South Asian J. Zool., vol. 44(1), pp 43-52, 2026 DOI: https://dx.doi.org/10.17582/sajz/2026/44.1.43.52

# Plasmid Borne Antibiotic Resistance Factors in Indigenous *Shigella boydii* Isolated from Diarrheal Patients in Azad Jammu and Kashmir, Pakistan

**Basharat Ahmed[1,2], Farah Rauf Shakoori[1], Syed Shahid Ali[1], Ansar Ahmed Abbasi[2], Madiha Khalid[3] and Abdul Rauf[2]***

[1]*Institute of Zoology, University of the Punjab, Quaid-i-Azam Campus, Lahore, Pakistan*
[2]*Department of Zoology, University of Azad Jammu and Kashmir, Muzaffarabad, Azad Jammu and Kashmir, Pakistan*
[3]*Department of Biotechnology, Women University of Azad Jammu and Kashmir, Bagh, Pakistan*

**Article Information**
**Received 15 May 2025**
**Revised 29 May 2025**
**Accepted 06 June 2025**
**Available online (early access)**
**Published 14 May 2026**

**Authors' Contribution**
**BA: Conceptualization, methodology, original draft preparation**
**FRS: Writing, revision, editing, validation, resources**
**SSA: Conceptualization, supervision, data curation**
**AAA: Sofware**
**MK: Investigation**
**AR: Writing, review, editing, supervision**

**Key words**
***Shigella boydii*, Antibiotic resistance, R-plasmid, Shigellosis, Multiple drug resistance, Antimicrobial resistance determinants, Plasmid profile**

## ABSTRACT

The antimicrobial susceptibility patterns for 110 *Shigella boydii* isolated from diarrhoeal patients admitted to hospitals in Azad Kashmir Pakistan were analyzed to determine their plasmid profile and changing trends in response to twenty antibiotics. Susceptibility of the isolated bacteria was determined against various antibiotics by antibiotic disc diffusion method. Plasmid DNA was isolated from the multiple antibiotic resistant strains of *S. boydii.* and was transferred to plasmid-less and sensitive to antibiotic strain of *Escherichia coli* HB101. The transformed cells were then examined for their sensitivity to ampicillin (100 µg/ml), chloramphenicol (100 µg/ml) and sulfamethoxazole-trimethoprim (100 µg/ml). The isolates showed highest resistance against penicillin followed by carbenicillin, ampicillin, tetracycline, erythromycin, ceftizoxime, kanamycin, co-trimoxazole, piperacillin, amoxicillin, amikacin, streptomycin, nalidixic acid, gentamicin, chloramphenicol, cephalothin and ceftriaxone. All *S. boydii* isolates were sensitive to cefixime, ciprofloxacin and enoxacin. Multiple drug resistance (MDR) was observed ranging from three to ten drugs and was resistant to three or more antibiotics at level as high as 300µg/ml. The resistant isolates showed different patterns of antibiotics resistance. The most common pattern was PCaA (penicillin, carbenicillin, ampicillin). The plasmids were observed in (*28.0 %*) MDR strains of *S. boydii* which were found resistant to three or more antibiotics. The number of plasmids varied from one to seven. Analysis of plasmid DNA of *S. boydii* revealed that all the strains contained a heterogenous population of plasmids ranging between 23.1 kb to 2.0 kb. Based on molecular weight, the pattern of different plasmids was also very diverse. Depending on the number of plasmids, individual strains were grouped into nine different plasmid patterns. The plasmids (23.1 Kb and <23.1 Kb) could only confer ampicillin, chloramphenicol and sulfamethoxazole-trimethoprim resistance to the competent cells of *E. coli* HB101.

## INTRODUCTION

Shigellosis is a public health problem in many parts of the world including Pakistan not only for morbidity but also for growth retardation and malabsorption. *Shigella* is a non-motile, nonspore-forming, facultative anaerobic Gram-negative bacterium. *Shigella* is a rod-shape and is lactose-fermenting bacterium causing dysentery (Yang *et al*., 2005). There are 4 species of *Shigella* classified on the basis of biochemical serological differences. Serogroup A: *S. dysenteriae* (12 serotypes), Serogroup B: *S. flexneri* (6 serotypes), Serogroup C: *S. boydii* (23 serotypes) and Sergoroup D: *S. sonnei* (1 serotype) (Niyogi, 2005). The *Shigella* genome includes a virulence plasmid that encodes conserved primary virulence determinants. The virulence plasmid encodes the ~30 kb Mxi-Spa type III secretion system (TTSS) and invasion plasmid antigens (Ipa proteins) required for invasion of the colonic and rectal epithelial cells (Sansonetti, 2001). In addition to

* Corresponding author: itsabdulrauf@gmail.com
1013-3461/2026/0001/0043 $ 0.00/0

Copyright 2026 by the authors.
This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (https://creativecommons.org/licenses/by/4.0/).

the plasmid, many chromosomal genes, such as those encoded by the *Shigella* pathogenicity island (SHI)-1 and SHI-2, also contribute to virulence (Moss *et al*., 1999). *Shigella boydii* are responsible for gastroenteritis that may progress to mucoid bloody diarrhoea. *S. boydii* (23 serotypes) is mainly endemic to the Indian subcontinent. In developing countries, shigellosis is widespread and causes extensive outbreaks. In industrialized countries, this disease has become rare, and it currently occurs as sporadic cases in migrant workers or those who travel to developing countries and is limited to epidemic episodes among children in daycare centers, individuals in custodial institutions, and homosexual men (Niyogi, 2005). Consequently, although shigellosis is a major public health concern, there is a great disparity between developing countries (over 163.2 million cases each year) and developed ones (1.5 million cases). Epidemics usually occur in areas with crowding and poor sanitary conditions, where transmission from person to person is common or when the organisms contaminate the food or water (Kotloff *et al*., 1999). Dramatic outbreaks may also occur, particularly in the context of humanitarian disasters (wars, refugee camps). Shigellosis is not the most frequent cause of diarrhoeal disease, but its dysenteric form is the most severe: each year, it kills between 600,000 and one million people, mostly children in developing countries. Those infected with *Shigella* develop diarrhoea, fever, and stomach cramps starting a day or two after the exposure to the bacterium. In some persons, especially young children and the elderly, the diarrhoea can be so severe that the patient needs to be hospitalized.

