

# Characterization of *Shigella* Strains in Iran by Plasmid Profile Analysis and PCR Amplification of *ipa* Genes

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To characterize *Shigella* clinical strains, we studied 82 *Shigella* strains recovered from 719 stool samples of patients with bloody diarrhea in Shiraz, Iran, over the period from April to October 2003. Serological assay classified the *Shigella* isolates as follows: 61 (74.39%) *Shigella sonnei* isolates, 16 (19.51%) *Shigella flexneri* isolates, 3 (3.65%) *Shigella boydii* isolates, and 2 (2.43%) *Shigella dysenteriae* isolates. In an antibiogram test, all *Shigella* strains were susceptible to ceftazidime, ciprofloxacin, and ceftriaxone. They showed high degrees of sensitivity to nalidixic acid, gentamicin, cephalothin, and amikacin. Approximately 90.24% of the *Shigella* isolates were resistant to co-trimoxazole. The plasmid profile patterns of all strains were determined by a modified alkaline lysis method. The average number of plasmid bands for each strain was 9.5. By plasmid profile analysis we identified 56 genotypes among all isolates and 42, 14, 3, and 2 genotypes among the *S. sonnei*, *S. flexneri*, *S. boydii*, and *S. dysenteriae* strains, respectively. PCR assays showed that all isolates were positive for two virulence genes, *ipaBCD* and *ipaH*. In conclusion, these data mandate local monitoring of drug resistance and its consideration in the empirical therapy of *Shigella* infections. These results also demonstrate that plasmid profile analysis is more reliable than antibiotic susceptibility pattern analysis for the identification of *Shigella* epidemic strains isolated in Iran.

Shigellosis is an acute gastroenteritis that is one of the most common causes of morbidity and mortality in children with diarrhea in developing countries. The disease is caused by microorganisms belonging to the genus *Shigella*. The annual number of *Shigella* episodes throughout the world is estimated to be 164.7 million, with 69% of all deaths attributable to shigellosis involving children less than 5 years of age (16). The disease is highly contagious due to its low infectious dose (7). Epidemics usually occur in areas with crowding and poor sanitary conditions (9, 12, 15, 26). Essential events in the pathogenesis of *Shigella* infections include bacterial invasion of epithelial cells, escape from the phagosome, and induction of apoptosis in macrophages (14). Shigellosis is caused by any of the four species of *Shigella*, namely, *Shigella dysenteriae*, *S. flexneri*, *S. boydii*, and *S. sonnei*. Except for *S. sonnei*, each species contains multiple serotypes, based on the structure of the O antigen (23). Thus far, at least 47 serotypes of *Shigella* have been recognized (30). The factors affecting the emergence or decline of epidemic shigellosis are not clear, and shigellae are generally believed to have only a human or a primate host. Recently, the World Health Organization has emphasized the need to understand the disease burden and epidemiology of *Shigella* infections in developing countries (30).

The identification of *Shigella* species is important because of both their clinical and their epidemiological implications. Serological testing is also needed for the identification of *Shigella* isolates (29). Understanding of the antibiotic resistance pat-

terns of shigellae and molecular characterization of plasmids and other genetic elements are also epidemiologically useful. Comparison of plasmid profiles is a useful method for assessing the possible relatedness of individual clinical isolates of a particular bacterial species for epidemiological studies (11). The present study was designed to isolate *Shigella* strains from clinical samples of patients with bloody diarrhea by culture methods and characterize them by appropriate biochemical, serological, and antibiogram tests. It was also designed to genetically characterize the isolates by using molecular techniques, such as plasmid profile analysis and PCR. Furthermore, this study was carried out to investigate the reliability of drug sensitivity patterns and plasmid profiles for the discrimination of epidemic strains of *Shigella* spp. isolated from epidemics of bacillary dysentery.

## MATERIALS AND METHODS

**Patients and samples.** During a period of 6 months from April to October 2003, stool specimens from 719 patients aged 2 months to 14 years were collected from three hospitals of the Shiraz University of Medical Sciences and transferred to a clinical microbiology laboratory. The inclusion criteria were as follows: the age of the patients was in the range of 2 months to 14 years, the patients had diarrhea that had lasted  $\leq 7$  days, and blood was evident by stool examination by an occult blood (OB) test.

