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Prevalence and antimicrobial susceptibility of *Salmonella* isolated from Korean slaughter pigs

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Abstract

During the period of December 2000 to November 2001, a total of 419(10.9%) *Salmonella* was isolated from 3,850 slaughtered pig samples(2,732 lymph nodes and 1,118 caecal contents) in Korea. Three hundred and seventy(13.5%) and 49(4.4%) *Salmonella* were isolated from lymph nodes and caecal contents, respectively. In the isolation ratio of *Salmonella* spp originated from each class of season, summer(15.7%), autumn(11.5%), spring(10.0%) and winter(8.3%), in order. As the result of serotyping, B group(67.5%) were the most common, in order of C_1(14.6%), D_1(6.0%), C_2(4.1%) and E_1(3.3%). 32 serovars were found, the major serotypes were as follows; *S typhimurium*(21.7%), *S schwarzengrund*(16.0%), *S derby*(15.8%), *S typhimurium* variant *copenhagen*(9.8%), *S enteritidis*(6.0%) and *S mbandaka*(6.0%). All of the isolates were susceptible to norfloxacin and ofloxacin. The isolates were resistant in order of doxycycline(69.2%), tetracycline(68.7%), penicliin(54.9%) and streptomycin(52.5%).

Key words : *Salmonella*, Serotype, Antimicrobial susceptibility, Pigs

Introduction

Salmonella is a diverse group comprising over 2,000 known serotypes and a facultative intracellular organism, typically colonizing reptiles, birds and mammals[1)]. These bacteria are widely distributed in nature, gaining entry to almost all aspects of the human food chain. As with other food-borne pathogens, control human infection with *Salmonella* spp depends primarily on maintenance of a high standard of food hygiene by both the producers and the consumers. In the United States, nontyphoidal *Salmonella* caused an estimated 1.4 million cases of human illness annually, with 95% of these illnesses being contracted by food borne transmission. Furthermore, it was estimated

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that nontyphoidal *Salmonella* was responsible for 9.7% of all outbreaks of food-borne illness in the Unites States. In many countries, efforts are now being made to reduce the incidence of *Salmonella* carriage and to identify contamination sources at the farm. Pork has been reported to be associated with as much as 15% of human cases of salmonellosis. It was estimated that 70% of carcass contamination resulted from infected pigs and that a *Salmonella* spp positive pig was 3-4 times more likely to result in a *Salmonella* spp positive carcass. The prevalence of *Salmonella* positive pigs reported at different countries differed considerably, however, *S typhimurium* had been reported the predominant serotype in pigs[2-8].

Emergence of antibiotic-resistant bacteria has become an increasing problem all over the world. Researchers think that this problem is because of overuse or misuse of antibiotics in not only human medicine but also veterinary medicine. In animals, studies on the prevalence of antibiotic-resistant *Salmonella* are carried out to improve management and husbandry practices and to decrease threats to the health of the consumers[9]. In human patients ill with *Salmonella*, isolation of the organism, determination of its serotype and drug resistant pattern and clinical assessment is often carried out to decide whether treatment for salmonellosis is appropriate and which drug should be administrated. The genetic resistance mechanism in bacteria often resides in mobile elements such as transposons or plasmid, and resistant microorganisms can be disseminated via fecal flora to other animals, particularly in intensive farming units. Antibiotic resistance in *Salmonella*, *Escherichia coli*, *Shigella* and other genera of the *Enterobacteriaceae* is often mediated by plasmids, some of which are self-transmissible, whereas others may be cotransferred by .conjugative plasmids. Bacteria of the genus *Salmonella* are almost those most often found to carry plasmids that encode drug resistance (R plasmids)[10,11]. Use of a single antobiotic may cause an increase in the frequency of bacteria resistant to other antibiotics because the R plasmid may encode resistance to additional antibiotics. This situation highlights the importance of establishing a routinely antibiotic resistance surveillance system. One of the best targets to use as an indicator of resistance evolution is *Salmonella* spp because it is ubiquitous and causes zoonosis[8,12-14].

The aim of this study was to determine the prevalence of *Salmonella* in both mesenteric lymph nodes and cecal contents of slaughter pigs in Korea and to determine the antimicrobial resistance patterns for these isolates.

