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In vivo R-plasmid transfer in a patient with a mixed infection of shigella dysentery

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SUMMARY

Transfer of shigella R-plasmids *in vivo* has seldom been demonstrated. Strains of *Shigella dysenteriae* type 1 and *Shigella flexneri* type 5b were isolated from a Bulgarian traveller who visited Vietnam and developed dysentery, which was treated with trimethoprim/sulfamethoxazole (TMP/SMZ) for a short time. Both species of shigellae are unusual in Bulgaria where strains of *S. sonnei* predominate. Both shigella strains were multiresistant to the same antimicrobial agents. Each strain contained a 48-kilobase plasmid that conferred the entire resistance phenotype to a susceptible *Escherichia coli*. Restriction endonuclease patterns of plasmid DNA from the respective strains were identical. Transmissible plasmids of the same resistance phenotypes and restriction patterns were isolated from the patient's colonic *E. coli*. Transconjugants hybridized to a dihydrofolate reductase type I-DNA probe. These studies support the hypothesis that R-plasmid transfer may occur between non-pathogenic, faecal strains and pathogenic shigellae, a process that may have been facilitated by inadequate treatment with TMP/SMZ at the onset of the illness.

INTRODUCTION

Shigellosis remains a major cause of morbidity and mortality throughout the world. Although strains of *Shigella sonnei* remain the major species isolated in Western countries, shigellosis due to *Shigella flexneri* and *Shigella dysenteriae* type 1 (*Shiga*) persists in developing countries [1, 2]. Each of these species has been reported to contain transferable plasmids which confer resistance to a broad range of antimicrobial agents including trimethoprim. Strains of *S. dysenteriae* 1 often contain a trimethoprim-resistant determinant and these strains have recently been associated with large epidemics especially in Southeastern Asia and Africa

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[3–5]. In Europe bacillary dysentery due to *S. dysenteriae* type 1 is uncommon, and in Bulgaria no strains of this serotype have been isolated since 1962.

In this report we described a case of bacterial dysentery due to a mixed infection with *S. dysenteriae* type 1 and *S. flexneri* type 5b. We characterized the two serotypes and their R-plasmids and documented *in vivo* transfer of these plasmids between shigella and *Escherichia coli* of the patient's faecal flora. Limited characterization of the two shigella isolates and their R-plasmids has been published previously [6].

CASE REPORT

A 66-year-old man visited Vietnam with a business friend at the end of 1988. While there, the patient admitted to eating local food whereas his friend brought food from home. Four days after his arrival he developed symptoms of severe acute dysentery with 24 watery stools the first day, with blood and mucus, cramping abdominal pain, tenesmus and fever up to 39·9 °C. He was treated for 2 days with trimethoprim/sulfamethoxazole. He remained toxic during his return to Bulgaria and was hospitalized immediately with persistent diarrhoea. Stool cultures yielded two different shigella serotypes – *S. dysenteriae* type 1 (strain 5236) and *S. flexneri* type 5b (strain 5237).

Strains were isolated, identified biochemically [7] (Table 1) and serologically by means of group- and type-specific antisera (Difco Products, Detroit, MI) in the National *Shigella* Reference Laboratory in Sofia, Bulgaria. Both strains were tested for virulence by the Sereny test using an inoculum of 10^6 bacteria per eye [8]. the guinea-pigs tested developed purulent keratoconjunctivitis 36 h after inoculation.

Strains were tested for antibiotic susceptibility by the disk method of Bauer and Kirby [9] using the following antimicrobials: ampicillin (Ap), chloramphenicol (Cm), ciprofloxacin (Cp), gentamicin (Gm), kanamycin (Km), nalidixic acid (Nx), streptomycin (Sm), sulfonamides (Su), tetracycline (Tc), trimethoprim (Tp). The MIC for trimethoprim was determined using a macrobroth dilution method with Mueller Hinton broth supplemented with thymidine [10].

Both the *S. dysenteriae* type 1 and the *S. flexneri* type 5b strains were resistant to streptomycin, sulfamethoxazole, tetracycline and trimethoprim. Both displayed high-level resistance to trimethoprim (MIC > 1500 μg/ml).

