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Comparison of antimicrobial resistance patterns and phage types of *Salmonella* Typhimurium isolated from pigs, pork and humans in Belgium between 2001 and 2006

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ABSTRACT

Infections with non-typhoid *Salmonella* represent a major problem in industrialized countries.
The emergence and spread of antimicrobial-resistant pathogens, among them *Salmonella*, has become a serious health hazard worldwide. One of the most commonly isolated non-typhoid *Salmonella* serovars in pigs, pork and humans is *Salmonella* Typhimurium. In this study the comparison of the incidences of resistance to nine antimicrobials, resistance patterns and phage types between *S.* Typhimurium isolated from pigs (n = 581), pork (n = 255) and humans (n = 1870) in Belgium in the period 2001 to 2006 was performed. Resistance to the antimicrobials ampicillin, chloramphenicol, streptomycin, sulfonamides and tetracycline was frequently observed and varied between 23.5% and 83.1%. Resistance ranged from 15.6% to 20.7% for the combination trimethoprim–sulfonamides and from 3.4% to 5.8% for nalidixic acid. Resistance to the critical important antimicrobials cephalosporins and fluoroquinolones was found sporadically (≤1.2%). Resistance to the different antimicrobials was observed to be similar in *S.* Typhimurium isolates from the various origins. Twenty-seven antimicrobial resistance patterns representing in total 75.2%, 89.0% and 89.6% of the isolates from pigs, pork and humans respectively were found to be common among the three groups and 73 combinations antimicrobial resistance pattern/phage type were found to be common among pork and human isolates, representing 70.1% of the pork isolates and 51.0% of the human isolates. The high percentage of isolates that have a common resistance pattern, and in a less pronounced way a common combination phage type/resistance pattern, are in agreement with the hypothesis of transfer of antimicrobial resistant *Salmonella* from pigs via the consumption of pork to humans as one of the possible pathways. The most prevalent combination in Belgium within both the pork isolates (7.4%) and the human isolates (13.2%) was *S.* Typhimurium DT104 resistant to ampicillin, chloramphenicol, streptomycine, sulfonamides and tetracycline.

1. Introduction

Infections with non-typhoid *Salmonella* are a major problem in industrialized countries (EFSA, 2010; Mølbak et al., 1999; Scallan et al., 2011). In the United States, it is estimated that yearly 1 million illnesses, 19,000 hospitalizations and 400 deaths are due to non-typhoid *Salmonella* (Scallan et al., 2011). Most human *Salmonella* infections result in a self-limiting gastrointestinal illness

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characterized by diarrhea, fever and abdominal cramps that do not warrant antimicrobial therapy. However, these infections can also lead to life-threatening systemic infections that require targeted chemotherapy (Helms, Vastrup, Gerner-Smidt, & Mølbak, 2002; Mølbak, 2005). The human health consequences of antimicrobial drugs resistant *Salmonella* and other food-borne pathogens were reviewed by Mølbak (2005). In summary, the author lists five important consequences : i) reduced efficacy of first-line empirical antimicrobial treatment, ii) limited choice of treatment agents after diagnosis, iii) increased selection of resistant *Salmonella* after treatment of patients with antimicrobial drugs for other reasons (*e.g.* pneumonia), iv) horizontal transmission of resistance genes and v) increased virulence. Administration of antimicrobial drugs in human and veterinary medicine for the prevention and treatment of bacterial diseases creates a selection pressure that favors the survival of antimicrobial resistant strains (Aarestrup, 1999, 2005). Food producing animals may represent a reservoir of antimicrobial resistant bacteria. During slaughtering of the animals or during processing, meat can become contaminated with feces (containing antimicrobial resistant bacteria), whereas subsequently the food chain can act as a vector for the transfer of resistant bacteria and resistance genes to humans (Angulo, Nargund, & Chiller, 2004; Claycamp & Hooberman, 2004; Mølbak, 2004). Via this way, antimicrobial resistant bacteria may represent a food safety risk. Most *Salmonella* infections (90%–95%) result from the ingestion of contaminated foods, such as poultry meat, pork, beef, eggs, milk, seafood and fresh produce (EFSA, 2008; INVS, 2004; Scallan et al., 2011). However infection with *Salmonella* through direct contact with carrier animals on farms and in slaughterhouses can also occur (Fey et al., 2000; Hendriksen, Orsel, Wagenaar, Miko, & van Duijkeren, 2004). Worldwide, one of the most commonly isolated non-typhoid *Salmonella* serovars in pigs, pork and humans is *Salmonella* Typhimurium (Astorga Marquez et al., 2007; Boyen et al., 2008; Perugini et al., 2010).

