Antibiotic resistance of *Salmonella* spp. isolates from pigs in the Czech Republic

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ABSTRACT: A total of 126 *Salmonella* spp. isolates from pigs belonging to 13 serotypes (Typhimurium, Derby, Infantis, Enteritidis, Agona, Kaapstad, London, Montevideo, Bredeney, Give, Oritamerin, Schwarzengrund and Tennessee) were tested for sensitivity to 14 antibiotics. Resistance to 1–8 antibiotics was demonstrated in 64 isolates (59.8%), classified into seven serotypes with the most frequent being *Salmonella typhimurium* (*n* = 54). *S. typhimurium* strains were found to be the most resistant to streptomycin (91.5%), sulphonamides (88.1%), ampicillin (86.4%), tetracycline (84.7%) and chloramphenicol (83.0%), displaying the ACSSuT phenotype. In all strains of this phenotype (*n* = 27), the gene for integrase (*int1*) and resistance genes bla_{PSE-1}, *floR, aadA2, sul1* and *tetG* were detected by PCRs. In some of the strains, additional resistance to amoxycillin/clavulanic acid, sulphamethoxazole/trimethoprim, nalidixic acid and enrofloxacin was found.

Keywords: *Salmonella* serotypes; pig; *S. typhimurium*; phage type DT104; antibiotic; multiresistance; genes of resistance

Widespread occurrence of multiresistant clones of *Salmonella enterica* serotype Typhimurium, predominantly of the phage type DT104, in human populations, foodstuffs and in various species of domestic and wild animals represents a serious problem with regard to public health protection. Swine herds and pork are considered as principal S*almonella typhimurium* sources for humans, especially of its multidrug resistant phage types (Baggesen and Wegener, 1994; Baggesen and Aarestrup, 1998; Molbak et al., 1999). In these strains, R-type ACSSuT with chromosomally coded resistance to ampicillin, chloramphenicol, streptomycin, sulphonamides and tetracycline is predominantly detected (Threlfall et al., 1994; Glynn et al., 1998; Poppe et al., 1998; Akkina et al., 1999; Humphrey, 2001; Davis et al., 2002). In addition to the pentaresistant phenotype, resistance to trimethoprim and ciprofloxacin (Threlfall et al., 1997) was observed. Increasing occurrence of multiresistant strains results in antibiotic treatment failure in both humans and animals and transmission of antibiotic resistance to other bacteria (Cloeckaert and Schwarz, 2001).

Worldwide increase of *S. typhimurium* has been observed in pig herds since the mid 1990s. This serotype is, in most European countries, the dominant agent causing subclinical salmonellosis in pigs. The infected pigs shed *Salmonella* through feces during the whole fattening period, and the bacteria are then transmitted to slaughterhouses. Contamination of swine carcasses and the slaughter line with Salmonellae via the intestinal content and cut lymph nodes poses a potential health risk for humans (Swanenburg et al., 2001). In some European countries, *Salmonella* control

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programmes in slaughtered pigs and in pig herds have therefore been implemented (Nielsen et al., 2001; Van Winsen et al., 2001).

In the Czech Republic, pentaresistant ACSSuT *S. typhimurium* strains of phage type DT104 were for the first time isolated in humans in 1996 (Karpiskova et al., 1999). However, a retrospective study confirmed the occurrence of these multiresistant epidemic strains *S. typhimurium* in cattle in the Czech Republic as early as 1990 (Faldynova et al., 2003). Nowadays their incidence has reached more than half of all isolated strains of serotype Typhimurium, which accounts for approximately 3% of salmonellosis etiology in humans. Raw materials and foodstuffs of animal origin are the most common vehicles of infection (Sramova et al., 2003). A pentaresistent clone *S. typhimurium* ACSSuT phage type DT104 was also isolated from cecum, mesenteric lymph nodes, and carcass surfaces of slaughtered pigs originating from latently infected herds (Sisak et al., 2004).

This study was focused on the determination of antibiotic resistance among *Salmonella* spp. strains isolated from pigs, as well as a detailed characterisation of multiresistance in *S. typhimurium* strains.

