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Plasmid typing and antibiotic susceptibility of *Salmonella* Enteritidis isolates

by

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Abstract

Isolates of Salmonella *Enteritidis of various origin (human stools, poultry and poultry products, other animals, meat, beach and surface water) were subjected to plasmid typing and antimicrobial susceptibility tests. A large majority of the strains (80% of human, 71% of non-human origin) harboured one single plasmid (56 kb). The remaining group presented a great variety of plasmid profiles that were infrequently encountered. Most of these profiles included also the 56 kb plasmid. Plasmid-free strains were found in 8.6% of the human and 13.4% of the non-human isolates; beach and surface water isolates were relatively more often plasmid-free. 92% of the human and non-human strains proved to be antibiotic sensitive. An increasing number of nalidixic acid monoresistant strains was noted among human and non-human isolates. Gentamicin resistance was acquired by two strains on a total of 339 investigated; all strains displayed susceptibility to norfloxacin. Transconjugation experiments highlighted the role of R^+ plasmids in the acquisition of antibiotic resistance. The virulence plasmid (56 kb) could be co-transconjugated with a cryptic 83 kb plasmid. Plasmid typing and antibiotic sensitivity testing do not contribute to a further differentiation of the majority of* S. *Enteritidis isolates.*

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Key-words

Antibiotic resistance, plasmids, *Salmonella* Enteritidis, transconjugation.

Introduction

The current epidemiologic picture of salmonellosis is considered by Tauxe (1) as a postmodern paradox : large numbers of human beings become ill after consuming food from apparently healthy animals that are colonized with *Salmonella*. Reporting of *Salmonella* infections in humans is indeed increasing on both sides of the Atlantic (2-4). *S.* Enteritidis is recognized now as the most common serovar causing *Salmonella* food-poisoning. It is suggested that this phenomenon is related to consumption of eggs and poultry which harbour the organism (5-7).

To confirm this hypothesis, typing of the epidemic strain(s) is of paramount importance. Although phage typing has proven its usefulness, phage types can be further subdivided by plasmid typing (8). As rapid methods for the extraction and analysis of plasmid DNA have become available, plasmid typing has become very attractive. In this study, 339 isolates of *S.* Enteritidis from Belgian human and non-human sources were subjected to plasmid typing. The antibiotic sensitivity patterns and the presence of R^+ plasmids were investigated. In addition the transferability of R^+ plasmids was analyzed.

Materials and Methods

Strains of *S.* Enteritidis were isolated from various sources : stools from patients with diarrhoea (n = 220), beach water (n = 37), poultry and poultry products (n = 39), meat products (n = 10), animals (n = 17), surface water (n = 10), litter (n = 2), pastry (n = 1), fish-salad (n = 1), unknown (n = 2).

The cultures were serotyped at the Belgian National Reference Centre for Salmonella and Shigella (Brussels). There was no obvious epidemiological relatedness between these isolates.

Plasmid extraction was performed following the alkaline lysis procedure of Birnboim and Doly (9). All plasmid DNA's were electrophoresed

on 0.6% agarose gels (Sea Kem ME agarose) by using a Bio-Rad Mini Sub Cell horizontal electrophoresis apparatus.

Electrophoresis was carried out at 40 V for 4 h. in TBE buffer (90 mM Tris, 90 mM borate, 2.5 mM EDTA; pH 8.0). Gels were stained with ethidium bromide (0.5 µg/ml) and photographed under UV-transillumination with a MP4 Polaroid Landcamara. Molecular Weight Markers type II (Boehringer) were included as molecular weight references.

Isolates were tested by the disc diffusion method (10) for susceptibility to the following antimicrobial agents (Diagnostics Pasteur) : streptomycin 10 IU, tetracycline 30 IU, chloramphenicol 30 µg, kanamycin 30 IU, ampicillin 10 µg, sulphonamides 200 µg, trimethoprim 5 µg, gentamicin 10 IU, nalidixic acid 30 µg, norfloxacin 5 µg. For abbreviations of antibiotics see legend Table 4.