This study reports on the comparison of the incidence of resistance, resistance patterns and phage types between *S.* Typhimurium isolated from pigs, pork and humans in Belgium in the period 2001 to 2006. Via this comparison, it is evaluated whether these phenotypic data are in agreement with the hypothesis of transfer of resistant *S.* Typhimurium from pigs to humans via consumption of pork.

2. Materials and methods

2.1. Isolation, serotyping and phage typing

Porcine *Salmonella enterica* subspecies *enterica* serovar Typhimurium isolated in Belgium between 2001 and 2006 from living pigs (n = 581) in the framework of the national *Salmonella* surveillance program and from pork (n = 255) during veterinary inspections by the Federal Agency for the Safety of the Food Chain (FASFC) were included in the study, next to human *S.* Typhimurium (n = 1870) provided by clinical laboratories from patients. The number of *S.* Typhimurium isolated per year and per origin is shown in Table 1. The pig strains were isolated from feces of either ill (*e.g.* diarrhea) or healthy animals. The pork isolates originated from samples taken at slaughter houses and meat cutting plants. Some of the pig and pork strains originated from the same pig farm, slaughterhouse or meat-cutting plant respectively. The human strains were mainly retrieved from feces (>95% of the human isolates), but also from blood, urine, wound or other body fluids from patients. Detection of *Salmonella* was performed according to the ISO 6579 standard. The serotype was determined by association of somatic O and flagellar H-antigenes according to the Kauffmann-White scheme (Kauffmann, 1996; SIPH, 2007). Phage typing was performed by the National Reference Centre for Phage Typing from the Scientific Institute of Public Health (WIV-ISP) according to the recommendations of the Public Health Laboratory Service from London (PHLS) (SIPH, 2007; Threlfall & Frost, 1990).

Table 1
Number of *S.* Typhimurium per year isolated from pigs (n = 581), pork (n = 255) and humans (n = 1870) between 2001 and 2006 in Belgium.

Year	Pigs (n)	Pork (n)	Human (n)
2001	96	57	308
2002	142	46	320
2003	18	26	314
2004	28	59	308
2005	139	56	304
2006	158	11	316

2.2. Antimicrobial susceptibility testing

For the pig isolates, antimicrobial susceptibility tests were performed by the Veterinary and Agrochemical Research Centre (CODA-CERVA) by means of the disk diffusion test using Rosco Neo-Sensitabs (Rosco, Roskilde, Denmark). Susceptibility tests of the human and pork isolates were executed by the Scientific Institute for Public Health (SIPH) using respectively the disk diffusion test according to Kirby Bauer and the E-test® (Bio-Mérieux). The susceptibilities to the following antimicrobial agents were determined for all *S.* Typhimurium isolates: ampicillin, cephalosporins (pig isolates: ceftiofur, pork isolates: ceftriaxone, human isolates: cefotaxime), chloramphenicol, fluoroquinolones (pig isolates: enrofloxacine, pork and human isolates: ciprofloxacine), nalidixic acid, streptomycin, sulfonamides, the combination trimethoprim–sulfonamides and tetracycline. The interpretation of the susceptibility tests occurred according to the directives described by the Clinical and Laboratory Standards Institute (CLSI), at that time National Committee on Clinical Laboratory Standards (NCCLS, 1999).