Conjugation experiments were performed using a susceptible *E. coli* K12 strain (*E. coli* C600 *lac*$^+$*rec*$^-$*rif*^{r}), as a recipient. Equal volumes of overnight broth cultures of the donor and of the recipient strains were mixed on Luria Agar and incubated 18 h at 37 °C. Transconjugants were selected on MacConkey agar containing 100 μg/ml rifampicin combined with tetracycline (25 μg/ml), or streptomycin (25 μg/ml), or trimethoprim (16 μg/ml). and subcultured twice on the initial selective media. All resistance markers of the two shigella strains were transferred *en bloc* suggesting the presence of a conjugative R-plasmid containing a cluster of all the resistance genes.

To characterize further the R-plasmids, extrachromosomal DNA was obtained by the method of Birnboim and Doly [11]. Agarose gel electrophoresis was performed as previously described [12]. Plasmids of known molecular mass

Table 1. *Biochemical characteristics of shigella strains*

Characteristics	*Shigella dysenteriae* type 1 Strain #5236	*Shigella flexneri* type 5b Strain #5237
D-Adonitol	−	−
L-Arabinose	−	−
Arginine dehydrolase	−	−
Citrate	−	−
Dulcitol	−	−
D-Glucose, gas	−	−
Hydrogen sulphide	−	−
Indole	−	+
Lactose	−	−
Lysine decarboxylase	−	−
Malonate	−	−
Maltose	−	+
D-Mannitol	−	+
Methyl Red	+	+
Motility	−	−
Mucate	−	−
myo-Inositol	−	−
ONPG	+	−
Ornithine decarboxylase	−	−
Oxidase	−	−
Phenylalanine deaminase	−	−
Raffinose	−	−
D-Rhamnose	−	−
Salicin	−	−
D-Sorbitol	−	−
Sucrose	−	−
Trehalose	+	+
Urease	−	−
Voges-Proskauer	−	−
D-Xylose	−	−

naturally occurring in *E. coli* V517 [13] as well as DNA molecular-weight markers II and III (λ/*Hin*d III and λ/*Eco*R I/*Hin*d III, Boehringer Mannheim, Indianapolis, IN) were used as controls for molecular size.

The parent *S. dysenteriae* 1 strain contained 6 plasmids and *S. flexneri* strain contained 7 plasmids of different sizes. The parent-transconjugant pairs had a 48-kb R-plasmid that encoded all the resistance determinants. R-plasmid DNA from transconjugants was subjected to endonuclease digestion analysis using several restriction enzymes: *Bam*H II, *Bss*H II, *Eco*R I, *Hae* III, *Pst* I, *Pvu* II, *Rsa* I, *Sal* I, and *Xba* I (Boehringer-Mannheim). Digestion was performed according to the manufacturer's directions. The specific patterns of *Pvu* II and *Bss*H II are shown in Fig. 1 and indicate that both R-plasmids (pSB5236 and pSB5237) generate restriction fragments similar in size.

The type of dihydrofolate reductase (DHFR)-mediating trimethoprim resistance was determined by using dot blot hybridizations of transconjugants. Reactions were performed on nylon filters using DNA probes as previously