**Bacterial culture and isolation.** All OB-positive samples were inoculated on xylose-lysin-desoxycholate agar and incubated at 37°C for 24 h. Non-lactose-fermenting colonies were picked from the culture plates and were subjected to further analysis by biochemical tests for the identification and isolation of possible *Shigella* colonies. The isolates, which were confidently identified as *Shigella* spp., were stored at -70°C for further studies. Two reference strains, *Shigella flexneri* ATCC 12022 and *Shigella sonnei* ATCC 9290, were used as control strains for comparison purposes.

**Serological tests.** The serotypes of all *Shigella* isolates were determined with commercially variable polyclonal antisera (Mast Co., Merseyside, United Kingdom) against all *Shigella* serotypes. The *Shigella* strains were subcultured on MacConkey agar plates, and serological tests were performed by the slide agglutination method, as described previously (28).

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TABLE 1. PCR primers used in this study to detect *Shigella* specific virulence genes

Primer	Description	Sequence	Amplicon size (bp)
<i>ipaH</i> -f	Forward primer for <i>ipaH</i>	5'-GTT CCT TGA CCG CCT TTC CGT TAC CGT C-3'	619
<i>ipaH</i> -b	Backward primer for <i>ipaH</i>	5'-GCC GGT CAG CCA CCC TCT GAG AGT AC-3'	
<i>ipaBCD</i> -f	Forward primer for <i>ipaBCD</i>	5'-GCT ATA GCA GTG ACA TGG-3'	612
<i>ipaBCD</i> -b	Backward primer for <i>ipaBCD</i>	5'-ACG AGT TCG AAG CAC TC-3'	

**Antimicrobial susceptibility testing.** The susceptibilities of all isolates to different antibiotics were determined by the disk diffusion method, as recommended by the CLSI (formerly the National Committee for Clinical Laboratory Standards) (20), with commercial antimicrobial disks (Mast Co.). The antibiotic disks used in this study were gentamicin (30 µg), nalidixic acid (30 µg), cephalothin (30 µg), ceftriaxone (30 µg), ceftazidime (30 µg), ciprofloxacin (5 µg), co-trimoxazole (25 µg), and amikacin (30 µg).

**Plasmid DNA extraction.** Plasmid DNA was extracted from the *Shigella* spp. by the alkaline lysis method of Birnboim and Doly (4). The extracted plasmid DNA was separated by horizontal electrophoresis in a 0.8% agarose slab gel in Tris-acetate-EDTA buffer at room temperature and 60 V for 4 h. After electrophoresis, the gel was stained with ethidium bromide and video images were obtained by a gel documentation system. The molecular masses of the unknown plasmid DNA were assessed by comparison of their mobilities with those of a supercoiled DNA ladder (Gibco-BRL-England) with known molecular masses. The Photo Capt  $M_w$  program was used to determine the molecular weights of the plasmid bands and to analyze the plasmid profiles.

**Analysis of similarity among strains and construction of a dendrogram.** The similarities among the isolates on the basis of their plasmid profiles were analyzed with NTSYS-PC software (Numerical Taxonomy and Multivariate Analysis System, version 2.02) for dendrogram construction. The matrix of the similarity of coefficients was subjected to unweighted pair group method analysis to generate dendrograms by use of the average linkage procedure.

**PCR assays.** Detection of the *ipa* genes was performed by amplifying both the *ipaH* and the *ipaBCD* genes by PCR. The primers sequences used were reported previously (2, 13) and were obtained from TIB MOLBIOL Syntheselabor GmbH (Berlin, Germany). Descriptions and the sequences of the PCR primers used in this study are given in Table 1. Other enzymes and chemicals were provided by Cinnagen Chemical Company (Tehran, Iran). Amplification was performed in a thermal cycler (Eppendorf, Germany) by the methods described by Aranda et al. (2) and Faruque et al. (13). The expected sizes of the amplicons were ascertained by electrophoresis in a 1.5% agarose gel with an appropriate molecular size marker (100-bp DNA ladder; MBI Fermentas, Lithuania).