2.3. Statistical analysis

For statistical analysis, all isolates that showed intermediate resistance were grouped with the antimicrobial resistant strains. Resistance and phage type data from 2001 to 2006 for *S.* Typhimurium of each origin of isolation (pigs, pork and humans) were merged. Confidence intervals on incidences of resistance for each antimicrobial were calculated as “binomial exact confidence intervals” (Clopper & Pearson, 1934). The significance of the differences, for each antimicrobial, between incidences of resistance in the *S.* Typhimurium of three origins was calculated via a Poisson regression using Stata version 11.0 (StataCorp, 2007). The level of significance was set at 5% and the presence of extra-binomial variability was tested. Because of

Table 2
Incidence of antimicrobial resistance, with 95% confidence interval, in *S.* Typhimurium isolated from pigs (n = 581), pork (n = 255) and humans (n = 1870) between 2001 and 2006 in Belgium.

	Pig	Pork	Human
Ampicillin	48.5^{b} (44.4–52.7)	35.3^{a} (29.4–41.5)	50.3^{b} (48–52.6)
Chloramphenicol	26.3^{a} (22.8–30.1)	23.5^{a} (18.5–29.2)	28.8^{a} (26.7–30.9)
Streptomycin	42.3^{a} (38.3–46.5)	55.3^{b} (49–61.5)	83.1^{c} (81.3–84.8)
Sulfonamides	58.9^{b} (54.7–62.9)	42.3^{a} (43.1–55.7)	54^{b} (51.7–56.2)
Tetracycline	56.5^{a} (52.3–60.5)	45.9^{a} (39.6–52.2)	56.5^{a} (54.2–58.7)
Trimethoprim–Sulfonamides	20.7^{b} (17.4–24.2)	19.6^{ab} (14.9–25)	15.6^{a} (13.9–17.3)
Nalidixic acid	3.4^{a} (2.1–5.3)	3.9^{a} (1.9–7.1)	5.8^{a} (4.8–6.9)
Cephalosporins	1.2 (0.5–2.5)	0.4 (0–2.2)	0.6 (0.3–1.1)
Fluoroquinolones	1.2 (0.5–2.5)	0.4 (0–2.2)	0.5 (0.3–1)

$^{a, ab, b, c}$ Entries within the same row with the same letter are not significantly different (Bonferroni correction used).

Table 3
Distribution of antimicrobial resistance patterns in *S.* Typhimurium isolated from pigs (n = 581), pork (n = 255) and humans (n = 1870) between 2001 and 2006 in Belgium.

