

Prevalence and Characteristics of *Salmonella* in Retail Poultry and Pork Meat in the Czech Republic in 2013–2014

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Abstract

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The EN ISO 6579/2002 guideline was used for the detection of *Salmonella* in retail poultry and pork meat in the Czech Republic in 2013 and 2014. The laboratory confirmed isolates were further typed (slide agglutination, phage typing, resistance to antimicrobial agents, PCR for the detection of selected genes encoding plasmid mediated quinolone resistance). Out of 176 poultry and 223 pork meat samples, 24 (13.6%) and 6 (2.7%) were positive for the detection of *Salmonella* spp., respectively. In *Salmonella* isolates from poultry, 14 serotypes were differentiated with *S. indiana*, *S. enteritidis* and *S. 6,7:-:1,5* being the most common serotypes. *S. typhimurium* and its monophasic variant *S. 4,[5],12:i:-* were predominant in pork meat. The overall resistance to one antimicrobial agent at least was high in both groups of isolates – 50% (poultry) and 71.4% (pork). No *Salmonella* isolate was confirmed to carry any of the selected PMQR genes. The study showed a higher prevalence of *Salmonella* in poultry, but pork meat also poses a risk to consumers.

Keywords: food; PCR; serotyping; phage typing; antimicrobial resistance

Salmonella is still among the most frequently reported zoonotic agents causing food-borne infections worldwide. Infections caused by non-typhoid *Salmonella* are mostly self-limiting. Nevertheless, 5% of patients will develop bacteraemia which requires antimicrobial treatment (PARRY & THRELFALL 2008). Although poultry meat and eggs have been described as the most common sources of *Salmonella* (ZHAO *et al.* 2001; EFSA 2013), pork meat is also responsible for a substantial part of infections. Moreover, some rather large-scale outbreaks connected with pork meat and products have been reported recently in Europe (BONE *et al.* 2010; GOSSNER *et al.* 2012). Different serotypes have been proved to be connected with poultry and pork production (EFSA 2013).

The use of typing methods enables the acquisition of surveillance data, detection of outbreaks

and identification of possible links to vehicles and sources of infection. Concern about various food-stuffs contaminated with *Salmonella* has received considerable attention because of the increased incidence of antimicrobial resistant strains. Whilst some serotypes such as the predominant serotype Enteritidis show moderate resistance or none at all, multidrug resistant strains are typical of some other serotypes (ÁLVAREZ-FERNÁNDEZ *et al.* 2012). Resistance to cephalosporins and quinolones has been most worrisome (GLENN *et al.* 2013; WONG *et al.* 2013; MOHAMED *et al.* 2014).

To date, no reports have been published about the comparison of *Salmonella* isolates from poultry and pork meat in the Czech Republic (CZ). The objectives of this study were to determine the prevalence of *Salmonella* spp. in poultry and pork meat from the

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retail market in the Czech Republic and to compare their characteristics including the level of antimicrobial resistance. Due to the fact that quinolones are the most commonly used antimicrobial agents in poultry flocks (WASYL *et al.* 2014) we also performed the polymerase chain reaction targeting the most common plasmid-mediated quinolone resistance genes (PMQR).

MATERIAL AND METHODS

Meat samples. Altogether, 176 randomly collected samples of fresh and frozen poultry meat (128 samples of chicken meat, 24 samples of hen meat, and 24 samples of turkey meat) and 223 samples of fresh pork meat were randomly purchased in retail outlets around the country in 2013 and 2014. The poultry meat samples originated from 6 countries – Czech Republic (159), Slovakia (7), Germany (4), Poland (3), France (2), and Brazil (1). Poultry meat originated from 15 Czech and 7 foreign producers (abattoirs). The pork meat samples were obtained in 5 countries – Czech Republic (182), Poland (29), Austria (9), Spain (2), and Denmark (1).

Sample processing and *Salmonella* confirmation. Samples of poultry and pork meat were collected from retail outlets and transported cooled into the laboratory where they were processed immediately. Poultry samples were processed according to EN ISO 6579/2002 (Microbiology of food and animal feeding stuffs – Horizontal method for detection of *Salmonella* spp.) – neck skin samples of 25 g were homogenised in 225 ml buffered peptone water and incubated at 37°C for 24 hours. One millilitre of each pre-enriched sample was transferred into a selective enrichment medium Mueller-Kauffmann Tetrathionate-Novobiocin Broth and 0.1 ml to Rappaport-Vassiliadis Broth (Oxoid, UK) and incubated at 37 and 42°C, respectively, for 24 hours. Finally, XLD (Oxoid, UK) and Rambach media (Merck, Germany) were inoculated and incubated. Pork and turkey meat was swabbed with 3M sponges (3M, USA) and further processed also according to EN ISO 6579/2002. All suspect colonies with typical growth characteristics were confirmed by the genus *Salmonella* specific PCR (OLSEN *et al.* 1995).