Shigellosis is one of the acute enteric diseases for which antimicrobial therapy is generally required to manage infection and reduce fecal excretion of the bacterium to prevent further transmission. Antimicrobial resistance pattern, plasmid profile and serotype correlation of *Shigella* strains have been reported from several countries (Dutta *et al*., 2002; Lin and Chang, 1992; Olukoya and Oni, 1990; Albert *et al*., 1990). Although *Shigella* spp. is intrinsically susceptible to all antibiotics that are active against Gram-negative bacilli, under antibiotic pressure, they have progressively acquired resistances to commonly recommended drugs (Hirose *et al*., 2005; Toro *et al*., 2005). The antibiotics commonly used for treatment are ampicillin, trimethoprim/ sulfamethoxazole, nalidixic acid, or ciprofloxacin. Appropriate treatment kills the *Shigella* bacteria and shortens the illness. Unfortunately, some *Shigella* bacteria have become resistant to antibiotics and using antibiotics to treat shigellosis can actually make the germs more resistant in the future (Nisa *et al*., 2022). Indiscriminate use of the drugs and horizontal gene transfer has led to *Shigella* species becoming resistant to commonly used antibiotics (Noriega *et al*., 1999). A high proportion of the resistant strains were found to be resistant to some of the most commonly used antibiotics such as tetracycline and streptomycin (Kotloff *et al*., 2005). Most of the *Shigella* strains isolated from patients are resistant to ampicillin, sulfamethoxazole-trimethoprim, and nalidixic acid (Hossain *et al*., 1998).

MDR in *Shegella* continues to pose a significant public health challenge particularly in developing countries. Whole genome sequencing and bioinformatic approaches unveiled more than 500 global plasmid entities and more than 1000 plamid mediated gene clusters from global databases. Asad *et al*. (2025) identified 28 antimicrobial resistance genes from nine antibiotic classes, with 75% orignating from plasmids.

The overuse of antibiotics is considered the main factor in the emergence and dissemination of antibiotic resistance. In order to ensure appropriate treatment, continual surveillance is required to determine which antibiotics are still active. The people in Azad Kashmir Pakistan face health hazards because of poor sanitation practices. The present study was aimed to investigate the virulence factors in locally isolated *S. boydii* and their possible role in infection. The object also was to suggest preventive measures. The strains of *S. boydii* resistant to commonly used antibiotics have been screened for plasmid DNA. The isolated plasmids have been characterized through biochemical and physical characteristics. The plasmid-less *E. coli* HB101 strains were transformed with plasmid DNA of multiple drug resistant (MDR) *S. boydii* isolates to identify the plasmid (s) involved in antibiotic resistance. Attempts have been made to determine any correlation between plasmid profile and serotype patterns of isolated shigella strains.

## MATERIALS AND METHODS

This study was carried out in Azad Kashmir, which is a mountainous region and located 140 km. north-east of Islamabad (Pakistan). Approximately 4.3 million people live in the state of Azad Kashmir comprising rural and urban populations.

*Bacterial strains*

*Shigella boydii* strains were isolated from stools of patients suffering from diarrhoea admitted at different hospitals of Azad Kashmir (Pakistan), over a 5-year period. The samples were obtained from children (aged 0-5 years) and adults. The study subjects were both male and female. For the isolation of *S. boydii* a loop full of stool was mixed with 10 ml of sterile buffered peptone water and incubated at 37°C for 24 h. After incubation a loop full of culture

was streaked on the SSA and MacConkey agar plates and were incubated at 37°C for 24 h. Non-lactose fermenting colonies (i.e., colorless) on MacConkey agar plates were inoculated on XLD agar and incubated at 37°C for 24 h. After incubation, red colonies with 2-4 mm diameter were marked and suspected colonies were subjected to subsequent Gram staining. All plates were incubated aerobically at 37 ºC for 24 h. From amongst the suspected *S. boydii* from both SSA and MacConkey agar, the non-lactose-fermenting (NLF) colonies were biochemically identified by Urea test, Triple Sugar Iron (TSI) test, Sulphide Indole Motility (SIM) test, and Simmons Citrate Agar – Microbial Utilization test. Serotyping was determined by Kligler's Iron Agar (KIA, DIFCO) test.

*Chemicals and media*

Chemicals and antibiotics used were obtained from Sigma Chemicals Co. and were of molecular biology grade. The culture media were purchased from DIFCO (USA). LB medium was used for the cultivation of bacteria and Muller Hinton agar DIFCO was used for susceptibility testing. Antibiotic susceptibility discs used were from OXOID, England. Antibiotics used in these studies were amikacin (Ak), amoxicillin (Am), ampicillin (A), carbenicillin (Ca), cefixime (Cfm), ceftizoxime (Cxm), ceftriaxone (Cz), cephalothin (Cl), chloramphenicol (C), ciprofloxacin (Cip), co-trimoxazole (Co), enoxacin (E), erythromycin (Er), gentamicin (G), kanamycin (K), nalidixic acid (Na), penicillin (P), streptomycin (S), sulfamethoxazole-trimethoprim (SxT) and tetracycline (T). All solutions were sterilized by Millipore (0.45mμ) filters and refrigerated.

*Antimicrobial sensitivity testing*

Antibiotic susceptibility tests of the collected strains of *S. boydii* were performed by antibiotic disc diffusion method (Bauer *et al*., 1966) using filter paper discs. The minimum inhibitory concentrations of fifteen commonly used antibiotics were determined by agar dilution method using MICs: 25μg/ml, 50μg/ml, 100μg/ml, and 300μg/ml. MIC was defined as the lowest concentration on which the growth appeared. Reference strains *Escherichia coli* ATCC 25922 and *Pseudomonas aeruginosa* ATCC 27853 were tested regularly as controls according to the National Committee for Clinical Laboratory Standards (1993).

*Plasmid DNA isolation*

Plasmid DNA was isolated from the multiple antibiotics resistant strains according to Birnboim and Doly (1976) to separate, identify and purify the plasmid DNA through agarose gel (Meyers *et al*., 1976). The plasmid DNA was purified by removal of RNA present in the solution with the help of RNase. To estimate the size of plasmid DNA, DNA Marker (Lambda DNA cut with *Hin*d-III) was used. After gel electrophoresis, plasmid DNA was stained with florescent, intercalating dye, ethidium bromide. DNA bands were visualized under UV transilluminator. Photographs of the gel were positioned over a short-wave UV light source that was taken with the help of gel documentation system GDS-5000 (UVP) and the images of DNA bands were obtained. Various plasmids DNA bands were individually cut out of the gel with a sharp razor, extracted, and purified by the usual molecular biological techniques (Weislander, 1979).