	Pigs no[a] (%)[b]	Pork no[a] (%)[b]	Human no[a] (%)[b]
A. Shared patterns in isolates from pigs, pork and humans			
Amp Chl Str Tet Su TSu	21 (3.6)	6 (2.4)	75 (4.0)
Amp Chl Tet Su TSu	10 (1.7)	1 (0.4)	1 (0.1)
Amp Str Tet Su TSu	4 (0.7)	9 (3.5)	79 (4.2)
Amp Chl Str Tet Su	53 (9.1)	30 (11.8)	391 (20.9)
Amp Chl Str Su	4 (0.7)	1 (0.4)	2 (0.1)
Amp Chl Str Tet	36 (6.2)	1 (0.4)	1 (0.1)
Amp Chl Tet Su	3 (0.5)	2 (0.8)	1 (0.1)
Amp Str Tet Su	18 (3.1)	5 (2.0)	106 (5.7)
Amp Tet Su TSu	5 (0.9)	1 (0.4)	4 (0.2)
Str Tet Su TSu	2 (0.3)	7 (2.7)	26 (1.4)
Tet Su TSu	14 (2.4)	6 (2.4)	2 (0.1)
Amp Str Su	2 (0.3)	6 (2.4)	41 (2.2)
Str Su TSu	4 (0.7)	5 (2.0)	24 (1.3)
Str Tet Su	9 (1.5)	12 (4.7)	30 (1.6)
Amp Tet Su	4 (0.7)	1 (0.4)	3 (0.2)
Su TSu	30 (5.2)	7 (2.7)	3 (0.2)
Amp Str	5 (0.9)	7 (2.7)	42 (2.2)
Amp Su	8 (1.4)	1 (0.4)	7 (0.4)
Amp Tet	5 (0.9)	5 (2.0)	12 (0.6)
Str Su	7 (1.2)	13 (5.1)	69 (3.7)
Str Tet	3 (0.5)	7 (2.7)	124 (6.6)
Amp	10 (1.7)	3 (1.2)	38 (2.0)
Nal	1 (0.2)	2 (0.8)	5 (0.3)
Str	3 (0.5)	17 (6.7)	371 (19.8)
Su	66 (11.4)	6 (2.4)	5 (0.3)
Tet	28 (4.8)	21 (8.2)	75 (4.0)
Susceptible to all tested antimicrobials	82 (14.1)	45 (17.6)	139 (7.4)
Subtotal	437 (75.2)	227 (89.0)	1676 (89.6)
B. Common patterns in isolates from pigs and pork			
Nal Su	1 (0.2)	1 (0.4)	0 (0.0)
Subtotal	1 (0.2)	1 (0.4)	0 (0.0)
C. Common patterns in isolates from pigs and humans			
Amp Chl Nal Str Su TSu	4 (0.7)	0 (0.0)	1 (0.1)
Amp Chl Nal Str Tet Su	3 (0.5)	0 (0.0)	19 (1.0)
Amp Chl Str Tet Su Cef	1 (0.2)	0 (0.0)	2 (0.1)
Chl Str Su TSu	1 (0.2)	0 (0.0)	5 (0.3)
Amp Nal Str Tet	1 (0.2)	0 (0.0)	1 (0.1)
Amp Su TSu	17 (2.9)	0 (0.0)	1 (0.1)
Nal Tet Su	2 (0.3)	0 (0.0)	1 (0.1)
Amp Chl Tet	2 (0.3)	0 (0.0)	2 (0.1)
Amp Str Tet	52 (9)	0 (0.0)	15 (0.8)
Chl Str	1 (0.2)	0 (0.0)	1 (0.1)
Tet Su	37 (6.4)	0 (0.0)	5 (0.3)
Subtotal	121 (20.8)	0 (0.0)	53 (2.8)
D. Common patterns in isolates from pork and humans			
Amp Chl Str Su TSu	0 (0.0)	1 (0.4)	7 (0.4)
Amp Str Su TSu	0 (0.0)	1 (0.4)	25 (1.3)
Nal Su TSu	0 (0.0)	1 (0.4)	2 (0.1)
Amp Chl Str	0 (0.0)	6 (2.4)	2 (0.1)
Chl Str Su	0 (0.0)	1 (0.4)	1 (0.1)
Str TSu	0 (0.0)	3 (1.2)	2 (0.1)
Nal Tet	0 (0.0)	2 (0.8)	4 (0.2)
Subtotal	0 (0.0)	15 (5.9)	43 (2.3)
E. Patterns only observed in isolates from pigs			
Amp Chl Str Tet Su TSu Fluo	2 (0.3)	0 (0.0)	0 (0.0)
Amp Chl Str Tet Su Fluo Cef	1 (0.2)	0 (0.0)	0 (0.0)
