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Plasmid profile analysis and antimicrobial susceptibility patterns of shigella isolates from Nigeria

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SUMMARY

In an epidemiological survey, plasmid profiles and antimicrobial susceptibility testing of 100 shigella isolates in Lagos, Nigeria was done. All the isolates were sensitive to nalidixic acid, nitrofurantoin and ciprofloxacin. The commonest antimicrobial susceptibility pattern was resistance to ampicillin, colistin sulphate, co-trimoxazole, streptomycin and tetracycline. All but 4 of 100 isolates screened contained one or more plasmids. Plasmid profile analysis distinguished more strains than did antimicrobial susceptibility patterns. A total of 36 isolates was able to transfer resistance plasmids to *Escherichia coli* K-12 by conjugation. Using *in vitro* transformation, seven isolates transferred resistance. These plasmids specified resistance to tetracycline, streptomycin, sulphonamide, trimethoprim and ampicillin.

INTRODUCTION

In most developing countries, diarrhoea constitutes a major cause of morbidity and mortality. One of the aetiological agents of diarrhoea is shigella. In many parts of the world, plasmid profile analysis has been used as an epidemiological tool in investigating outbreaks of infectious disease. Plasmid profile analysis may aid in the identification of a source of infection, differentiating strains or evaluating the efficacy of control measures [1–3]. The usefulness of plasmid profile analysis for typing shigella strains in Nigeria has not been evaluated. In addition, there has been no previous study on plasmid profiles in relation to antimicrobial susceptibility patterns of shigella from this part of the World. This report seeks to determine: (a) whether plasmid analysis could be used to discriminate between shigella isolates for epidemiological studies, (b) antimicrobial susceptibility patterns of isolates in relation to their plasmid content.

MATERIALS AND METHODS

Bacteriology

Shigella flexneri, *S. sonnei*, *S. dysenteriae* and *S. boydiii* isolates were obtained from the National Institute for Medical Research or isolated from various private hospitals in Lagos between 1988 and 1989. The strains were isolated from cases of endemic shigellosis in Lagos. Isolation and identification of pathogens was carried out using standard procedures [4, 5].

Antimicrobial susceptibility testing

A disk-diffusion technique was used to determine susceptibility patterns. The inoculum was prepared by suspending five colonies of each pure bacterial culture into a sterile bijou bottle containing 5 ml of Mueller–Hinton (MH) broth (Oxoid)) and incubating overnight at 37 °C. After dilution of the culture to about 10^{-4}, a sterile cotton swab was dipped into the adjusted inoculum and used to inoculate MH agar. The inoculated plate was allowed to dry, and antimicrobial disks that contained the following agents were applied; (Oxoid) ampicillin 25 μg, colistin sulphate 10 μg, co-trimoxazole 25 μg, streptomycin 25 μg, tetracycline 50 μg, nalidixic acid 30 μg, nitrofurantoin 20 μg, Compound sulphonamide 300 μg, ciprofloxacin 5 μg; (MAST Laboratories), kanamycin 10 μg, gentamicin 10 μg, chloramphenicol 10 μg. Plates with antibiotic disks were incubated for 24 h. *E. coli* ATCC 25922 was used to control sensitivity and medium.

Plasmid isolation

Plasmid DNA was isolated by a modification of the technique of Birnboim and Doly [6]. Colonies were scraped from Brain Heart Infusion (Oxoid) agar plates into 0·5 ml of 50 mM glucose, 10 mM-EDTA, Tris (pH 8·0), before treatment with lysozyme (Sigma) and detergent, alkaline denaturation and ethanol precipitation. Isolates giving doubtful or negative results were retested as described by Meyers and co-workers [7]. Electrophoresis was carried out on 0·8% horizontal agarose slab gels in Tris-borate buffer. Gels were stained with ethidium bromide 0·5 μg/ml for 45 min and photographed under ultraviolet light. *Escherichia coli* V517, a strain carrying plasmid molecular weight standards, was provided by Dr J. Crossa (Oregon Health Sciences University, Portland, Oregon, USA). Strain V517 contained eight plasmids having the following molecular size in kilobases (Kb): 55·5, 7·4, 5·7, 5·3, 4·0, 3·1, 2·8, 2·2. Other *E. coli* K12 carrying plasmids of molecular weights ranging from 52 Kb to 120 Kb were from our own collection.

