTTGTA	Variable	White <i>et al.</i> (2001)
hep51	GATGCCATCGCAAGTACGAG		
IntI2-F2	GATCCTGCCATCATTGAGTA	Within class 2 integron	This study
IntI2-R2	AGGGGAAGCCGAAGTTTCC		
TEM-F	ATAAAATTCTTGAAGACGAAA	1080 bp	Weill <i>et al.</i> (2004)
TEM-R	GACAGTTACCAATGCTTAATC		
SHV-F	TTATCTCCCTGTTAGCCACC	795 bp	Weill <i>et al.</i> (2004)
SHV-R	GATTTGCTGATTCGCTCGG		
OXA-F	TCAACTTTCAGATCGCA	591 bp	J02967
OXA-R	GTGTGTTTAGAATGGTGA		
OXA-F-2	ATTAAGCCCTTTACCAAACCA	890 bp	J02967
OXA-R-2	AAGGGTTGGGCGATTTTGCCA		
CTX-M-F	CGCTTTGCGATGTGCAG	550 bp	Bonnet <i>et al.</i> (2000)
CTX-M-R	ACCGCGATATCGTTGGT		

mobile genetic elements such as plasmids, transposons, insertion sequences and genomic islands has been the major factor in the development of multidrug-resistant strains (Rowe-Magnus *et al.*, 2002). The matter became more serious after the discovery of another mechanism for the dissemination of resistance, involving integrons (Stokes & Hall, 1989). Integrons are genetic elements that acquire and exchange exogenous DNA, known as gene cassettes, by a site-specific recombination mechanism (Stokes & Hall, 1989). The most notable gene cassettes identified within integrons are those conferring resistance to antibiotics. The increasing incidence of multidrug-resistant micro-organisms has led to tremendous interest in the genetics and mechanisms of resistance evolved by bacteria to counteract the effects of antimicrobial agents. Recent reports have determined the molecular basis of multidrug-resistance (MDR) phenotypes of *Shigella* spp. in Australia (McIver *et al.*, 2002), Ireland (DeLappe *et al.*, 2003), Korea (Oh *et al.*, 2003), Italy (Mammìna *et al.*, 2005) and Brazil (Peirano *et al.*, 2005). However, there are no reports related to the molecular basis of these resistances in Japan. Hence, the aim of this study was to characterize the genetic basis of MDR phenotypes in clinical isolates of *Shigella* spp. in Japan.

METHODS

Bacterial strains. Twenty-six strains of *Shigella* spp. (*Shigella sonnei*, *n*=17; *Shigella flexneri*, *n*=8; *Shigella boydii*, *n*=1) were characterized in this study. These strains were the entire complement of the collection from stool samples of sporadic diarrhoeic patients in Hiroshima prefecture, Japan, from 2000 to 2004 and sent to the Division of Biological Science, Hiroshima City Institute of Public Health, for further analysis. The isolates were identified and serotyped by standard procedures (Murray *et al.*, 1995). Selected resistant strains were stored in Luria-Bertani broth containing 50% glycerol at -80 °C until use.

Antimicrobial susceptibility testing. Antibiotic susceptibility testing was performed using the Kirby-Bauer disc susceptibility

method according to National Committee for Clinical Laboratory Standards guidelines (NCCLS, 2002). *Shigella* spp. were tested for susceptibility to various antibiotics, including: ampicillin (10 µg), chloramphenicol (30 µg), ciprofloxacin (5 µg), kanamycin (30 µg), gentamicin (10 µg), nalidixic acid (30 µg), streptomycin (10 µg), trimethoprim (5 µg), tetracycline (30 µg) and cefotaxime (5 µg).

Bacterial DNA preparation, PCR and DNA sequencing of integrons. Preparation of the bacterial DNA template and PCR conditions for the detection of class 1 integrons have been described previously (Ahmed & Shimamoto, 2004). The class 1 integron primers 5'-CS and 3'-CS (Table 1) (Lévesque *et al.*, 1995), which amplify the region between the 5'-conserved segment (5'-CS) and 3'-CS of class 1 integrons, were used. For the detection of class 2 integrons, PCR was performed with the primer pair hep74 and hep51, specific to the conserved regions of class 2 integrons, as described elsewhere (DeLappe *et al.*, 2003). Two other primers were then designed according to the preliminary DNA sequencing results for class 2 integrons (IntI2-F2 and IntI2-R2; Table 1). These primers were located within the PCR fragment and were used for complete sequencing of both DNA strands of the whole class 2 integron segment. PCR products were subjected to electrophoresis in a 1.0% agarose gel, stained with ethidium bromide and visualized under UV light. The PCR fragment was purified from the agarose gel using a QIAquick Gel Extraction kit (Qiagen). Both DNA strands of the PCR product were sequenced using an ABI automatic DNA sequencer (model 3730xl; Perkin-Elmer).

