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Plasmid profiles of *Shigella* and *Salmonella spp.* isolated from diarrhoeic humans in Ibadan, Nigeria

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Summary

Clinical isolates of *Shigella flexneri, S. dysentariae, S. boydii* and *Salmonella spp.* were screened for the presence of plasmids. Most of the isolates harboured more than one plasmid ranging in molecular size from 1.3 to 36.1 x 10^6 daltons. Very large plasmids were not encountered.

Résumé

Ou a fait un test chezles isolatés à clinique de *Shigella flexneri, S. dysenteriae, S. boydii* et *Salmonella spp.* pour la présence de clés plasmides. La plupart des specimènes preleves portaient en leur seiu plus d'un plasmide ayant de dimensions moleculaires qui varient entre 1.3 à 36.1 x 10^6 daltonnes. Des plasmides ayant des trés grandes dimensions n'etaient pas découverts.

Introduction

Plasmids are extrachromosomal genetic elements found virtually in all bacterial species. Those haboured by members of the *Enterobacteriaceae* mediate the transfer of a variety of genetic determinants including those for drug resistance, haemolysin and enterotoxin synthesis, colicinogeny, heavy metal tolerance, resistance to ultraviolet light irradiation, carbohydrate fermentation, hydrogen sulphide synthesis and other metabolic characters[1,2].

Plasmid profile analysis has been used as an epidemiological tool in investigating outbreaks of enteric diseases[3], and as a finger print for differentiation of strains or identifying the source of infection[4]. It has also been demonstrated that plasmid profile analysis is as specific as phage-typing in identifying related organisms[5]. Over the past decade and a half a number of techniques have been developed for the isolation and characterization of plasmid DNA[6,7,8]. These techniques basically involve rapid isolation of plasmid DNA from bacteria and the use of agarose gel electrophoresis to detect and estimate the molecular weights of plasmids.

In Nigeria, there are very few reports on plasmid profiling of most pathogenic organisms. This paper describes the plasmid screening of *Shigella* and *Salmonella spp.* isolated in Ibadan, Nigeria.

Materials and methods

Bacterial isolate

Human faecal samples were collected from diarrhoeic patients in the out-patient department of three Government Hospitals in Ibadan between January 1987 and December 1988. The methods used to isolate and identify the organisms followed those of Edwards and Ewing[9]. A total of fifty-three bacterial isolates comprising of twenty-four *Shigella flexneri*, four *S. dysenteriae*, three *S. boydii*, nine *Salmonella typhi* and thirteen other *Salmonella spp.* A multiple plasmid containing *Escherichia coli* strain (v517) obtained from Dr. O. Olukoya (National Institute for Medical Research Yaba, Lagos) was employed as size reference.

Isolation of plasmids

Plasmid DNA was isolated by a modification of the method of Birnboim and Dolly[7]. Bacteria were grown overnight on Brain Heart Infusion (Oxoid, England) agar plates. They were later suspended in 0.5ml, 50mM glucose, 10mM EDTA, Tris (pH 8.0) and treated with lysozyme (Sigma). This was followed by treatment with detergent (SDS), alkaline denaturation and ethanol precipitation. Electrophoresis was carried out on 0.8% agarose slab gels in Tris-borate buffer. The gels were allowed to run for 4-5 hrs at 5 Vcm (constant voltage). Gels were later stained with 0.5mg./µl. ethidium bromide

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for 1 hr. at room temperature and photographed under ultraviolet light.

Results

Of the fifty-three isolates screened, twenty-eight were found to be harbouring plasmids. These include fifteen *Shigella flexneri*, three *S. dysenteriae*, two *S. boydii*, two *S. typhi* and six *Salmonella spp.* Twenty-four of these contained two or more plasmids while three *Shigella flexneri* and one *Salmonella typhi* isolates have one plasmid each. Some strains contain as many as eight or more plasmids. The molecular weights of the plasmids vary between 1.3 and 36.1 daltons. The plasmids x 10^6 patterns of the strains harbouring plasmids are shown in Table 1 below.