E. coli 14 R 525, coding chromosomally resistance to nalidixic acid, was chosen as receptor culture in transconjugation experiments. Appropriate *S.* Enteritidis donor cultures and the receptor were cultured in Brain Heart Infusion broth : 2 ml of the donor culture was mixed with 2 ml of *E.coli* culture and incubated 24 h at 37°C. Potential transconjugates were isolated from Mueller-Hinton plates containing nalidixic acid and the corresponding antibiotics to which the donor strain is resistant. Transconjugates were identified and tested for antibiotic susceptibility and plasmid content.

Results

On a total of 220 human stools isolates, 175 strains (80%) present a same plasmid profile consisting of one single large plasmid with a size of 56 kb (Table 1). With 19 isolates, no plasmids are detected. The remaining 26 isolates exhibit 25 different plasmid profiles (from a single plasmid to up to six plasmids) of whom 17 profiles contain the 56 kb plasmid. All but one profile (76/56) is represented only once. The size of the individual plasmids range from 1.9 kb to 87 kb.

One plasmid profile (56 kb) predominates also among the strains of non-human origin, regardless the nature of the source (Table 2). On 119 cultures investigated, this profile is encountered with 84 isolates (71%). In total 16 isolates were found plasmid-free. However isolates from certain sources are relatively more exempt from plasmids : beach water 22%, surface water 20% of the isolates. In addition to the prevailing 56

TABLE 1

Distribution of plasmid profiles among S. Enteritidis isolates of human origin

Plasmid profile *	Number of strains
1. no plasmids	19
2. single plasmid	
83	1
72	1
71	1
68	1
56	175
36	1
3. two plasmids	
83/56	1
79/56	1
78/56	2
72/56	1
71/56	1
68/56	1
56/41	1
56/2.1	1
56/2.0	1
9.6/3.9	1
4.5/2.4	1
2.5/2.3	1
4. three plasmids	
87/56/4.4	1
76/56/18	1
72/56/1.9	1
68/14/4.9	1
56/4.4/2.6	1
56/2/1.9	1
5. four plasmids	
79/56/9.4/4	1
6. six plasmids	
58/56/4.1/3.5/2.3/2.1	1

* size of plasmids expressed in kb

TABLE 2
Distribution of plasmid profiles among S. Enteritidis isolates of non-human origin

Origin	Plasmid profile*	Number of strains
Beach water	absence	8
	83	1
	56	28
Poultry and poultry products	absence	3
	65	2
	62	1
	56	31
	72/62	1
	56/2.2/2.0	1
Meat products	absence	1
	71	1
	56	8
Animal	absence	2
	71	1
	56	6
	2.1	1
	56/2.1	2
	56/2.0	2
	56/2.9/2.2	2
	76/56/2.2/2.0	1
Surface water	absence	2
	56	7
	56/38	1
Miscellaneous	68	1
	56	4
	56/2.2	1

* size of plasmids expressed in kb

TABLE 3

Identical rarely occurring plasmid profiles with S. Enteritidis of different origin

Plasmid profile*	Origin of the isolates
83	human stools / beach water
71	human stools / faeces of a duck (zoo) / sample of minced meat
68	human stools / unindentified animal
65	poultry / chicken skin
56/2.1	human stools / panther (zoo) / guinea-pig (zoo)
56/2.0	human stools / barn-owl (zoo) / faeces of an ibis (zoo)
56/2.9/2.2	faeces of a grebe (zoo) / liver, egg-yolk of a parrot (zoo)

* size of plasmids expressed in kb

TABLE 4

Antibiotic sensitivity patterns of S. Enteritidis isolates

Human origin		Non-human origin	
Sensitivity pattern	Number of strains	Sensitivity pattern	Number of strains
Sensitive	202	sensitive	109
R^+ : T	1	R^+ : A	1
R^+ : A	2	R^+ : Nal	7
R^+ : Nal	5	R^+ : S(I)*	1
R^+ : S(I)*	2	R^+ : S Su	1
R^+ : Asu	1		
R^+ : S Su Gt	1		
R^+ : S A Su Tp	1		
R^+ : S T Su Tp	2		
R^+ : S T A Su	1		
R^+ : S C K A Su Tp	1		
R^+ : S T C A Su Tp Gt	1		