Screening for β -lactamase-encoding genes. *Shigella* spp. were tested for the β -lactamase-encoding genes *bla*_{TEM}, *bla*_{SHV}, *bla*_{OXA} and *bla*_{CTX-M} by PCR, as described previously (Bonnet *et al.*, 2000; Siu *et al.*, 2000; Weill *et al.*, 2004). Universal primers for the β -lactamase-encoding genes were used (Table 1).

PFGE analysis. All isolates of *Shigella* spp. were analysed by PFGE with the restriction enzyme *Xba*I using standardized methods (Centers for Disease Control and Prevention, 2000). The DNA was separated by PFGE using the GenePath system (Bio-Rad). Following electrophoresis, gels were stained with 0.5 µg ethidium bromide ml⁻¹, illuminated under UV light and photographed. Macrorestriction fragment patterns were analysed according to the

Table 2. Characteristic features of *Shigella* spp. used in this study

No.	Strain name	Species	Date of isolation	Resistance phenotype*	Integron	ESBL
1	Sh00005	<i>S. sonnei</i>	14/02/00	SM, TC, TMP	Class 2 (<i>dfrA1</i> , <i>sat1</i> , <i>aadA1</i>)	—
2	Sh00006	<i>S. flexneri</i>	02/06/00	SM, TC	—	—
3	Sh00007	<i>S. boydii</i>	05/06/00	SM, TC	—	—
4	Sh00008	<i>S. sonnei</i>	24/10/00	SM, TC, TMP	Class 2 (<i>dfrA1</i> , <i>sat1</i> , <i>aadA1</i>)	—
5	Sh00010	<i>S. sonnei</i>	27/10/00	SM, TC, TMP	Class 2 (<i>dfrA1</i> , <i>sat1</i> , <i>aadA1</i>)	—
6	Sh01002	<i>S. flexneri</i>	12/01/01	SM, TC, TMP, AM	Class 2 (<i>dfrA1</i> , <i>sat1</i> , <i>aadA1</i>)	<i>bla</i> _{OXA-30}
7	Sh01003	<i>S. sonnei</i>	04/04/01	SM, TMP	Class 2 (<i>dfrA1</i> , <i>sat1</i> , <i>aadA1</i>)	—
8	Sh01005	<i>S. flexneri</i>	14/06/01	SM, KM, TC, AM, CP	—	<i>bla</i> _{OXA-30}
9	Sh01006	<i>S. flexneri</i>	05/11/01	SM, TC, AM	—	<i>bla</i> _{OXA-30}
10	Sh01007	<i>S. sonnei</i>	05/12/01	SM, TC, TMP, NA	Class 2 (<i>dfrA1</i> , <i>sat1</i> , <i>aadA1</i>)	—
11	Sh01008	<i>S. sonnei</i>	09/12/01	SM, TC, TMP, NA	Class 2 (<i>dfrA1</i> , <i>sat1</i> , <i>aadA1</i>)	—
12	Sh01009	<i>S. sonnei</i>	19/12/01	SM, TC, TMP, NA	Class 2 (<i>dfrA1</i> , <i>sat1</i> , <i>aadA1</i>)	—
13	Sh01010	<i>S. sonnei</i>	19/12/01	SM, TC, TMP, NA	Class 2 (<i>dfrA1</i> , <i>sat1</i> , <i>aadA1</i>)	—
14	Sh01011	<i>S. sonnei</i>	19/12/01	SM, TMP, NA	Class 2 (<i>dfrA1</i> , <i>sat1</i> , <i>aadA1</i>)	—
15	Sh01012	<i>S. sonnei</i>	24/12/01	SM, TC, TMP, NA	Class 2 (<i>dfrA1</i> , <i>sat1</i> , <i>aadA1</i>)	—
16	Sh02001	<i>S. sonnei</i>	07/06/02	SM, TC, TMP	Class 2 (<i>dfrA1</i> , <i>sat1</i> , <i>aadA1</i>)	—
17	Sh02002	<i>S. sonnei</i>	10/09/02	SM, TMP, AM, CP	Class 1 (<i>estX</i> , <i>aadA1</i>)	—
18	Sh02003	<i>S. sonnei</i>	13/11/02	SM	—	—
19	Sh03001	<i>S. sonnei</i>	08/10/03	SM, TC, TMP	Class 2 (<i>dfrA1</i> , <i>sat1</i> , <i>aadA1</i>)	—
20	Sh03002	<i>S. sonnei</i>	07/10/03	SM, TC, TMP	Class 2 (<i>dfrA1</i> , <i>sat1</i> , <i>aadA1</i>)	—
21	Sh04005	<i>S. sonnei</i>	12/05/04	SM, TC, TMP, NA	Class 2 (<i>dfrA1</i> , <i>sat1</i>)	—
22	Sh04006	<i>S. flexneri</i>	13/06/04	SM, TC, TMP, AM, NA, CP	Class 2 (<i>dfrA1</i> , <i>sat1</i> , <i>aadA1</i>)	<i>bla</i> _{OXA-30}
23	Sh04007	<i>S. flexneri</i>	13/06/04	SM, TC, TMP, AM, NA, CP	Class 2 (<i>dfrA1</i> , <i>sat1</i> , <i>aadA1</i>)	<i>bla</i> _{OXA-30}
24	Sh04008	<i>S. flexneri</i>	14/06/04	SM, KM, TC, TMP, AM, NA, CP	Class 2 (<i>dfrA1</i> , <i>sat1</i> , <i>aadA1</i>)	<i>bla</i> _{OXA-30}
25	Sh04009	<i>S. flexneri</i>	15/06/04	SM, TC, TMP, AM, NA, CP	Class 2 (<i>dfrA1</i> , <i>sat1</i> , <i>aadA1</i>)	<i>bla</i> _{OXA-30}
26	Sh04010	<i>S. sonnei</i>	22/11/04	SM, TC, TMP, AM, NA, CP	Class 2 (<i>dfrA1</i> , <i>sat1</i> , <i>aadA1</i>)	—