## RESULTS

**Cases of shigellosis.** Among 719 children with diarrhea, 243 (34%) OB-positive patients from the ages of 2 months to 13 years were enrolled in our study. Seventy-one percent of the 243 patients were under 5 years of age. There were 132 males (53.6%) and 112 females (46.4%). Fever (94%), vomiting (71.6%), abdominal pain (68.8%), and convulsion (27%) were the most common presenting symptoms. Based on the biochemical and bacteriological properties, 82 (34.3%) of the isolates were confirmed to be *Shigella*.

***Shigella* serotypes.** According to the results of the serological tests, 61 (74.39%) of the isolates were identified as *Shigella sonnei*, 16 (19.51%) were identified as *Shigella flexneri*, 3 (3.1%) were identified as *Shigella boydii*, and 2 (2.43%) were identified as *Shigella dysenteriae*.

**Antibiotic susceptibility analysis.** The antimicrobial activities of eight commonly used antibiotics against all the *Shigella* isolates were determined by the Kirby-Bauer method and according to the recommendations of the CLSI (20). The results of the antibiotic susceptibility tests for four species of *Shigella* isolates are shown in Table 2. In total, of the 82 isolates, 2.43% were resistant to gentamicin, 4.87% were resistant to nalidixic

acid, 3.65% were resistant to amikacin, 7.31% were resistant to cephalothin, and 90.24% were resistant to co-trimoxazole. Resistance to ceftriaxone, ceftazidime, and ciprofloxacin was not detected. Multiple resistances with the patterns of nalidixic acid, co-trimoxazole, and cephalothin resistance or nalidixic acid, co-trimoxazole, and amikacin resistance were seen in 1.21 and 3.65% of all the isolates, respectively. A total of 6.09% of the strains were sensitive to all antibiotics under study. The remaining isolates were resistant to one or two antibiotics.

**Plasmid profile analysis.** In this study only small plasmids, which appeared as bright bands mostly below the band of chromosomal DNA on the gel, were used in the typing analysis because large plasmids tend to be lost during cell storage and subculturing or plasmid extraction. Analysis of plasmid DNA revealed that all 82 isolates harbored multiple plasmids, with an average of 9.5 plasmids (range, 5 to 14 plasmids) in each isolate of all strains and a mean of 10, 8, 10, and 9 plasmids in each isolate of *S. sonnei*, *S. flexneri*, *S. boydii*, and *S. dysenteriae*, respectively (Table 3). Figure 1 shows the plasmid patterns of some representative strains of the isolates. In total, 52 different plasmid profile patterns were revealed among all isolates. Plasmid analysis identified 42, 14, 3, and 2 genotypes among the *S. sonnei*, *S. flexneri*, *S. boydii*, and *S. dysenteriae* strains, respectively. The sizes of the plasmids from among all isolates ranged from 1 to 21 kb. Plasmids of 2 to 3 kb were the most frequently detected and were seen in about 96.34% of the isolates, while plasmids of 15 kb were detected in only 2.43% of all isolates.

**Genetic similarity among the isolates.** The genetic similarities among the 82 *Shigella* strains based on their plasmid patterns are represented by the dendrogram shown in Fig. 2. Similarities ranged from 55% to 100%. The organisms were

TABLE 2. Antimicrobial resistance patterns of *Shigella* isolates from Iran

Antimicrobial agent <sup>a</sup>	Resistance rate (%)				All <i>Shigella</i> spp.
	<i>S. sonnei</i>	<i>S. flexneri</i>	<i>S. boydii</i>	<i>S. dysenteriae</i>	
SXT	90.16	75	100	100	90.24
CEF	8.19	6.25	0	0	7.31
NAL	4.91	6.25	0	0	4.87
AMK	1.63	12.5	0	0	3.65
GEN	1.63	6.25	0	0	2.43
CAZ	0	0	0	0	0
CIP	0	0	0	0	0
CRO	0	0	0	0	0
SXT-CEF	6.55	0	0	0	4.87
SXT-NAL	1.63	0	0	0	1.21
SXT-AMK	6.25	0	0	0	1.21
AMK-GEN	6.25	0	0	0	1.21
SXT-CEF-NAL	6.25	0	0	0	1.21
SXT-NAL-AMK	4.91	0	0	0	3.65

<sup>a</sup> SXT, co-trimoxazole; CEF, cephalothin; NAL, nalidixic acid; AMK, amikacin; GEN, gentamicin; CAZ, ceftazidime; CIP, ciprofloxacin; CRO, ceftriaxone.

