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Assessment of Bacterial Contamination of Raw Meats Sold in Korea, 2007

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2007년 한국에서 판매된 식육의 미생물 오염도 평가

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요 약

본 연구는 인간의 건강과 식품의 오염을 예방하기 위한 유용한 자료를 얻기 위하여 육류(소고기, 닭고기, 돼지고기)로부터 분리된 staphylococci를 가지고 한국에서 광범위하게 사용되는 6가지 항생제와 daptomycin, linezolid, quinupristin/dalfopristin and tigecycline과 같은 4가지의 신항생제에 대한 항생제 감수성 시험을 수행하였다. 이에 더하여 육류의 세균 오염실태를 조사하기 위하여 총 세균수와 대장균수를 측정하였다. 그 결과 147개의 육류 샘플 중 48%의 샘플에서 일반세균수의 기준에 부적합한 결과가 나타났으며, 대장균수에 대해서는 31%의 샘플에서 기준에 부적합한 결과가 나타났다. 또한 육류의 91%에서 stapylococci가 검출되었으며 이들 staphylococci는 Coagulase Negative Staphylococci (CNS)와 *S. aureus*로 동정되었다. 분리된 staphylococci의 5.4%가 methicillin (oxacillin)에 내성을 나타내었고, 특히 mupirocin (MIC_{90}, >12 μg/mL)과 gentamicin (MIC_{90}, 64 μg/mL)에 높은 내성을 나타내었다. 그러나 신항생제에 대해서는 methicillin 내성 staphylococci를 포함한 모든 staphylococci 분리균주가 높은 감수성을 나타내었다. 결론적으로 본 연구의 결과들은 육류가 소비자들의 건강에 중요한 위험요소로 작용할 수 있음을 보여주고 있다.

Key words : antimicrobial resistance, minimum inhibitory concentration (MIC), microbiological quality, raw meats, staphylococci

INTRODUCTION

In recent decades, antimicrobial resistance and reduced sensitivity in bacteria have become a major public health problem for many countries (Centers for Disease Control and Prevention, 1993; Diekema and Jones, 2001; Kim *et al.*, 2001; Jones, 2003).

The extended use and misuse of antibiotics in agriculture, stock-farming and the treatment of human diseases has lead to a rapid increase in bacteria which are resistant to antimicrobial agents. Food is an excellent source of pathogenic microorganisms among

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the general population and particularly in immuno-compromised individuals; therefore the consumption of contaminated food can facilitate the transfer of antibiotic-resistant bacteria to the intestinal tract of consumers very efficiently. The transfer of resistance genes between non-pathogenic bacteria and pathogenic or opportunist bacteria is known to occur within the intestine (Valsangiacomo *et al.*, 2000; Jones *et al.*, 2002; Kitai *et al.*, 2005; Yucel *et al.*, 2005).

The consumption of raw meats has increased from 22.5 kg to 31.9 kg per individual in 1989 and 2005 respectively, in Korea (Agricultural and Forestry Statistics, 2005). Interestingly, the incidences of food poisoning have also increased, from 55 cases to 93 cases in 1995 and 2001, respectively. Raw meats and meat products were exhibited as a major cause (24.8%) according to Hwang *et al.* (2004). Staphylococci are one of the most important causes of food-borne illness and are frequently found in raw meats for human consumption. Most infections are caused by coagulase-positive staphylococci, including *Staphylococcus aureus* (Jordan, 1996; Lowy, 1998).

Antimicrobial agents are existed and widely used in the treatment and control of staphylococcal infections. However, when microorganisms develop resistance to commonly used treatment methods, serious complications can result for the very young, geriatric, and the immuno-compromised members of society. The possibility of transmission of methicillin-resistant. *S. aureus* (MRSA) through food is very high in immuno-compromised individuals. When a few cells of *S. aureus* enter an immuno-competent organism, they are destroyed by the gastric juices. Thus the entry of *S. aureus* in an immuno-compromised patient can result in the release of these bacteria into the circulatory system and thereby cause infections that may evolve to septicaemia (Kluytmans *et al.*, 1995; Jones *et al.*, 2002).