*AM, ampicillin; CP, ciprofloxacin; KM, kanamycin; NA, nalidixic acid; SM, streptomycin; TC, tetracycline; TMP, trimethoprim.

criteria of Tenover *et al.* (1995); isolates having PFGE patterns with differences in one to three bands were considered to be closely related.

Plasmid isolation, probe preparation and Southern blot hybridization. Plasmids were isolated from the most recent *bla*_{OXA-30}-positive strain of *Shigella flexneri* (Sh04009; Table 2) using the alkaline lysis method described by Sambrook & Russell (2001). Horizontal gel electrophoresis was carried out using 0.7% agarose gel in 1% TAE buffer at 100 V for 1 h. After agarose gel electrophoresis, the DNA fragments in the gel were transferred to Hybond-N⁺ nylon membranes (Amersham Biosciences) according to the manufacturer's instructions. The DNA fragment containing the whole *bla*_{OXA-30} gene (890 bp) was amplified by PCR using the *bla*_{OXA-30} primers OXA-F-2 and OXA-R-2 (Table 1) and purified as described above. The purified fragment was labelled with alkaline phosphatase using an AlkPhos Direct Labelling System (Amersham Biosciences) and subsequently used as a DNA probe. All hybridization steps were carried out according to the manufacturer's protocol. Hybridization was performed at 55 °C for 12 h and the hybridized DNA was detected using the CDP-Star chemiluminescent signal generation system (Amersham Biosciences) according to the manufacturer's instructions.

Conjugation experiments. A mating-out assay was carried out as described previously (Ahmed *et al.*, 2005) using the *bla*_{OXA-30}-positive strain of *Shigella flexneri* (Sh04009) as the donor strain and a rifampicin-resistant mutant of *Escherichia coli* HB101 obtained *in*

vitro (Sambrook & Russell, 2001) as the recipient strain. Transconjugants were selected on Mueller–Hinton agar containing 200 µg rifampicin ml⁻¹ and 100 µg ampicillin ml⁻¹.

Computer analysis of the sequence data. A similarity search was carried out using BLAST (<http://www.ncbi.nlm.nih.gov/blast/>).