Table 1: Number and size of different plasmids among *Shigella* and *Salmonella spp.*

Isolate Laboratory No.	Identification	Number of Plasmids Harboured	Size of Plasmids x 10^6 daltons			
Y001	*Shigella flexneri*$_3$	2	7.8	1.5		
Y002	*Shigella flexneri*$_1$	8	7.8	3.2	2.6	1.9
			1.7	1.6	1.5	1.3
Y003	*Shigella flexneri*$_6$	8	7.8	5.5	3.5	2.6
			1.9	1.7	1.5	1.3
Y004	*Shigella flexneri*$_2$	8	7.8	5.5	4.8	4.5
			3.5	2.6	1.9	1.7
Y005	*Shigella dysenteriae*$_1$	9	16.3	9.2	7.8	5.2
			3.7	3.2	1.9	1.7
			1.6			
Y006	*Shigella boydii*	10	16.3	9.7	7.8	6.5
			5.5	4.4	3.2	1.9
			1.6	1.5		
Y007	*Shigella flexneri*$_2$	8	28.1	18.9	7.8	4.8
			1.9	1.6	1.5	1.3
Y0019	*Shigella flexneri*$_2$	2	1.9	1.3		
Y0020	*Shigella flexneri*$_6$	4	8.4	1.7	1.5	1.3
Y0021	*Shigella boydii*	3	7.8	4.2	1.7	
Y0021	*Shigella flexneri*$_6$	3	9.2	1.6	1.3	
Y0026	*Shigella dysenteriae*$_3$	5	28.6	18.9	1.7	
			1.5	1.3		
Y0027	*Shigella dysenteriae*$_1$	2	2.3	1.9		
Y0028	*Shigella flexneri*	1	1.9			
Y0030	*Shigella boydii*	4	5.5	4.8	3.2	
			1.9			
Y0032	*Shigella flexneri*	6	22.1	7.8	5.5	
			3.8	1.7	1.5	
Y0033	*Shigella flexneri*	3	7.8	1.7	1.5	
Y0034	*Shigella flexneri*	1	1.9			
Y0036	*Shigella flexneri*	2	1.8	1.5		
Y0037	*Shigella flexneri*	1	3.6			
Y0049	*Shigella flexneri*	7	11.1	7.8	4.2	
			1.9	1.8	1.6	
			1.5			
Z008	*Salmonella spp.*	9	20.2	9.7	7.8	
			5.5	3.8	2.9	

Table *contd.*

			2.8	2.6	1.3
Z0011	*Salmonella spp.*	2	16.9	8.3	
Z0055	*Salmonella spp.*	5	5.5	4.8	3.2
			1.9	1.6	
Z0061	*Salmonella typhi*	4	36.1	5.5	1.7
				1.5	
Z0065	*Salmonella spp.*	6	9.2	8.3	4.2
			3.2	1.9	1.7
Z0066	*Salmonella typhi*	1	36.1		
Z0067	*Salmonella spp.*	4	36.1	9.2	8.3
				4.2	

Discussion

This investigation revealed the presence of plasmids in twenty-one of the thirty-one *Shigella* and seven of the twenty-two *Salmonella* isolates. The molecular weights of the plasmids were found to range between 1.3 to 36.1 x 10^6 daltons. Most of the plasmids have molecular weights of less than 10 x 10^6 daltons (Table 1). Similar studies[10] failed to demonstrate large molecular weight plasmids in all the strains of enteric pathogens isolated in Lagos (Nigeria). These findings are at variance with the work of Tacket *et al*[11] who found that most profiles of *Shigella* isolates in Bangladesh were characterised by the presence of large molecular weight plasmids (110-200 megadaltons). This difference may be justified by the fact that different strains are responsible for enteric diseases in different communities. It also emphasizes the value of plasmid profile studies as an epidemiological tool in differentiating strains and identifying source of infection. Many of the isolates used in this work contain a number of plasmids which were identical in size thereby indicating that they may be related genetically. This is suggestive of a common source of infection of most patients from whom these isolates were recovered. It is of interest also to note that the presence of 36.1 x 10^6 daltons plasmids (the biggest) was restricted to the *Salmonella* isolates whereas none of the *Shigella* isolates carried plasmids larger than 29 x 10^6 daltons.

Initial studies[12] have shown that these isolates were resistant to many of the commonly used antibiotics. It is then obvious that some of these plasmids are R-factors which code for antibiotic resistance exhibited by these isolates. Plasmids containing antibiotic resistant pathogens constitute very serious problems to man and animals because their plasmids are easily transferred among strains in the community. Thus the disease they cause may be more difficult to treat with available drugs.

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