*Transformation*

All the isolates were tested for the ability to transfer their determinants. *E. coli* HB101 (plasmid less and sensitive to antibiotics) were transformed with different individually isolated plasmids. For this, 5 μl of plasmid DNA of multiple drug resistant (MDR) *S. boydii* was added to competent cells of *E. coli* HB101, prepared, incubated on ice for 30 min and then at 42 ºC for two min. One ml of pre-warmed LB broth was then added to this mixture and re-incubated at 37ºC at 60 rpm for 80 min. The whole mixture was then spread on two different Luria-Bertani agar plates containing ampicillin (100 μg/ml), chloramphenicol (100 μg/ml) sulfamethoxazole-trimethoprim (100 μg/ml) and incubated at 37ºC overnight (Sambrook *et al*., 1983).

## RESULTS

During the study period, out of 110 *S. boydii,* in 1994, 27 (12.0%) strains were recovered, whereas this number was 20 (11.9 %), 21 (11.0 %), 14 (11.5%) and 28 (13.6 %) in 1995, 1996, 1997 and 1998, respectively. *S. boydii* decreased from 12.0 % in 1994 to 11.9 % in 1995, 11.0 % in 1996 but again increased up to 11.5% in 1997 and the highest number (13.6 %) was recovered in 1998.

Over all the highest proportion of stool specimens infected with *S. boydii* were in the age group of >50-60 years (27.3%) followed in >60 years (23.1%), >30-40 years (20.6%), >10-20 years (17.6%), >20-30 years (16.7%), >40-50 years (14.3%) and >5-10 years (13.6%). The lowest infestation was observed in the age group >0-5 years (9.5%).

*Antimicrobial sensitivity testing*

Table I shows that the overall 65.4% *S. boydii* isolates were resistant to penicillin (P) followed by 51.8% to tetracycline (T), 49.1% to erythromycin (Er), 47.3% to ampicillin (A), 46.4% to ceftizoxime (CXM), 42.7%

**Table I. Occurrence of antibiotics resistance of 110 *S. boydii* at four different concentrations, isolated from stools of patients with diarrhea in Azad Kashmir.**

| Antibiotics | No. of resistant isolates at | | | |
|---|---|---|---|---|
| | 25 µg/ml | 50 µg/ml | 100 µg/ml | 300 µg/ml |
| Amikacin (Ak) | 36(32.7%) | 34 (30.9%) | 15 (13.6%) | 3 (2.7%) |
| Ampicillin (A) | 52(47.3%) | 50 (45.4%) | 30 (27.3%) | 12 (10.9%) |
| Amoxicillin (Am) | 35(31.8%) | 33 (30.0%) | 17 (15.4%) | 2 (1.8%) |
| Carbenicillin (Ca) | 47(42.7%) | 46 (41.8%) | 27 (24.5%) | 13 (11.8%) |
| Cefixime (Cef) | 00(0.0%) | 00 (0.0%) | 00 (0.0%) | 00 (0.0%) |
| Ceftizoxime (CXM) | 51(46.4%) | 49 (44.5%) | 24(21.8%) | 9 (8.1%) |
| Ceftriaxone (Cz) | 21(19.1%) | 17 (15.4%) | 6 (5.4%) | 1 (0.9%) |
| Cephalothin (Cl) | 26(23.6%) | 23 (20.9%) | 7 (6.4%) | 2 (1.8%) |
| Chloramphenicol (C) | 29(26.4%) | 25 (22.7%) | 9 (8.2%) | 3 (2.7%) |
| Ciprofloxacin (Cip) | 00(0.0%) | 00(0.0%) | 00 (0.0%) | 00 (0.0%) |
| Co-trimoxazole (Co) | 39(35.4%) | 37 (33.6%) | 12 (10.9%) | 4 (3.6%) |
| Enaxacin (E) | 00(0.0%) | 00 (0.0%) | 00 (0.0%) | 00 (0.0%) |
| Erythromycin (Er) Gentamicin (G) | 54(49.1%) | 52 (47.3%) | 16 (14.5%) | 8 (7.2%) |
| Kanamycin (K) | 30(27.3%) | 27 (24.5%) | 8 (7.2%) | 5 (4.5%) |
| Nalidixic acid (Na) | 43(39.1%) | 41(37.3%) | 14 (12.7%) | 7 (6.4%) |
| Penicillin (P) | 24(21.8%) | 22(20.0%) | 10 (9.1%) | 3 (2.7%) |
| Sulfamethoxazole-Trimethoprim (SxT) | 72(65.4%) | 69 (62.7%) | 45 (40.9%) | 17(15.4%) |
| Streptomycin (S) | 37(33.6%) | 35 (31.8%) | 11 (10.0%) | 4 (3.6%) |
| Tetracycline (T) | 34(30.9%) | 29 (26.4%) | 12 (10.9%) | 3 (2.7%) |
| | 57(51.8%) | 54 (49.1%) | 28 (25.4%) | 12 (10.9%) |

to carbenicillin, (Ca), 39.1% to kanamycin (K), 35.4% to co-trimoxazole (Co), 33.6% to sulfamethoxazole-trimethoprim (SxT), 32.7% to amikacin (Ak), 31.8 % to amoxicillin (Am), 30.9% to streptomycin (S), 27.3% to gentamicin (G), 26.4% to chloramphenicol (C), 23.6% to cephalothin (Cl), 21.8% to nalidixic acid (Na), and 19.1% to ceftriaxone (Cz). All *S. boydii* isolates were sensitive to cefixime (Cfm), ciprofloxacin (CIP) and enoxacin (E).

The MICs of twenty antibiotics against 110 strains of *S. boydii* are shown in a comparative account of the antibiotics resistance of isolates at four levels 25µg/ml, 50µg/ml, 100µg/ml and 300µg/ml in Table I. Generally, the isolates showed the highest frequency of resistance against penicillin (P) at all the four levels. The lowest frequency of resistance was against ceftriaxone (Cz) at all the four levels of antibiotics screened. At 100µg/ml level the isolates showed a considerable decrease in the resistance frequency of almost all the antibiotics tested. Multiple drug resistance was observed in this study ranging from three to ten drugs. Out of 584 isolates, screened for antibiotic resistance, 31% were resistant to three or more antibiotics at 25µg/ml, 26% were resistant to three or more antibiotics at 50µg/ml, 11% were resistant to three or more antibiotics at 100µg/ml and 4% were resistant to three or more antibiotics at 300µg/ml. The resistant isolates showed different patterns of antibiotics resistance. The most common pattern was PCaA at all the four levels shown in Table II.

Total 75 strains of *S. boydii* were processed for isolation of plasmids and 21 (28.0 %) of *S. boydii* carried plasmids. These were resistant against three or more antibiotics. The number of plasmids varied from one to seven. The plasmid pattern was determined by the presence or absence of a single plasmid within a group of strains.