S: steptomycin; T: tetracycline; C: chloramphenicol; K: kanamycin;
A: ampicillin; Su: sulphonamides; Tp: trimethoprim; Gt: gentamicin;
Nal: nalidixic acid
* (I): intermediate resistance

kb profile, 14 different patterns are infrequently observed with isolates of non-human origin : 8 patterns are composed of more than one plasmid, with the 56 kb plasmid present in 7 of them. The size of the individual plasmids ranges from 2.0 to 83 kb.

There is little similarity between the rarely occurring plasmid patterns from human and non-human origin. Only in a limited number of cases, a same profile is observed with isolates of different origin (Table 3). Human isolates harbouring a single plasmid of 83 kb and of 68 kb were also isolated from respectively beach water and an unidentified animal. A human isolate carrying a 71 kb plasmid was also cultured from the faeces of a duck and a sample of minced meat. Human strains with the plasmid patterns 56/2.1 and 56/2.0 were isolated twice from different zoo animals. A poultry isolate carrying a 65 kb plasmid was also cultured from a chicken skin. A specific three plasmid profile (56/2.9/2.2) was found with two different zoo animals.

Antibiotic sensitivity was tested against 10 antibiotics. A great majority of the isolates of human (92%) and non-human origin (92%) were found to be sensitive (Table 4). The isolation rate for strains monoresistant to nalidixic acid is remarkably higher than for the other resistance patterns : nalidixic acid resistance is seen with 5 human, 6 beach water and 1 meat sample isolates. A greater variation of resistance patterns is observed with isolates of human origin compared to their non-human counterparts. In addition multiresistance is seldom reported in the latter group : one culture only shows resistance to streptomycin and sulphonamide (Table 4). Gentamicin is no longer 100% effective against *S.* Enteritidis : two human isolates acquired gentamicin resistance as part of a multiresistance (R^+ : S Su Gt and R^+ : S T C A Su Tp Gt). Norfloxacin was the only antimicrobial agent tested to which all the isolates proved susceptible.

Table 5 outlines the association between plasmid profile and antibiotic resistance pattern. Human isolates generate more associations than the non-human isolates. While analyzing table 5, it must be kept in mind that the 56 kb plasmid is the Enteritidis-serovar linked plasmid which does not code for resistance (11). The association 56 kb – nalidixic acid resistance was recovered several times : 4x with human isolates, 5x with beach water isolates, 1x with a meat isolate. Nalidixic acid resistance was also found in plasmid-free strains. Monoresistance to ampicillin is found with three different plasmid profiles : 56/44; 2.5/2.3; 76/56/2.2/2.0. The intermediate resistance behaviour against streptomycin is associat-

ed with two single plasmids : 36 and 68 kb. Although presenting different resistance patterns (R^+ : S Su Gt and R+ : S T C A Su Tp Gt) the two gentamicin resistant cultures exhibit a same plasmid profile : 78/56. On the other hand, a same resistance pattern (R^+ : S T Su Tp) is associated with two different plasmid profiles : 79/56/9.4/4.0 and 87/56/4.4. A multiresistant culture (R^+ : S C K A Su Tp) harbours only the serovar-linked plasmid (56 kb).

In order to determine if antibiotic resistance is conjugative, a conjugation experiment between a selected number of *S.* Enteritidis isolates and a *E.coli* receptor strain was carried out. Table 6 summarizes the results. The receptor culture is able to acquire large plasmids (83, 78, 76 kb). At the same time, the antibiotic resistance pattern of the donor isolate is fully transmitted with one exception, whereby a Su-resistance is lost during transconjugation. The virulence-associated plasmid (56 kb) does not tend to be transconjugated frequently; only on one occasion *E.coli* acquired the 56 kb plasmid in association with a 83 kb plasmid.