MATERIAL AND METHODS

Bacterial strains, serotyping and phage-typing. A total of 126 isolates were collected over a period 2001–2004. These were isolated from cecum, mesenteric lymph nodes and carcass swabs of slaughtered pigs originating from 28 herds, or were isolated from feces and organs of pigs that had died in the herds under examination. Isolated *Salmonella* were typed by agglutination with monovalent O and H anti-*Salmonella* sera (BIO-RAD, France), and classified into serotypes according to Kauffman-White system. Phage types of *S. typhimurium* strains were determined using a set of 32 typing phages CPHA London, UK (Anderson et al., 1977).

Antibiotic susceptibility testing. All isolates were tested by the disc diffusion method based on NCCLS (2002) for sensitivity to the following 14 antibiotics (OXOID Ltd., UK): ampicillin (A, 10 μg), amoxycillin/clavulanic acid (AMC, 30 μg), apramycin (APR, 15 μg), colistin (CT, 10 μg), sulphamethoxazole/trimethoprim (SXT, 25 μg), cefotaxime (CTX, 30 μg), enrofloxacin (ENR, 5 μg), gentamicin (CN, 10 μg), neomycin (N, 30 μg), streptomycin (S, 10 μg), tetracycline (T, 30 μg), chloramphenicol (C, 30 μg), nalidixic acid (NA, 30 μg) and sulphonamides (Su, 300 μg). Inhibition zones of particular antibiotics were assessed after a 24 hours culture at 37°C on Mueller-Hinton agar (OXOID Ltd., UK)

PCR reactions. Genes encoding resistance to antibiotics in *S. typhimurium* strains of the phenotype ACSSuT were identified by specific PCR reactions, as described previously (Faldynova et al., 2003).

For detection of the integrase gene (*int1*) and genes encoding resistance to ampicillin (bla_{PSE-1}), chloramphenicol (*floR*), streptomycin (*aadA2*), sulfonamides (*sul1*), tetracycline (*tetG*), six gene specific PCR reactions were developed. The amplification products were detected by electrophoresis in 1.5% agarose gel, supplemented with ethidium bromide and visualized under UV light.

RESULTS

The results of the sensitivity assessment of *Salmonella* spp. serotypes isolated from pigs are shown in Table 1. A total of 126 field isolates were classified into the following 13 serotypes: Typhimurium (n = 59), Derby (n = 23), Infantis (n = 15), Enteritidis (n = 7), Agona (n = 6), Kaapstad (n = 4), London (n = 3), Montevideo (n = 3), Bredeney (n = 2), Give (n = 1), Oritamerin (n = 1), Schwarzengrund (n = 1) and Tennessee (n = 1).

Resistance to 1–8 antibiotics was demonstrated in 64 isolates belonging to serotypes Typhimurium, Derby, Infantis, Enteritidis, Agona, Schwarzengrund and Oritamerin. The isolates of the serotypes Kaapstad, London, Montevideo, Bredeney, Give and Tennessee were sensitive to all antibiotics used. The largest number of resistant strains were observed in the serotype Typhimurium (n = 54). The highest resistance was found in streptomycin (n = 54), sulphonamides (n = 52), ampicillin (n = 51), tetracycline (n = 50), chloramphenicol (n = 49). Resistance to amoxycillin/clavulanic acid (n = 11), sulphamethoxazole/tripetoprim (n = 8) and to fluoroquinolones – nalidixic acid (n = 7), enrofloxacin (n = 4) was low, and resistance to gentamicin (n = 1) and neomycin (n = 1) was sporadic. All strains were sensitive to apramycin, colistin and cefotaxime. *S. derby* isolates exhibited resistance types SSu (n = 1), CN SSu (n = 1) and CSSuT AMC (n = 1). *S. infantis* isolates showed resistance of the type A C (n = 1), C Su SXT (n = 1) and CSSuT

Table1. Assessment of sensitivity to antibiotics in *Salmonella* spp. serotypes isolated from pigs

