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Phenotypic and genotypic typing of *Salmonella enterica* serovar Enteritidis isolates from poultry farms environments in Tunisia

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Abstract - *Salmonella enterica* serovar Enteritidis is one of the major *Salmonella* serovars which may cause animal infections and human salmonellosis. In this study, two hundred forty five samples (faeces, water and environmental swabs) were taken from eight poultry farms localized in different geographical areas of Tunisia. We found *Salmonella* serovar Enteritidis (16 strains), *Salmonella typhimirium* (2), *Salmonella scharzengrund* (2), and *Salmonella braenderup* (1). *Salmonella* Enteritidis strains were characterized by pulsed field gel electrophoresis (PFGE) analysis, plasmid analysis and antibiotic resistance profiles. *Xba*I PFGE analysis revealed two PFGE types and plasmid profiling identified four plasmid types. The majority of isolates were susceptible to all antibiotic tested. The combined use of phenotypic and genotypic methods indicates the spread of a particular *Salmonella* Enteritidis clone. This clone is highly related to a major world-wide clone identified in many other countries.

Key words: *Salmonella* Enteritidis; poultry farms; clonally related.

INTRODUCTION

Salmonella enterica serotype Enteritidis is one of the major *Salmonella* serovars which may cause human salmonellosis and animal infections in Tunisia and in many other countries (Medici *et al*., 2003; Liebana *et al*., 2004; Ben Aissa *et al*., 2007). The main sources of human infections are foods of animal origin, such as poultry, eggs, eggs products, milk, beef and pork (Suzuky, 1994; Boonmar *et al*., 1998). Previous studies have reported that poultry can become contaminated with *S. enteritidis* at the farm level. These organisms can be transferred to the production facility and can become a source of contamination for the final products (Bryan and Doyle, 1995; Hoover *et al*., 1997). Accordingly, it's interesting to have available phenotypic and genotypic markers that can be used in epidemiological investigations for *S. enteritidis* isolates from avian sources. Traditional methods for identifying and typing *S. enteritidis* include biotyping, serotyping, and phage typing and are not efficiently discriminative due to the instability of phenotypic traits (Thong *et al*., 1995; Liebana *et al*., 2001). However, DNA based methods such as plasmid analysis, IS*200* restriction fragment length polymorphism; ribotyping and pulsed field gel electrophoresis (PFGE) have proven to be efficient for genotypic characterization of many serovar Enteritidis strains (Millemann *et al*., 1995; Lin *et al*., 1996; Liebana *et al*., 2001; Pang *et al*., 2007). These methods have provided useful insight into evolution and epidemiological relationships between strains belonging to the same serotype and phage type (Thong *et al*., 1995; Lin *et al*., 1996; Nauerby *et al*., 2000).

The aim of this study was to investigate the occurrence of *Salmonella* spp. in the environments of poultry farms in Tunisia, as well as to characterize strains of serovar Enteritidis by plasmid analysis, PFGE and antibiotic sensitivity testing.

MATERIALS AND METHODS

Sample collection and bacterial isolates. Two hundred forty five samples (faeces, water and environmental swabs), taken at eight poultry farms localized in different geographical areas of Tunisia (Tunis, Ben Arous and Nabeul), were examined in this study. Samples were processed according to international norms for *Salmonella* ISO 6579, 2002 and Annex D, 2006.

Each sample was pre-enriched in Buffered Peptone Water at 37 °C for 18 h and then 0.1 ml of pre-enriched mixture was inoculated onto modified semi solid Rappaport Vassiliadis agar (MSRV) with 0.01% novobiocin and incubated at 41.5 °C for 24 h. Where opaque growth was seen on MSRV, a 1 µl loop from the edge of the opaque zone was inoculated onto Xylose Lysine Desoxycholate (XLD) agar. In addition, aliquots of 0.1 ml and 1

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ml of the pre-enrichment were respectively transferred in 10 ml of Rappaport Vassiliadis broth and 10 ml of Muller-Kauffmann broth, and incubated at 41.5 °C (Rappaport Vassiliadis) and at 37 °C (Muller-Kauffmann) for 18-24 h. Afterwards, broth cultures were streaked onto both XLD agar and Brilliant Green agar, and plates were incubated at 37 °C for 18-24 h. Plates were then examined to identify *Salmonella* presence. Colonies presumptively identified as *Salmonella* were confirmed by API 20E (Bio-Mérieux, Marcy l'Etoile, France).