In *S. boydii,* the analysis of plasmid DNA revealed that all the strains contained a heterogeneous population of plasmids ranging between 23.1 kb to 2.0 kb, (Fig. 1, Table III). The molecular size of all plasmids was determined by comparison with a bacteriophage lambda DNA digest with *Hind*-III. The most dominant plasmids were 2.3 Kb, 6.5 Kb, 2.0 Kb, >4.3 Kb, 4.3Kb, 23.1 Kb, <23.1 Kb and <6.5 Kb. The frequency with which they were encountered was 66.7%, 61.9 %, 61.9 %, 57.1%, 57.1 %, 47.6 %, 23.8 % and 23.8 %, respectively. Other plasmids were observed in lesser frequency. The frequency of 9.4 Kb plasmid was 14.3 %, for <4.3 Kb it was 9.5 % and for >2.3 Kb it was 4.8 %.

Based on molecular weight, the pattern of different plasmids was also very diverse. Depending on the number of plasmids, individual strains were grouped into nine different plasmid patterns, designated P1-P9, for 21 strains. Four strains (19.0 %) had pattern P1 (5 plasmids), three strains (14.3 %) had pattern P2 (2 plasmids), whereas three strains (14.3 %) had pattern P3 (6 plasmids), while

another group of three strains (14.3 %) had pattern P4 (4 plasmids), two strains (9.5 %) had pattern P5 (5 plasmids), whereas two strains (9.5 %) had pattern P6 (4 plasmids), while another group of two strains (9.5 %) had pattern P7 (6 plasmids),whereas one strain (4.8 %) had P8 (3 plasmids) and the remaining one strain (4.8 %) had pattern P9 (2 plasmids).

**Table II. Multiple antibiotic resistance patterns occurring in *S. boydii* isolated from stools of patients with diarrhea in Azad Kashmir.**

| Antibiotics resistance patterns | Percent of resistant isolates at | | | |
|---|---|---|---|---|
| | 25 µg/ml | 50 µg/ml | 100 µg/ml | 300 µg/ml |
| P, Ca, A | 31 | 26 | 11 | 4 |
| P, A, T | 27 | 25 | 9 | 3 |
| P, Ca, A, T | 24 | 23 | 7 | 3 |
| P, A, T, Er | 22 | 21 | 7 | 3 |
| P, Ca, A, Er | 20 | 19 | 5 | 2 |
| P, Ca, A, CXM | 18 | 16 | 5 | 2 |
| P, Ca, A, T, Er | 15 | 14 | 4 | 1 |
| P, C, A, T, CXM | 13 | 10 | 3 | 1 |
| P, Ca, T, CXM, K | 10 | 7 | 2 | 1 |
| P, Ca, A, T, K, Co | 10 | 7 | 2 | 1 |
| P, A, T, Er, K, Co | 8 | 5 | 1 | 1 |
| P, Ca, A, T, Er, Co, SxT, Am | 7 | 4 | 1 | - |
| P, A, C, Er, K, Co, Am, Ak, S, Na | 2 | 2 | - | - |
| P, Ca, A, T, Co, Am, Ak, S, Na, G | 1 | 1 | - | - |
| P, Ca, A, K, Am, Na, G, C, Cl, Cz | 1 | 1 | - | - |

A, Ampicillin; AK, Amikacin; Am, Amoxicillin; Ca, Carbenicillin; Cef, Cefixime; CXM, Ceftizoxime; CZ, Ceftriaxone; Cl, Cephalothin; C, Chloramphenicol; Co, Co-trimoxazole; Er, Erythromycin; G, Gentamicin; K, Kanamycin; Na, Nalidixic acid; P, Penicillin; SxT, Sulfamethoxazole-Trimethoprim; S, Streptomycin; T, Tetracycline.

*Transfer of antimicrobial resistance determinants and antimicrobial sensitivity testing*

Of the 21 S. boydii strains, the plasmids of 18 strains were processed for transformation into *E. coli* HB101 separately for ampicillin (MIC-100 µg/ml), chloramphenicol (MIC-100 µg/ml) and sulfamethoxazole-trimethoprim (MIC-100 µg/ml), plasmids of 11 strains (61.1 %) for only ampicillin, 9 (50.0 %) for chloramphenicol, and 8 (44.4%) for sulfamethoxazole-trimethoprim resistance. Of the 18 transformations, 15 (83.3 %) were successfully accomplished as *E. coli* HB101 acquired antibiotic resistance to ampicillin, chloramphenicol and sulfamethoxazole-trimethoprim. Plasmids of three strains (no. BSb-721, BSb-785 and BSb-7004) were successfully transferred to *E. coli* Hb101 shown by the acquisition of resistance to ampicillin, and plasmids of another three strains (no. BSb-736, BSb-7003 and BSb-7008) with chloramphenicol resistance were also successfully introduced into *E. coli* HB101. Plasmids of 11 strains resistant to ampicillin, 9 strains resistant to chloramphenicol, and 8 strains resistant to sulfamethoxazole-trimethoprim were also successfully introduced into *E. coli* HB101.

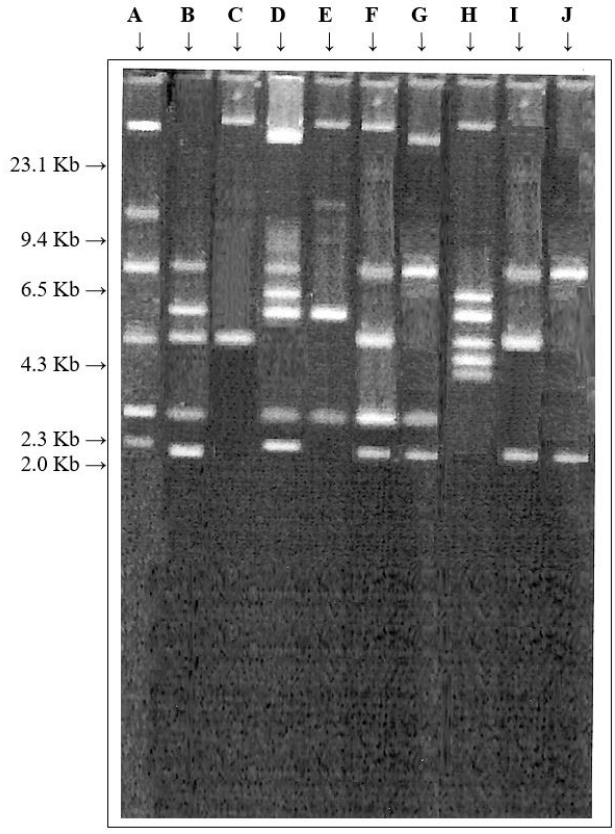


Fig. 1. Plasmid profile of representative *S. boydii* strains isolated from fecal samples of patients with gastroenteritis in Azad Kashmir. (Lane A, marker λ DNA cut with *Hind* III; Lane B, BSb-721; Lane C, BSb-736; Lane D, BSb-741; Lane E, BSb-756; Lane F, BSb-785; Lane G, BSb-796; Lane H, BSb-7003; Lane I, BSb-7004; Lane J, BSb-7008). (B, author's name; Sb, *S. boydii*; digits, sample number).