Materials and methods

Sample collection and *Salmonella* isolation procedure

During the period of December 2000 to November 2001, 2,732 mesenteric lymph nodes and 1,118 cecal contents of slaughter pigs were collected in Busan. Five grams of lymph nodes were submerged in boiling water for 10 sec to decontaminate the surface. And then they were macerated with 45 mℓ buffered peptone water(BPW) and incubated at 37℃ for 18 to 24 hrs. Aliquots of 0.1 mℓ of BPW were transferred to 10 mℓ of Rappaport-Vassiliadis R10 broth(Difco, Detroit, USA) and incubated at 42℃ for 18 to 24hrs. Portions of 5 g of caecal contents were gently stirred with 45 mℓ BPW and incubated at 37℃ for 18 to 24 hrs. One mℓ of BPW were transferred to 9 mℓ of selenite cystine broth (Difco) and incubated at 37℃ for 18 to 24hrs. One loop of enrichment broth was streaked onto the XLD agar (Difco) and SS agar(Difco) plates and then incubated at 37℃ for 18 to 24hrs. A total of five suspect colonies were examined with biochemical tests and VITEK GNI (bioMérieux, France) identification system[15].

Serotyping and antimicrobial susceptibility

Comfirmed *Salmonella* isolates were serotyped using *Salmonella* O and H antisera according to the procedures of the manufacturers(Difco, USA; Denka Seiken, Japan)[16,17]. All of the *Salmonella* isolates were tested for their antimicrobial susceptibility to 19 antimicrobial agents by the disk diffusion methods[18,19]. Antimicrobial susceptibility testing was conducted with the following antimicrobials (concentration): amikacin(30 μg), ampicillin(10 μg), amoxicillin(30 μg), carbenicillin(100 μg), cefazolin(30 μg), cephalothin(30 μg), chloramphenicol(30 μg), ciprofloxacin(5 μg), colistin(10 μg), doxycycline (30 μg), gentamicin(10 μg), kanamycin(30 μg), nalidixic acid(30 μg), neomycin(30 μg), norfloxacin(10 μg), ofloxacin(5 μg), streptomycin (10 μg), tetracycline(30 μg) and the combination of trimethoprim and sulfamethoxazole(23.75/1.25 μg). Strains resistant to four or more antimicrobial agents were considered multiresistant.

Results and Discussion

Although the prevalence of *Salmonella* spp in slaughter pigs might not accurately predict the prevalence of the other sample on individual farm, it might be possible to infer the prevalence of *Salmonella* among the farms. At present study, *Salmonella* spp were isolated from 13.5%(370 / 2,732) of mesenteric lymph nodes and 4.4%(49 / 1,118) of cecal contents in slaughter pigs(Table 1). A previous study conducted in Korea identified 85(23.1%) of the 367 mesenteric lymph nodes of slaughtered pigs as positive for *Salmonella.* In an American study, 50% of mesenteric lymph nodes collected from pig carcasses were positive for *Salmonella*[8]. This study demonstrated that the prevalence of *Salmonella* spp in mesenteric lymph nodes (13.5%) was higher than that in cecal contents(4.4%). Seasonal isolation rate of *Salmonella* was higher in summer(17.3% in mesentric lymph nodes; 7.6% in cecal contents) than in winter(12.9% in mesentric lymph nodes; 2.9% in cecal contents).

The commonly isolated serogroups were as follows; serogroup B, C1, D1, C2, E1, G2 and E3 in descending order of frequency. Among a total 11 serogroups isolated from slaughter pigs, serogroup B was the most frequently isolated(291 of 419 isolates; 69.5%). The top

Table 1. Results of one-year *Salmonella* survey in slaughter pigs

Samples	No(%) of *Salmonella* isolates/samples				Total
	Spring	Summer	Fall	Winter	
Mesenteric lymph nodes	152 / 1,293 (11.8)	91 / 526 (17.3)	70 / 472 (14.8)	57 / 441 (12.9)	370 / 2,372 (13.5)
Cecal contents	11 / 333 (3.2)	8 / 105 (7.6)	19 / 300 (6.3)	11 / 380 (2.9)	49 / 1,118 (4.4)

Table 2. Prevalence of *Salmonella* serogroups isolated from slaughter pigs

Rank	Serogroup(%) of *Salmonella* isolates		
	Mesenteric lymph nodes	Cecal contents	Total
1	B (66.0)	B (75.5)	B (67.5)
2	C_1 (14.9)	C_1 (12.2)	C_1 (14.6)
3	D_1 (6.2)	E_1 (6.1)	D_1 (6.0)
4	C_2 (4.6)	D_1 (4.1)	C_2 (4.1)
5	E_1 (3.0)	G_2 (2.0)	E_1 (3.3)
Other	6 other serogroups (4.9)	-	6 other serogroups (4.3)
No of total *Salmonella* isolates	370	49	419