Antibiotics	*S. typhimurium*	*S. derby*	*S. infantis*	*S. agona*	*S. enteritidis*	*S. kaapstad*	*S. london*	*S. montevideo*	*S. bredeney*	*S. give*	*S. schwarzengrund*	*S. oritamerin*	*S.tennessee*
	n = 59	*n* = 23	*n* = 15	*n* = 7	*n* = 6	*n* = 4	*n* = 3	*n* = 3	*n* = 2	*n* = 1	*n* = 1	*n* = 1	*n* = 1
A, 10 μg	**51**	0	**1**	**1**	0	0	0	0	0	0	0	0	0
AMC, 30 μg	**11**	**1**	0	0	0	0	0	0	0	0	0	0	0
APR, 15 μg	0	0	0	0	0	0	0	0	0	0	**1**	0	0
CT, 10 μg	0	0	0	0	0	0	0	0	0	0	0	0	0
SXT, 25 μg	**8**	0	**1**	0	0	0	0	0	0	0	**1**	0	0
CTX, 30 μg	0	0	0	0	0	0	0	0	0	0	0	0	0
ENR, 5 μg	**4**	0	0	0	0	0	0	0	0	0	**1**	0	0
CN, 10 μg	**1**	**1**	0	0	0	0	0	0	0	0	**1**	0	0
N, 30 μg	**1**	0	0	0	0	0	0	0	0	0	0	0	0
S, 10 μg	**54**	**3**	**1**	0	**1**	0	0	0	0	0	**1**	**1**	0
T, 30 μg	**50**	**1**	**1**	0	0	0	0	0	0	0	**1**	0	0
C, 30 μg	**49**	**1**	**3**	0	0	0	0	0	0	0	0	0	0
NA, 30 μg	**7**	0	0	0	0	0	0	0	0	0	**1**	0	0
Su, 300 μg	**52**	**3**	**2**	0	0	0	0	0	0	0	**1**	0	0
Number of resistant	**54**	**3**	**3**	**1**	**1**	0	0	0	0	0	**1**	**1**	0

A – ampicillin; AMC – amoxycillin/clavulanic acid, APR – apramycin, CT – colistin, SXT – sulphamethoxazole/trimethoprim, CTX – cefotaxime, ENR – enrofloxacin, CN – gentamycin, N – neomycin, S – streptomycin, T – tetracycline, C – chloramphenicol, NA – nalidixic acid, Su – sulphonamides

(*n* = 1). Resistance to ampicillin was only found in the isolate of *S. enteritidis*. Both isolates *S. agona* and *S. oritamerin* were resistant to streptomycin. In one isolate of *S. schwarzengrund*, multiresistance of the type APR SXT ENR CN S T NA was found.

Resistance to particular antibiotics of *S. typhimurium* strains isolated from pigs is shown in Figure 1. The highest percentage of resistance was observed in streptomycin (91.5%), sulphonamides (88.1%), ampicillin (86.4%), tetracycline (84.7%)

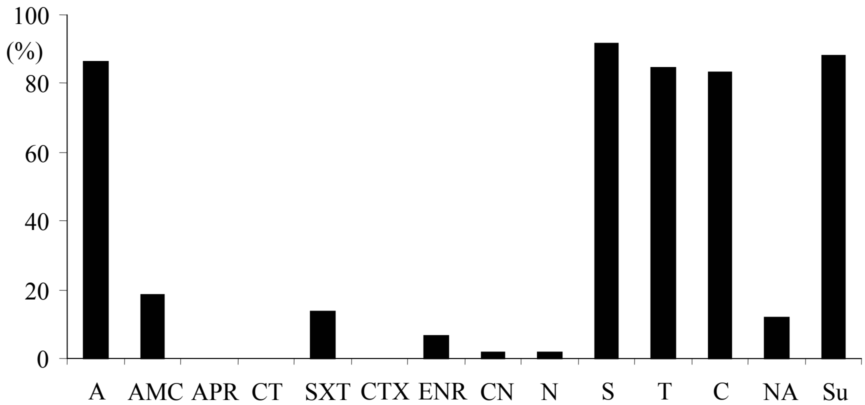

Figure 1. Percentage of resistant *S. typhimurium* strains isolated from pigs

Table 2. Occurrence of antibiotic resistance among phage types of *Salmonella typhimurium* strains isolated from pigs