Amp Chl Nal Str Tet Su Cef	1 (0.2)	0 (0.0)	0 (0.0)
Amp Chl Nal Su TSu	1 (0.2)	0 (0.0)	0 (0.0)
Amp Chl Str Tet Su Fluo	1 (0.2)	0 (0.0)	0 (0.0)
Amp Chl Nal Str Tet	4 (0.7)	0 (0.0)	0 (0.0)
Amp Chl Nal Str Su	1 (0.2)	0 (0.0)	0 (0.0)
Tet Su TSu Fluo	1 (0.2)	0 (0.0)	0 (0.0)
Amp Chl Nal Str	1 (0.2)	0 (0.0)	0 (0.0)
Amp Chl Tet TSu	1 (0.2)	0 (0.0)	0 (0.0)
Amp Tet Fluo Cef	1 (0.2)	0 (0.0)	0 (0.0)
Su TSu Fluo	1 (0.2)	0 (0.0)	0 (0.0)
Chl Str Tet	1 (0.2)	0 (0.0)	0 (0.0)
Su Cef	3 (0.5)	0 (0.0)	0 (0.0)
Tet TSu	2 (0.3)	0 (0.0)	0 (0.0)
Subtotal	22 (3.8)	0 (0.0)	0 (0.0)
F. Patterns only observed in isolates from pork			
Amp Chl Str TSu	0 (0.0)	2 (0.8)	0 (0.0)
Amp Nal Str Cef	0 (0.0)	1 (0.4)	0 (0.0)
Chl Nal Fluo	0 (0.0)	1 (0.4)	0 (0.0)
Chl Nal Su	0 (0.0)	1 (0.4)	0 (0.0)
Chl Tet Su	0 (0.0)	1 (0.4)	0 (0.0)
Chl Nal	0 (0.0)	1 (0.4)	0 (0.0)
Chl	0 (0.0)	5 (2.0)	0 (0.0)
Subtotal	0 (0.0)	12 (4.7)	0 (0.0)
G. Patterns only observed in isolates from humans			
Amp Chl Nal Str Tet Su TSu Fluo Cef	0 (0.0)	0 (0.0)	1 (0.1)
Amp Chl Nal Str Tet Su TSu Fluo	0 (0.0)	0 (0.0)	5 (0.3)
Amp Chl Str Tet Su TSu Cef	0 (0.0)	0 (0.0)	1 (0.1)
Amp Chl Nal Str Tet Su TSu	0 (0.0)	0 (0.0)	5 (0.3)
Amp Chl Nal Str Tet Su Fluo	0 (0.0)	0 (0.0)	3 (0.2)
Amp Str Tet Su TSu Cef	0 (0.0)	0 (0.0)	1 (0.1)
Amp Chl Nal Tet Su TSu	0 (0.0)	0 (0.0)	1 (0.1)
Amp Nal Str Tet Su TSu	0 (0.0)	0 (0.0)	7 (0.4)
Amp Nal Str Su TSu	0 (0.0)	0 (0.0)	1 (0.1)
Chl Str Tet Su TSu	0 (0.0)	0 (0.0)	4 (0.2)
Amp Str Tet Fluo Cef	0 (0.0)	0 (0.0)	1 (0.1)
Amp Str Tet Su Cef	0 (0.0)	0 (0.0)	1 (0.1)
Amp Nal Str Tet Su	0 (0.0)	0 (0.0)	23 (1.2)
Nal Str Tet Su TSu	0 (0.0)	0 (0.0)	2 (0.1)
Amp Chl Nal Tet	0 (0.0)	0 (0.0)	1 (0.1)
Amp Chl Su TSu	0 (0.0)	0 (0.0)	1 (0.1)
Amp Nal Str Su	0 (0.0)	0 (0.0)	3 (0.2)
Amp Nal Tet Su	0 (0.0)	0 (0.0)	1 (0.1)
Amp Str Tet Cef	0 (0.0)	0 (0.0)	1 (0.1)
Chl Str Su Cef	0 (0.0)	0 (0.0)	1 (0.1)
Chl Str Tet Su	0 (0.0)	0 (0.0)	4 (0.2)
Amp Str Tet TSu	0 (0.0)	0 (0.0)	3 (0.2)
Nal Str Tet Su	0 (0.0)	0 (0.0)	3 (0.2)
Amp Tet Cef	0 (0.0)	0 (0.0)	1 (0.1)
Amp Nal Str	0 (0.0)	0 (0.0)	2 (0.1)
Nal Str Su	0 (0.0)	0 (0.0)	3 (0.2)
Str Tet TSu	0 (0.0)	0 (0.0)	2 (0.1)
Nal Str	0 (0.0)	0 (0.0)	9 (0.5)
Nal Str Tet	0 (0.0)	0 (0.0)	5 (0.3)
Str Cef	0 (0.0)	0 (0.0)	1 (0.1)
Tet Cef	0 (0.0)	0 (0.0)	1 (0.1)
Subtotal	0 (0.0)	0 (0.0)	98 (5.2)
Grand Total	581 (100)	255 (100)	1870 (100)