Serotyping of *Salmonella* isolates. *Salmonella* isolates were serotyped by the slide agglutination method with commercial antisera (Denka Seiken, Japan; BioRad, France) and the final antigenic

structure was obtained according to the Kauffmann-White-Le Minor scheme (GRIMONT & WEILL 2007).

Phage typing of *Salmonella* serotypes Enteritidis, Typhimurium and monophasic 4,[5],12:i:-. Phage typing was performed according to previously published protocols (ANDERSON *et al.* 1977; WARD *et al.* 1987) using HPA Colindale sets of phages.

Antimicrobial susceptibility testing. Antimicrobial susceptibility testing was performed by a disk diffusion method on Mueller-Hinton medium using CLSI guidelines (CLSI 2012). The spectrum of agents used was: AMP – ampicillin (10 µg), AMC – ampicillin/clavulanic acid (30 µg), CTX – cefotaxime (30 µg), CHL – chloramphenicol (30 µg), MEM – meropenem (10 µg), STR – streptomycin (10 µg), KAN – kanamycin (30 µg), GEN – gentamicin (10 µg), N – neomycin (30 µg), S3 – sulphonamides (300 µg), SXT – sulfamethoxazole/trimethoprim (25 µg), TMP – trimethoprim (5 µg), TET – tetracycline (30 µg), NAL – nalidixic acid (30 µg), CIP – ciprofloxacin (5 µg), ENR – enrofloxacin (5 µg), CT – colistin (10 µg), and ATM – aztreonam (30 µg). *Escherichia coli* CCM 3954 was used as the control strain.

PCR for the detection of PMQR. Polymerase Chain Reaction for the detection of *qnrA*, *qnrB*, *qnrS* genes (CATTOIR *et al.* 2007), *qepA* gene (YAMANE *et al.* 2008), and *aac(6′)-Ib-cr* (PARK *et al.* 2006) was also performed in 7 poultry isolates showing phenotypic resistance to nalidixic acid.

RESULTS

Altogether, 24 and 6 *Salmonella* positive samples out of 176 (13.6%) and 223 (2.7%) were detected in poultry and pork meat, respectively. Two different *Salmonella* strains were isolated from one pork meat sample, thus 31 *Salmonella* isolates were obtained in total. Four positive isolates were from countries other than the Czech Republic (Tables 1 and 2). The 24 *Salmonella* isolates from poultry meat originated from 9 producers (Table 1). In poultry meat 14 serotypes were determined with a maximum of 4 isolates of one serotype (Table 1). In pork meat 4 serotypes were detected with Typhimurium being the most frequent one (Table 2). Altogether, 9 isolates (3 Enteritidis, 4 Typhimurium, and 2 4,[5],12:i:-) were subtyped by phage typing and resulted in 8 different phage types (Tables 1 and 2). Antimicrobial susceptibility testing showed that 37.5% (9) and 57.1% (4) of *Salmonella* isolates from poultry and

Table 1. Characteristics of *Salmonella* spp. isolates from poultry meat at retail

Source	Country of origin	Producer	Serotype	Phage type	Antimicrobial resistance pattern
Chicken meat (cooled)	CZ	A	6,7:-:1,5	–	STR-S3-TET-NAL
	CZ	A	indiana	–	susceptible
	CZ	A	indiana	–	susceptible
	CZ	A	indiana	–	susceptible
Chicken meat (frozen)	CZ	B	agona	–	susceptible
Chicken meat (cooled)	CZ	B	infantis	–	TET-NAL
Hen meat (cooled)	CZ	C	enteritidis	8	susceptible
	CZ	C	enteritidis	8	susceptible
Hen meat (frozen)	CZ	D	6,7:-:1,5	–	STR-S3-TET-NAL
Chicken meat (frozen)	CZ	D	6,7:-:1,5	–	STR-S3-TET-NAL
Hen meat (frozen)	CZ	D	9,12:l,v:-	–	susceptible
Chicken meat (frozen)	CZ	D	agona	–	susceptible
	CZ	D	braenderup	–	STR-S3-TET-NAL
Hen meat (frozen)	CZ	D	derby	–	susceptible
Chicken meat (frozen)	CZ	D	infantis	–	STR-S3-TET-NAL
Hen meat (frozen)	CZ	D	ohio	–	susceptible
	CZ	D	ohio	–	susceptible
	CZ	D	tennessee	–	susceptible
	CZ	D	typhimurium	U302	susceptible
	CZ	E	kentucky	–	susceptible
Turkey meat (cooled)	CZ	F	newport	–	AMP-AMC-TET
Chicken meat (frozen)	France	G	indiana	–	susceptible
Turkey meat (frozen)	Poland	H	virchow	–	AMP-AMC-STR-NAL
Chicken meat (frozen)	Slovakia	I	enteritidis	3	NAL

pork meat, respectively, were resistant to one agent, at least. In the group of poultry isolates the most widespread resistances were to nalidixic acid (77.8% of the resistant isolates). In pork meat resistance to sulphonamides and tetracycline (100%) was most

often detected. Antimicrobial resistance patterns are summarised in Tables 1 and 2. No *Salmonella* strain carrying plasmid-mediated quinolone resistance genes *qnrA*, *qnrB*, *qnrS*, *qepA*, or *aac(6')-Ib-cr* was detected.