***Salmonella* serotyping.** *Salmonella* isolates were serotyped at the National Centre for Enteropathogenic Bacteria at Pasteur Institute, Tunisia, according to the Kauffmann-White serotyping scheme with the use of antiserum (BioRad, France, Marnes la coquette). Serotyping was performed by the slide agglutination method to identify the somatic O antigen and flagellar H antigen.

Antimicrobial susceptibility testing. Antibiotic susceptibility was determined by the disk diffusion method on Mueller-Hinton agar as described by the Comité de l'Antibiogramme de la Société Française de Microbiologie (www.sfm.2007). The following antimicrobial agents were tested: amoxicillin, ticarcillin, cefoxitin, cefotaxime, ceftazidime, azetronam, gentamicin, kanamycin, streptomycin, tetracycline, chloramphenicol, colistin, nalidixic acid, ciprofloxacin, trimethoprim-sulfamethoxazol, and fosfomycin. *Escherichia coli* ATCC 25922 was used as the control.

Isolation of plasmid DNA. Plasmid DNA was isolated by the alkaline lysis method as described by Woodford *et al*. (1993) with slight modification for application to Gram negative bacteria. Briefly, all strains were grown overnight in 5 ml of Brain Heart Infusion broth at 37 °C, after which 2 ml of the cultures was harvested and washed with TE1 (100 mM Tris-HCl, 100 mM EDTA, pH 8) at 8000 *x g* at 4 °C for 10 min. Cells were suspended in 200 µl of TEG buffer (10 mM Tris-HCl, 1 mM EDTA, 50 mM of sucrose, pH 8.0) containing lysozyme (Sigma) 1 mg/ml and RNase (Sigma) 10 µg/ml, then incubated at 37 °C for 30 min. The cells were lysed by the addition of 300 µl of alkaline sodium dodecyl sulfate (0.2 M NaOH, 1% sodium dodecyl sulfate), and the samples were incubated at 56 °C for 1 h. After addition of 200 µl of 3 M potassium acetate (pH 4.8), the samples were left on ice for 20 min. The samples were centrifuged at 12000 *x g* for 5 min at 4 °C and the precipitated debris was removed by decanting the supernatant into a fresh tube. An equal volume (approximately 500 µl) of phenol-chloroform-isoamyl alcohol (25:24:1) was added, and samples were again spun for 5 min. The DNA from 400 µl of the supernatant was precipitated with 2 volumes of cold ethanol at -20 °C during overnight, and then centrifuged at 12000 *x g* at 4 °C for 20 min. The DNA pellets were dried at 37 °C for 1 h and suspended in 25 µl of TE (10 mM Tris-HCl, 1 mM EDTA, pH 8.0). DNA samples were analyzed by electrophoresis in 1X TBE buffer at 80 V for 2 h on 0.7% agarose gels. The approximate molecular sizes of plasmids were determined by comparison with known-size plasmids from *Escherichia coli* V 517 (53.7, 7.2, 5.4, 5, 4, 3, 2.6 and 2 kb).

Pulsed field gel electrophoresis (PFGE). Preparation and digestion of genomic DNA using *Xba*I restriction endonuclease (Amersham Bio-sciences, Orsay, France) were performed as described previously (Liebana *et al.*, 2001). Electrophoresis was performed with CHEF DR III system (BioRad laboratories, Richmond, CA) using the following conditions: pulse times ranged from 12 s to 40 s during 20 h at 6.0 V/cm at 14 °C. DNA banding patterns were compared visually using the criteria of Tenover *et al*. (1995).

