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ANALYSIS OF ANTIBIOTIC RESISTANCE AMONG SALMONELLA STRAINS ISOLATED FROM PIG IN VIETNAM

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SUMMARY

This study aimed to examine the susceptibility to 16 antimicrobial agents of a total of 102 Salmonella strains isolated from slaughter pig in Vietnam. No strain was found resistant to Amoxicillin clavulanic acid, Cefalexin, Ceftazidime, Cefotaxime, Ceftiofur nor Cefoxitin. 53% of the strains were resistant to at least one antibiotic, 48% of the strains were found resistant to Tetracycline, 39.2% to Sulfamide and 35.3% to Streptomycine. Multiresistance to six antibiotics (AM, TE, S, GM, SSS, TMP) was found for both S. Derby and S. Typhimurium.

KEY WORDS: Salmonella, S. Derby, S. Typimurium, Antibiotic, Antimicrobial resistance, slaughterhouse, pig, Vietnam.

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INTRODUCTION

Salmonellosis is one of the most frequent foodborne disease, representing an important public health problem worldwide (D'Aoust, J.Y., 1997). Foods most often associated with the transmision of Salmonella include those of animal origin, such as beef, pork and poultry (Jay, 2000).

Contamination can occur at multiple steps along the food chain, including production, processing, distribution, and retail marketing, and handling preparation (Shaohua et al., 2003). Infected pigs are usually asymptomatic carriers and may shed Salmonella through their feces during the whole fattening period. Contamination of swine carcasses and the slaughter line with Salmonella via the intestinal content and cut lymph nodes poses a potential health risk for humans (Swanenburg et al., 2001). In Vietnam, Salmonella has been described as an important issue in pig slaughterhouses with a very high carcass prevalence (Vo et al. 2006; Le Bas et al. 2008).

The use of antibiotics for animal disease treatment and prevention, as well as for growth promoting feed additives, has led to a serious increase in multiple antibiotic-resistant bacteria, including zoonotic pathogens, which can be transmitted to human via the food chain (Moelleing, 1998, Tollefson and Miller, 2000; WHO, 1997).

The aim of this study was to determine the incidence of antimicrobial resistance among Salmonella strains isolated from slaughter pig in Vietnam.

MATERIALS AND METHODS

Bacterial strains:

A total of 102 Salmonella strains representing 15 serotypes (Table 1) were analyzed for antimicrobial resistance. The strains were taken out of 125 Salmonella strains

isolated from pig feces, swab samples and water from a pig slaughterhouse in Hanoi, during a previous study in 2005 (Le Bas et al., 2006).

Antimicrobial susceptibility tests:

Antimicrobial susceptibility of the Salmonella strains was determined by the Disc diffusion method using Mueller Hinton agar in accordance with the standards of the CA-SFM, 2005 (an Antibiogram Committee created by the French Society for Microbiology). After incubating at 370C for 24h, the MacFaland machine was used to produce a standardized inoculum McF 0.5 (105 CFU/ml), then, the inoculum suspension was diluted to 107CFU/ml. Finally, the inoculum solution was spread onto a Mueller Hinton agar plate using a cotton swab.

Antimicrobial agents tested and their corresponding concentrations were as follows: Ampicillin (10 ⌠g), the combination of Amoxicillin and Clavulanic acid (20/10 ⌠g), Cefalexin (30 ⌠g), Chloramphenicol (30 ⌠g), Ceftazidime (30 ⌠g), Cefotaxime (30 ⌠g), Ceftiofur (30 ⌠g), Cefoxitin (30 ⌠g), Tetracycline (30IU), Streptomycin (10IU), Gentamycin (15 ⌠g/10IU), Nalidixic acid (30 ⌠g), Sulfamide (200 ⌠g), Trimetroprim (5 ⌠g), Enrofloxacin (5 ⌠g), Ciprofloxacin (5 ⌠g). E.coli 7624 was used as a control strain. Zone size diameters were red using an automated scanner camera and were interpreted according to the CA-SFM guidelines. Results were given in terms of inhibition diameter.

RESULTS

The results of antimicrobial resistance tests are presented in table 1. Strains were classified as susceptible, intermediate or resistant. No strain was found resistant to Amoxicillin plus clavulanic acid, Cefalexin, Ceftazidime, Cefotaxime, Ceftiofur and Cefoxitin. 53% of the strains were resistant to at least one antibiotic. The highest propostion of strains (49 out of 102) were resistant to Tetracycline (48%), followed by 39.2% (40 out of 102) with resistance to Sulfamides; 35.3% (36 out of 102) to Streptomycine; 28.4 % (29 out of 102) to Chloramphenicol. Some strains were resistant to Ampicillin (19.6%) and only 2 strains (2%) were resistant to Enrofloxacin and Ciprofloxacin.