It is of great concern that food-borne pathogens are exhibiting resistance to antibiotics commonly used for treatment of human bacterial infections (Abuin *et al.*, 1994; Rota *et al.*, 1996; Meng *et al.*, 1998). Recent studies have suggested that the use of antimicrobial agents as a feed additive (eg. avorpacin and virginiamycin) in food-producing animals has contributed to the increase in antibiotic-resistant bacterial prevalence in food of animal origin, and consequently, in community and in nosocomial isolates (Bower and Daeschel, 1999; Wegener *et al.*, 1999; WHO, 2001; Barber *et al.*, 2003). Furthermore, the emergence and increase of antimicrobial agent resistance and consequent reduction of effective antibiotics as a therapeutic measure for the treatment and control of infections in humans, have warranted an increased need for research and development of newer and better antimicrobial agents (Wenzel, 2004; Fritsche *et al.*, 2005; Nathwani, 2005).

The present study aimed at evaluating the microbiological quality and rates of food-isolated staphylococci that were resistant to certain antibiotics that are both normally, and rarely used in clinical therapy, with particular emphasis on methicillin and vancomycin.

MATERIALS AND METHODS

1. Sample collection

A total of 147 raw meats (55 from beef, 21 from chicken and 71 from pork) were purchased from butcheries and markets in Korea during the period of February to June 2007. These were then transferred under refrigerated conditions to the laboratories for further research and analysis.

2. Microbiological quality

For total bacteria, *E. coli* count and isolation of staphylococci, meat samples (1 g) were homogenized and diluted with 1 mL of buffered peptone water (BPW) and seeded onto Nutrient agar (Difco, USA) and Desoxycholate agar (Difco, USA), For selective isolation of staphylococci, the test medium was *Staphylococcus* Medium No. 110 (Difco, USA). The plates were incubated aerobically at 37°C for 24 hours and 48 hours. *E. coli* count was determined as enumeration of rose-red colonies that were large and flat. Staphylococci isolated from *Staphylococcus* Medium No. 110, were identified by Samkwang Medical Laboratories (SML).

3. Antimicrobial agents

The following ten antimicrobial agents were provided by their manufacturers for use in this study: oxacillin (Sigma, USA), vancomycin (Lilly, USA), teicoplanin (Gruppo Lepetit S.p.A., Italy), ciprofloxacin (Il-Dong, Korea), gentamicin (Kuk-Je, Korea), mupirocin (Hanol, Korea), daptomycin (Cubist Pharmaceuticals, Lexington, MA, USA) linezolid (Pharmacia, Kalamazoo, MI, USA), quinupristin/dalfopristin (Rhone-Poulenc Rorer, West Malling, Kent ME, UK) and tigecycline (Wyeth Pharmaceuticals, Philadelphia, PA, USA).

Table 1. Microbiological limits of raw meats based on HACCP* and KFDA**

Criterion	Microbiological quality (CFU***/g)		
	Beef	Chicken	Pork
Total bacteria	$< 10^6$	$< 10^5$	$< 10^6$
Coliform bacteria	$< 10^4$	$< 10^4$	$< 10^4$
Escherichia coli	< 100	$< 10^4$	$< 10^3$

*Hazard Analysis Critical Control Point
**Korea Food and Drug Administration
***Colony forming unit

4. Antimicrobial susceptibility

The staphylococci isolates were grown overnight on Mueller Hinton broth (Difco, USA) at 37°C for 24 hours and were tested for susceptibility to ten antimicrobial agents. MICs (minimum inhibitory concentration) of the various antibiotics were determined by the agar dilution method according to the guidelines established by the National Committee for Clinical Laboratory Standards (NCCLS, 2003). For susceptibility of daptomycin, the test medium was Mueller Hinton agar, adjusted to contain physiological levels of calcium (50 mg/L) as recommended by Fuchs *et al.* (2000). MIC was defined as the lowest concentration producing no visible growth. The isolates were categorized as susceptible and resistant according to NCCLS guidelines (2003 and 2005) and each drug description.

