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Study of plasmid screening amongst pathogenic bacteria isolated in Nigeria

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Summary

Clinical isolates of *Neisseria gonorrhoeae, Campylobacter jejuni, Escherichia coli, Shigella dysenteriae, Shigella boydii, Yersinia* spp. and *Salmonella* spp. were screened for the presence of plasmids. Approximately 80% of these strains harboured plasmids ranging in molecular weight from 1.0 to 45 × 10^6 daltons.

Résumé

On a fait un test chez les isolés à clinique de *Neisseria gonorrhoeae, Campylobacter jejuni, Escherichia coli, Shigella dysenteriae, Shigella boydii, Yersinia* spp., et *Salmonella* spp. pour la présence de clés plasmides. On a fait ce test chez quarante-cinq strains. Proche de 80% de ces strains ont eu les plasmides avec le poids moléculaire de 1.0 à 45 × 10^6 daltons.

Introduction

Plasmids are extrachromosomal elements capable of autonomous replication. They were first found in *Enterobacteriaceae*, but have since been identified in almost every bacterial group where they have been investigated. Plasmids can be conjugative or non-conjugative. Bacterial plasmids specify a wide variety of biological functions. Some of these include resistance to antibiotics [1,2], to heavy metals [3,4], to ultraviolet radiation [5] and to colicins [6]. Others include the ability to catabolize various compounds [7,8], production of enterotoxin [9] and haemolysin [10].

The rapid development of techniques for studying bacterial plasmids has given the epidemiologist new tools with which to study bacterial infections. The availability of techniques for the rapid isolation of plasmid DNA from bacteria, and the use of agarose gel electrophoresis to detect plasmids and to estimate their molecular weights, have enhanced the study of bacterial infection in many clinical microbiological laboratories [11–15]. Restriction endonuclease digests of plasmids DNA make it possible to 'type' plasmids.

A number of methods are being used in epidemiological studies of infections by bacteria e.g. antibiogram, biotyping, serotyping, phage typing and lately plasmid profiles. However, plasmid profiles have been found to be one of the best characteristics for routine identification of bacteria originating from the same clone [16]. Thus, many workers [17,18] have employed plasmid profiles when studying the epidemiology of pathogenic bacteria.

Material and methods

Escherichia coli K-12 strains carrying plasmids of known molecular weights (molecular weights in parenthesis) were obtained from Dr J. R. Guest (University of Sheffield) or were from our own collection pBR 322 (2.6 × 10^6), Rp4 (36 × 10^6), PGS9 (17.3 × 10^6), pDOK1 (13.3 × 10^6), pBR 325 (3.6 × 10^6). Clinical isolates were: *Escherichia coli* (five strains), *Salmonella* spp. (six strains), *Yersinia* (one strain), *Shigella* spp. (nine strains), *Campylobacter* spp. (14 strains), *Neisseria gonorrhoeae* (10 strains).

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Growth media

Mueller-Hinton (Difco Laboratories, Detroit, MI, U.S.A.) or Brain Heart Infusion broth were used for culturing all strains. Bacteria were normally grown in 5 ml broth at 37°C or 42°C (for *Campylobacter*) for 16–18 h. *Neisseria gonorrhoeae* cultures were scraped from chocolate agar medium.

Plasmid DNA isolation procedure

This was carried out by the method of Birnboim and Doly [12], but with certain modification. Since our foremost objective was to screen for the presence of plasmids, certain procedures seemed unnecessary. Treatment with RNase was omitted, since the concentration of NaOH used was sufficient to remove RNA. As the phenol required re-distillation, chloroform alone was used instead of the phenol-chloroform mixture.

Bacteria were grown overnight. One and a half millilitres of the culture was poured into an Eppendorf tube and centrifuged at 8000 × *g* for 2 min. The medium was removed by aspiration and the bacterial pellet was resuspended in 100 µl of an ice-cold solution of: 50 mM glucose, 10 mM Tris (pH 8.0). The mixture was hand-held for 5 min at room temperature. Then 200 µl of freshly prepared 0.25 N NaoH, 1% sodium dodecyl sulphate (SDS) were added. The tube was closed and the contents were mixed by inverting the tube rapidly two or three times. The tube was held on ice for 5 min before 150 µl of an ice-cold solution of potassium acetate (pH 4.8) were added. The tube was held on ice for 5 min and then centrifuged for an additional 5 min at 4°C. The supernatant was transferred to a clean tube and an equal volume of chloroform was added. The contents were mixed on vortex mixer and then centrifuged for 2 min. The supernatant was transferred to a new tube and two volumes of 95% ethanol were added to precipitate the DNA.