TABLE 3. Number of different plasmid profiles and antimicrobial susceptibility patterns among *Shigella* isolates from Iran

Species	Mean no. of plasmids per isolate (no. of isolates)	No. of different plasmid profiles (no. of isolates)	No. of different antimicrobial susceptibility patterns (no. of isolates)
<i>S. sonnei</i>	9.5 (61)	42 (61)	8 (61)
<i>S. flexneri</i>	9 (16)	14 (16)	5 (16)
<i>S. boydii</i>	10 (3)	3 (3)	1 (3)
<i>S. dysenteriae</i>	9 (2)	2 (2)	1 (2)
All isolates	9.5 (82)	56 (82)	11 (82)

clustered into nine groups, with more than 85% similarity between the groups. Occasional isolates showed similarities with other isolates of less than 60%.

**Detection of *Shigella* virulence genes by PCR assay.** The results of DNA amplification by the PCR method based on the primers used in this study showed the presence of a 619-bp fragment for the *ipaH* gene and a 612-bp fragment for the *ipaBCD* gene in the DNA preparations obtained from all isolates. These data showed that all *Shigella* isolates were positive for invasive genes.

## DISCUSSION

Bacillary dysentery caused by members of the genus *Shigella* is prevalent in many countries with temperate climates. It is a disease of children from 6 months to 10 years of age, although it can affect susceptible individuals of any age who are subject to poor sanitation (25). In our study most of the patients (71%) were under 5 years of age. Similar results were reported from a survey of *Shigella* infections in the United States from 1974 to 1980, in which the age group with the highest rate of infection comprised children less than 5 years of age (5). This reflects the fact that in general young children are less likely than older children to practice good hygienic habits. Serotyping of the isolates showed that *S. sonnei* was the most frequent species (74.39%) isolated in the period of our study. Most cases of dysentery, especially those due to *S. sonnei* infection, are mild and do not require antibiotic therapy (1). More than half of our patients (50.6%) showed mild clinical symptoms. However, the severity of the clinical symptoms is related to the bacterial species, the age of the patients, the immunity of the patient, and the inoculum dose. While Iran is a developing country, the prevalence of *S. sonnei* in Iran is similar to that shown by the results of some of studies that have been done in Israel, the United States, Canada, and other developed countries. It has been found that *S. sonnei* is the predominant species in those countries and is more common in children than in adults (3, 13). However, in Taiwan and Bangladesh the infections are mostly caused by *S. flexneri* (8, 13, 27). It has been suggested that factors involved in natural selection may have been the main reason for these discrepancies (22).

Antimicrobial resistance patterns are valuable as a guide to empirical therapy, as a typing method, and as an indicator of the dissemination of antimicrobial resistance determinants (10). By analyzing trends in the resistance patterns of the various *Shigella* species, we found that *S. sonnei* is currently signifi-

cantly more resistant than the other *Shigella* species (Table 2). This finding is of special importance, because at present *S. sonnei* is the predominant species in Iran. According to our findings and other reports, the rate of resistance to the antimicrobial agents used to treat shigellosis in young children, namely co-trimoxazole, has reached 87.5 to 94% (3, 10, 18, 21). However, the overall susceptibility patterns of the test strains focus on the fact that the strains were not frequently exposed to expanded- or broad-spectrum antibiotics. Therefore, due to the lack of variability in susceptibility patterns, the antimicrobial resistance pattern was not a useful epidemiological marker in our study.