Amp: ampicillin; Cef: cephalosporins; Chl: chloramphenicol; Fluo: fluoroquinolones; Nal: nalidixic acid; Su: Sulfonamides; Str: streptomycine; Tet: tetracycline; TSu: combination trimethoprim–sulfonamides.

[a] Number of *S.* Typhimurium observed with a particular resistance pattern.

[b] Percentage of the total number of *S.* Typhimurium isolated from pigs, pork and humans with a particular resistance pattern.

the low incidence of resistance for cephalosporins and fluoroquinolones, these two antimicrobials were withdrawn from the analysis.

3. Results

3.1. Phage types

A clearly defined phage type was available for 1706 human isolates (out of 1870) and 204 pork isolates (out of 255). For the other isolates involved in the study, either no phage type was determined (pig isolates), an unusual phage type was obtained or the strains were not typable. Thirty-two and 112 different phage types were observed in the pork and the human *S.* Typhimurium isolates, respectively. The most prevalent phage types in the human isolates were DT104 (23.0%), DT120 (17.8%) and DT193 (10.6%). These were also the most

prevalent types found in pork: DT104 (17.6%), DT120 (10.5%) and DT193 (10.5%).

3.2. Phenotypic resistance to the individual antimicrobials

Resistance to the antimicrobials ampicillin, chloramphenicol, streptomycin, sulfonamides and tetracyclines was frequently observed among *S.* Typhimurium isolates from all origins and varied between 23.5% and 83.1% (Table 2). Resistance ranged from 15.6% to 20.7% for the combination trimethoprim–sulfonamides and from 3.4% to 5.8% for nalidixic acid. Resistance to cephalosporins and fluoroquinolones was found sporadically (≤1.2%). Via statistical analysis for each antimicrobial, the significance of the differences between the incidences of resistance in *S.* Typhimurium isolated from pigs, pork and human was calculated (Table 2). For chloramphenicol, tetracycline and nalidixic acid, no significant differences were found between the incidences of resistance in *S.* Typhimurium from different origins. A significant difference was observed for streptomycin between *S.* Typhimurium isolated from pigs and pork ($p = 0.035$), pork and humans ($p < 0.001$) and pigs and humans ($p < 0.001$) and also a significant difference for the combination trimethoprim–sulfonamides between *S.* Typhimurium isolated from pigs and humans ($p = 0.027$). Similar significant differences in resistance were observed for ampicillin and sulfonamides between *S.* Typhimurium isolated from pigs and pork ($p = 0.026$, $p = 0.005$, respectively) and between *S.* Typhimurium isolated from pork and humans ($p = 0.006$, $p = 0.030$, respectively).

3.3. Phenotypic antimicrobial resistance pattern

From the 581 pig isolates, 255 pork isolates and 1870 human isolates, respectively 85.9%, 82.4%, and 92.6% were resistant to at least one antimicrobial. On average 31.2%, 26.7%, and 43.9% of pig, pork and human isolates, respectively, were resistant to at least 4 antimicrobials (multi-resistant). One strain isolated from humans was resistant to all tested antimicrobials.