Table 3. Distribution of serotypes of *Salmonella* isolates

Serotypes	No of *Salmonella* isolated from		Total (n=3,850)
	Mesenteric lymph nodes (n=2,732)	Cecal contents (n=1,118)	
Typhimurium	80	11	91
Schwarzengrund	62	5	67
Derby	53	13	66
Typhimurium variant copenhagen	39	2	41
Enteritidis	23	2	25
Mbandaka	23	2	25
Litchfield	13	0	13
Ardwick	12	0	12
Braenderup	9	0	9
Rissen	6	3	9
Agona	8	0	8
London	6	2	8
Kedougou	6	0	6
Ruiru	6	0	6
Bradenburg	1	3	4
Newport	4	0	4
Bredeney	0	3	3
Tennessee	2	1	3
Lomita	2	0	2
Thomasville	2	0	2
Anatum	1	0	1
Assinie	1	0	1
Cubana	0	1	1
Eimsbuettel	1	0	1
Havana	1	0	1
Kinshasa	1	0	1

Table 3. Continue

Serotypes	No of *Salmonella* isolated from		Total (n=3,850)
	Mesenteric lymph nodes (n=2,732)	Cecal contents (n=1,118)	
Langensalza	0	1	1
Montevideo	1	0	1
Orion	1	0	1
Raus	1	0	1
Senftenberg	1	0	1
Westhampton	1	0	1
Untypable	3	0	3*
Total	370	49	419

* Three *Salmonella* isolates were sero-grouped but not serotyped.

Table 4. Results of antimicrobial susceptibility test of *Salmonella* isolated from slaughter pigs

Antimicrobial	No of isolates with indicated antimicrobial susceptibility					
	Mesenteric lymph node isolates (n=370)			Cecal content isolates (n=49)		
	R	I	S	R	I	S
Amikacin	6	40	324	1	2	46
Ampicillin	59	0	311	5	0	44
Amoxicillin	0	2	368	0	0	49
Carbenicillin	67	244	59	9	31	9
Cefazolin	0	21	349	1	0	48
Cephalothin	1	48	321	1	2	46
Chloramphenicol	60	0	310	6	2	41
Ciprofloxacin	1	7	362	0	0	49
Colistin	0	6	364	0	1	48
Doxycycline	251	7	112	39	2	8
Gentamicin	1	3	366	0	0	49
Kanamycin	10	154	206	5	8	36
Nalidixic acid	51	14	305	4	1	44
Neomycin	9	202	159	4	19	26
Norfloxacin	0	0	370	0	0	49
Ofloxacin	0	0	370	0	0	49
Streptomycin	190	118	62	29	7	13
Trimethoprim/sulfamethoxazole	48	5	317	0	2	47
Tetracycline	250	3	117	38	2	9

R=Resistant, I=Intermediate, S=Susceptible three serogroups, B, C1 and D1, accounted for 88.1% of all isolates with known serogroups(Table 2).

From the 3,850 pig samples, 419 *Salmonella* were isolated and belonged to 31 serotypes. The most prevalent serotypes were *S typhimurium*, *S schwarzengrund*, *S derby*, *S typhimurium* variant *copenhagen*, *S enteritidis* and *S mbandaka* which were isolated from 21.7%, 16.0%, 15.8%, 9.8%, 6.0% and 6.0% of the slaughter pigs in Korea, respectively. Although *S enteritidis* and *S typhimurium* were the most frequent food-poisoning bacteria in Korea, *S enteritidis* was found only 6.0% in this study. However, *S typhimurium* was the most dominant serotype in Korean pigs and of alarming significance in public health. *S choleraesuis* was the prevalent serotype in some country and frequently isolated in the

Table 5. Antimicrobial resistance patterns of *Salmonella*(n = 315) according to its serotypes

Antimicrobials	No(%) of resistant strains					
	Typhimurium (n=91)	*Schwarzengrund* (n=67)	*Derby* (n=66)	*Typhimurium variant copenhagen* (n=41)	*Enteritidis* (n=25)	*Mbandaka* (n=25)
Aminoglycosides						
Amikacin	1 (1.1)	2 (3.0)	1 (1.5)	1 (2.4)	0	0
Gentamicin	0	0	0	0	0	2 (8.0)
Kanamycin	0	9 (13.4)	1 (1.5)	0	1 (4.0)	0
Neomycin	0	6 (9.0)	1 (1.5)	1 (2.4)	1 (4.0)	0
Streptomycin	84 (92.3)	64 (95.5)	22 (33.3)	40 (97.6)	2 (8.0)	0
β-lactams						
Amoxicillin	0	0	0	0	0	0
Ampicillin	41 (45.1)	12 (17.9)	1 (1.5)	4 (9.8)	0	1 (4.0)
Carbenicillin	43 (47.3)	13 (19.4)	4 (6.1)	6 (14.6)	0	2 (8.0)
Cefazolin	0	1	0	0	0	0
Cephalothin	1 (1.1)	1 (1.5)	0	0	0	0
Chloramphenicol	39 (42.9)	15 (22.4)	0	7 (17.1)	0	0
Colistin	0	0	0	0	0	0
Tetracyclines						
Doxycycline	88 (96.7)	66 (98.5)	56 (84.8)	41 (100)	8 (32.0)	2 (8.0)
Tetracycline	88 (96.7)	67 (100)	56 (84.8)	41 (100)	8 (32.0)	2 (8.0)
Quinolones						
Nalidixic acid	46 (50.5)	1 (1.5)	0	5 (12.2)	3 (12.0)	0
Ciprofloxacin	0	0	1 (1.5)	0	0	0
Norfloxacin	0	0	0	0	0	0
Ofloxacin	0	0	0	0	0	0
Trimrthoprim/ sulfamethoxazole	40 (44.4)	1 (1.5)	1 (1.5)	3 (7.3)	0	0