Phage type	Antibiotic profile	Number of strains
DT104 (*n* = 51)	ACSSuT	27
	ACSSuT AMC	8
	ACSSuT SXT	3
	ACSSuT NA	3
	ACSSuT ENR NA	2
	ACSSuT AMC ENR NA	1
	ACSSuT SXT ENR NA	1
	ACSSuT AMC SXT	1
	ASSu SXT	1
	SSu	1
	sensitive	3
DT110 (*n* = 2)	ACSSuT AMC	1
	S	1
DT120 (*n* = 2)	ACSSuT AMC SXT	2
DT194 (*n* = 1)	ASSuT CN N	1
DT195 (*n* = 1)	S	1
U302 (*n* = 1)	sensitive	1
RDNC* (*n* = 1)	sensitive	1

*reacts but does not conform

and chloramphenicol (83.1%). A lower percentage of resistance was exhibited in the isolated strains towards amoxycillin/clavulanic acid (18.6%), sulphamethoxazole/trimethoprim (13.6%), nalidixic acid (11.9%) and enrofloxacin (6.8%). A very low percentage of resistance was found to gentamicin (1.7%) and neomycin (1.7%).

The assessment of antibiotic resistance among phage types of *S. typhimurium* isolates from pigs is shown in Table 2. The highest number of strains resistant to two, four, five, six, seven and eight antibiotics at the same time was observed in the prevalent phage type DT104 (*n* = 51). More than half of the strains (*n* = 27) of this phage type exhibited

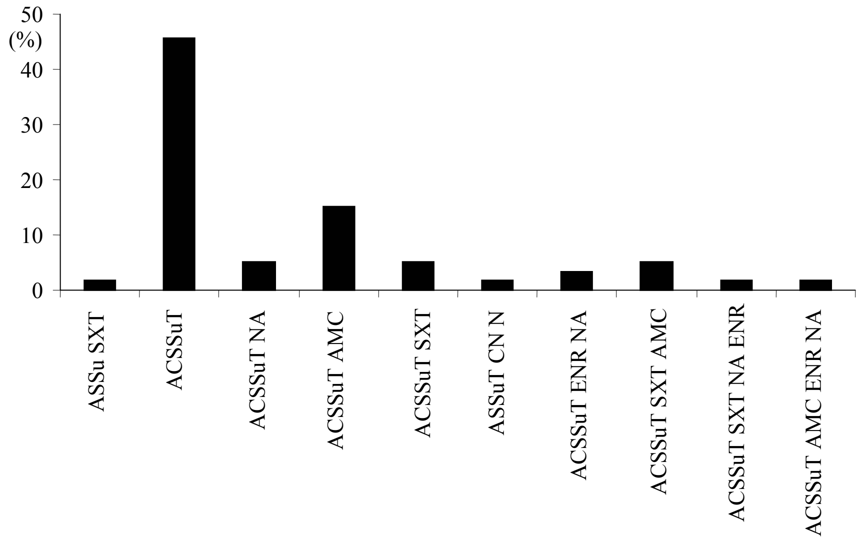

Figure 2. Prevalence of multiresistant phenotypes in *S. typhimurium* strains isolated from pigs

pentaresistant antibiotic profile ACSSuT. In addition to this typical pentaresistance, resistance to amoxycillin/clavulanic acid (n = 8), sulphamethoxazole/trimethoprim (n = 3) or nalidixic acid (n = 3) was observed. Furthermore, pentaresistance was associated with resistance ENR NA (n = 2), SXT AMC (n = 1), AMC ENR NA (n = 1) and SXT NA ENR (n = 1). Resistance to the type SSu and ASSu SXT was found in two isolates. Only three strains of this phage type were sensitive to all the antibiotics used. Multiresistance to the type ACSSuT AMC and monoresistance to streptomycin were detected in both strains of phage type DT110. Both strains of the phage type DT120 had the same antibiotic profile ACSSuT SXT AMC. Multiresistance ASSuT CN N was further detected in one isolate of phage type DT194, and resistance to streptomycin in one isolate of phage type DT195. Both strains of phage type U302 and RDNC were sensitive to all antibiotics used.

The prevalence of multiresistance to 4–8 antibiotics in *S. typhimurium* isolates from pigs is shown in Figure 2. The phenotype ACSSuT (45.8 %) was the most prevalent type of multiresistance, followed by ACSSuT AMC (13.6%), ACSSuT SXT (5.1%), ACSSuT NA (5.1%), ACSSuT SXT AMC (5.1%) and ACSSuT ENR NA (3.4%). The prevalence of multiresistance in ASSuT CN N, ACSSuT SXT NA ENR and ACSSuT AMC ENR NA was very low (1.7%).