The distribution of antimicrobial resistance patterns is summarized in Table 3, showing that a total of 54, 42 and 76 different resistance patterns were observed among the pig, pork and human isolates, respectively. Taken together, they represent 99 different antimicrobial resistance patterns. Twenty-seven antimicrobial resistance patterns represented 75.2%, 89.0% and 89.6% of the pig, pork and human isolates respectively. Resistance to cephalosporins and fluoroquinolones was not observed in the 27 shared antimicrobial resistance patterns. One antimicrobial resistance pattern was found only in pig and pork isolates, eleven antimicrobial resistance patterns were found only in pig and in human isolates, while 7 were detected only in pork and human isolates. Fifteen, 7 and 31 antimicrobial resistance patterns were observed uniquely among the isolates from pigs, pork and humans, respectively. The antimicrobial resistance pattern "*Amp Chl Str Tet Su*", associated with the *Salmonella* genomic island found among *S.* Typhimurium DT104, was the second most frequently observed antimicrobial resistance pattern in *Salmonella* isolates from pork (11.8%) and humans (20.9%) while it was the third most frequent in pig isolates (9.1%).

3.4. Combination antimicrobial resistance pattern/phage type

A total of 129 and 465 different resistance pattern/phage type combinations were observed among the pork and human isolates, respectively. All together 521 different combinations were detected (Annex 1). Seventy-three combinations were found both in pork and human isolates, representing 70.1% of the pork isolates and 51.0% of the human isolates. The most prevalent combination in both the pork (7.4%) and human isolates (13.2%) was *S.* Typhimurium DT104 resistant to ampicillin, chloramphenicol, streptomycin, tetracycline and sulfonamides.

4. Discussion

Antimicrobial resistance is an increasing problem for human health (Alcaine, Warnick, & Wiedmann, 2007; ECDC/EMEA, 2009; Septimus & Kuper, 2009; Threlfall, Ward, Frost, & Willshaw, 2000). In this study, the observed incidences of resistance in *S.* Typhimurium from pigs, pork and humans show that the majority of the isolated strains (between 82.4% and 92.6%) are resistant to at least one antimicrobial and also that multi-resistance is ubiquitous in these bacterial populations in Belgium (between 26.7% and 43.9%). Despite that for most of the *Salmonella* infections antimicrobial treatment is not required, it can be life saving for invasive infections. Fluoroquinolones and extended spectrum cephalosporins are the preferred drugs-of-use for the treatment of invasive *Salmonella* infections in adults and children, respectively (Angulo, Johnson, Tauxe, & Cohen, 2000; Astorga Marquez et al., 2007; Helms et al., 2002). The resistance data on *S.* Typhimurium demonstrate that a low level of resistance (≤1.2%) to these critical antimicrobials is already observed in the *S.* Typhimurium population in Belgium during the studied period. It is therefore important that these critical important antimicrobials are carefully used both in human and veterinary medicine and also that their resistance is continually monitored. Besides the direct risk for human health of antimicrobial resistant *Salmonella*, these bacteria may also be a source of resistance genes that can be passed to human commensal and pathogenic bacteria and as such represent an indirect risk. Although several *in vitro* and *in vivo* studies have demonstrated horizontal gene transfer between food-bacteria and human gut bacteria (Dahl et al., 2007; Gay et al., 2006; Kruse & Sørum, 1994; Salyers, Moon, & Schlesinger, 2007; Smet et al., 2010), the public health importance of this transfer remains a subject of debate (Bester & Essack, 2010).