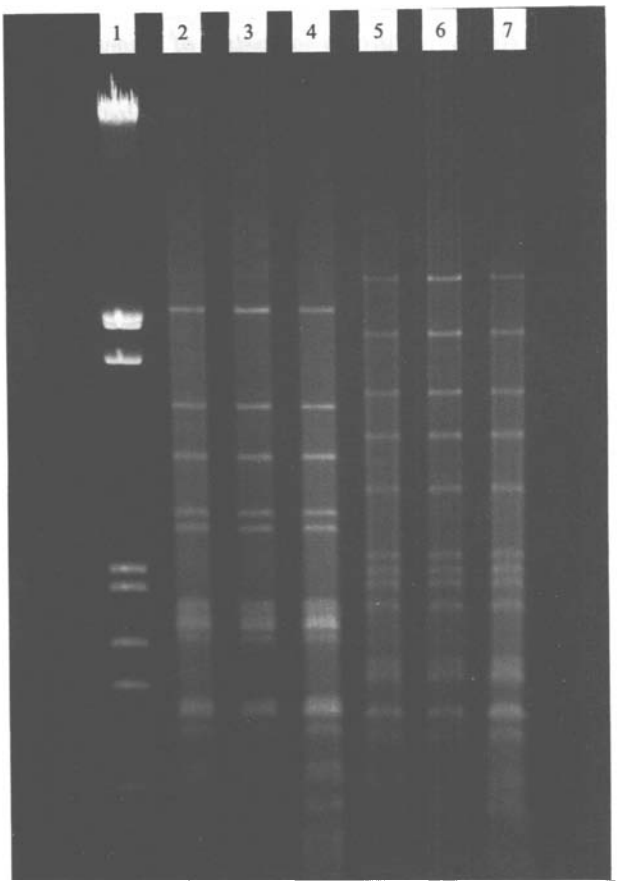

Fig. 1. Restriction endonuclease analysis of R-plasmids from transconjugants of *S. dysenteriae* type 1 #5236, *S. flexneri* 5b #5237, and *E. coli* #75 from the patient's faecal flora. Lane 1. DNA molecular standard III. Lanes 2–4. *Pvu* II digestion of R-plasmid DNA from *S. dysenteriae* type 1, *S. flexneri* 5b. and *E. coli* strains. Lanes 5–8. *Bss*H II digestion of R-plasmid DNA from the same strains respectively.

described [14]. The type I probe was a 500-bp *Hpa* I fragment of pFE872. Th type II probe was an 800-bp *Eco*R I fragment of pUC4-12. Each probe was labelle with γ-[^{32}P] dCTP by randomly primed hexanucleotides (DuPont, NEN Researc Products, Boston, MA) [15], and hybridized overnight at 48 °C under stringer conditions [16]. The hybridization assays showed a strong signal with DHFRI f both shigella transconjugants.

These results indicate a high probability of identity between the R-plasmi found in the *S. dysenteriae* 1 and *S. flexneri* 5b respectively. To investigate whethe the normal gut flora of our patient contained other resistant bacteria whi harboured the same R-plasmid, we studied 100 strains of *E. coli* recovered fro the stool of the patient. Eighty-three of these strains grew on trimethoprin containing media (16 μg/ml) and had the same antibiogram as the shigel isolates-Sm, Su, Tc, Tp resistant. Ten of these trimethoprim-resistant strains of *coli* were studied further. All contained an R-plasmid with the same characteristi as the shigella R-plasmids. They transferred *en bloc* all resistance markers, had th

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same molecular mass and restriction patterns (Fig. 1) and showed high-level trimethoprim resistance mediated through a DHFRI.

The occurrence of the same R-plasmid in two different *Shigella* species and many isolates of *E. coli* in the patient's gut strongly suggest the *in vivo* transfer of the R-plasmid. Unfortunately, under the circumstances it is impossible to determine whether transfer occurred from *E. coli* to shigella or in the reverse direction.

These findings provide further evidence that bacteria of the enteric flora, pathogenic or not, may serve as reservoirs of resistance genes that may be transferred to susceptible enteric microorganisms. Selective pressure by antibiotics may facilitate the process and ensure the persistence of the resistant strains. Examples of *in vivo* transfer of R-plasmids among Enterobacteriaceae have been reported by other authors [17–19].

The present study re-emphasizes the problem of importation of strains of *S. dysenteriae* type 1 into industrialized countries from certain developing countries where this species is endemic. With increasing travel between industrialized and developing countries, the epidemiology of shigellosis will probably become more complex and require molecular tools for insightful analysis. Plasmid analysis and other molecular techniques can serve as useful epidemiological tools to monitor the movement of pathogenic bacteria and their plasmids [2, 3, 20]. Limited and judicious use of antimicrobials for the prophylaxis and therapy of bacterial infections offer the most practical solution for interrupting the cycle of R-plasmid transmissibility.