The gene for integrase *int1* and resistance genes bla_{PSE-1}, *floR, aadA2, sul1* and *tetG* were detected in PCRs of all 27 strains of *S. typhimurium* isolated from pigs with the pentaresistant phenotype ACSSuT (Figure 3).

DISCUSSION

A compulsory programme of *Salmonella* monitoring in slaughtered pigs and swine herds, as well as surveillance of antibiotic resistance in causative agents of these zoonoses, has recently been introduced in EU member states because of a growing public health concern (Anonymous, 2003). Implementation of WHO global salm-surv external duality assurance system – EQUAS (Petersen et al., 2002) has also been an important initiative for improving the quality of *Salmonella* serotyping and antimicrobial susceptibility testing worldwide.

Our study confirmed a high incidence of antibiotic resistance among *Salmonella* spp. serotypes isolated from pigs in the Czech Republic (Table 1, Figure 1). The results are comparable with data from the USA and Canada, where the most resistant serotypes in pigs to the most commonly used an-

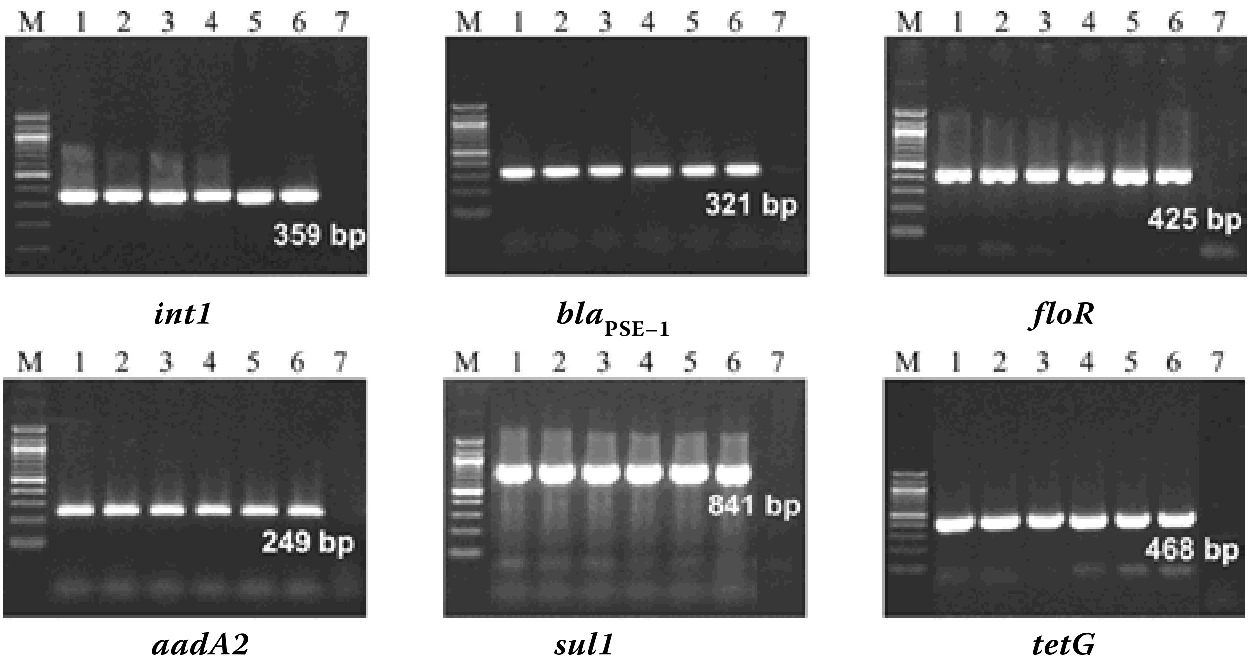

Figure 3. Gene-specific PCRs in six pentaresistant strains ACSSuT of *Salmonella typhimurium* isolated from pigs. Lane M – 100 bp ladder; lanes 1–6, multidrug resistant *S. typhimurium* field strains; lane 7, negative control