Table 1: Antimicrobial resistance of Salmonella strains

Antimicrobial		*Salmonella strains (n=102)*					
Agents	*Abbreviation*	*Susceptible*		*Intermediate*		*Resistant*	
		n	*%*	*n*	*%*	*n*	*%*
Ampicillin	*AM*	*82*	*80.4*	*0*	*0*	*20*	*19.6*
Amoxicillin plus clavulanic acid	*AMC*	*96*	*94.1*	*6*	*5.88*	*0*	*0.0*
Cefalexin	*CN*	*101*	*99.0*	*1*	*0.98*	*0*	*0.0*
Chloramphenicol	*C*	*68*	*66.7*	*5*	*4.90*	*29*	*28.4*
Ceftazidime	*CAZ*	*102*	*100*	*0*	*0*	*0*	*0.0*
Cefotaxime	*CTX*	*102*	*100*	*0*	*0*	*0*	*0.0*
Ceftiofur	*XNL*	*102*	*100*	*0*	*0*	*0*	*0.0*
Cefoxitin	*FOX*	*89*	*87.3*	*13*	*12.7*	*0*	*0.0*
Tetracycline	*TE*	*49*	*48.0*	*5*	*4.90*	*49*	*48.0*
Streptomycin	*S*	*46*	*45.1*	*20*	*19.6*	*36*	*35.3*
Gentamycin	*GM*	*94*	*92.2*	*0*	*0*	*8*	*7.8*
Nalidixic acid	*NA*	*72*	*70.6*	*12*	*11.8*	*18*	*17.6*
Sulfamides	*SSS*	*57*	*55.9*	*5*	*4.90*	*40*	*39.2*
Trimetroprim	*TMP*	*74*	*72.5*	*17*	*16.7*	*11*	*10.8*
Enrofloxacin	*ENR*	*100*	*98.0*	*0*	*0*	*2*	*2.0*
Ciprofloxacin	*CIP*	*100*	*98.0*	*0*	*0.00*	*2*	*2.0*

In Table 2, the proportion of resistant strains is represented for each serotype. S.Derby and S. Typhimurium were resistant to eight kinds of antibiotics; S. Anatum, S. Enteritidis, S. Agona, S. Luberhurt and S. Kedougou were resistant to four kinds of antibiotics; S. London was resistant to three kinds of antibiotics; S. PolyII and S. Saint-Paul were resistant to two kinds of antibiotics and S. Newport was resistant to only one kind of antibiotic. On the other hand, S. Stanley, S. Weltervrenden, S. Dumfries and S. Weston were sensitive to all 16 antibiomicrobial agents.

Table 2: Distribution (%) of antimicrobial resistance of Salmonella serotypes

Serotypes	N*	Antimicrobial resistance** (%)															
		AM	AMC	CN	C	CAZ	CTX	XNL	FOX	TE	S	GM	NA	SSS	TMP	ENR	CIP
S.Derby	56	8.9	0	0	48	0	0	0	0	52	25	1.8	23	32	5.4	0	0
S.Typhimurium	11	73	0	0	9.1	0	0	0	0	73	73	64	27	73	64	0	0
S.Saint paul	11	0	0	0	0	0	0	0	0	0	9.1	0	0	9.1	0	0	0
S.Anatum	5	60	0	0	0	0	0	0	0	100	100	0	0	100	0	0	0
S.London	3	0	0	0	0	0	0	0	0	33	33	0	0	33	0	0	0
S.Enteritidis	3	100	0	0	0	0	0	0	0	100	100	0	0	100	0	0	0
S.Agona	2	0	0	0	0	0	0	0	0	50	0	0	100	0	0	100	100
S.Lamberhurt	2	50	0	0	0	0	0	0	0	100	100	0	0	100	0	0	0
S.Stanley	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
S.Weltervrenden	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
S.Dumfries	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
S.Kedougou	1	0	0	0	100	0	0	0	0	100	100	0	0	100	0	0	0
S.Newport	1	0	0	0	0	0	0	0	0	0	0	0	0	100	0	0	0
S.Weston	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
S.PolyII	1	0	0	0	0	0	0	0	0	0	100	0	0	0	100	0	0

*Number of strain isolates with this serotype
**AM= Ampicillin; AMC= Amoxicillin + clavulanic acid; CN= Cephalexin; C= Chloramphenicol; CAZ= Ceftazidime; CTX= Cefotaxime; XNL= Ceftiofur; FOX= Cefoxitin; TE= Tetracyclin; S= Streptomycine; GM= Gentamycine; NA= Nalidixic acid; SSS= Sulfamides; TMP= Trimetroprime; ENR= Enrofloxacine; CIP= Ciprofloxacin

In this study, most of the isolates were S. Derby (56 out of 102), followed by S. Typhimurium (11 out of 102). From a total of 11 S. Typhimurium isolates, three of them (3/11) were resistant to only one antimicrobial agent. The prevalence of multiresistance to 5-7 antibiotics in S. Typhimurium isolates from pigs is shown in table 4a. The most common pattern was resistance to AM, TE, S, GM, SSS and TMP (36.4%).