In some multiple plasmid strains (no. BSb-741, BSb-756 and BSb-796), all the DNA bands of different molecular sizes were cut out of the gel, extracted, purified and then successfully transferred to *E. coli* HB101 individually. The plasmids (23.1 Kb and <23.1 Kb) could only confer ampicillin, chloramphenicol and sulfamethoxazole-trimethoprim resistance to the competent cells of *E. coli* HB101.

**Table III. Transformation of plasmids of *S. boydii* into *E. coli* Hb101.**

| Sample no. | No. of plasmids | Molecular weight of plasmids which were individually transferred to *E. coli* HB101. | Transformed plasmids that conferred antibiotic resistance |
|---|---|---|---|
| 741 | 6 | <23.1Kb, 6.5Kb, <6.5Kb, >4.3Kb,2.3Kb,2.0Kb | <23.1Kb. |
| 756 | 4 | 23.1Kb,9.4Kb,>4.3Kb,2.3Kb | 23.1Kb. |
| 796 | 4 | <23.1Kb,6.5Kb,2.3Kb,2.0Kb | <23.1Kb |

## DISCUSSION

*Shigella* is a leading cause of shigellosis (bacillary dysentery) worldwide and a major cause of diarrhoeal disease in developed and developing countries (Soebel *et al*., 1998). Antimicrobial resistance is now recognized as an increasingly global problem (Tenvor and Hughes, 1996). Resistance to commonly-prescribed antibiotics is an expanding global problem and has been observed in both developed and developing countries. Because of introduction of new antimicrobial agents is usually followed sooner or later by emergence of bacterial resistance to these drugs (Patwary, 1994). The problem of changing resistance patterns in *S. boydii* will remain an ongoing threat for both developed and developing countries. In the present study, it was noted that among the *Shigella* spp. isolated, (12.1%) isolates were *S. boydii* strains.

Shigellosis is primarily a childhood disease in both developed and developing countries, whereas epidemic shigellosis affects all age groups including Pakistan (Keusch and Bennish, 1991; Ahmed and Shakoori, 1996). However, the information about the etiology and drug sensitivity pattern of bacterial strains is lacking due to the lack of diagnostic facilities. In this study, *S. boydii* decreased from (12.0 %) in 1994 to (11.9 %) in 1995, (11.0 %) in 1996 but again increased up to (11.5 %) in 1997 and the highest number (13.6 %) was recovered in 1998. The highest proportion of stool specimens infected with *S. boydii* were in the age group of >50-60 years (27.3 %). The lowest infestation was observed in the age group >0-5 years (9.5 %). Almost similar results were reported by Ahmad *et al*. (2003) who recorded shigellosis in all age groups, but slightly higher in the age groups of >10-20 and 20-30 years. Khalil *et al*. (1998) reported highest infestation of *Shigella* in the age groups of 18-23 and 24-35 years. Similarly, Bhattacharya *et al*. (2005) reported that the majority (79%) of *Shigella* species were isolated from children aged less than five years in a recent study in Eastern Nepal.

In this study, *S. boydii* isolates were resistant to penicillin followed by tetracycline, erythromycin, ampicillin, ceftizoxime, carbenicillin, kanamycin, co-trimoxazole sulfamethoxazole-trimethoprim, amikacin, amoxicillin, streptomycin, gentamicin, chloramphenicol, cephalothin, nalidixic acid, and ceftriaxone. All *S. boydii* isolates were sensitive to cefixime, ciprofloxacin and enoxacin. However, the resistance to these commonly used antibiotics, especially tetracycline has already been documented from several parts of the world including Pakistan (Ahmed *et al*., 2003; Ahmed and Shakoori, 2001) and may reflect widespread overuse of the antibiotics especially, tetracycline. Analogous results were presented in a previous study by Dutta *et al*. (2002) who reported serovars of *S. dysenteriae* and *S. boydii* from Kolkata. Most of the *Shigella* isolates were multidrug resistance (MDR) i.e., resistant to antimicrobials like ampicillin, chloramphenicol, tetracycline, nalidixic acid and co-trimoxazole, amoxicillin. Ansaruzzaman *et al*. (2005) in a study in Bangladesh observed that the isolates were all susceptible to ampicillin, sulfamethoxazole-trimethoprim, nalidixic acid, ciprofloxacin and mecillinam but eight exhibited resistance to tetracycline.

The MICs of twenty antibiotics against one hundred and ten strains of *S. flexneri* are shown in a comparative account of the antibiotics resistance of isolates at four levels 25µg/ml, 50µg/ml, 100µg/ml and 300µg/ml in Table I. Generally, the isolates showed the highest frequency of resistance against penicillin at all the four levels. The lowest frequency of resistance was against ceftriaxone at all the four levels of antibiotics screened. At 100µg/ml level the isolates showed a considerable decrease in the resistance frequency of almost all the antibiotics tested. MDR was observed in this study ranging from three to ten drugs. Out of one hundred and ten isolates, screened for antibiotic resistance, 31% were resistant to three or more antibiotics at 25µg/ml, 26% were resistant to three or more antibiotics at 50µg/ml, 11% were resistant to three or more antibiotics at 100µg/ml and 4% were resistant to three or more antibiotics at 300µg/ml (Ahmed and Shakoori, 1996). Ahmad and Shakoori (1996) reported highest frequency of resistance against septran at 50 and 100µg/ml. Chloramphenicol resistance was 88.8%. In a recent study in Pakistan Ahmad and Shakoori (2001) documented

50% resistance of *Shigella* strains and Ahmad *et al*. (2003) reported 14.3% resistance of *Shigella* strains against chloramphenicol in Northern Areas of Pakistan. The resistant isolates showed different patterns of antibiotics resistance. The most common pattern was PCaA at all the four levels. Analogous results were reported by other investigators in many countries including Pakistan (Ahmed *et al*., 2003; Ahmed and Shakoori, 2001).