RESULTS AND DISCUSSION

1. Microbiological quality

A total of 147 raw meat samples were investigated for total bacteria, coliform and *E. coli* count. The

Table 2. Bacterial traits by sources isolated from raw meats (beef, chicken and pork)

Samples	Bacteria	Distribution of bacterial count (CFU*/g)				
		$< 10^2$	$10^2 \sim < 10^3$	$10^3 \sim < 10^4$	$10^4 \sim < 10^5$	$> 10^5$
Beef (%) (n=55)	Total	–	2 (3.7)	12 (21.8)	28 (50.9)	13 (23.6)
	Staphylococcus	5 (9.1)	6 (10.9)	27 (49.1)	12 (21.8)	5 (9.1)
	Coliform	17 (30.9)	19 (34.5)	12 (21.8)	5 (9.1)	2 (3.7)
	E. coli	32 (58.2)	17 (30.9)	5 (9.1)	1 (1.8)	–
Chicken (%) (n=21)	Total	–	1 (4.8)	5 (23.8)	9 (42.8)	6 (28.6)
	Staphylococcus	4 (19.0)	4 (19.0)	4 (19.0)	5 (24.0)	4 (19.0)
	Coliform	5 (23.8)	7 (33.4)	3 (14.3)	2 (9.5)	4 (19.0)
	E. coli	14 (66.7)	4 (19.0)	2 (9.5)	1 (4.8)	–
Pork (%) (n=71)	Total	–	2 (2.8)	9 (12.7)	27 (38.0)	33 (46.5)
	Staphylococcus	5 (7.0)	15 (21.1)	19 (26.8)	20 (28.2)	12 (16.9)
	Coliform	16 (22.5)	17 (24.0)	15 (21.1)	14 (19.7)	9 (12.7)
	E. coli	35 (49.3)	15 (21.1)	17 (24.0)	4 (5.6)	–

*Colony forming unit

microbiological quality of raw meat samples was determined according to Table 1. Of the total bacteria count, 71 (48%) meat samples exhibited unacceptable quality. The quality of pork samples was exceptionally lower than other meat samples in terms of its total bacteria count. Coliforms were detected in 74% of raw meat samples whereas 45% raw meat samples were found to be contaminated with *E. coli* 36 (24%) and 45 (31%) meat samples were shown to be of unacceptable quality in terms of its coliform and *E. coli* count, respectively. 21% of pork samples showed 3 times greater contamination of coliforms than that of other meat samples. It is believed that this may be attributable to the presence of faeces during the slaughtering process. Furthermore, unacceptable quality in terms of all the bacterial counts (total, coliform and *E. coli*) was highest exhibited in the pork samples (13%). Interestingly, most chicken samples generally exhibited low counts compared to the other meat samples (Table 2 and 3). However, it must be noted that the rate of excellent quality ($<10^3$ CFU/g) in this study was found to be lower than other published studies on raw meats (Castillo *et al.*, 1999; Palumbo *et al.*, 1999; Delmore *et al.*, 2000).

Table 3. Detection of unacceptable quality in raw meat samples tested

Bacteria	Beef (n=55)	Chicken (n=21)	Pork (n=71)
Total	13 (23.6)	6 (28.6)	52 (73.2)
Coliform	7 (12.7)	6 (28.6)	23 (32.4)
E. coli	23 (41.8)	1 (4.8)	21 (29.6)
Total, Coliform	6 (10.9)	6 (28.6)	20 (28.2)
Coliform, *E. coli*	6 (10.9)	1 (4.8)	15 (21.1)
Total, *E. coli*	7 (12.7)	1 (4.8)	15 (21.1)
Total, Coliform, *E. coli*	5 (9.1)	1 (4.8)	13 (18.3)