Discussion

In recent years, Salmonella gastro-enteritis gains a lot of importance. The Belgian National Reference Centre for Salmonella recorded an increase of the number of human isolates from 6.092 in 1986 to 12.008 in 1996. At the same time Enteritidis has become the most important serovar: representatives of this serovar increased from 4.9% (1986) to 51% (1996) of all serovars combined. Similar tendencies are also observed elsewhere (2-4). Is this phenomenon due to a spectacular spread of one single clone or are multiple clones involved ? The plasmid typing results from this study are likely to support the hypothesis of a single clone spread : the majority of the isolates of human and non-human origin display a same plasmid profile consisting of one single plasmid of 56 kb; in addition the isolates are antibiotic susceptible. On the other hand, a minority of the isolates present a great variety of plasmid profiles which are infrequently encountered. The data from the Belgian study are in accordance with data from Germany (12), USA (13), Italy (2), and England (8).

The size of the serovar-related plasmid was estimated in this study to be 56 kb. However, a variety of other values for this plasmid were reported : 55 kb (13, 14), 54 kb (11, 15), 40 MDa (16), ± 40 MDa (17), 38.9 MDa (5), 37 MDa (12, 18), 36 MDa (19), 34.5 MDa (2), 34 MDa (20), 38

TABLE 5
Association between plasmid and antibiotic resistance pattern

Human isolates		Non-human isolates	
Plasmid profile*	Antibiotic resistance pattern**	Plasmid profile	Antibiotic resistance pattern**
68	S (I)***	68	S (I)
56	Nal	56	Nal
56	S C K A Su Tp	absence	Nal
absence	Nal	absence	S Su
36	S (I)	76/56/2.2/2.0	A
83/56	A Su		
78/56	S Su Gt		
78/56	S T C A Su Tp Gt		
72/56	S A Su Tp		
56/44	A		
2.5/2.3	A		
87/56/4.4	St Su Tp		
76/56/18	T		
79/56/9.4/4.0	S T Su Tp		
58/56/4.1/3.5/2.3/2.1	S T A Su		

* size of plasmids expressed in kb
** abbreviations of antibiotics: see legend Table 4
*** (I): intermediate resistance

TABLE 6
Transconjugation between S. Enteritidis and E. Coli

Donor: *S.* Enteritidis		Receptor: *E. Coli*	
Plasmid profile*	Antibiotic resistance pattern **	Plasmid profile	Antibiotic resistance pattern**
76/56/18.2	T	76	T
78/56	S T C A Su Tp Gt	78	S T C A Su Tp Gt
83/56	A Su	83/56	A Su
78/56	S Su Gt	78	S Gt
56	S C K A Su Tp	–	S C K A Su Tp

* size in plasmids expressed in kb
** abbreviations of antibiotics: see Table 4

MDa (21 - 23). As these values differ only slightly and given the experimental error, one may suggest that these plasmids are identical or highly clonal related. Evidence for this concept comes from restriction enzyme digest studies on these plasmids. Martinetti *et al* (14) found identical restriction patterns for the 55 kb plasmid. Brown *et al.* (21) came to the same conclusion for the 38 MDa plasmid of various phage types; identical patterns were also found for the 36 MDa plasmid from 4 different Japanese geographical sources (19).

The serovar-related plasmid seems to be responsible for mice virulence (18); however this plasmid does not seem to be associated with virulence for chicks (19).

In addition to the 56 kb plasmid, *S.* Enteritidis can acquire large plasmids (87, 83, 79,... kb) and small plasmids (4.9, 3.9, 2.0, 1.9 kb). A greater variety of multi-plasmid profiles is observed with isolates of human origin; no multi-plasmid profiles were seen with beach water isolates. It is likely that a human or animal passage favours the acquisition of other plasmids. On the other hand, environmental stress (sea, surface water...) can facilitate plasmid curing; indeed beach and surface water isolates constitute the largest group of plasmid-free isolates (20% versus ± 10% for isolates of human and animal origin).