RESULTS

Occurrence of *Salmonella* serovars

Twenty-one *Salmonella* isolates were collected from the eight poultry farms; sixteen were identified as *Salmonella enterica* serovar Enteritidis (*Salmonella* Enteritidis). These isolates were detected from samples originated from four out of the eight farms. The other non *Salmonella* Enteritidis isolates were:

TABLE 1 - Phenotypic and genotypic characteristics of *S*. Enteritidis isolates from poultry farms in Tunisia

Strain	Source	Farm/ Region[a]	Resistance profiles[b]	PFGE type	Plasmid profile (kb)	Fingerprint[c]
S1	Environment	A/BA	Am, S, N, Cip	X2	P2 (40)	F1: X2-P2
S2	Environment	A/BA	Am, T, N, Cip, F	X2	P4 (54)	F2: X2-P4
S3	Faeces	A/BA	Am, S, N, Cip, Sxt, F	X2	P1 (-)	F3: X2-P1
S4	Faeces	A/BA	Am, T, N, Cip, F	X2	P3 (2,3,4)	F4: X2-P3
S5	Environment	A/BA	Susceptible	X1	P4 (54)	F5: X1-P4
S6	Environment	B/BA	Ch	X1	P4 (54)	F5: X1-P4
S7	Environment	B/BA	Ch, S	X1	P4 (54)	F5: X1-P4
S8	Faeces	B/BA	S, T, Sxt, N	X1	P4 (54)	F5: X1-P4
S9	Faeces	B/BA	Susceptible	X1	P4 (54)	F5: X1-P4
S10	Faeces	B/BA	G	X1	P4 (54)	F5: X1-P4
S11	Faeces	C/Nab	Susceptible	X1	P4 (54)	F5: X1-P4
S12	Faeces	C/Nab	Susceptible	X1	P1 (-)	F6: X1-P1
S13	Faeces	C/Nab	Susceptible	X1	P4 (54)	F5: X1-P4
S14	Faeces	C/Nab	Susceptible	X1	P1 (-)	F6: X1-P1
S15	Faeces	C/Nab	Am, S, Cip	X1	P3 (2,3,4)	F7: X1-P3
S16	Environment	D/Nab	Susceptible	X1	P4 (54)	F5: X1-P4

[a] BA: Ben Arous region, Nab: Nabeul region.

[b] Am: amoxicillin, S: streptomycin, N: nalidixic acid, Cip: ciprofloxacin, T: tetracycline, F: fosfomycin, Sxt: trimethoprim-sulfamethoxazol, Ch: chlormaphenicol, G: gentamicin.

[c] Fingerprint is composed of PFGE type/plasmid profile.

Salmonella typhimurium (2 strains), *Salmonella scharzengrund* (2 strains) and *Salmonella braenderup* (1 strain).

Antibiotic susceptibility

Ten distinctive antimicrobial resistance profiles were identified among the 16 *Salmonella* Enteritidis isolates (Table 1). Seven strains were susceptible to all the antimicrobial agents tested. Nine *Salmonella* Enteritidis isolates exhibited resistance to at least one antimicrobial, from which six were resistant to three or more of tested antibiotics. *Salmonella* Enteritidis isolates displayed resistance most often to amoxicillin and nalidixic acid and were susceptible to third-generation cephalosporins (cefotaxime and ceftazidime) and azetronam.

Plasmid profile analysis

Plasmid analysis showed that 13 strains carried one to three plasmids with molecular sizes ranging from 2 to 54 kb (Table 1). Ten strains possessed only the 54-kb plasmid. Three strains were plasmid free. According to plasmid profiles, strains were classified into 4 clusters (P1 to P4).

PFGE patterns

*Xba*I-PFGE analysis of 16 isolates generated two pulsotypes containing 9 to 12 DNA restriction fragments. The most prevalent genotype was the pulsotype X1, encompassing 12 isolates, which were isolated from four farms. The second pulsotype X2 was detected in a unique farm (Farm A) and contained four isolates (Table 1, Fig. 1).