(n)=number of total samples
(%)=percentage of samples unsatisfactory

2. Antimicrobial susceptibility

To investigate the rates of food-isolated staphylococci that were resistant to antibiotics, 133 staphylococci were isolated from raw meat samples. The antimicrobial susceptibilities of these staphylococci were tested against six antibiotics, which are in widespread clinical use, and four new antimicrobials namely daptomycin, linezolid, quinupristin/dalfopristin and tigecycline, which are rarely used or have not yet entered clinical use in Korea. Furthermore, these new antimicrobials have been utilized against

Table 4. Prevalence of *Staphylococcus* and methicillinresistant *Staphylococcus* in raw meats

Microorganisms	Samples (%)			Total (n=147)
	Beef (n=55)	Chicken (n=21)	Pork (n=71)	
Staphylococcus	50 (90.9)	17 (81.0)	66 (93.0)	133 (90.5)
Methicillin-resistant *Staphylococcus*	3 (2.3)	0 (0.0)	5 (3.8)	8 (6.0)

(n)=number of total samples

Table 5. Minimum inhibitory concentration (MIC) of methicillin-resistant staphylococci isolated from raw meat samples

Strains	MIC (μg/mL)									
	OXA	VAN	TEI	CIP	GEN	MUP	SYN	LNZ	DAP	TIG
BS 07-08	8	2	4	0.25	4	>128	2	1	2	0.5
BS 07-18	16	2	2	0.25	8	>128	16	1	1	0.5
BS 07-26	4	1	4	0.125	4	128	1	2	0.5	0.5
PS 07-11	8	1	2	0.3	4	128	2	2	0.5	0.5
PS 07-21	16	0.5	2	0.1	<0.06	>128	2	0.5	0.5	0.5
PS 07-27	16	2	2	0.1	8	>128	4	2	4	0.5
PSA 07-65	16	2	4	0.1	8	>128	1	1	2	0.5
PS 07-66	8	2	8	0.3	4	128	2	1	0.5	0.5

OXA: oxacillin, VAN: vancomycin, TEI: teicoplanin, CIP: ciprofloxacin, GEN: gentamicin, MUP: mupirocin, SYN: synercid (quinupristin/dalfopristin), LNZ: linezolid, DAP: daptomycin, TIG: tigecycline, BS: CNS isolated from beef samples, PS: CNS isolated from pork samples, PSA: *S. aureus* isolated from pork samples infections caused by multidrug-resistant, Gram-positive bacteria, particularly vancomycin-resistant enterococci (VRE) and methicillin-resistant staphylococci (Meka and Gold, 2004; Ross *et al.*, 2004; Wesson *et al.*, 2004). Resistance to oxacillin (methicillin) was observed in 3 (2.3%) and 5 (3.8%) of the total number of staphylococci isolated from beef and pork, respectively (Table 4). SML identified these methicillin-resistant staphylococci as 5 CNS and 1 *S. aureus*. Methicillin-resistant staphylococci isolates were highly resistant to mupirocin (MIC value, ≥128 μg/mL). Furthermore, 2 isolates among methicillin-resistant staphylococci exhibited resistance to quinupristin/dalfopristin (MIC value, ≥4 μg/mL) despite the agent not being used in veterinary medicine.

Several investigations have indicated that the use of avoparcin in animal husbandry has contributed to the increased pool of vancomycin-resistant enterococci in animals (Wegener *et al.*, 1999; WHO, 2001). It is thus believed that the extended usage of the streptogramin antibiotic, virginiamycin, as a growth promoter in the commercial animal husbandry may have contributed to the development of cross-resistance against quinupristin/dalfopristin. However, vancomycin, which is the antibiotic of choice for the treatment of methicillin-resistant *S. aureus* infections (Mulazimoglu *et al.*, 1996; Klein *et al.*, 1998; Schentag, 2001), was found to be active against methicillin-resistant staphylococci in this study (Table 5).