Plasmid-free isolates are thought to be less virulent or avirulent because of the absence of the virulence plasmid. How could they be involved in human gastro-enteritis ? One explanation lies in the loss of the virulence plasmid during transport to and conservation in the laboratory, whereby the presence of sugars in the medium could exert a curing effect. Other factors than plasmids can also be involved in pathogenicity.

Although one plasmid profile is dominating, some rare profiles may also have epidemiological relevance. A same rare profile has been found in isolates from human stools, animals, beach water and minced meat (Table 3). The demonstration of a same profile from different zoo animals indicates a certain degree of cross-contamination between animals at a same zoo. Recently, we investigated a foodborne outbreak in Belgium, attributed to a *S.*Enteritidis epidemic strain with a rare plasmid profile; such kind of outbreaks were also reported from Spain (11) and USA (13, 24).

A great majority of the *S.* Enteritidis isolates are antibiotic susceptible. Increasing monoresistance to nalidixic acid is noted. This resistance is probably chromosomally encoded because nalidixic acid resistant isolates are either plasmid-free or carry only the serotype-related plasmid (56 kb). This plasmid associated with the large majority of susceptible isolates , is usually not considered as a R^+ plasmid. Tables 5 and 6 evoke the role plasmids may play in antibiotic resistance. However, as already mentioned, chromosomally encoded resistance may not be discarded: in addition to nalidixic acid resistant isolates , a strain (R^+: S Su) was found to be plasmid-free, a strain (R^+ : S C K A Su Tp) harboured only the serovar linked plasmid (56 kb).

With the emergence of multiresistant isolates, newer antimicrobial agents are needed. Resistance to gentamicin, 100% active for a long time, has been detected in two isolates. All the strains were still susceptible to norfloxacin, a quinolone that actually shortens the course of clinical disease and terminates the excretion of *Salmonella* in the stools (25).

It is clear from the conjugation experiments that *S.* Enteritidis may carry large conjugative plasmids. As the virulence plasmid is considered to be non-conjugative (19), it is rather surprising to see this plasmid being co-transconjugated with a 83 kb plasmid. This co-transconjugation sounds disquieting because other Gram-negative bacteria may acquire the *S.* Enteritidis virulence plasmid by this mechanism.

As *S.* Enteritidis foodborne infections tend to increase, discriminatory typing methods are needed for the investigation of these outbreaks. Does plasmid typing fulfils the criterion of a sensitive typing method ? In the case of *S.* Enteritidis, the successful application seems rather limited. As one plasmid profile predominates among the virulent strains, differentiation between strains of various sources is hardly achieved. Only in the case of a rare plasmid profile, plasmid typing may prove useful. Application of other molecular typing methods also leads to the same concept of a highly clonal distribution of *S.* Enteritidis (26, 27), diminishing molecular type variation among isolates derived from foodborne outbreaks.

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Samenvatting

Salmonella Enteritidis stammen werden verder gekarakteriseerd door plasmide typering en antibiotica-resistentie. De stammen waren van verschillende oorsprong (stoelgang bij humane diarree, kippen, kiphoudende voedingswaren, andere dieren, vlees, strand- en oppervlaktewater). De meerderheid van de stammen (80% humane, 71% niet-humane) droegen 1 enkele plasmide (56 kb). Bij de overige isolaties werd een brede waaier van weinig frequente plasmide profielen gevonden, waarvan de meeste toch ook de 56 kb-plasmide omvatten. Bij 8,6% humane en 13,4% niet-humane isolaties konden geen plasmiden worden gedetecteerd; hierbij waren de strand- en oppervlaktewater isolaties sterk vertegenwoordigd. Een meerderheid van de stammen (92%) was antibiotica-gevoelig. Een stijgend aantal isolaties (zowel humane als niet-humane) waren nalidixinezuur monoresistent. Op een totaal van 339 waren amper 2 stammen gentamicine resistent. Alle isolaties waren gevoelig voor norfloxacine. De rol van R^+-plasmiden bij het ontstaan van antibiotica-resistentie werd door transconjugatie experimenten aangetoond. De *S.* Enteritidis virulentie plasmide (56 kb) kon samen met een cryptische 83 kb plasmide worden gecotransfereerd naar een *E. coli* stam. Plasmide typering en antibiotica gevoeligheidsbepaling dragen niet echt bij tot verdere subdifferentiering van de meerderheid van *S.* Enteritidis isolaties.