Genetic transfer

Conjugation was performed [8] using *E. coli* K-12 J53-2 (F^- *pro met*) Rif^r or KH 802 (F^- *metB hsdRk gal lac*) Rif^r as recipients, and selecting transconjugants on minimal agar plates supplemented with amino acids and antibiotics as required. For the selection of resistant strains, antibiotics were added to the media as follows: ampicillin, 50 μg/ml; nalidixic acid, 50 μg/ml; tetracycline, 25 μg/ml streptomycin 20 μg/ml, trimethoprim 50 μg/ml; rifampicin 100 μg/ml. Trimethoprim resistance was tested as described previously [9, 10]

Transformation was carried out [11] using *E. coli* K-12 HB 101 (*ara*$^-$14, *galk* 2, *hsd*520, *lacyl*, *leu*, *mtl*-1, *proA2*, *recA13*, *rpsL20*, *supE44 thi xyl-5*) as recipient and plasmid pBR322 as the positive control. Co-transformation of resistance determinants was confirmed by testing all transformants against each antibiotic to which the donor strain was resistant. DNA extracts from transformants were obtained as described above and subjected to electrophoresis. Transformation was confirmed as positive only when resistant transformants were shown to contain a plasmid(s) of a size similar to that found in the original isolate.

Table 1. *The incidence of resistance to nine antimicrobial agents among 100 shigellae from Nigeria*

Antibiotics	Percentage of resistant isolates
Ampicillin	70
Colistin-sulphate	91
Gentamicin	10
Co-trimoxazole	74
Streptomycin	71
Ciprofloxacin	0
Tetracycline	89
Nalidixic acid	0
Nitrofurantoin	0

Table 2. *Antibiotic resistance patterns*

Antibiotic resistance patterns	No and % exhibiting patterns	No and % of isolates with resistance plasmid	Resistant phenotype of transformation/ transconjugants (No)
1. Ap Ct Sxt Sm Tc	44	17	Tc (5), TC Sm (6), Tc Su Sm (1) Sm Tm (5) Ap Tc Sm (5T*)
2. Ap Ct Sxt Tc	12	5	Tc (3), Tm (2)
3. Ap Ct Sxt Sm, Tc Gm	8	3	Tc Sm (1), Tc Su Sm (2) (2T*)
4. Ct Tc	8	2	Tc (2)
5. Ct Sxt, Sm Tc	5	2	Tc Su Sm (1) Sm Tm (1)
6. Ct Sm Tc	5	2	Tc Sm (1), Tc (1)
7. Ap Ct Sxt	4	—	—
8. Sm Tc	4	2	Tc Sm (1) Tc (1)
9. Sxt Sm Tc	3	1	Sm Tm (1)
10. Ct	3	—	—
11. Ap Ct Sxt Sm	2	2	Tm, Sm Tm (1)
12. Gm	2	—	—

Ap, ampicillin; Ct, colistin-sulphate; Sxt, co-trimoxazole; Sm, streptomycin; Tc, tetracycline; Gm, gentamicin; *T, transformable.

RESULTS

The 100 isolates examined included 48 *S. flexneri*; 36 *S. dysentriae*; 9 *S. boydii*; 7 *S. Sonnei*.

All the strains were sensitive to nalidixic acid, nitrofurantoin and ciprofloxacin. Many of these organisms were also resistant to broad-spectrum antibiotics (Tables 1 and 2). The most common antimicrobial susceptibility pattern was resistance to ampicillin, colistin sulphate, co-trimoxazole streptomycin and tetracycline (44%). *S. flexneri* isolates showed 7 resistance patterns while *S. dysenteriae*, *S. boydii* and *S. sonnei* showed 6, 3 and 2 respectively (Table 3). No isolate was sensitive to all the agents tested.

