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Plasmid screening amongst *Aeromonas* species and *Plesiomonas shigelloides* isolated from subjects with diarrhoea in Lagos, Nigeria

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Summary

Fifty-three *Aeromonas* strains and 16 *Plesiomonas shigelloides* isolated from subjects with diarrhoea in Lagos were screened for the presence of plasmids. Nine (17%) of the *Aeromonas* strains and one (6.3%) of the *P. shigelloides* harboured one or more plasmids, ranging in size from 2.4 to 16.8 MDa. As has been documented in other enteropathogens, the possibilities are that these plasmids code for some factors to enhance the virulence of their hosts.

Résumé

Cinquante-trois variétés d'*Aeromonas* et seize de *Plesiomonas shigelloides* étant isolés des malades ayant la diarrhée à Lagos étaient étudiés pour la présence des plasmides. Neuf (17%) parmi les variétés d'*Aeromonas* et un (6.3%) de *P. shigelloides* avaient un ou plus de plasmides, avec les tailles de 2.4 à 16.8 MDa. Comme il était documenté chez d'autres entéropathogènes, il y a des possibilités que ces plasmides codent certains facteurs pour favoriser la virulence de leur hôtes.

Introduction

Plasmids are extra chromosomal deoxyribonucleic acids (DNA), capable of stable autonomous replication. Their recognition was in the early 1950s soon after the discovery of conjugation in *Escherichia coli* by Lederberg [1]. Interest in plasmid studies is primarily due to their ability to code for genetic determinants, and thus confer some phenotypic properties or biological functions on their hosts. Properties that have been shown to be plasmid-mediated in bacterial strains include resistance to antimicrobial agents [2,3], virulence [4], enterotoxin production [5], and adherence to mammalian cells [6].

Another area where plasmids have been widely used is in the epidemiological studies of bacterial infections. For instance, among penicillinase-producing *Neisseria gonorrhoeae* (PPNG), two distinct types of penicillin resistance are now recognized. One with a molecular weight of 4.4 MDa is associated with isolates of Far Eastern origin, while plasmids with molecular weights of 3.2 MDa are linked with West Africa [7,8]. More recently, Olukoya *et al.* [9] employed plasmids analysis to study the epidemiology of some pathogenic bacteria in Nigeria.

At present, there are a few reports in the literature on plasmids in *Aeromonas* or *Plesiomonas* strains. Shotts *et al.* [10] reported plasmid-mediated antibiotic resistance in *A. hydrophila* isolated from fish, while Olsen and Wright [11] reported that *A. salmonicida* can be the recipient of resistance-mediating plasmids from *Enterobacteriaceae* and the pseudomonads. Similarly, Holmberg *et al.* [12] reported that many *Plesiomonas* strains in the U.S.A. possessed plasmids with molecular weights of 150 MDa. Recently, Nolte *et al.* [13] reported that an isolate of *P. shigelloides* from a patient with proctitis and fatal septicaemia possessed a plasmid greater than 100 MDa. In Nigeria, we are not aware of any report on plasmids in *Aeromonas* or *Plesiomonas*. Hence,

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this study was carried out to determine the prevalence of plasmids in these organisms.

Materials and methods

Bacterial strains

Fifty-three strains of *Aeromonas* and 16 of *P. shigelloides* were isolated from subjects with diarrhoea and were screened for plasmids in this study. The *Aeromonas* strains were biotyped using the scheme of Popoff [14], and consisted of 20 *A. hydrophila*, seven *A. sobria* and 26 *A. caviae*.

Isolation of plasmid DNA

The method used for the isolation of plasmid DNA was the rapid alkaline lysis method of Birnboim and Doly [15], based on the principle of the alkaline denaturation of linear chromosomal DNA while covalently closed circular DNA remains double stranded. Basically, it involves the gentle lysis of the bacterial cells with lysozyme, and centrifugation to remove the bulk of the chromosomal DNA.

Agarose gel electrophoresis

Electrophoresis of DNA was carried out on horizontal agarose slab gels in Tris–borate buffer (89 mM Tris–borate, 89 mM boric acid, 2 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 before electrophoresis. Loaded gels were subjected to electrophoresis at 100 mA for 3 h. The gels were then stained with ethidium bromide (0.5 μg/ml) solution in water for 45 min. The DNA was visualized by transmitted short-wave ultraviolet light, and the distance migrated by plasmid DNA was measured in millimetres.

Control strain

Escherichia coli V517 carrying eight plasmids of molecular weight standards pVA 517A–11 [16] was included in the study as a control. It was provided by Dr J. Crossa of Oregon Health Sciences University, Portland, Oregon, U.S.A.

Results

Table 1 shows the number of isolates harbouring one or more plasmids in this study. The molecular weights of the plasmids range from 2.4 to 16.8 MDa. Of the 20 *A. hydrophila* strains examined, three (15%) harboured plasmid. Out of these three *A. hydrophila* strains that were positive, two harboured similar plasmids of 2.4 MDa while one possessed a larger plasmid of 6.7 MDa. Similarly, nine (34.6%) of 26 *A. caviae* strains and one (6.3%) of 16 *P. shigelloides* strains examined harboured at least one plasmid.

It is interesting to note that isolates with multiple plasmids in this study included an *A. caviae* with plasmids 3.0 and 5.3 MDa, and a *P. shigelloides* strain with plasmids 9.7 and 16.8 MDa.

Discussion

The results of this study showed that only a few of the *Aeromonas* and *Plesiomonas* isolates from subjects with diarrhoea in our environment harboured plasmids, with molecular weights ranging from 2.4 to 16.8 MDa. Specifically, 15% of *A. hydrophila* strains examined were positive while 34.6% of *A. caviae* and 6.3% of *P. shigelloides* were also positive. However, no plasmid was detected in seven isolates of *A. sobria* examined.

There appeared to be some interesting patterns in the occurrence of plasmids among the strains studied. For instance, the plasmid of 2.4 MDa occurred with equal frequencies among *A. hydrophila* and *A. caviae* (Table 1). Similarly, the plasmid of 8.4 MDa was found only in *A. caviae*, while plasmids of 9.7 and 16.8 MDa were restricted to *P. shigelloides*. However, while these findings tend to suggest species-relatedness of some plasmids, more elaborate studies would be needed before definite conclusions could be drawn.

In the literature, very little information is available on plasmids in *Aeromonas* species or *P. shigelloides* [10–13]. In recent times however, plasmid studies have provided a better understanding of the pathogenesis and epidem-

Table 1. *Aeromonas* and *Plesiomonas* strains from subjects with diarrhoea harbouring one or more plasmids

Strain no.	Species	Plasmid size (MDa)
832	*A. caviae*	2.4
1430	*A. caviae*	2.4
1653	*A. hydrophila*	2.4
1708	*A. hydrophila*	2.4
234*	*A. caviae*	3.0
		5.3
156	*A. caviae*	6.4
2269	*A. hydrophila*	6.7
65	*A. caviae*	8.4
596	*A. caviae*	8.4
577*	*P. shigelloides*	9.7
		16.8

*Strains harbouring more than one plasmid.

iology of many infectious diseases [2,4,6–8,10]. Our strains were isolated from subjects with diarrhoea, and so plasmids in some of them might have contributed significantly to diarrhoea caused by such strains. It is most probable therefore that further plasmid studies in *Aeromonas* and *Plesiomonas* may provide clues to a number of controversies currently surrounding their enteropathogenic status.

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