Table 3a: Resistance patterns of Salmonella Typhimurium

List	Antimicrobial resistance combination	S. typhimurium (n=11)	
		n	%
1	AM + C + TE + S + SSS	1	9.1
2	AM + TE + S + GM + SSS + TMP	4	36.4
3	AM + TE + S + GM + NA + SSS + TMP	3	27.3

In contrast, 17 out of 56 isolates of S. Derby were resistant to only one antimicrobial agent. The prevalence of multiresistance to 2-6 antibiotics in S. derby isolates from pigs is shown in table 3b. Most S. derby isolates were resistant to Chloramphenicol and Tetracyclin (23.2%), follow by Chloramphenicol, Tetracyclin and Nalidixic acid (21.4%). Multiresistance of S.derby to 6 antibiotics (AM, TE, S, GEN, SSS and TMP) were found (1.8%)

Table 3b: Resistance patterns of Salmonella Derby

List	Antimicrobial resistance combination	S. Derby (n=56)	
		n	%
1	AM + SSS	4	7.1
2	C + TE	13	23.2
3	S + SSS	6	10.7
4	C + TE + NA	12	21.4
5	S + SSS + TMP	2	3.6
6	C + TE + NA + S	1	1.8
7	AM + TE + S + GEN + SSS + TMP	1	1.8

DISCUSSION

Some studies in Vietnam report antimicrobial resistance among Salmonella, but very few for Salmonella isolated from animal (Trung et al., 2007; Van et al., 2007).

Our results confirm previous study on Salmonella resistance rate isolated from raw food: Van et al. found an average of 50.5% of resistant isolates against at least one antibiotic, close to the 53% we found. In accordance with the resistances described by salmonella isolated from pork by Van et al., our Salmonella isolates were mainly resistant against tetracycline, sulfonamides, streptomycin, chloramphenicol and ampicillin (Van et al. 2007). Trung et al. detected similar phenotypic resistant profiles by S. Typhi in Vietnam, with resistances against chloramphenicol, ampicillin, tetracycline and trimethotprim/sulfamids (Trung et al., 2007). These results confirm that antibiotic resistance is widespread in food from animal origin and in pork for commonly used antibiotics in Vietnam, i.e. mainly tetracycline, sulfonamides/trimethoprim, streptomycin, chloramphenicol and ampicillin. Similar resistance patterns observed by S. Typhi suggest that the same antibiotics may be commonly used for human treatments in Vietnam, and/or that resistance has already been transmitted through plasmid conjugation between food, animal pathogens and human pathogens, as suggested by Van et al. The high resistance of S. Enteritidis isolates against tetracycline has already been described in Malaysia (Son et al., 1995).

17.6% of our isolates were resistant and 11.8% intermediate to Nalidixic acid. In the USA and Canada, resistance to ciprofloxacin was not observed either in Salmonella isolates from pigs, food animals, or food (Gebreyes et al., 2004; Johnson et al., 2005). In Spain, 0.7% and 4.0% of Salmonella strains were found resistant with Ciprofloxacin and Enrofloxacin respectively (Mateu, et al., 2000). Resistance to quinolones is widely distributed and increasing in Europe (Molbak, 1999; Murphy, 2001), while the absence of resistance to most of the Quinolones in studies in Vietnam suggests that this antibiotic class is not commonly used yet, especially in livestock production.

In this study, the most resistant serotypes were S. Typhimurium and S. Derby, in accordance with data from other studies (Farrington et al., 2001; Gebreyes et al., 2000; Rajic et al., 2004; Sisak et al., 2004). The emergence of S. Typhimurium multi-drug resistance (including aminoglycosides, chloramphenicol, penicillins, sulfonamides, tetracycline, trimethroprim, cephalosporin and fluoroquinolone) has already been reported (Aarestrup et al., 1997; Dunne et al., 2000; Threlfall et al., 1998), especially for multiresistant epidemic strains of S. Typhimurium DT104 (Threlfall et al., 1998).

Increased use of antimicrobial agents in agriculture has been described as an important contributing factor for the emergence of multi-drug resistance strains in humans (Cohen and Tauxe, 1996; WHO, 1997).