RESULTS AND DISCUSSION

Emergence of multidrug-resistant *Shigella* spp. in Japan

Antimicrobial therapy causes marked symptom improvement and shortens the duration of illness in *Shigella* infection (Salam & Bennish, 1991; Mahoney *et al.*, 1993). Previously, various types of antimicrobial agent have been effective for the treatment of shigellosis, but options for treatment of this disease are now becoming limited due to the emergence of multidrug-resistant strains of *Shigella*. Over the last decade, *Shigella* spp. have shown a pattern of steadily increasing resistance to antibiotics worldwide (Replogle *et al.*, 2000; Lee *et al.*, 2001; McIver *et al.*, 2002; Oh *et al.*, 2003). In Japan, many *Shigella sonnei* isolates were recently found to have MDR phenotypes to nalidixic acid,

Fig. 1. PFGE patterns of *Shigella* spp. after *Xba*I digestion. (a) PFGE patterns of *Shigella sonnei* isolates. (b) *Shigella flexneri* isolates (lane 18–25) and a single isolate of *Shigella boydii* (lane 26). M, Size markers (DNA from *Salmonella* Braenderup H9812 digested with *Xba*I).

tetracycline and trimethoprim/sulfamethoxazole (Hirose *et al.*, 2005). In the present study, many isolates of *Shigella* spp., especially those isolated recently, were shown to have MDR phenotypes against ampicillin, streptomycin, trimethoprim, tetracycline, nalidixic acid and ciprofloxacin (Table 2).

PFGE patterns showed limited diversity within most clusters of the same species (Fig. 1). Over the last few years, similar MDR patterns in *Shigella* spp. have been reported in Ireland (DeLappe *et al.*, 2003), India (Pazhani *et al.*, 2004), Spain (Navia *et al.*, 2005), Turkey (Ozmert *et al.*, 2005) and the USA (Sivapalasingam *et al.*, 2006). Hence, in order to ensure appropriate treatment for shigellosis, continual surveillance is required to determine which antibiotics are still active.

Analysis of integron gene cassettes

The capture and spread of antibiotic resistance determinants by integrons underlies the rapid evolution of MDR phenotypes among diverse, Gram-negative clinical isolates (Rowe-Magnus *et al.*, 2002). Integrons encode an enzyme, termed integrase, that allows them to capture antibiotic-resistance gene cassettes (Stokes & Hall, 1989). Of the different classes of MDR integrons that have been identified, integron classes 1 and 2 are the most common in Gram-negative bacteria (White *et al.*, 2001). The structure of class 1 integrons includes the 5'- and 3'-CS and a variable region. The organization of class 2 integrons is similar to that of class 1, but they are associated with transposon Tn7 and are known to carry three classic gene cassettes, dihydrofolate reductase (*dfrA1*), streptothricin acetyltransferase (*sat1*) and aminoglycoside adenyltransferase (*aadA1*), conferring resistance to trimethoprim, streptothricin and streptomycin/spectinomycin, respectively (Hansson *et al.*, 2002).

In this study, PCR and DNA sequencing results identified only one type of class 1 integron in a single isolate (Sh02002) of *Shigella sonnei* (Table 2). This isolate had a unique PFGE pattern (Fig. 1, lane 11). This integron was 1904 bp and contained two gene cassettes. One was a probable esterase/lipase (*estX*) and the other was an aminoglycoside adenyltransferase (*aadA1*), which confers resistance to streptomycin and spectinomycin. Similar class 1 integrons of about 1955 bp have been identified in *Shigella sonnei* isolated in Ireland (DeLappe *et al.*, 2003). Moreover, in a recent report, a class 1 integron harbouring only one gene cassette, *aadA1*, was detected in a single isolate of *Shigella sonnei* from Brazil (Peirano *et al.*, 2005). Two types of class 2 integron were identified in this study. One was the classic type (2158 bp) and carried the three conserved resistance gene cassettes of class 2 integrons, *dfrA1*, *sat1* and *aadA1*. This type was detected in both *Shigella sonnei* (14 isolates) and *Shigella flexneri* (five isolates). The other type was shorter (1313 bp) and carried only two gene cassettes, *dfrA1* and *sat1*. This integron was detected in an isolate (Sh04005) of *Shigella sonnei* that was isolated in 2004 (Table 2). The PFGE pattern of this isolate differed from that of the other cluster (Fig. 1, lane 14). This short integron has also been detected previously (but at a size of 1371 bp) in a single isolate of *Shigella sonnei* from Ireland (DeLappe *et al.*, 2003). The low incidence of class 1 integrons and the high incidence of class 2 integrons in *Shigella* spp. in Japan consolidate previous results related to the incidence of these integrons in *Shigella* spp. from other countries such as Australia (McIver *et al.*, 2002), Ireland (DeLappe *et al.*, 2003), Korea (Oh *et al.*, 2003), Italy (Mammìna *et al.*, 2005) and Brazil (Peirano *et al.*, 2005). Although other mechanisms of resistance are possible, streptomycin and trimethoprim resistance are most likely attributable to expression from the genes contained in the corresponding integrons (Table 2).