Table 2. Characteristics of *Salmonella* spp. isolates from pork meat at retail

Country of origin	Serotype	Phage type	Antimicrobial resistance pattern
CZ	9,12:l,v:-	–	susceptible
CZ	typhimurium	DT206	susceptible
CZ	4,[5],12:i:-	DT193	AMP-STR-S3-TET
Poland	4,[5],12:i:-	U	AMP-STR-S3-TET
CZ	agona	–	susceptible
CZ	typhimurium	DT208	STR-S3-TET-TMP
CZ	typhimurium	DT120	AMP-STR-KAN-N-S3-TET

DISCUSSION

The extent of retail meat contamination is an important parameter to monitor considering the impact on public health. *Salmonella* is a zoonotic agent usually transmitted via products of animal origin with poultry and pork meat being the most common sources (EFSA 2013). In our study, we detected 13.1% of poultry meat positive for *Salmonella*. The European Food Safety Authority reported 10% prevalence in broiler meat in the Czech retail market in 2011 (EFSA 2013). In our study the highest number of *Salmonella* isolates was obtained from hen meat (10 isolates out of 24 samples – 41.7%). Hen meat is not covered by the EFSA report, which might be the reason why the prevalence number reported to EFSA from the Czech Republic is lower than in our survey. Processing of hens at slaughterhouses usually differs from processing of broilers especially in the cooling procedure. Whereas hens are cooled in chilled water, broilers are cooled by chilled air, which is supposed to be the more hygienic method which protects the carcasses from further contamination at slaughterhouse. Broiler meat was positive in 9.4% of samples in our study, which is very close to the prevalence of 10% officially reported by EFSA in the retail market (EFSA 2013). Some non-European countries (Vietnam, Pakistan) reported the prevalence of *Salmonella* in retail chicken meat to be about 40% (SOOMRO *et al.* 2010; TA *et al.* 2014). The significantly lower number of positive samples in Europe might be caused by the implementation of the national control programmes in poultry flocks coordinated by the European Union and obligatory for all member states. However, a Greek study showed the prevalence as high as 39.5% (ZDRAGAS *et al.* 2012). Nonetheless, the reported prevalence rates in retail poultry meat are much higher than in poultry flocks (EFSA 2013). Turkey meat in our study showed a prevalence of 8.3% (2/24), which is in accordance with results from other European countries, where the highest prevalence at a retail level in 2010 was reported from Austria (14.6%) (EFSA 2012). Altogether, the positive samples originated from 9/22 producers (abattoirs), which means poultry samples from 13 producers were tested negative for *Salmonella* spp., 6 of them repeatedly. Positivity for *Salmonella* spp. detection at a particular producer ranged from 0 to 35.5% (producer D).

The occurrence of *Salmonella* in pork meat described in this study (2.7%) is in accordance with

data from other European countries. The prevalence of *Salmonella* in retail pork meat was reported to be from 0 to 5.2% in Europe in 2011. Nonetheless, no data at a retail level were officially reported from the Czech Republic (EFSA 2013). In Germany, the prevalence of *Salmonella* in pork meat at retail was 0.4% (SCHWAIGER *et al.* 2012).

In the long term, Enteritidis, Infantis, Virchow, and Hadar have been the most emergent serotypes in poultry flocks (ANTUNES *et al.* 2003; EFSA 2012, 2013). In our study, 14 different serotypes were described in 24 isolates from poultry meat. Only three and two isolates of the serotypes Enteritidis and Infantis were revealed, respectively. No isolate of the serotype Hadar was detected. Unexpectedly, some serotypes usually connected with pigs such as Agona, Derby, and Typhimurium (EFSA 2013; KEROUANTON *et al.* 2013) were found in poultry meat at retail, therefore the results indicate a possible contamination at various levels of processing. However, in an Australian study, *S. Typhimurium* was the second most common serotype detected in chicken neck skin at a retail level (FEARNLEY *et al.* 2011). *S. Indiana* found in four chicken meat samples is considered rare both in Czech Republic and in Europe. However, we registered an increased number of human cases caused by this serotype showing the same macrorestriction profile as the strains from chicken meat (MYŠKOVÁ *et al.* 2013). Also, a serotype defective in expressing the first flagellar phase 6,7:-:1,5 was detected in three samples of poultry meat in this study. The same serotype has recently been noted in human, pig, and poultry isolates in the Czech Republic (unpublished data). According to the Commission Regulation No. 1086/2011 (ANONYMOUS 2011), fresh poultry meat placed on the market during their shelf-life must not contain specifically the serotypes Enteritidis and Typhimurium including its monophasic variant 4,[5],12:i:-. Other serotypes are not specified, although they are capable of causing human illness equally. In our study, only 16.7% of positive isolates from fresh poultry meat were of the serotypes mentioned in the regulation.

