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Phage type conversion in *Salmonella enterica* serotype Enteritidis caused by the introduction of a resistance plasmid of incompatibility group X (IncX)

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SUMMARY

The plasmid pOG670, a 54 kb, conjugative plasmid that specifies resistance to ampicillin and kanamycin and belonging to the incompatibility group X (IncX), was transferred into 10 isolates of *Salmonella enterica* serotype Enteritidis belonging to 10 different phage types (PT1, 2, 3, 4, 8, 9, 9b, 10, 11 and 13). Acquisition of the plasmid by these strains did not result in the loss of any resident plasmids but resulted in phage type conversion in 8 of the 10 strains (PT1, 2, 4, 8, 9, 9b, 10 and 11). The observed changes in phage type were found to result from the loss of sensitivity to 3 of the 10 typing phages used (phages 3, 5 and 7). Where the conversion resulted in a change to a defined phage type, both the new and original PTs belonged to the same, previously described, evolutionary lines. Enteritidis PTs 1, 4 and 8, commonly associated with poultry world-wide, were converted to PTs 21, 6 and 13a respectively. The results indicate a different route for phage type conversion Enteritidis from others reported in the literature and, although IncX plasmids are not normally present in PT8 or PT13a, may suggest a possible mechanism/link connecting these phage types.

INTRODUCTION

The traditional methods of strain identification based on serotyping and phage typing have been used in the epidemiological study of salmonella for many years and, despite the increasing use of typing methods based on molecular biological techniques, continue to provide the basis for the study of salmonella epidemiology. Phage typing has been mainly used to differentiate isolates within serotypes Typhi [1], Typhimurium [2], Virchow [3], Hadar [4] and Enteritidis [5].

The dramatic increase in the incidence of human cases of salmonellosis caused by Enteritidis in many countries around the world [6] was shown by the use of phage typing to be caused predominantly by strains of phage type (PT) 4 in Europe [7] and PT8 and PT13a in the USA and Canada [8, 9]. Phage typing of isolates of Enteriditis has been used to provide the basis for epidemiological analysis and 44 phage types are now recognized [10].

Although phage typing has remained the method of choice, being rapid, reproducible, discriminatory and cost effective [11], there have been reports of phage type conversion caused by the acquisition or the loss of plasmid DNA in *S. typhimurium* [12, 13] and Typhi [14]. The conversion of Enteritidis PT 4 to PT 24 following acquisition of an IncN, drug resistance plasmid was reported by Frost and colleagues in 1989 [15] and it was recently shown that PT4 could be converted to PT8 following lysogenization with phage 2 from the Enteritidis typing scheme [16].

The IncX R-plasmid, pOG670, and a cointegrate

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(pOG669) formed between this plasmid and the virulence plasmid of Typhimurium have previously been described by Platt and Smith [17]. During a study of the virulence determining properties of these plasmids [18], it was noted that the plasmids, after conjugal transfer into Enteritidis recipients, altered the phage type. The results of the work reported in this paper confirmed that these plasmids were responsible for the observed changes in phage type and, furthermore, allowed the changes in phage reaction to be ascertained.

MATERIALS AND METHODS

Bacterial strains and culture media

Ten isolates of Enteritidis of different phage types were taken from the strain collection of the Department of Veterinary Microbiology, Royal Veterinary and Agricultural University, Copenhagen. All strains had been tested for sensitivity to ampicillin and kanamycin and found to be sensitive. Plasmid content of the strains was determined as described by Christensen and collegaues [19]. Strains, phage types and plasmid profiles are listed in Table 1. A strain of *Escherichia coli* J53-2, resistant to rifampin, served as the donor strain for plasmid transfers [17]. All strains were grown on LA agar or in LB broth at 37 °C. Selection for plasmid transfer was carried out on MacConkey agar containing the appropriate antibiotics.

Conjugal transfer of pOG670

The donor strain *E. coli* K12 J53-2, containing pOG670, and the recipient strains were cultured overnight at 37 °C in LB broth. 0·1 ml of each strain were swabbed over the surface of an LA agar plate and incubated for 4 h at 37 °C. Growth from the surface of the plate was harvested with a sterile swab and resuspended in sterile PBS. Dilutions of the suspension were plated on MacConkey agar containing 50 μg/ml ampicillin (Sigma) to select for transfer of pOG670 and colicin E2 to eliminate the donor [20]. Colonies that were *lac*-and amplicillin resistant were replated for purity and verified serologically as salmonellae using salmonella poly-O antiserum (Difco, Michigan, USA). The plasmid DNA content of transconjugants was verified as described previously [19].

Phage typing

Phage typing of Enteritidis strains both with and without pOG670 was carried out using the method of Ward and colleagues [5].

RESULTS

Conjugal transfer of pOG670

The conjugal transfer of pOG670 was successful in each of the 10 strains of Enteritidis examined and no displacement of resident plasmids was observed in the transconjugants.

Phage typing

The typing reactions obtained from phage typing of the Enteritidis strains and their pOG670 transconjugants are shown in Table 1. In each case, introduction of pOG670 caused restriction of phages 3, 5 and 7. After the acquisition of pOG670, the strain of Enteritidis PT1 converted to PT21, Enteritidis PT2 converted to PT22, Enteritidis PT4 converted to PT6, Enteritidis PT8 converted to PT13a, Enteritidis PT9 and PT9b converted to untypable, Enteritidis PT10 converted to PT13, and Enteritidis PT11 converted to PT34. Enteritidis PT3 and PT13 were unaffected by the introduction of pOG670.