All but 4 of 100 isolates screened contained one or more plasmids of different molecular size. Some isolates contained as many as eight plasmids. The sizes of the plasmids range from 1·5 Kb to 180 Kb. The majority of the plasmids were smaller than 9 Kb. Seventy of the 100 isolates of all species contained a plasmid between

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Table 3. *Number of different plasmid profiles and antimicrobial susceptibility patterns among shigella isolates in Lagos, Nigeria*

	No of different plasmid profiles (No of isolates)	No of different resistance patterns (No of isolates)
S. flexneri	38 (48)	7 (48)
S. dysenteria	27 (36)	6 (36)
S. boydii	7 (9)	3 (9)
S. sonnei	5 (7)	2 (7)

180 Kb and 225 Kb. All three colistin-sulphate resistant (Ct^r) and one colistin-sulphate plus tetracycline resistant (Ct^r Tc^r) isolates lacked plasmids (Table 2). Plasmid profile distinguished more strains than did antimicrobial susceptibility pattern (Table 2). A plasmid profile pattern was considered different from another when it consistently differed with regard to one or more plasmid bands.

All of the 100 isolates were used in conjugation experiments. A total of 36 isolates were able to transfer resistance plasmids to *E. coli* K-12. The presence of most of these plasmids was also confirmed by transformation using either total cellular extracts or all gel bands of the particular isolate.

The R plasmids isolated in this study ranged size between 9 Kb and 84 Kb. The antibiotic resistance determinants specified by conjugative plasmids were Tc^r (12), Tc^r Sm^r (9), Sm^r Tm^r (8) and Tc^r Su^r Sm^r (4) Tm^r (3). Using transformation, it was possible to transfer resistance to ampicillin, tetracycine and streptomycin (Ap Tc Sm) from seven *S. dysenteriae* isolates. The conjugation and transformation techniques were reproducible as long as the appropriate antibiotics were added to the growth media to maintain plasmids carrying antibiotic resistance genes harboured by the donors.

The highest rate of transfer was found amongst *S. dysenteriae* where 27 (75%) isolates transferred plasmids whereas 7 (14·5%) *S. flexneri*, 1 (11·1%) *S. boydii* and 1 (14·3%) *S. sonnei* transferred R plasmids. The frequency of transfer varied from 10^{-2}–10^{-6}.

DISCUSSION

Plasmid profile analysis, antimicrobial susceptibility testing and studies on drug resistance determinants were carried out on shigella isolates from Nigeria.

The usefulness of plasmid profile analysis for typing strains of shigella in this environment has not been previously evaluated. This study indicates that a large number of clones which are not differentiated by antimicrobial susceptibility pattern are responsible for shigellosis in Nigeria. This is in contrast to findings in the developed countries where one or few clones may account for shigellosis in a community [12, 13]. When the dominant profile is known for a given locality, the identification of a distinctively different plasmid profile can be very useful.

This study has also revealed that plasmid profiles differentiated specifically among these isolates. This may be due to the presence of a large number of different endemic strains or a high rate of plasmid transfer or instability amongst local strains or both. Similar to the findings of Tacket and co-workers [12] for

Bangladesh, plasmid profile analysis may be more useful in epidemiological studies in rural areas of Nigeria with less diverse populations.

Antimicrobial resistance patterns revealed a total of 12 patterns (Table 2). The widespread resistance [14] observed in the developing countries could be due to the indiscriminate and widespread use caused by the over-the-counter availability of antiboitics as well as the higher exposure of people to enteric flora in places with poor sanitation. This is further confirmed by the number of R-factors isolated in this study.

The present study demonstrated that shigellae isolated in Nigeria harboured conjugative plasmids. As previously reported [15, 16, 17] R – plasmid mediated antibiotic resistance was especially common in *S. dysenteriae* (75%). The plasmid profiles of *S. dysenteriae* and *S. flexneri* (where rate of R plasmid transfer was considerably lower) showed no striking distinction as diversified plasmid profiles spread through each group. The inability to obtain ampicillin resistant transconjugants may be an indication that the genes are chromosomally-borne in these isolates or that the plasmids are unstable in the recipient. It was, however, interesting to note that plasmids carrying ampicillin resistant genes from seven isolates were transformable. The use of transformation enabled us to detect potentially non-self-transmissible plasmids. Perhaps triparental crosses with conjugative plasmids may yield more R factors. Further investigations suggest that about 70% of shigella isolates from farm animals transferred ampicillin, chloramphenicol and tetracycline resistance (ApCTc) at high frequencies (unpublished observations).

This is the first report on the plasmid analysis of shigella isolates from Nigeria. This approach to epidemiological investigation will enable us to compare our local isolates of pathogenic bacteria with those from other parts of the world. In addition it is now imperative that the use of antimicrobials in developing countries be reappraised.

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