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REFERENCES

1. Pal SC, Segupto PG, Sen D, Bhattacharya SK, Deb BC. Epidemic shigellosis due to *S. dysenteriae* 1 in South Asia. Indian J Med Res 1989; **89**: 57–64.
2. Haider K, Kay BA, Talukder KA, Hug MI. Plasmid analysis of *Shigella dysenteriae* type 1 isolates obtained from widely scattered geographical locations. J Clin Microbiol 1988; **26**: 2083–6.
3. Frost JA, Vandepite J, Rowe B, Threlfall EJ. Plasmid characterization in the investigation of an epidemic caused by multiply resistant *Shigella dysenteriae* type 1 in Central Africa. Lancet 1981; ii: 1074–6.
4. Haider K, Chatkaeomorakot A, Kay BA, et al. Trimethoprim resistance gene in *Shigella dysenteriae* 1 isolates obtained from widely scattered locations of Asia. Epidemiol Infect 1990; **104**: 219–28.
5. Benish ML, Salam MA, Hossain MA, et al. Antimicrobial resistance of *Shigella* isolates in Bangladesh, 1983–1990: increasing frequency of strains multiply resistant to ampicillin, trimethoprim sulfamethoxazole, and nalidixic acid. J Infect Dis 1992; **14**: 1055–60.
6. Bratoeva M, Raitchev G. A case of shigellosis caused by *Shigella dysenteriae* type 1 and *Shigella flexneri* type 5b. J Hyg Epidemiol Microbiol Immunol 1991; **35**: 35–9.
7. Edwards P, Ewing W. Identification of Enterobacteriaceae. Minneapolis: Burgess Public Co, 1972.
8. Day NP, Scotland SM, Rowe B. Comparison of an HEp-2 tissue culture test with the Sereny test for detection of enteroinvasiveness in *Shigella* spp. and *Escherichia coli*. J Clin Microbiol 1981; **13**: 596–7.
9. Bauer AF, Kirby WMM, Sherris JC, Turck M. Antibiotic susceptibility testing by standardized single disk method. Am J Clin Pathol 1966; **45**: 493–6.

10. Jones RN, Barry AL, Gavan TL, Washington JA. Susceptibility tests: microdilution and macrodilution broth procedures. In: Lennette EH, Ballows, A, Hausler WJ, Shadomy HJ eds. Manual of clinical microbiology. 4th edn. Washington, DC: American Society for Microbiology, 1985: 972–7.
11. Birnboim HC, Doly J. A rapid alkaline extraction procedure for screening recombinant plasmid DNA. Nucleic Acid Res 1977; **7**: 1513–23.
12. John JF, Twitty JA. Plasmids as epidemiologic markers in nosocomial Gram-negative bacilli: experience at a university hospital and review of the literature. Rev Infect Dis 1986; **8**: 693–704.
13. Macrina FL, Kopecko DJ, Jones KR, Ayers DJ, McCowen SM. A multiple plasmid-containing *Escherichia coli* strain: convenient source of size reference plasmid molecules. Plasmid 1978; **1**: 417–20.
14. Fling ME, Walton L, Elwell LP. Monitoring of a plasmid-encoded, trimethoprim resistant dihydrofolate reductase gene: detection of a new resistant enzyme. Antimicrob Agents Chemother 1982; **22**: 882–8.
15. Feinberg A, Vogelstein B. A technique for radiolabeling DNA restriction endonuclease fragments to high specific activity. Anal Biochem 1983; **132**: 6–13.
16. Maniatis T. Cloning manual. 2nd edn, Cold Spring Harbor: Cold Spring Harbor Laboratory. 1983.
17. Farrar WF, Edison M, Guerry P, Falkow S, Drusin LM, Roberts RR. Interbacterial transfer of R factor in the human intestine: *in-vivo* acquisition of R-factor-mediated kanamycin resistance by a multiresistant strain of *Shigella sonnei*. J Infect Dis 1972; **126**: 27–33.
18. Tauxe RV, Cavanagh TR, Cohen ML. Interspecies gene transfer *in vivo* producing an outbreak of multiply resistant shigellosis. J Infect Dis 1989; **160**: 1062–70.
19. Threlfall EJ, Ward LR, Rowe B, Robinson-Brown R. Acquisition of resistance by *Salmonella typhi in vivo*. The importance of plasmid characterization. Lancet 1982: i: 740.
20. Strockbine NA, Parsonnet J, Greene K, Kiehlbanch JA, Wachsmuth IK. Molecular epidemiologic techniques in analysis of epidemic and endemic *Shigella dysenteriae* type 1 strains. J Infect Dis 1991; **163**: 406–9.