Résumé

Une étude des profils plasmidiques et de la résistance aux antibiotiques a été réalisée sur des souches de *S.* Enteritidis de différentes sources (coprocultures humaines, poulets, denrées alimentaires à base de poulet, autres animaux, viande, eaux de plage et eaux de surface). La majorité des souches (80% d'origine humaine, 71% d'origine non-humaine) contiennent un seul plasmide de 56 kb. Chez les autres souches, un éventail de profils plasmidiques a été décelé, comprenant presque toujours le plasmide de 56 kb. Une minorité de souches (8,6% d'origine humaine, 13,4% d'origine non-humaine) étaient exemptes de plasmides. Les souches isolées des eaux de plage et des eaux de surface étaient plus sensibles à la perte des plasmides. La plupart des souches (92%) étaient sensibles aux antibiotiques, néanmoins une tendance à la monorésistance à l'acide nalidixique se dégage. Une résistance à la gentamicine a été notée pour 2 souches sur un total de 339 souches étudiées. Toutes les souches étaient sensibles à la norfloxacine. Des expériences de transconjugaison indiquaient le rôle important des R^+-plasmides dans l'acquisition de la résistance aux antibiotiques. Le plasmide de virulence de *S.* Enteritidis (56 kb) était co-transférable avec un plasmide cryptique (83 kb). La majorité des souches de *S.* Enteritidis ne peuvent pas être différenciées en utilisant le typage de plasmide et l'antibiogramme.