Combination of fingerprinting profiles

The use of various typing methods identified different groups of clones. Therefore, their results could be combined to obtain a detailed overall fingerprint type. A combination of results described above (plasmid profiles and PFGE patterns) allowed clear differentiation of seven groups (F1-F7), among which the combined type F5 (X1-P4) was the most frequent, found in nine strains. Of the combined types, five were present as a single isolate (Table 1).

DISCUSSION

In bacteria, epidemiological studies are based on many phenotypic and genotypic methods. The phenotypic methods are based on the comparison of phenotypic traits exhibited by different strains of the same species or serotype. These traits are generally showed as unstable and varied according to the environmental conditions as well as to the stability degree of the genetic pool of the strain. However, genotypic methods investigate strains at the genomic level, therefore were considered more reliable.

In our study, phenotypic typing of *Salmonella* isolates from the eight poultry farms showed that *S*. Enteritidis was the most prevalent serovar (16 out of 21 *Salmonella* strains). The other non *S.* Enteritidis isolates were: *S. typhimurium* (2), *S. scharzengrund* (2) and *S. braenderup* (1). This finding is concordant with a recent study published by Ben Aissa *et al*. (2007) confirming that *S*. Enteritidis is the most common serotype isolated from animal origin (especially poultry) in Tunisia during the last decade.

Antibiotic resistance profiles were employed in many previous studies as a typing method for several *Salmonella* serotypes (Moore *et al*., 2003). In this report, we found that antibiogram typing is not a very useful tool for subtyping *S. enteritidis* strains, because the majority of isolates were susceptible to all antibiotic tested. It is interesting to note that six of all isolates were multi-drug resistant. The presence of these strains may be due to therapeutic practices at the farm level (Olah *et al.*, 2004). However, an overview of our results supports in part the reported findings that most of the *S. enteritidis* strains are susceptible to a wide range of antimicrobial agents (Ling *et al*., 1998).

Analysis of plasmid profiles has proven to be efficient for typing several *Salmonella* serovars (Baggessen *et al*., 2000; Malorny *et al*., 2001; Michael *et al*., 2006). However, other workers (Martinetti and Altwegg, 1990; Morris *et al*., 1992, Liebana *et al*., 2001) have reported that this method presents serious limitations. Indeed, it has been shown that the vast majority of serovars Enteritidis strains carry just the serospecific virulence plasmid of ca.54 kb (Poppe *et al*., 1993; Nauerby *et al*., 2000; Rychlick

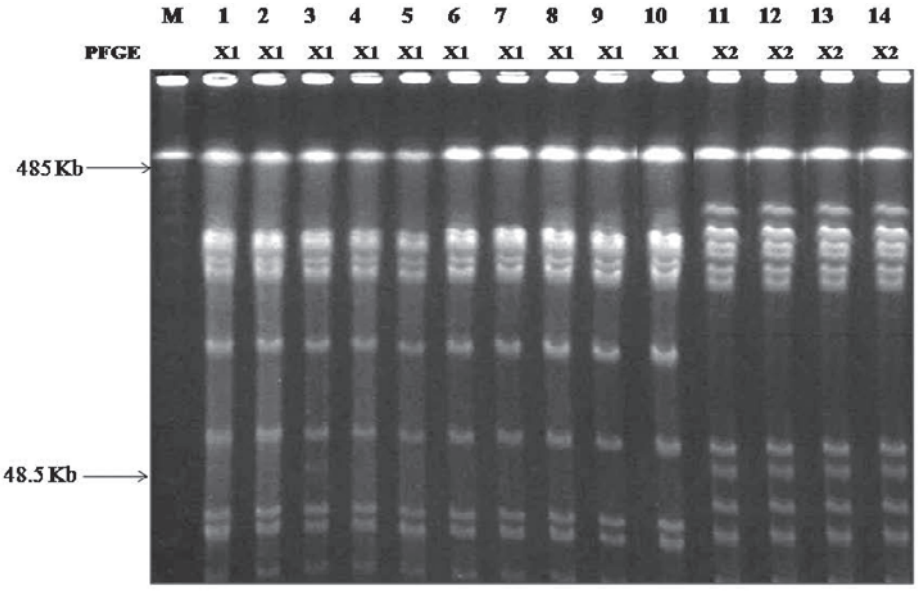

FIG. 1 - Pulsed field gel electrophoresis (PFGE) patterns of *XbaI*-digested genomic DNA of *Salmonella* Enteritidis strains obtained from Tunisian poultry farms. Lane M: lambda ladder marker for PFGE; lanes 1 to 10: strains S5 to S14, respectively; lanes 11 to 14: strains S1 to S4, respectively.