Comparison of plasmid profiles is a useful method for assessment of the possible relatedness of individual clinical isolates of a particular bacterial species for epidemiological studies (11, 17, 24). In the present study, plasmid profiling could distinguish the 82 clinical isolates of *Shigella*. All isolates harbored plasmids, with an average of 9.5 plasmids in each isolate (range, 5 to 14 plasmids). Most profiles were characterized by the presence of small plasmids of 2 to 3 kb, 4 to 5 kb, and 7 to 9 kb. They were detected in about 96.34% (79 of 82), 87.2% (71 of 82), and 85.36% (70 of 82) of all isolates, respectively. However, the plasmids of 15 to 16 kb were detected in only 2.43% of all isolates. These data show that the former plasmids have a high rate of persistence in almost all *Shigella* strains, while the plasmids of 15 to 16 kb have a low degree of stability and so can be lost more easily. According to the data shown in Table 3, the plasmid profiles distinguished more strains than the antimicrobial susceptibility patterns did. These data indicate that shigellosis in the patients seen in Shiraz, Iran, is caused by a large number of clones which are not differentiated

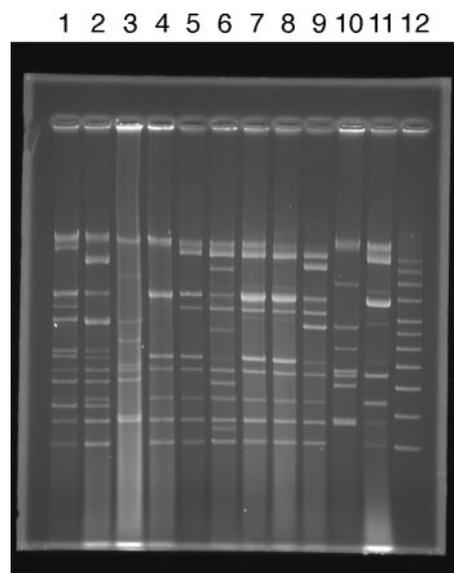


FIG. 1. Plasmid profiles of 11 representative *Shigella* isolates in 0.8% agarose gel. Lane 1, *S. flexneri* (strain 1); lane 2, *S. sonnei* (strain 1); lane 3, *S. flexneri* (strain 2); lane 4, *S. sonnei* (strain 2); lane 5, *S. sonnei* (strain 3); lane 6, *S. sonnei* (strain 4); lane 7, *S. sonnei* (strain 5); lane 8, *S. sonnei* (strain 6); lane 9, *S. sonnei* (strain 7); lane 10, *S. flexneri* (strain 3); lane 11, *S. sonnei* (strain 8); lane 12, supercoiled DNA ladder (the marker).