In Vietnam, antibiotic use is not regulated

in both human and veterinary medicines. Futhermore, the farmers usually lack of knowledge about antibiotic use, like withdrawing periods, for instance. Various percentages of resistant strains have been described in other studies on slaughter pigs: 93.5% of the caecal isolates on slaughter pigs were found multi-drug resistant (Farrington et al., 2001), while Rajic et al. reported in Alberta 46.6% of the strains resistant against at least one of the 18 antibiotics tested, with an absence of resistance to cephalosporins and fluoroquniolones and a frequent resistance for ampicillin and tetracycline, comparable to our results (Rajic et al., 2004). But a higher proportion of our isolates, especially for S. Typhimurium were resistant to Trimethoprim, which is an important antimicrobial to treat human salmonellosis. In Czech, a study found 6 out of 27 Salmonella strains (22%) resistant to at least one antibiotic. The S. Typhimurium DT104 were all found pentaresistant (Sisak et al., 2004). In Spain, a study reported high resistance rates of 65 Salmonella strains to various antimicrobial agents, including tetracycline (84.6%), streptomycin (69.2%), neomycin (63.0%), sulfonamides (61.5%), ampicillin (53.8%), and amoxicillin (53.8%) (Astorga et al. 2007) and a recent investigation in Lao reported for 59 pig isolates resistance rates to tetracycline, streptomycin, ampicillin, sulfamethoxazole-trimethoprim, chloramphenicol, amoxicillin-clavulanic acid and nalidixic acid from 24%, 22%, 14%, 5%, 2%, 2% and 2%, respectively, globally lower than in our study (Boonmar et al. 2008).

Even though our results showed lower resistance rates than in certain studies in European countries, they indicate that the uncontrolled market and use of antibiotics in Vietnam has led to high rates of antibioresistance already of Salmonella strains by pig, including important antibiotics for human treatment. The possible transfer of resistance to human pathogens also represents a threat to public health. Therefore, this should alert veterinarian and public health authorities to increase the awareness in antibiotic usage. Surveillance network of antibiotic resistant food-borne pathogens should be implemented.

ACKNOLEDGEMENTS

We would like to thank the UBM Laboratory staff in AFSSA for their invaluable contribution. This study resulted from a partnership between NIVR and CIRAD in the frame of PRISE research consortium in Vietnam.

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OCCURRENCE OF LISTERIA MONOCYTOGENES IN RETAIL PORK MEAT IN VIETNAM

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ABSTRACT

The aim of this study was to evaluate the occurrence of L. monocytogenes in refrigerated minced pork meat sold in Hanoi retail shops and supermarkets. Among 134 samples, 34.3% were found positive for L. monocytogenes and 71.6% for Listeria spp. A significant difference was found between the supermarkets tested ($p<0.05$) and between the slaughterhouses supplying the retailers tested. Among the 47 isolates serotyped, 1/2b (n=42, 89.4%), 3b (n=3, 6.4%) and 4a (n=2, 4.3%) were identified. The results show that the incidence of L. monocytogenes in this study is high compared to most of the literature, although it is admitted that minced raw pork meat is known to be at risk and few studies reported similar incidence. Serotypes 1/2a and 1/2b are responsible of sporadic listeriosis cases, so that pork in Vietnam can represent a potential risk for sporadic cases, through cross-contaminations, and especially because some traditional Vietnamese products are made of raw pork meat. Our results confirmed that both slaughtering and processing/packaging are the most probable sources of contamination with L. monocytogenes. It should be further investigated which step is the main contributor to the final contamination of raw meat.

Keywords: Listeria monocytogenes; Pork; Meat; Retail; Vietnam

INTRODUCTION

In a context of high concern for food safety in Vietnam, studies on Listeria monocytogenes (L. monocytogenes) on food from animal origin are scarce. In 2006, 23% of chicken meat samples (National Institute of Hygiene and Health, personal communication) and 24% of Seafood samples have been found positive for L. monocytogenes in HoChiMinh city (Thu, 2007).

The aim of this study was to evaluate the occurrence of L. monocytogenes in refrigerated minced pork meat sold in Hanoi retail shops and supermarkets. L. monocytogenes is known for its ability to survive to refrigeration, which is even considered as a risk factor for its development in food products (Le Monnier and Leclercq, 2009), and chilling and cutting tends to increase the contamination of pork meat (Thévenot et al., 2006). Minced meat is more susceptible to be contaminated than unprocessed meat, since cross-contaminations can occur (Uyttendaele et al., 1999). In Hanoi, most of the meat is sold in markets without refrigeration, but the part of meat sold through the cold chain is increasing, in supermarkets or retail shops offering better storage conditions and advertising for better quality products. Hanoi's consumers usually consider that supermarkets' products are safer (Figuié et al., 2004).

MATERIAL AND METHODS

Sampling

Among a list, we selected at random 8 supermarkets and 7 retail shops in Hanoi. In total, 134 packed minced meat samples were taken at random between April and September 2008. During sampling, the origin of the meat, name and localisation of the abattoir, were recorded.

Detection method