tibiotics were Typhimurium and Derby (Gebreyes et al., 2000; Farrington et al., 2001; Gebreyes and Altier, 2002; Rajic et al., 2004). The obtained data were also in accordance with data from our previous study, when we assessed the occurrence of *Salmonella* serotypes and their resistance to antibiotics in slaughtered pigs (Sisak et al., 2004). The fact that various types resistance were found in strains of serovars Derby, Infantis, Schwarzengrund, Enteritidis, Agona and Oritamerin confirms that the antibiotic resistance in isolates from pigs is not restricted to the serotype Typhimurium. In accordance with other authors, we confirmed correlation between high prevalence of antibiotic resistance and the serotype Typhimurium (Baggesen and Aarestrup, 1998; Molbak et al., 1999; Gebreyes et al., 2000; Gebreyes and Altier, 2002; Yang et al., 2002). Only results from Spain and Brazil differed slightly when reporting the most frequent multiresistances in the serotypes Anatum and Agona (Mateu et al., 2002; Oliveira et al., 2002). Unlike the findings of Gebreys et al. (2000) and Gebreys and Altier (2002), we found a relatively low frequency of resistance towards amoxycillin/clavulanic acids (18.6%). Very low resistance to gentamicin and neomycin (1.7%), and a sensitivity of all *S. typhimurium* isolates to apramycin, colistin and cefotaxime has also been recorded by other authors (Gebreyes et al., 2000; Rajic et al., 2004). A high resistance rate to the commonly used antibiotics indicates the existence of a link between a long-term use of the above antibiotics for medication in swine herds and high number of isolates exhibiting a similar resistance pattern (van der Wolf et al., 1999; Farrington et al., 2001).

The most important finding in our *S. typhimurium* isolates from pigs was the occurrence of resistance to sulphamethoxazole/trimethoprim (13.6%) and fluoriquinolones, which are the most efficient antibiotics used in humans. Fluoroquinolones have been widely used in the Czech Republic since the early 1990s for the medication of farm animals, predominantly in pigs and poultry. We found a significant relationship between the resistance to nalidixic acid (11.9%) and enrofloxacin (6.8%), in contrast to the data from Brazil. Oliveira et al. (2002) found in *Salmonella* isolates of different serotypes isolated from pigs resistant to nalidixic acid (narrow-spectrum quinolone), while resistance to ciprofloxacin and norfloxacin (broad-spectrum quinolone) was not observed. In the USA and Canada, resistance to ciprofloxacin was not observed either in *Salmonella* isolates from pigs, food animals and food (Gebreyes et al., 2004; Rajic et al., 2004; Johnson et al., 2005). However, the situation in Europe is different, as with human and animal *Salmonella* isolates, the resistance to quinolones is widely distributed and is still on the increase (Malorny et al., 1999; Molbak et al., 1999; Murphy et al., 2001).

We demonstrated that the most frequent multiresistance in *S. typhimurium* isolates from pigs is to ampicillin, chloramphenicol, streptomycin, sulphonamides, tetracycline – phenotype ACSSuT which was only found in the prevalent phage type DT104 (Table 2, Figure 2). Using PCR, we detected integrase gene *int1* and resistance genes bla_{PSE-1}, *floR*, *aadA2*, *sul1* and *tetG* (Figure 3) in all of these isolates. Our results correspond with findings from other countries where dissemination of this pentaresistant phenotype has been described not only in the phage type DT104 *S. typhimurium*, but also in other phage types isolated from pigs (Baggesen and Aarestrup, 1998; Gebreyes et al., 2000; Yang et al., 2002). Besides the typical pentaresistance ACSSuT, eight other multiresistance phenotypes have been found, most frequently in the phage type DT104, followed by DT110, DT120 and DT194. These results correspond with the data obtained by Poppe et al. (1998), Gebreys et al. (2000), Gebreys and Altier (2002). Our results therefore confirm the similarity of antibiotic resistance in *Salmonella* strains originating from different geographical regions (Baggesen and Aarestrup, 1998; Molbak et al., 1999; Gebreyes and Altier, 2002; Yang et al., 2002), and demonstrate the stability of this multiresistant clone among *S. typhimurium* isolates.

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