The link between antimicrobial resistant *S.* Typhimurium from pigs and humans was already demonstrated, in 1998, in Denmark when molecular epidemiology and analysis of data from patients from an outbreak with quinolone resistant *S.* Typhimurium DT104 (25 confirmed cases, 11 patients hospitalized and 2 died) indicated that pork was the vehicle of infection and that the primary source was a Danish Swine Herd (Mølbak et al., 1999). This relation was also described by Zhao et al. (2003) revealing that clonal spread of resistant *S.* Newport between food animals and humans occurred. Their conclusions were based on comparison of pulsed field gel electrophoresis patterns (PFGE), antimicrobial resistance phenotypes, the presence of the *bla*CMY gene and the class 1 integron. In the present study, the incidences of antimicrobial resistance from pig strains were compared with those from pork and humans. Via statistical analysis, it was shown that incidences of resistance in *S.* Typhimurium from pigs, pork and humans isolated in Belgium between the period 2001 and 2006, did not differ significantly for 13 out of 21 differences tested. In total, the incidences of resistance in *S.* Typhimurium from different origins did not differ more than a factor 2 (ampicillin, chloramphenicol, streptomycin, tetracycline, sulfonamides, nalidixic acid and the combination trimethoprim–sulfonamides) or 3 (cephalosporins and fluoroquinolones). Interpretation of these observations is not straightforward, but as was also suggested by Chen et al. (2010), who reported similar findings in a comparative study on antimicrobial resistance in *S.* Schwarzengrund isolated from chicken meat and humans in Taiwan, transmission via meat products might be a reason.

Angulo et al. (2000) argued that if veterinary use of antimicrobials is responsible for the development of antimicrobial resistant *Salmonella* in animals which may be transmitted to humans, the patterns of antimicrobial resistance observed among *Salmonella* isolates collected from healthy animals can be expected to resemble

those from humans. The present study contains a detailed comparison of the antimicrobial resistance patterns of pig, pork and human strains and also of the combinations antimicrobial resistance pattern/phage type of pork and human strains in Belgium during the period 2001–2006. The results demonstrate that both the majority of the resistance patterns (between 75.2% and 89.6%) and in a less pronounced way the combinations antimicrobial resistance pattern/phage type (between 51.0% and 70.1%, with pig strains not included) were shared among the different groups. These data on their own do not prove a causal relationship between antimicrobial resistant *Salmonella* in pig feces, pork and humans. However, when taking prior knowledge into account, such as the known pathway of accidental contamination of pork with feces from pig intestines in slaughterhouses (Borch, Nesbakken, & Christensen, 1996; Botteldoorn, Heyndrickx, Rijpens, Grijspeerdt, & Herman, 2003), the occurrence of (resistant) *S.* Typhimurium in pigs feces and pork (reviewed by Boyen et al., 2008) and epidemiological studies (Mølbak et al., 1999; Wegener & Baggesen, 1996), one can suggest that these data are in agreement with the hypothesis of transfer of resistant *Salmonella* from pigs via the consumption of pork to humans as one the possible pathways. Although no source attribution studies were published for the Belgian situation, studies in other countries using microbial subtyping or expert opinion estimate the attributed part of salmonellosis to pork between 6% and 25% (EFSA, 2008; Hoffmann, Fischbeck, Krupnick, & McWilliams, 2007; NFI, 2010; Van Pelt et al., 1999).

A particular case concerns *S.* Typhimurium DT104 with antimicrobial resistance pattern "*Amp Chl Str Tet Su*", that has been shown to be the source of many food-borne outbreaks. In the literature, contaminated pork (Mølbak et al., 1999), milk (Walker et al., 2000), ground beef (Dechet et al., 2006; Ethelberg et al., 2007), lettuce (Horby et al., 2003), dried anchovy (Ling, Goh, Wang, Neo, & Chua, 2002) and raw-milk cheese (Cody et al., 1999; Villar et al., 1999) among other foods have all been identified as food vehicles for outbreaks of multidrug-resistant *S.* Typhimurium DT104. In the present study, *S.* Typhimurium DT104 was the most prevalent strain among pork (7.4%) and human isolates (13.2%) in Belgium. These results suggest that since its first notification in the United Kingdom in 1980, multi-resistant *Salmonella* Typhimurium DT104 remains an important public health risk.

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