et al., 2006). Similarly, a single 54 kb plasmid was also common in our *S.* Enteritidis isolates and was detected in 12 out of 16 strains. Furthermore, it is recognised that extrachromosomal DNA is an unstable genetic marker owing to its ability to integrate in the chromosome or the spontaneous curing under unselective conditions (Liebana *et al*., 2001, 2002). Thus, analysis of plasmid profiles seems to be insufficient to identify subtypes of *S.* Enteritidis.

PFGE has been successfully used for molecular characterization of *S. enteritidis* strains in comparison with other typing techniques (Liebana *et al*., 2002, 2004; Moore *et al*., 2003; Dionisi *et al*., 2006). Many restriction endonucleases were evaluated for their usefulness in PFGE such as *Xba*I, *Spe*I, *Avr*II, and *Not*I (Tsen *et al*., 1999; Malorney *et al*., 2001). Among these, *Xba*I was the most discriminative for the PFGE analysis of *S*. Enteritidis strains. In our study two *Xba*I-PFGE types (pulsotypes X1 and X2) were found within sixty *S.* Enteritidis isolates. Those types differed by more than four DNA fragments. Thus, they were considered unrelated strains based on the previously published consensus guidelines for PFGE interpretation (Tenover *et al*., 1995). The majority of *S*. Enteritidis isolates (12/16) belonged to the *Xba*I-PFGE X1 genotype. Strains of this cluster were isolated from four farms located in different geographical areas far apart from each other. This finding is in agreement with a study reported by Liebana *et al*. (2001) in which the majority of *S*. *enteritidis* strains isolated from English poultry farms belonged to a single *Xba*I-PFGE pattern. Similarly, other workers (Thong *et al*., 1995; Pang *et al*., 2005, 2007) have reported that the genome of *S.* Enteritidis strains isolated from human and animal origins in areas geographically far apart from (Germany, Malaysia, Switzerland, Thailand) each other were found to be highly similar by PFGE. Unfortunately, we have not included some of the reported strains as control strains in our PFGE to evaluate their genetic relatedness with our strains. However, a meticulous comparison of PFGE pattern X1 with published PFGE photo of reported Malaysian and Swiss strains (Thong *et al*., 1995) showed a high genetic similarity of our PFGE pattern X1 and the predominant PFGE pattern B of those strains. We can speculate that this international dissemination of major clone could be indicative of movement of the strains between these countries and may be a reflection of increased global travel (Thong *et al*., 1995). Furthermore, this phenomenon may be indicates limited genetic rearrangement or recombination in the genome of *S*. Enteritidis during the evolution (Pang *et al*., 2007). It is important to note that instability of plasmid added to independent phenomenon of acquisition of exogenous genetic elements such as integrons would have contributed to the occurrence of closely related strains with different phenotypic patterns.

In conclusion, the combined uses of phenotypic and genotypic methods indicate the emergence of a particular *S. enteritidis* clone in different poultry farms in Tunisia. Many strains belonging to this clone were resistant to at least one antibiotic. These antimicrobial resistant strains can pose a significant health risk for humans. It would be interesting to investigate more strains from more regions to confirm the clonality of Tunisian isolates and to determine the extent of antimicrobial resistant strains.

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REFERENCES

Baggesen D.L., Sandvang D., Aarestrup F.M. (2000). Characterization of *Salmonella enterica* serovar Typhimurium DT104 isolated from Denmark and comparison with isolates from Europe and the United States. J. Clin. Microbiol., 38: 1581-1586.