Characterization of *bla*_{OXA-30}

Resistance to ampicillin in Gram-negative bacteria is primarily mediated by β -lactamases, which hydrolyse the β -lactam ring and thereby inactivate the antibiotic (Livermore, 1995). Many different β -lactamases have been described, but TEM-, SHV-, OXA- and CTX-M-type β -lactamases are predominant in Gram-negative bacteria (Bradford, 2001). Hence, *Shigella* spp. were examined for the presence of these β -lactamase-encoding genes using universal primers for each group (Table 1). PCR screening results showed that all isolates of *Shigella* spp. were negative for TEM-, SHV- and CTX-M β -lactamases, while all isolates of *Shigella flexneri* (except for the oldest strain, Sh00006, which was isolated in 2000) were positive for OXA-type β -lactamase (Table 2). Interestingly, the negative strain, Sh00006, had a unique PFGE pattern that distinguished it from the other *Shigella flexneri* strains by one missing band (Fig. 1, lane 23). In contrast, all isolates of *Shigella sonnei* were negative for OXA-type β -lactamase. PCR and DNA sequencing results using primers for the whole *bla*_{OXA} gene (OXA-F-2 and OXA-R-2; Table 1) showed that this OXA-type β -lactamase was 100% identical to *bla*_{OXA-30}, which was originally discovered in *Shigella flexneri* strains isolated from Hong Kong and Shanghai, China, in 2000 (Siu *et al.*, 2000) and was then detected in an isolate of *Salmonella enterica* serotype Typhimurium from an Australian patient (Hanson *et al.*, 2002). OXA-type β -lactamases confer resistance to ampicillin and cephalothin and are characterized by their high hydrolytic activity against oxacillin and cloxacillin (Bradford, 2001). *bla*_{OXA-30} is closely related to *bla*_{OXA-1}, differing in only one amino acid at codon 131 (AGA→GGA; Arg to Gly), and confers more resistance to cefepime than to ceftazidime (Siu *et al.*, 2000). Southern blot hybridization using the most recent *bla*_{OXA-30}-positive strain of *Shigella flexneri*, Sh04009, showed that *bla*_{OXA-30} was located in the chromosome (Fig. 2). The conjugation experiment results also supported the chromosomal location of *bla*_{OXA-30}, as no transconjugants were obtained on Mueller–Hinton agar containing rifampicin and ampicillin. The chromosomal location of *bla*_{OXA-30} has been reported in *Shigella flexneri* strains isolated from Hong Kong and Shanghai (Siu *et al.*, 2000). These results may explain the persistence of *bla*_{OXA-30} in *Shigella flexneri* in overcoming the effects of ampicillin, which was among the antibiotics recommended by the World Health Organization for the treatment of bacillary dysentery (World Health Organization, 1995).

PFGE patterns in relation to resistance phenotypes, integrons and *bla*_{OXA-30}

With the exception of the above-mentioned unique PFGE patterns for some strains, macrorestriction analysis by *Xba*I showed either indistinguishable or similar PFGE patterns (one or two band differences) among strains of the same species (Fig. 1). These PFGE patterns were associated with similar resistance phenotypes and the predominance of class 2 integrons in most strains of *Shigella sonnei* and *Shigella*

Fig. 2. Agarose gel electrophoresis (a) and Southern blot hybridization (b) of the *bla*_{OXA-30}-positive strain of *Shigella flexneri*, Sh04009. M, *Salmonella enterica* serotype Typhimurium LT2 plasmid (90 kb) used as size marker; Sf, the plasmids of *Shigella flexneri*, including part of its chromosome. The hybridization signal is located in the chromosome.

flexneri and with *bla*_{OXA-30} in all strains (except one) of *Shigella flexneri* (Table 2). These data suggest a clonal relatedness of strains of the same species, especially those isolated from the same locality (Hiroshima prefecture). Similar limited diversity in strains of *Shigella* spp. has been reported previously in Ireland (DeLappe *et al.*, 2003), India (Pazhani *et al.*, 2004) and Japan (Terajima *et al.*, 2004).

Conclusions

In this study, we have characterized the molecular basis of the MDR phenotype of *Shigella* spp. for the first time in Japan. Our study focused on the analysis of class 1 and class 2 integron gene cassettes and also on β -lactamase-encoding genes. Class 2 integrons were found to be predominant in *Shigella* spp. and *bla*_{OXA-30} is reported for the second time in *Shigella flexneri*.

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