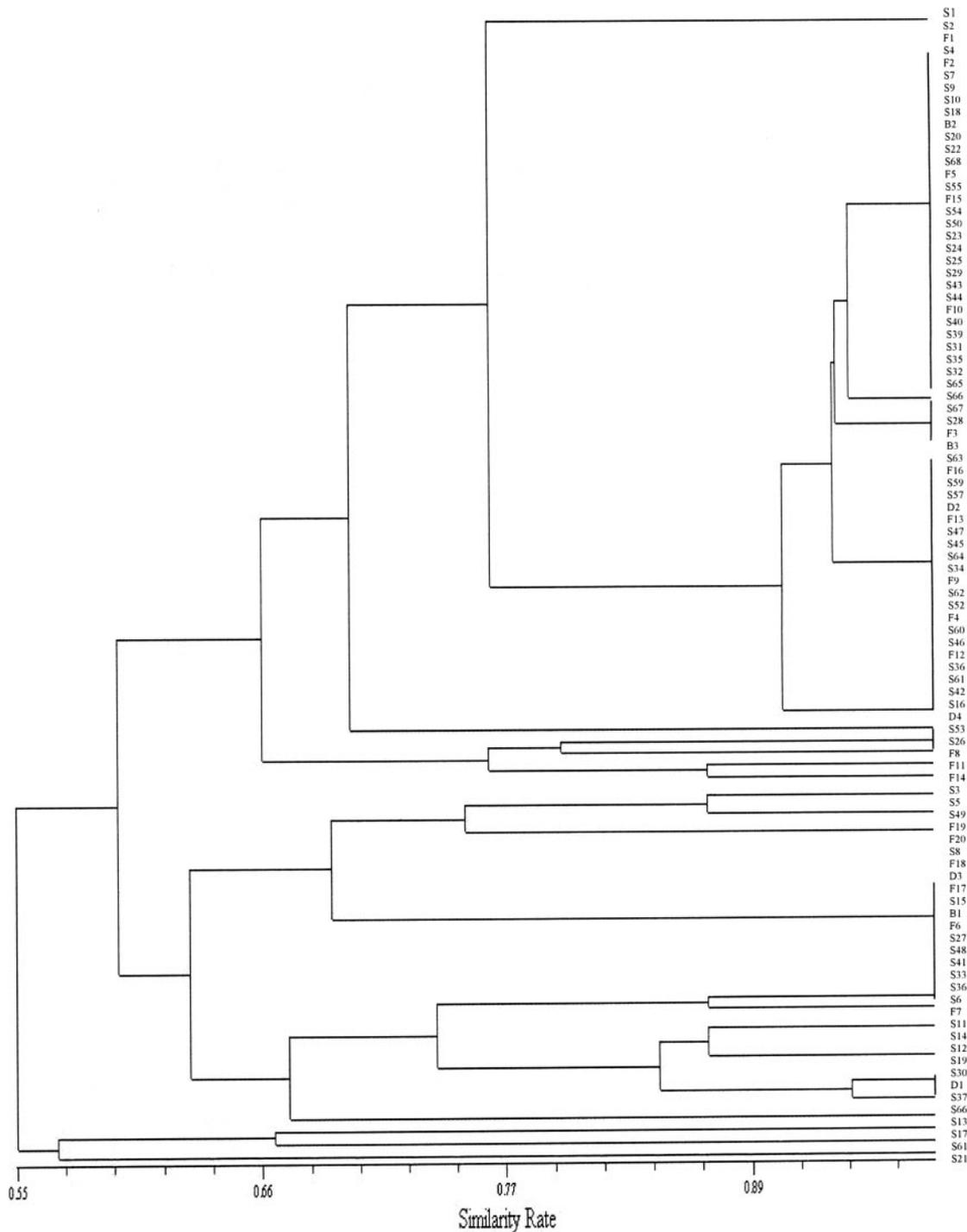


FIG. 2. Similarity among the *Shigella* isolates on the basis of their plasmid profiles. F, *S. flexneri*; S, *S. sonnei*; D, *S. dysenteriae*; B, *S. boydii*.

by their antimicrobial susceptibility patterns. This is similar to the results of studies done in Bangladesh but are in contrast to experiences in the developed world, where one or a few clones account for shigellosis in a community (6, 27). The similarity among the isolates on the basis of their plasmid profiles was also analyzed with NTSYS-PC, version 2.02K, software (Fig. 2). As seen in the dendrogram, similarities ranged from 55% to 100%. The organisms were clustered into nine groups with

more than 85% similarity (possible similarity levels at which organisms may be considered identical). Occasional isolates showed similarities with other isolates of less than 60%.

Invasiveness is an important property of pathogenic *Shigella* species. In this study we reviewed all of the strains for invasive characteristics by PCR to detect the two virulence genes, *ipaH* and *ipaBCD*. Based on the PCR results, all isolates were invasive. It is now well documented that copies of these genes are

located on the large invasive plasmids (19). Our data showed that all *Shigella* isolates in this study harbored the large invasive plasmid. However, the largest plasmid that we could detect was approximately 25 kb, which was detected in only two (2.1%) of the isolates. This is because large plasmids (>15 kb) are usually unstable and cannot be used for plasmid profile analysis. Only small plasmids below the band of chromosomal DNA on the gel are suitable for analysis.

In conclusion, these data mandate local monitoring of resistance and its consideration in empirical therapy of *Shigella* infections, especially those caused by *S. sonnei*. The results also demonstrate that within the species *S. sonnei* and *S. flexneri*, the plasmid profiles distinguished more strains than the antimicrobial susceptibility pattern did. However, more studies with more isolates are needed to make the same conclusion for *S. boydii* and *S. dysenteriae*.