Table 6. Multidrug resistance patterns of the most frequently occurring *Salmonella* serotypes

Resistance patterns	No of resistant strains	Resistance patterns	No of resistant strains	Resistance patterns	No of resistant strains
S typhimurium		*S schwarzengrund*		*S derby*	
DT[1]	4	ST	1	DT	35
DST	30	CbDT	1	DST	15
CDST	1	DST	45	AnCbDT	1
CbDST	2	ADST	1	CbDST	2
DNaST	6	AnDST	2	CxDST	1
ACbDST	4	CDST	4	DKNT	1
DNaSxtST	2	CBDST	2	ACbDSSxtT	1
ACCbDST	1	ACCbDT	1		
CDNaSxtST	2	ACDSSxtT	1		
ACCbDNaSxtST	34	ACCbDKNT	1		
AAnCCbDNaSxtST	1	ACCbDKST	3		
ACCbCfDNaSxtST	1	ACCbDKNST	4		
		ACCbCfCzDKNNaST	1		
S typhimurium variant *copenhagen*		*S enteritidis*		*S mbandaka*	
DT	1	Na	2	Cb	1
DST	27	DT	7	G	2
AnDST	1	KNS	1	T	1
CDST	3	DNaST	1	AD	1
CbDST	2			CbDT	1
DNST	1				
DNaST	1				
CbDNaST	1				
ACCbDST	1				
ACCbDNaSxtST	3				

[1]Antibiotics : A; ampicillin, An; amikacin, C; chloramphenicol, Cb; carbenicillin, Cf; cephalothin, Cx; ciprofloxacin, Cz; cefazolin, D; doxycycline, G; gentamicin, K; kanamycin, N; neomycin, Na; nalidixic acid, Sxt; trimethoprim/sulfamethoxazole, S; streptomycin, T; tetracycline
[2]Number of isolates resistant to the antimicrobial agents

diseased pigs[8,13], but during the course of this study no *S choleraesuis* was recovered from Korean pigs(Table 3).

The numbers of the 419 *Salmonella* isolates that were susceptible, intermediate or resistant to the antimicrobial agents employed were shown in Table 4. The main concern is the existence of *Salmonella* isolates with a decreased susceptibility to fluoroquinolones, frist choice drugs to treat salmonellosis in humans. All the isolates were susceptible to the fluoroquinolone antibiotics, norfloxacin and ofloxacin. However, it was worth to note that 13% of isolates were resistant to nalidixic acid and 8 isolates were intermediate or resistant to ciprofloxacin. Decreasing ciprofloxacin susceptibility demonstrated the major problem of fluoroqiunolones resistance in *Salmonella* originated from animals.

Antimicrobial susceptibility tests showed that most isolates were susceptible to amoxicillin(417 of 419 isolates; 99.5%), gentamicin(415; 99.0%), colistin(412; 98.3%), ciprofloxacin(411; 98.1%), cefazolin(397; 94.7%), amikacin(370; 88.3%), cephalothin (367; 87.6%), the combination of trimethoprim and sulfamethoxazole(364; 86.9%), ampicillin (355; 84.7%) and nalidixic acid(349; 83.3%). Antimicrobials to which the highest percentage of isolates were resistant were doxycycline(69.2%), tetracycline(68.7%), penicillin(54.9%) and streptomycin(52.3%). Although it was prohibited from the use of chloramphenicol in food animals, 66 isolates (15.8%) were resistant to chloramphenicol and resulting presumably from the spread of multiresistant *Salmonella* strains. The isolates were high resistant to streptomycin (51.4%), being compared to other aminoglycosides. Maybe, it was accorded to the consumption of the aminoglycosides.

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