DISCUSSION

Phage typing of isolates of Enteritidis is routinely carried out in Denmark using the 10 typing phages described in the scheme by Ward and colleagues [5]. In this study, we have demonstrated the ability of the IncX plasmid pOG670 to alter the phage type of strains of Enteritidis. The plasmid pOG670 was first described in wild type Typhimurium isolated from cattle in Scotland in 1985 [17] and is readily transferable to different serotypes of salmonella [18]. The facility of the latter observation reflects the widespread, natural distribution of IncX plasmids among salmonellae of different serotypes including those isolated in the pre-antibiotic era [22, 23]. Examination of the changes in sensitivity to the individual phages used indicated that the effect of the plasmid was to restrict the reactions of Enteritidis typing phages 3, 5 and 7. According to the description of the typing scheme by Ward and colleagues [5], phage 3 was a lysogenic phage isolated from a wild

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Table 1. *Plasmid profiles and phage type reactions of strains of Enteritidis and their derivatives containing the IncX resistance plasmid, pOG670*

Strain designation	Plasmids (kb)	Phage reactions										Phage type
		1	2	3	4	5	6	7	8	9	10	
664	57	ol*	scl	cl	ol	ol	scl	cl	scl	ol	ol	1
664,pOG670	57; 54	ol	scl	–	ol	–	scl	–	scl	ol	ol	21
560	57	ol	–	cl	cl	cl	3+	cl	< ol	cl	cl	2
560,pOG670	57; 54	ol	–	–	ol	–	3+	–	ol	ol	ol	22
561	57	ol	–	–	–	–	–	–	ol	–	cl	3
561,pOG670	57; 54	ol	–	–	–	–	–	–	cl	–	cl	3
GR16485	57	–	scl	cl	ol	cl	scl	cl	scl	ol	ol	4
GR16485,pOG670	57; 54	–	scl	–	ol	–	scl	–	scl	scl	ol	6
GR18188	57	–	–	scl	ol	cl	scl	scl	scl	ol	ol	8
GR18188,pOG670	57; 54	–	–	–	ol	–	scl	–	scl	ol	ol	13a
569	57	–	–	cl	–	cl	–	cl	–	–	–	9
569,pOG670	57; 54	–	–	–	–	–	–	–	–	–	–	NT
HBH95	85	–	–	cl	–	cl	–	–	–	–	–	9b
HBH95,pOG670	85; 54	–	–	–	–	–	–	–	–	–	–	NT
570	90	–	–	–	ol	2+	scl	–	–	ol	–	10
570,pOG670	90; 54	–	–	–	ol	–	scl	–	–	cl	–	13
571	80	–	–	2+	–	cl	–	–	scl	–	cl	11
571,pOG670	80; 54	–	–	–	–	–	–	–	scl	–	< ol	34
573	57	–	–	–	cl	–	3+	–	–	cl	–	13
573,pOG670	57; 54	–	–	–	cl	–	scl	–	–	cl	–	13

* ol, opaque lysis; scl, semi-confluent lysis; cl, lysis; 2+, 21–80 plaques; 3+, 81–100 plaques; –, no reaction.

strain of Enteritidis whereas phage 7 was isolated from a sewage sample. Phage number 5 was an adapted phage obtained by host induced modification of phage number 3.

Isolates of Enteritidis with phage type (PT) 4 predominate in Europe [7] with PT1 also being common in Denmark [21] and while PT8 and PT13a are predominant in the USA and Canada [8, 9]. Enteritidis PT1, which is sensitive to all 10 phages, was converted to PT21 by the acquisition of pOG670, a change involving only the restriction of phages 3, 5 and 7. The same changes in phage restriction in Enteritidis PT4 resulted in conversion to type 6. Strains of Enteritidis PT4 have been previously shown to convert to PT7 by spontaneous mutation [24] or, following the introduction of an IncN plasmid, to convert to PT24 [15]. More recently it was demonstrated that PT4 could also convert to PT8 by lysogenic conversion [16].

Most of the phage type changes described here resulted in a change to a defined phage type (PT1 to 21, 2 to 22, 4 to 6, 8 to 13a, 10 to 13, 11 to 34). These changes, apart from PT34 which has not yet been assigned a lineage, were found to be wholly compatible with the evolutionary clonal lineages of Enteritidis phage types based on IS200 distribution described previously [24, 25] in that the phage type changes observed were all within clonal lineages. It must, however, be noted that phage type conversions in Enteritidis have been described which involve crossing clonal lineages [16], suggesting that the phylogenetic framework is not rigid.

The strain of Enteritidis PT8 was converted to PT13a following the introduction of pOG670 and may indicate that PT13a may be derived from PT8 (or vice versa) by a phage restriction mechanism similar to that conferred by plasmid pOG670. Further work is required to elucidate the mechanism of phage restriction encoded by pOG670 and the possibility that the same mechanism exists in PT8/PT13a strains, either encoded by other plasmids, phages or chromosomal genes. However, the phage type conversions described here, taken together with the previously reported PT4 to PT8 conversion [16], suggest a possible mechanism for the epidemiological differences observed in Europe, where PT4 is predominant, and in North America, where PT8 and PT13a are most commonly isolated.

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