Bacteria have evolved numerous strategies for resisting the action of antibiotics and antibacterial agents. In many hospital units, exploitation of antibiotics is very intensive and this generates an enormous selective pressure for bacteria to acquire the means by which they may become antibiotic-resistant. Resistance to a particular agent may be accomplished by more than one resistance mechanism (Haribage *et al*., 2003). Multiple drug resistance in *Shigella* has complicated the situation in recent years (Gosh and Sehgal, 1998). The genes for resistance to ampicillin, chloramphenicol, spectinomycin, and tetracycline formed a linkage group located on the chromosome of the strains of all serotypes (Casalino *et al*., 1994). The drug resistance in bacterial population is may be due to a genetic and non-genetic mechanism. Regarding genetic mechanism most drug resistant microbes emerged as a result of genetic changes and subsequent processes by antimicrobial drugs. The drug resistance may be chromosomal DNA or plasmid DNA mediated. The plasmid mediated drug resistance is caused due to the presence of drug-resistant gene(s) harboring on the plasmid DNA. These gene(s) confer the drug resistance phenomenon in the host organism (Meyers *et al*., 1976).

In view of the overall high incidence of multiple drug resistance (MDR) among the *S. boydii*, the possibility of presence of R-plasmid was explored. This study revealed that (28.0 %) isolates of *S. boydii* carried plasmids. These were found resistant to three or more antibiotics used in this research work. The number of plasmids varied from one to seven. The number of plasmids varied from one to seven. *Shigella* species usually harbor a heterogeneous population of plasmids ranging in number from 2 to as many as 10 (Ansaruzzaman *et al*., 2005). In this study the analysis of plasmid DNA revealed that all the strains contained a heterogeneous population of plasmids ranging between 23.1 kb to 2.0 kb. Based on molecular weight, the pattern of different plasmids was also very diverse. Depending on the number of plasmids, individual strains were grouped into nine different plasmid patterns and were found among (MDR) *S. boydii* strains. These results are comparable with the results of a previous study by Ansaruzzaman *et al*. (2005) where they observed the multiple plasmids of different molecular sizes, of which the most common were 140, 3.4, 2.7 and 1.4 MDa. Two Bangladeshi isolates contained additional 1.2 and 1.6 MDa plasmids, and one of these isolates harbored a further plasmid of approximately 62 MDa. Antibiotic resistance did not correlate with the presence of any particular plasmid. The results of our study are also comparable with the results of Farshad *et al*. (2006) who observed that all the *Shigella* spp. Isolated from Iran 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 plasmids in each isolate of *S. boydii*. 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. Similar results were reported in a previous study by Gebre-Yohannes and Drasar (1997) who showed that patterns of small plasmids of less than 15 kb, were similar within each of the individual *S. boydii* serotypes. Plasmids of about 3·3–3·7 kb were found in all strains of serotypes 2 and 4. Plasmids of about 4·3–4·6 kb were found in about 86% of strains. Serotypes 1, 2 and 3 were characterized by plasmids of about 5·6–5·7 kb. The 6·4·6–7 kb plasmid was found consistently in serotypes 1, 2, 3, 5, 8, 12 and 13 which were resistant to SSu or had an SSu resistance component in their phenotypes. Large plasmids (155–186 kb) were found in most S. boydii strains. Although pathogenicity tests are not used as criteria for the classification of members of the Enterobacteriaceae, the invasiveness of the isolates in the Sereny test, the presence of a 140 MDa plasmid (Sansonetti *et al*., 1982) and *Shigella* enterotoxin 2 (sen) gene and ipaH gene associated with invasiveness of the strains provide additional evidence that these isolates are representative of *Shigella.* The large plasmid was found to contain a gene conferring virulence, the large plasmid was very unstable and easily lost, only a small number of strains of *Shigella* species were found to have the large virulence plasmid (Vargas *et al*., 1999). In addition, Ahamed and Giri (2021) reported that 97% *Shigella* species harbored at least one plasmid. The number of plasmids varied from 1 to 9. The continuing emergence of drug resistant *Shigella* is narrowing considerably the efficacy of commonly used antibiotics in the treatment of shigellosis (Ahmad and Shakoori, 2001). Ahamed and Giri (2021) have shown that Shigella develops resistance through plasmid mediated quinolone resistance gene (*PMQR*) and quinolone resistance determining region (*QRDR*), effelux pumps gene (*gyrA, gyrB, ParC, ParE*, gyrase, topoisomerase IV) and mutations in drug binding regions.

The plasmids allow the movement of genetic material, including antimicrobial resistance genes between bacterial species and genera (Sherley *et al*., 2004). In the present report, the plasmids of (MDR) S. boydii strains,

were processed for transformation into *Escherichia coli* HB101 separately for ampicillin (MIC-100 µg/ml), chloramphenicol (MIC-100 µg/ml) and sulfamethoxazole-trimethoprim (MIC-100 µg/ml). The transformations of (83.3 %) were successfully accomplished as *Escherichia coli* HB101 acquired antibiotic resistance to ampicillin, chloramphenicol and sulfamethoxazole-trimethoprim. The plasmids (23.1 Kb and <23.1 Kb) could only confer ampicillin, chloramphenicol and sulfamethoxazole-trimethoprim resistance to the competent cells of *Escherichia coli* HB101. Similar results were observed where the 6·4·6–7 kb plasmid was found consistently in S. boydii which were resistant to SSu or had an SSu resistance component in their phenotypes (Ghosh and Sehgal, 1998). The conjugative drug resistance plasmids, most often coding for three or less drugs, were found in about 26% of drug resistant strains. R-factors, coding for AT resistance (in types 2 and 8), and ASSuT resistance (in type 4), were compatible with all reference plasmids tested. Plasmids belonging to incompatibility groups X and N were found in serotypes 5 and 10, respectively. To date there is no broadly available vaccine against *Shigella,* but several candidates are being evaluated in preclinical and clinical studies (Raso *et al*., 2023).

## CONCLUSIONS AND RECOMMENDATIONS

Since the main route of transmission of shigellosis is through water, food and also person-to-person contact, the prevention and control strategies essentially include provision of safe water supply and adequate sanitation facilities, maintenance of good personal hygiene and food safety. Hand washing with plenty of water and soap is the most important single effective preventive strategy against shigellosis. It is emphasized that hands should be washed before eating, before feeding children, after defecation and after disposal of children's excreta. These measures are further reinforced in epidemic situations, the stringent control measures need to be instituted through simple but effective health education messages to the common masses.

## DECLARATIONS

*Acknowledgements*

The funds for this study were provided by the University of Azad Jammu & Kashmir, Muzaffarabad and University of the Punjab, Lahore.