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#### REFERENCES

- Ahmetagic, S., E. Jusufovic, J. Petrovic, V. Stojic, and Z. Delibegovic. 2003. Acute infectious diarrhea in children. *Med. Arhiv* 57:87–92.
- Aranda, K. R., U. Fagundes-Neto, and I. C. Scaletsky. 2004. Evaluation of multiplex PCRs for diagnosis of infection with diarrheagenic *Escherichia coli* and *Shigella* spp. *J. Clin. Microbiol.* 42:5849–5853.
- Ashkenazi, S., I. Levy, V. Kazaronovski, and Z. Samra. 2003. Growing antimicrobial resistance of *Shigella* isolates. *J. Antimicrob. Chemother.* 51:427–429.
- Birnboim, H. C., and J. Doly. 1979. A rapid alkaline extraction procedure for screening recombinant plasmid DNA. *Nucleic Acids Res.* 7:1513–1523.
- Blaser, M. J., R. A. Pollard, and R. A. Feldman. 2003. *Shigella* infections in the United States, 1974–1980. *J. Infect. Dis.* 147:771–775.
- Boyce, J. M., J. M. Hughes, A. R. Alim, M. Khan, K. M. Aziz, J. G. Wells, and G. T. Curlin. 1982. Patterns of *Shigella* infection in families in rural Bangladesh. *Am. J. Trop. Med. Hyg.* 31:1015–1020.
- Bradbury, W. C., A. D. Pearson, M. A. Marko, R. V. Congi, and J. L. Penner. 1984. Investigation of a *Campylobacter jejuni* outbreak by serotyping and chromosomal restriction endonuclease analysis. *J. Clin. Microbiol.* 19:342–346.
- Chiou, C. S., W. B. Hsu, H. I. Wei, and J. H. Chen. 2001. Molecular epidemiology of a *Shigella flexneri* outbreak in a mountainous township in Taiwan, Republic of China. *J. Clin. Microbiol.* 39:1048–1056.
- Craun, G. F., R. L. Calderon, and M. F. Craun. 2005. Outbreaks associated with recreational water in the United States. *Int. J. Environ. Health Res.* 15:243–262.
- DeLappe, N., F. O'Halloran, S. Fanning, G. Corbett-Feeney, T. Cheasty, and M. Cormican. 2003. Antimicrobial resistance and genetic diversity of *Shigella sonnei* isolates from western Ireland, an area of low incidence of infection. *J. Clin. Microbiol.* 41:1919–1924.
- Dutta, S., K. Rajendran, A. Roy, S., Chatterjee, P., Dutta, G. B. Nair, S. K. Bhattacharya, and S. I. Yoshida. 2002. Shifting serotypes, plasmid profile analysis and antimicrobial resistance pattern of *Shigella* strains isolated from Kolkata, India during 1995–2000. *Epidemiol. Infect.* 129:235–243.
- El-Gendy, A., N. El-Ghorab, E. M. Lane, R. A. Elyazeed, N. I. A. Carlin, M. M. Mistry, B. A. Kay, S. J. Savarino, and L. F. Peruski, Jr. 1999. Identification of *Shigella flexneri* subserotype 1c in rural Egypt. *J. Clin. Microbiol.* 37:873–874.
- Faruque, S. M., R. Khan, M. Kamruzzaman, S. Yamasaki, Q. S. Ahmad, T. Azim, G. B. Nair, Y. Takeda, and D. A. Sack. 2002. Isolation of *Shigella dysenteriae* type 1 and *S. flexneri* strains from surface waters in Bangladesh: comparative molecular analysis of environmental *Shigella* isolates versus clinical strains. *Appl. Environ. Microbiol.* 68:3908–3913.
- Guichon, A., D. Hersh, M. R. Smith, and A. Zychlinsky. 2001. Structure-function analysis of the *Shigella* virulence factor IpaB. *J. Bacteriol.* 183:1269–1276.
- Hossain, M. A., M. Rahman, Q. S. Ahmed, M. A. Malek, R. B. Sack, and M. J. Albert. 1998. Increasing frequency of mecillinam-resistant *Shigella* isolates in urban Dhaka and rural Matlab, Bangladesh: a 6 year observation. *J. Antimicrob. Chemother.* 42:99–102.