In pork meat, 4 different serotypes were found. Although the majority of the serotypes were typical of pigs – Typhimurium, 4,[5],12:i:-, and Agona (PRENDERGAST *et al.* 2009), one sample was positive for two different strains with one of them being of a monophasic serotype 9,12:l,v.- first described in Israel (SECHTER & CAHAN 1984), which was also detected in poultry meat and is very rare in this

country and in the European Union. However, this rare serotype caused a local outbreak in this country in 2011 (MYŠKOVÁ *et al.* 2012). Our results suggest that both poultry and pork meat carried the same *Salmonella* serotype and therefore it might have been the source of both sporadic and outbreak related cases in the human population.

The variety of phage types detected in this survey also demonstrates that the contamination of retail meat occurs from different sources and at diverse levels. Phage types PT8 (Enteritidis), DT104 (Typhimurium), and DT193 (4,[5],12:i:-) are the predominant phage types in human isolates in the Czech Republic (MYŠKOVÁ *et al.* 2014). Interestingly, no isolate of the phage type DT104, which started to dominate in human infections caused by the serotype Typhimurium in the 1990's, was obtained in this study. However, the phage types described in this study are not considered rare and were detected also in other studies dealing with *Salmonella* isolates from retail meat (WHITE *et al.* 2001; PRENDERGAST *et al.* 2009).

We detected 37.5% of the isolates from poultry and 57.1% from pork meat being resistant to one or more antimicrobials. Overall resistance to one antimicrobial agent at least in foodstuffs of animal origin at a retail level is reported to be about 50–80% in some countries (THU HAO VAN *et al.* 2007; YANG *et al.* 2010) and have been rising (ÁLVAREZ-FERNÁNDEZ *et al.* 2012). The higher level of *Salmonella* resistance in pork meat isolates might be in connection with the fact that *S. Typhimurium* and the monophasic variant as serotypes connected with pig breeding are described to tend to be highly resistant (THRELFALL 2000; HOPKINS *et al.* 2010). Seven isolates resistant to quinolones (nalidixic acid) were detected in poultry meat isolates compared to zero in pork meat. This result might reflect the use of quinolone antibiotics (mainly enrofloxacin) in poultry flocks. A high prevalence of resistance to quinolones and fluoroquinolones in poultry has been reported from various countries (RAHMANI *et al.* 2013; LUKASZ *et al.* 2014). The resistance of salmonellae to quinolone is usually caused by mutations in gyrase and topoisomerase genes, however plasmid mediated resistance to this group of antibiotics has been of great concern recently mainly because quinolones and fluoroquinolones together with cephalosporins are used in the treatment of severe salmonella infections (STRAHILEVITZ *et al.* 2006). Moreover, an increased number of hospitalisations and invasive infections that are caused by emerging resistance of

foodborne pathogens has been confirmed (VERRAES *et al.* 2013). However, no isolate carrying any tested PMQR gene was confirmed in this study. Resistance patterns detected in this study in pork meat isolates are in accordance with a previous study conducted in the Czech Republic aimed at pigs (SISÁK *et al.* 2006). The overall high resistance to sulphonamides and tetracycline was revealed both in poultry and pork meat and is in accordance with other studies (CARRAMINANA *et al.* 2004). Tetracycline resistance is associated with several types of efflux pumps; therefore this resistance is widespread (HORIYAMA *et al.* 2011). Resistance to sulphonamides is caused either by mutations in chromosomal DNA or by an acquisition of *sul1*, *sul2*, or *sul3* genes mediated by transposons and plasmids which enable an extensive dissemination (KOZAK *et al.* 2009).

CONCLUSION

Despite the implementation of remedial programmes in poultry flocks in European countries, the prevalence of salmonellae in poultry meat at a retail level is still high. The variety of serotypes and phage types indicates contamination at different levels of processing. The serotypes found in fresh poultry meat at retail, which pose risk to consumers, do not correspond with those mentioned in the EU regulation. On the other hand, the prevalence of salmonellae in pork meat at a retail level is moderate and the serotypes also imply the pig origin only. Pork meat is likely to be a source of resistant strains; however, poultry meat seems to be a source of quinolone resistant strains, which may impede the treatment of severe salmonella infections in humans.

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