Ben Aissa R., Al-Gallas N., Troudi H., Belhadj N., Belhadj A. (2007). Trends in *Salmonella enterica* serotypes isolated from human, food, animal, and environment in Tunisia, 1994-2004. J. Infect., 20: 1-16.

Boonmar S., Bangtrakulnonth A., Pornrunangwong S., Terajima J., Watanabe H., Kaneko K.I., Ogawa M. (1998). Epidemiological analysis of *Salmonella enteritidis* from humans and broiler chickens in Thailand by phage typing and pulsed field gel electrophoresis. J. Clin. Microbiol., 36: 971-974.

Bryan F.L., Doyle M.P. (1995). Health risks and consequences of *Salmonella* and *Campylobacter jejuni* in raw poultry. J. Food. Prot., 58: 326-344.

Comité de l'antibiogramme de la Société Française de Microbiologie. Les Recommandations du Comité de l'Antibiogramme (CA-SFM) (edition January 2007). http://www.sfm.asso.fr/publi/general.php?pa=1 [accessed 20 December 2007].

Dionisi A.M., Carattoli A., Luzzi I., Magistrali C., Pezzotti G. (2006). Molecular genotyping of *Salmonella enterica* Abortusovis by pulsed field gel electrophoresis. Vet. Microbiol., 116: 217-223.

Hoover N.J., Kenney P.B., Amick J.D., Hypes W.A. (1997). Perhaps sources of *Salmonella* colonization in Turkey production. Poultry Sci., 76: 1232-1238.

Liebana E., Clouting C., Garcia-Migura L., Clifton-Hadley F.A., Lindsay E., Threlfall E.J., Davies R.H. (2004). Multiple genetic typing of *Salmonella* Enteritidis phagetypes 4, 6, 7, 8 and 13a isolates from animals and humans in the UK. Vet. Microbiol., 100: 189-195.

Liebana E., Garcia-Migura L., Clouting C., Clifton-Hadley F.A., Lindsay E., Threlfall E.J., McDowell S.W.J, Davies R.H. (2002). Multiple Genetic Typing of *Salmonella enterica* serotype Typhimurium isolates of different phage types (DT104, U302, D T204 b, and DT49) from animals and humans in England, Wales and Northern Ireland. J. Clin. Microbiol., 40: 4450-4456.

Liebana E., Guns D., Garcia-Migura L., Woodward M.J., Clifton-Hadley F.A., Davies R.H. (2001). Molecular typing of *Salmonella* serotypes prevalent in animals in England: Assessment of Methodology. J. Clin. Microbiol., 39: 3609-3616.

Lin A.W., Miguel A.U., Barrett T.J., Goldsby R. A. (1996). Application of Random Amplified polymorphic DNA analysis to differentiate strains of *Salmonella* Enteritidis. J. Clin. Microbiol., 34: 870-876.

Ling J.M., Koo I.C., Kam K.M., Cheng A.F. (1998). Antimicrobial susceptibilities and molecular epidemiology of *Salmonella enterica* serotype Enteritidis isolated in Hong Kong from 1986 to 1996. J. Clin. Microbiol., 36: 1693-1699.

Malorny B., Schroeter A., Bunge C., Hoog B., Steinbeck A., Helmuth R. (2001). Evaluation of molecular typing methods for *Salmonella enterica* serovar Typhimurium DT104 isolated in Germany from healthy pigs. Vet. Res., 32: 119-129.

Martinetti G., Altwegg M. (1990). rRNA gene restriction patterns and plasmid analysis as a tool for typing *Salmonella* Enteritidis. Res. Microbiol., 141: 1151-1162.

Medici D., Croci L., Delibato E., Di Pasquale S., Filetici E., Totti L. (2003). Evaluation of DNA extraction methods for use in combination with SYBR Green I Real-Time PCR to detect *Salmonella enterica* Serotype Enteritidis in Poultry. App. Environ. Microbiol., 69: 3456-3461.