*IRB approval and ethical statement*

Ethical issues including plagiarism, informed consent, double publication and/or submission, redundancy etc have been completely observed by the authors.

The ethical approval for his study was taken from Institutional Review Board, University of the Punjab before sample collection. The informed consent forms were signed by the patient or close relative of the patient for current procedure.

*Generative AI or AI-assisted technology statement*

The authors declare that no generative AI and AI assisted technology was used in the creation of this manuscript.

*Statement of conflict of interest*

The authors have declared no conflict of interest.

## REFERENCES

Ahamed, S.K.T. and Giri, N., 2021. Shigellosis and development of multiple antimicrobial resistance mechanisms of *Shigella* spp. *Biosci. Biotech. Res. Asia*, **18**: 703-718. https://doi.org/10.13005/bbra/2953

Ahmed, B. and Shakoori, A.R., 1996. Multiple antibiotic resistance among *Shigella* species, isolated from patients of shigellosis in Pakistan. *Punjab Univ. J. Zool*., **11**: 83-88.

Ahmed, B., Shakoori, A.R., 2001. Isolation and characterization of R-plasmid from antibiotic resistant clinical, isolates of *Shigella* species. *Pakistan J. Zool*., **33**: 99-104.

Ahmed, K., Shakoori, F.R. and Shakoori, A.R., 2003. Aetiology of Shigellosis in northern Pakistan. *J. Hlth. Popul. Nutr.,* **21**: 32-39.

Albert, M.J., Singh, K.V., Murray, B.E. and Erlich, J., 1990. Molecular epidemiology of *Shigella* infection in Central Australia. *Epidemiol. Infect*., **105**: 51-57. https://doi.org/10.1017/S0950268800047634

Ansaruzzaman, M., Sultana, M., Talukder, K.A., Alam, K., Matsushita, S., Safa, A., Khajanchi, B.K., Dutta, D.K., Islam, Z., Albert, M.J., Nair, G.B. and Sack, D.A., 2005. Isolation and characterization of provisional serovar *Shigella boydii* E16553 from diarrhoeal patients in Bangladesh. *J. med. Microbiol*., **54**: 477–480. https://doi.org/10.1099/jmm.0.45889-0

Asad, A., Nayeem, M.A.J., Mostafa, M.G., Begum, R., Faruque, S.N., Nusrin, S., Jahan, I., Hayat, S. and Islam, Z., 2025. Resistome phylodynamics of multidrug resistant shigella isolated from diarrheal patients. *Microbiol. Spectr*., **13**: e0163524. https://doi.org/10.1128/spectrum.01635-24

Bauer, A.W., Kirby, W.M., Sherries, J.C. and Turck, M., 1966. Antibiotic susceptibility testing by a standard single disk method. *Am. J. clin. Pathol*., **5**: 493-496. https://doi.org/10.1093/ajcp/45.4_ts.493

Bhattacharya, S., Khanal, B., Bhattarai, N.R. and Das, M.L., 2005. Prevalence of *Shigella* species and their antimicrobial resistance patterns in Eastern Nepal. *J. Hlth. Popul. Nutr*., **23**: 339-342.

Birnboim, H.C. and Doly, J., 1979. A rapid alkaline extraction procedure for screening recombinant plasmid DNA. *Nucl. Acids Res*., **7**: 1513-1523. https://doi.org/10.1093/nar/7.6.1513

Casalino, M., Nicoletti, M., Salvia, A., Colonna, B., Pazzani, C., Calconi, A., Mohamud, K.A. and Maimone, F., 1994. Characterization of endemic *Shigella flexneri* strains in Somalia: Antimicrobial resistance, plasmid profiles, and serotype correlation. *J. clin. Microbiol*., **32**: 1179-1183. https://doi.org/10.1128/jcm.32.5.1179-1183.1994

Dutta, S., Rajendran, K., Roy, S., Chatterjee, A., Dutta, P., Nair, G.B., Bhattacharya, S.K., Yoshida, S.I., 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. https://doi.org/10.1017/S0950268802007240

Farshad, S., Sheikhi, R., Japoni, A., Basiri, E. and Alborzi, A., 2006. Characterization of *Shigella* strains in Iran by plasmid profile analysis and PCR amplification of *ipa* genes. *J. clin. Microbiol*., **44**: 2879–2883. https://doi.org/10.1128/JCM.00310-06

Gebre-Yohannes, A., Drasar, B.S., 1997. Plasmid profiles of drug resistant *Shigella boydii* types 1-5, 8, 10, 12-14 from Ethiopia (1974-85). *Epidemiol. Infect*., **119**: 293-298. https://doi.org/10.1017/S0950268897008182

Ghosh, A.R. and Sehgal, S.C., 1998. *Shigella* infections among children in Andaman and archipelage of tropical islands in Bay of Bengal. *Epidemiol. Infect*., **121**: 43-48. https://doi.org/10.1017/S0950268898008978

Heritage, J., Chambers, P.M., Tyndall, C. and Buescher, E.S., 2003. SHV-34 an extended-spectrum beta lactamase conferring transferable resistance encoded by an epidemic plasmid. *J. Antimcrob. Chemother*., **52**: 1015-1017. https://doi.org/10.1093/jac/dkh017

Hirose, K., Terajima, J., Izumiya, H., Tamura, K., Arakawa, E., Takai, N., Watanabe, H., 2005. Antimicrobial susceptibility of *Shigella sonnei* isolates in Japan and molecular analysis of *S. sonnei* isolates with reduced susceptibility to fluoroquinolones. *Antimicrob. Agents Chemother*., **49**: 1203–1205. https://doi.org/10.1128/AAC.49.3.1203-1205.2005

Hossain, M.A., Rahman, M., Ahmed, Q.S., Malek, M.A., Sack, R.B. and Albert, M.J., 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. https://doi.org/10.1093/jac/42.1.99

Keusch, G.T. and Bennish, M.L., 1991. Shigellosis. In: *Bacterial infections of humans: Epidemiology and control* 2nd ed (eds. A.S. Evans and P.S. Brachman). Plenum, New York, pp. 593-620. https://doi.org/10.1007/978-1-4757-1211-7_29

Khalil, K., Khan, S.R., Mazhar, K., Kaijser, B. and Lindblom, G.B., 1998. Occurrence and susceptibility to antibiotics of *Shigella* species in stools of hospitalized children with bloody diarrhea in Pakistan. *Am. J. Trop. Med. Hyg*., **58**: 800–803. https://doi.org/10.4269/ajtmh.1998.58.800

Kotloff, K.L., Winickoff, J.P., Ivanoff, B., Clemens, J.D., Swerdlow, D.L., Sansonetti, P.J., Adak, G.K., Levine, M.M., 1999. Global burden of *Shigella* infections: Implications for vaccine development and implementation of control strategies. *Bull. World Hlth. Organ*., **77**: 651-666.