References

1. Tauxe RV. Salmonella a postmodern pathogen. J. Food Protection 1991:54:563-8.
2. Fantasia M, Filetici E, Anastasio MP, Marcozzi MD, Gramenzi MP, Aureli P. Italian experience in *Salmonella* Enteritidis 1978-1988 : charaterization of isolates from food and man. Int. J. Food Microbiol. 1991:12:353-62.
3. Mishu B, Koehler J, Lee LA, *et al.* Outbreaks of *Salmonella* Enteritidis infections in the United States, 1985-1991. J. Infect. Dis. 1994:169:547-52.
4. Rodrigue DC, Tauxe R V, Rowe B. International increase in *Salmonella* Enteritidis : a new pandemic ? Epidemiol. Infect. 1990:105:21-7.
5. Binkin N, Scuderi G, Novaco F, *et al.* Egg-related *Salmonella* Enteritidis, Italy 1991. Epidemiol. Infect. 1993:110:227-37.
6. Cowden JM, Chisholm D, O'Mahony M, Lynch D, Mawer SL, Spain GE, Ward L, Rowe B. Two outbreaks of *Salmonella* Enteritidis phage type 4 infection associated with the consumption of fresh shell-egg products. Epidemiol. Infect. 1989:103:47-52.
7. Hedberg CW, David MJ, White KE, MacDonald KL, Osterholm MT. Role of egg consumption in sporadic *Salmonella* Enteritidis and *Salmonella* Typhimurium infections in Minnesota. J. Infect. Dis. 1993:167:107-11.
8. Threlfall EJ, Rowe B, Ward LR. Subdivision of *Salmonella* Enteritidis phage types by plasmid profile typing. Epidemiol. Infect. 1989:102:459-65.
9. Birnboim HC, Doly J. A rapid alkaline extraction procedure for screening recombinant plasmid DNA. Nucleic Acids Res. 1979:7:1513-23.
10. Courvalin P., Goldstein F., Philippon A., Sirot J. editors. L'antibiogramme. Paris : MPC-Videom, 1985.
11. Rivera MJ, Rivera N, Castillo J, Rubio MC, Gómez-Lus R. Molecular and epidemiological study of *Salmonella* clinical isolates. J. Clin. Microbiol. 1991:29:927-32.
12. Schroeter A, Pietzsch O, Steinbeck A, *et al.* Epidemiologische Untersuchungen zum *Salmonella*-enteritidis Geschehen in der Bundesrepublik Deutschland 1990. Bundesgesundhbl. 1991:4/91:147-51.
13. Morris JGJr, Dwyer DM, Hoge CW, *et al.* Changing clonal patterns of *Salmonella* Enteritidis in Maryland : evaluation of strains isolated between 1985 and 1990. J. Clin. Microbiol. 1992:30:1301-3.
14. Martinetti G, Altwegg M. rRNA gene restriction patterns and plasmid analysis as a tool for typing *S.* Enteritidis. Res. Microbiol. 1990:141:1151-62.
15. Bichler LA, Nagaraja KV, Pomeroy BS. Plasmid diversity in *Salmonella* Enteritidis of animal, poultry and human origin. J. Food Protection 1994:57: 4-11.
16. Hadfield TL, Monson MH, Wachsmuth IK. An outbreak of antibiotic-resistant *Salmonella* Enteritidis in Liberia, West Africa. J. Infect. Dis. 1985:151:790-5.
17. Pohl P, Lintermans P, Marin M, Couturier M. Epidemiological study of *Salmonella* Enteritidis strains of animal origin in Belgium. Epidemiol. Infect. 1991:106:11-6.
18. Helmuth R, Stephan R, Bunge C, Hoog B, Steinbeck A, Bulling E. Epidemiology of virulence-associated plasmids and outer membrane protein patterns within seven common *Salmonella* serotypes. Infect. Immun. 1985:48:175-82.
19. Nakamura M, Sato S, Ohya T, Suzuki S, Ikeda S. Possible relationship of a 36-megadalton *Salmonella* Enteritidis plasmid to virulence in mice. Infect. Immun. 1985:47:831-3.
20. Stubbs AD, Hickmann-Brenner FW, Cameron DN, Lee L, Farmer JJ. Subdivision of *Salmonella* Enteritidis phage type 8 by plasmid profiles and additional typing phages. 90th ASM Annual Meeting, Anaheim 1990:Abstract N°. C-355.

21. Brown DJ, Threlfall EJ, Hampton MD, Rowe B. Molecular characterization of plasmids in *Salmonella* Enteritidis phage types. Epidemiol. Infect. 1993:110:209-16.
22. Coyle FF, Palmer SR, Ribeiro CD, Jones HI, Howard AJ, Ward L, Rowe B. *Salmonella* Enteritidis phage type 4 infection : association with hens' eggs. Lancet 1988:2:1295-7.
23. Stanley J, Burnens AP, Threlfall EJ, Chowdry N, Goldsworthy M. Genetic relationships among strains of *Salmonella* Enteritidis in a national epidemic in Switzerland. Epidemiol. Infect. 1992:108:213-20.
24. Dorn CR, Silapanuntakul R, Angrick EJ, Shipman LD. Plasmid analysis of *Salmonella* Enteritidis isolated from human gastroenteritis cases and from epidemiologically associated poultry flocks. Epidemiol. Infect. 1993:111:239-43.
25. Asperilla MO, Smego Jr RA, Scott LK. Quinolone antibiotics in the treatment of Salmonella infections. Rev. Infect. Dis. 1990:12:873-89.
26. Helmuth R, Schroeter A. Molecular typing methods for *S.* Enteritidis . Int. J. Food Microbiol. 1994:21:69-77.
27. Powell NG, Threlfall EJ, Chart H, Rowe B. Subdivision of *Salmonella* Enteritidis PT4 by pulsed-field gel electrophoresis : potential for epidemiological surveillance. FEMS Microbiol. Lett. 1994:119:193-8.