Michael G.B., Cardoso M., Rabsch W., Schawarz S. (2006). Phenotypic and genotypic differentiation of porcine *Salmonella enterica subsp*. *enterica* serovar Derby isolates. Vet. Microbiol., 118: 312-318.

Millemann Y., Lesage M.C., Chaslus-Dancla E., Lafont J.P. (1995). Value of plasmid profiling, ribotyping, and detection of IS*200* for tracing avian isolates of *Salmonella typhimurium* and *S. enteritidis*. J. Clin. Microbiol., 33: 173-179.

Moore J.E., Murray L., Fanning S., Cormican M., Daly M., Delappe N., Morgan B., Murphy PG. (2003). Comparison of phenotypic and genotypic characteristics of *Salmonella bredeney* associated with a poultry-related outbreak of gastroenteritis in Northern Ireland. J. Infect., 47: 33-39.

Morris J. G., Dwyer D.M., Hoge C.W., Stubbs A.D, Tilghamn D., Groves C., Israel E., Libonati J.P. (1992). Changing clonal patterns of *Salmonella* Enteritidis in Maryland: evaluation of strains isolated between 1985 and 1990. J. Clin. Microbiol., 30: 1301-1303.

Nauerby B., Pedersen K., Dietz H., Madsen M. (2000). Comparison of Danish Isolates of *Salmonella enterica* serovar Enteritidis PT9a and PT11 from Hedgehogs (*Erinaceus europaeus*) and humans by plasmid profiling and pulsed-field gel electrophoresis. J. Clin. Microbiol., 38: 3631-3635.

Olah P.A, Sherwood J.S., Elijah L.M., Dockter M.R., Doetkott C., Miller Z., Logue C.M. (2004). Comparison of antimicrobial resistance in *Salmonella* and *Campylobacter* isolated from turkeys in the Midwest USA. Food Microbiol., 21: 779-789.

Pang J.C., Chiu T.H., Choui C.S., Schroeter A., Tsen H.Y. (2005). Pulsed filed gel electrophoresis, plasmid profiles and phage types for the human isolates of *Salmonella enteritidis* obtained over 13 years in Taiwan. J. Appl. Microbiol., 99: 1472-1483.

Pang J.C., Chiu T.H., Helmuth R., Schroeter A., Guerra B., Tsen H.Y. (2007). A pulsed-field gel electrophoresis (PFGE) study that suggests a major world-wide clone of *Salmonella enterica* serovar Enteritidis. Int. J. Food. Microbiol., 116: 305-312.

Poppe C., McFadden K.A., Brouwer A.M., Demczuk W. (1993). Characterization of *Salmonella enteritidis* strains. Can. J. Vet. Res., 57: 176-184.

Rychlik D., Gregorova D., Hradecka G.H. (2006). Distribution and function of plasmids in *Salmonella enterica*. Vet. Microbiol., 112: 1-10.

Suzuky S. (1994). Pathogenicity of *Salmonella* Enteritidis in poultry. Int. J. Food. Micobiol., 21: 89-105.

Tenover F.C., Arbeit R.D., Goering R.V., Mickelsen P.A., Murray B.E., Persing D.H., Swaminathan B. (1995). Interpreting chromosomal DNA restriction patterns produced by pulsed-field gel electrophoresis: Criteria for bacterial strain typing. J. Clin. Microbiol., 33: 2233-2239.

Thong K.L., Ngeow Y.F., Altwegg M., Navaratnam P., Pang T. (1995). Molecular analysis of *Salmonella enteritidis* by pulsed-field gel electrophoresis and ribotyping. J. Clin. Microbiol., 33: 1070-1074.

Tsen H.-Y., Lin J.S., Hu H.H., Liu P.R., Wang T.K. (1999). Use of pulsed field gel electrophoresis as an epidemiological tool for analysis of sporadic associated strains of *Salmonella typhi* isolated in Taiwan. J. App. Microbiol., 86: 761-768.

Woodford N., Morrison D., Cookson B., George R.C. (1993). Comparison of high-level gentamicin resistant *Enterococcus faecium* isolates from different continents. Antimicrob. Agents. Chemother., 37: 681-684.