After mixing on a vortex mixer the tube was held at room temperature for 2 min. The tube was then centrifuged for 5 min at room temperature and then the supernatant was removed. The liquid in the tube was allowed to drain away by placing it in an inverted position on a paper towel. One millilitre of 70% ethanol was added after vortex mixing again briefly, the tube was centrifuged for 5 min. The supernatant was removed and the tubes were inverted to allow the pellet to dry. The pellet was resuspended in 50 µl of TE buffer (pH 8.0).

Agarose gel electrophoresis

Electrophoresis of DNA was carried out on 0.7–1% agarose slab gels in Tris-borate buffer (89 mM Tris-borate, 89 mM boric acid, 25 mM EDTA pH 8.0). A dye solution, consisting of bromophenol blue (0.25%) and sucrose (40% W/V) in water, was added to DNA samples prior to electrophoresis. Electrophoresis was carried out in a horizontal slab gel apparatus. The gels were run for 4–5 h at 5 V/cm (constant voltage). The gel was stained after electrophoresis by immersing the gel in water containing ethidium bromide (0.5 µg/ml) for 45 min at room temperature. The stained gel was visualized with a shortwave ultraviolet light transilluminator and the photograph of the plasmid bands on gel was taken.

Table 1. Plasmid patterns of the strains screened

Bacterial strains*	No. screened	No. harbouring plasmids	No. harbouring more than one plasmid
Escherichia coli	5	4	3
Salmonella spp.	6	6	5
Yersinia spp.	1	1	—
Shigella spp.	9	8	5
Campylobacter spp.	14	8	3
Neisseria gonorrhoeae	10	9	1

*Some of these strains were resistant to commonly prescribed antibiotics.

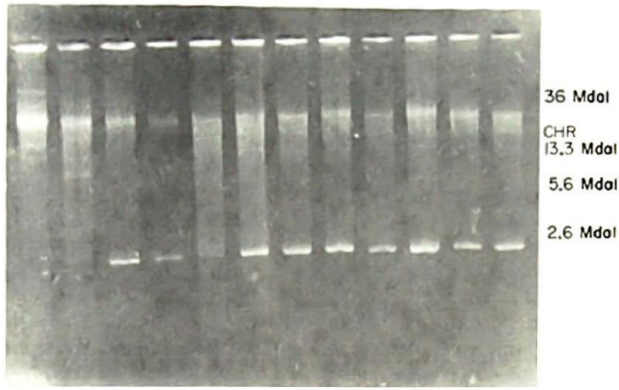

Fig. 1. Agarose gel electrophoresis of plasmids extracted from strains of *Neisseria gonorrhoeae* and *Escherichia coli*. From the left, columns 1–2, *Escherichia coli*; columns 3–12, *Neisseria gonorrhoeae* showing plasmid CHR chromosomal DNA.

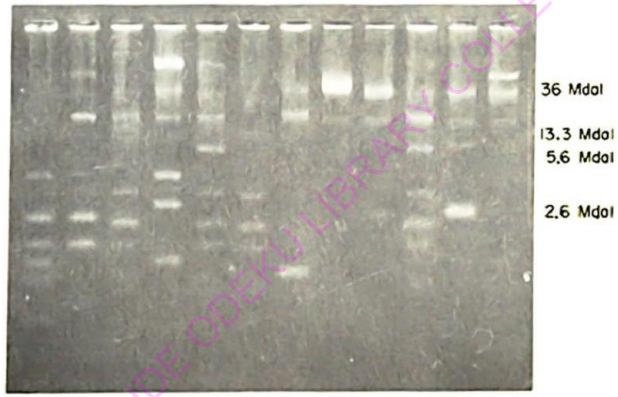

Fig. 2. Agarose gel electrophoresis of plasmids extracted from strains of *Escherichia coli*, and *Shigella*. From the left, columns 1–7, *Shigella* spp.; columns 8–12, *Escherichia coli* strains containing plasmids of known sizes.

Results and discussion

Figures 1 and 2 show agarose gel electrophoresis of plasmids from some of the bacterial strains. By using plasmids of known molecular weights, the molecular weights of the plasmids isolated from these bacteria were determined to be within the range of 1.0×10^6–45×10^6 daltons. The plasmid patterns of the strains screened are shown in Table 1.

Although more strains will have to be screened before unequivocal conclusion can be drawn, it is interesting to note that 80% of these clinical isolates harbour plasmids. Some of these plasmids probably code for antibiotic resistance. Future work in this area will include detail characterization of plasmids, genetic probing of resistance genes and the study of the functions of cryptic plasmids.

This is to our knowledge the first report on plasmid isolation that was done locally.

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