- Kotloff, K. L., J. P. Winickoff, B. Ivanoff, J. D. Clemens, D. L. Swerdlow, P. J. Sansonetti, G. K. Adak, and M. M. Levine. 1999. Global burden of *Shigella* infections: implications for vaccine development and implementation of control strategies. *Bull. W. H. O.* 77:651–666.
- Liu, P. Y., Y. J. Lau, B. S. Hu, J. M. Shyr, Z. Y. Shi, W. S. Tsai, Y. H. Lin, and C. Y. Tseng. 1995. Analysis of clonal relationships among isolates of *Shigella sonnei* by different molecular typing methods. *J. Clin. Microbiol.* 33:1779–1783.
- Mermel, L. A., S. L. Josephson, J. Dempsey, S. Parenteau, C. Perry, and N. Magill. 1997. Outbreak of *Shigella sonnei* in a clinical microbiology laboratory. *J. Clin. Microbiol.* 35:3163–3165.
- Nataro, J. P., J. A. Serowata, A. Fasano, D. R. Maneval, L. D. Guers, F. Noriega, F. Dubovsky, M. M. Levine, and J. G. Morris, Jr. 1995. Identification and cloning of a novel plasmid-encoded enterotoxin of enteroinvasive *Escherichia coli* and *Shigella* strains. *Infect. Immun.* 63:4721–4728.
- National Committee for Clinical Laboratory Standards. 2003. Performance standards for antimicrobial disc susceptibility tests, 8th ed. Approved standard M2-A8. National Committee for Clinical Laboratory Standards, Wayne, Pa.
- Oh, J. Y., H. S. Yu, S. K. Kim, S. Y. Seol, D. T. Cho, and J. C. Lee. 2003. Changes in patterns of antimicrobial susceptibility and integron carriage among *Shigella sonnei* isolates from southwestern Korea during epidemic periods. *J. Clin. Microbiol.* 41:421–423.
- Pan, T. M. 1997. Bacillary dysentery in Taiwan, 1995–1996. *Epidemiol. Bull.* 13:151–161.
- Simmons, D. A., and E. Romanowska. 1987. Structure and biology of *Shigella flexneri* O antigens. *J. Med. Microbiol. Rev.* 23:289–302.
- Stoll, B. J., R. I. Glass, M. I. Huq, M. U. Khan, H. Banu, and J. Holt. 1982. Epidemiologic and clinical features of patients infected with *Shigella* who attended a diarrheal disease hospital in Bangladesh. *J. Infect. Dis.* 146:177–183.
- Surdeanu, M., L. Ciudin, E. Pencu, and M. Straut. 2003. Comparative study of three different DNA fingerprint techniques for molecular typing of *Shigella flexneri* strains isolated in Romania. *Eur. J. Epidemiol.* 18:703–710.
- Szkal, D. D., G. Schneider, and T. Pal. 2003. A colony blot immune assay to identify enteroinvasive *Escherichia coli* and *Shigella* in stool samples. *Diagn. Microbiol. Infect. Dis.* 45:165–171.
- Tacket, C. O., N. Shahid, M. I. Huq, A. R. Alim, and M. L. Cohen. 1984. Usefulness of plasmid profiles for differentiation of *Shigella* isolates in Bangladesh. *J. Clin. Microbiol.* 20:300–301.
- Talukder, K. A., D. K. Dutta, A. Safa, M. Ansaruzzaman, F. Hassan, K. Alam, K. M. N. Islam, N. I. A. Carlin, G. B. Nair, and D. A. Sack. 2001. Altering trends in the dominance of *Shigella flexneri* serotypes and emergence of serologically atypical *S. flexneri* strains in Dhaka, Bangladesh. *J. Clin. Microbiol.* 39:3757–3759.
- Talukder, K. A., M. A. Islam, D. K. Dutta, F. Hassan, A. Safa, G. B. Nair, and D. A. Sack. 2002. Phenotypic and genotypic characterization of serologically atypical strains of *Shigella flexneri* type 4 isolated in Dhaka, Bangladesh. *J. Clin. Microbiol.* 40:2490–2497.
- World Health Organization. 1999. Generic protocol to estimate the burden of *Shigella* diarrhoea and dysenteric mortality. WHO/V&P/99.26. World Health Organization, Geneva, Switzerland.