Lin, S.R. and Chang, S.F., 1992. Drug resistance and plasmid profile pf *Shigellae* in Taiwan. *Epidemiol. Infect*., **108**: 87-97. https://doi.org/10.1017/S0950268800049530

Meyers, J.A., Sanchez, D., Elwell, L.P. and Falkow, S., 1976. Simple agarose gel electrophoresis method for the identification and characterization of plasmid deoxyribonucleic acid. *J. Bact*., **127**: 1529-1537. https://doi.org/10.1128/jb.127.3.1529-1537.1976

Moss, J.E., Cardozo, T.J., Zychlinsky, A., Groisman, E.A., 1999. The selC-associated SHI-2 pathogenicity island of *Shigella flexneri*. *Mol. Microbiol*., **33**: 74–83. https://doi.org/10.1046/j.1365-2958.1999.01449.x

National Committee for Clinical Laboratory Standards, 1993. *Performance standards for antimicrobial disk susceptibility tests.* Document M2-A4, 4th ed., 10(7). National Committee for Clinical Laboratory Standards, Villanova, Pa.

Nisa, I., Haroon, M.M., Driessen, A., Nijland, J., Rahman, H., Yasin, N., Hussain, M., Khan, T.A., Ali, A., Khan, S.A. and Qasim, M., 2022. Antimicrobial resistance of *Shigella flexneri* in Pakistani pediatric population reveals an increased trend of third-generation cephalosporin resistance.

*Curr. Microbiol*., **79**: 118. https://doi.org/10.1007/s00284-022-02805-9

Niyogi, S.K., 2005. Shigellosis. *J. Microbiol*., **43**: 133-143.

Noriega, F.R., Liao, F.M., Maneval, D.R., Ren, S., Formal, S.B. and Levine, M.M., 1999. Strategy for cross-protection among *Shigella flexneri* serotypes. *Infect. Immun*., **67**: 782-788. https://doi.org/10.1128/IAI.67.2.782-788.1999

Olukoya, D.K. and Oni, O., 1990. Plasmid profile analysis and antimicrobial susceptibility patterns of *Shigella* isolation from Nigeria. *Epidemiol. Infect*., **105**: 59-64. https://doi.org/10.1017/S0950268800047646

Patwary, A.K., 1994. Multidrug resistant *Shigella* infections in children. *J. Diorrhoel. Dis. Res*., **12**: 182-186.

Raso, H.H., Arato, V., Gasperini, G. and Micoli, F., 2023. Toward a *Shigella* vaccine: Opportunities and challenges to fight and antimicrobial-resistant pathogen. *Int. J. mol. Sci*., **24**: 4649. https://doi.org/10.3390/ijms24054649

Sambrook, J., Fritsch, E.F. and Maniatis, T., 1983. *Molecular cloning a laboratory manual*. Cold Spring Harbor Laboratory, New York. Volume 1: Sections 1.3-1.110.

Sansonetti, P.J., 2001. Microbes and microbial toxins: Paradigms for microbial–mucosal interactions III. Shigellosis: from symptoms to molecular pathogenesis. *Am. J. Physiol. Gastroint. Liver Physiol*., **280**: G319–G323. https://doi.org/10.1152/ajpgi.2001.280.3.G319

Sansonetti, P.J., Kopecko, D.J. and Formal, S.B., 1982. Involvement of a plasmid in the invasive ability of *Shigella flexneri. Infect. Immun*., **35**: 852-860. https://doi.org/10.1128/iai.35.3.852-860.1982

Sherley, M., Gordon, D.M., Collignon, P.J., 2004. Evolution of multi- resistance plasmids in Australian clinical isolates of *Escherichia coli*. *Microbiology*, **150**: 1539-1546. https://doi.org/10.1099/mic.0.26773-0

Soebel, J., Cameron, D.N., Ismail, J., Strockbine, N., Williams, M., Diaz, P.S., Westley, B., Ritmann, M., DiCristina, J., Ragazzoni, H., Tauxe, R.V. and Mintz, E.D., 1998. A prolonged outbreak of *Shigella sonnei* infections in traditionally observant Jewish communities in North America caused by a molecularly distinct bacterial subtype. *J. Infect. Dis.,* **177**: 1405-1409. https://doi.org/10.1086/517825

Surdeanu, M., Pencu, E., Tonciu, M., Mihai, I., Ciudin, L., 2000. Differentiation of *Shigella* strains by plasmid profile analysis, serotyping and phage typing. *Roman. Arch. Microbiol. Immunol*., **59**: 103-117.

Tenvor, F.C. and Hughes, J.M., 1996. The challenges of emerging infectious diseases: Development and spread of multiply-resistant bacterial pathogens. *J. Am. med. Assoc.,* **275**: 300–304. https://doi.org/10.1001/jama.1996.03530280052036

Toro, C.S., Farfan, M., Contreras, I., Flores, O., Navarro, N., Mora, G.C. and Prado, V., 2005. Genetic analysis of antibiotic-resistance determinants in multidrug-resistant *Shigella* strains isolated from Chilean children. *Epidemiol. Infect*., **133**: 81–86. https://doi.org/10.1017/S0950268804003048

Vargas, M., Gascon, J., Jimenez, D.A.M.T. and Vila, J., 1999. Prevalence of *Shigella* enterotoxins 1 and 2 among *Shigella* strains isolated from patients with traveler's diarrhea. *J. clin. Microbiol*., **37**: 3608–3611. https://doi.org/10.1128/JCM.37.11.3608-3611.1999

Weislander, L., 1979. A simple method to recover intact high molecular weight RNA and DNA after electrophoretic separation in low gelling temperature agarose gels. *Anal. Biochem*., **98**: 305-309. https://doi.org/10.1016/0003-2697(79)90145-3

Yang, F., Yang, J., Zhang, X., Chen, L., Jiang, Y., Yan, Y., Tang, X., Wang, J., Xiong, Z., Dong, J., Xue, Y., Zhu, Y., Xu, X., Sun, L., Chen, S., Nie, H., Peng, J., Xu, J., Wang, Y., Yuan, Z., Wen, Y., Yao, Z., Shen, Y., Boqin, Q.B., Hou, Y., Yu, J. and Jin, Q., 2005. Genome dynamics and diversity of *Shigella* species, the etiologic agents of bacillary dysentery. *Nucl. Acids Res*., **33**: 6445–6458. https://doi.org/10.1093/nar/gki954