Table 6. MIC distributions for ten antimicrobial agents of *Staphylococcus* isolated from beef samples

Strain (year)/ antibiotic	MIC (μg/mL)		Cumulative % inhibited at MIC (μg/mL)											
	50%	90%	0.06	0.12	0.25	0.5	1	2	4	8	16	32	64	128
Oxacillin	1	1	6	6	16	46	90	90	92	96	98	98	98	98
Vancomycin	1	2	6	6	10	10	54	98	98	98	98	98	98	98
Teicoplanin	2	4	8	8	10	12	24	64	94	96	98	98	98	98
Gentamicin	0.06	4	66	72	76	84	84	86	90	98	100	–	–	–
Ciprofloxacin	0.12	0.12	18	94	100	–	–	–	–	–	–	–	–	–
Mupirocin	0.5	>128	8	12	28	52	72	74	78	80	80	80	80	84
Daptomycin	0.5	1	8	12	22	60	90	98	98	98	98	98	98	98
Linezolid	1	2	6	6	6	10	56	98	98	98	98	98	98	98
Synercid	2	2	6	6	6	10	46	92	96	96	98	98	98	98
Tigecycline	0.5	0.5	10	10	12	98	98	100	–	–	–	–	–	–

MIC=Minimum inhibitory concentration

Table 7. MIC distributions for ten antimicrobial agents of *Staphylococcus* isolated from chicken samples

Strain (year)/ antibiotic	MIC (μg/mL)		Cumulative % inhibited at MIC (μg/mL)											
	50%	90%	0.06	0.12	0.25	0.5	1	2	4	8	16	32	64	128
Oxacillin	1	1	0	0	0	47	100	–	–	–	–	–	–	–
Vancomycin	2	2	0	0	0	0	18	94	100	–	–	–	–	–
Teicoplanin	2	4	0	0	0	0	6	65	94	100	–	–	–	–
Gentamicin	0.06	64	53	82	82	82	82	88	88	88	88	88	100	–
Ciprofloxacin	0.12	0.25	12	88	100	–	–	–	–	–	–	–	–	–
Mupirocin	1	2	0	0	29	41	82	94	94	94	94	94	94	100
Daptomycin	0.5	2	0	0	6	53	71	94	100	–	–	–	–	–
Linezolid	1	2	0	0	0	0	53	100	–	–	–	–	–	–
Synercid	1	2	0	0	0	0	59	94	100	–	–	–	–	–
Tigecycline	0.5	1	0	0	0	88	94	100	–	–	–	–	–	–

MIC=Minimum inhibitory concentration

Table 8. MIC distributions for ten antimicrobial agents of *Staphylococcus* isolated from pork samples

Strain (year)/ antibiotic	MIC (μg/mL)		Cumulative % inhibited at MIC (μg/mL)											
	50%	90%	0.06	0.12	0.25	0.5	1	2	4	8	16	32	64	128
Oxacillin	1	2	2	3	14	48	88	91	91	95	100	–	–	–
Vancomycin	2	2	5	5	5	8	45	100	–	–	–	–	–	–
Teicoplanin	2	8	3	3	3	5	17	58	88	95	100	–	–	–
Gentamicin	0.06	8	67	77	80	82	83	86	89	94	97	98	100	–
Ciprofloxacin	0.12	0.25	20	85	100	–	–	–	–	–	–	–	–	–
Mupirocin	0.5	>128	2	3	30	58	76	80	83	83	83	83	85	89
Daptomycin	1	2	2	5	11	38	80	97	98	100	–	–	–	–
Linezolid	2	2	3	3	5	11	41	100	–	–	–	–	–	–
Synercid	2	2	2	2	2	2	45	91	97	100	–	–	–	–
Tigecycline	0.5	0.5	3	3	14	95	97	100	–	–	–	–	–	–

MIC=Minimum inhibitory concentration

The ranges and MICs for 50% and 90% of all staphylococci isolates tested of the antimicrobial agents are shown in Table 6, 7 and 8. High resistance to mupirocin (MIC_{90}, >128 μg/mL) was observed in staphylococci isolated from beef and pork (Table 6 and 8). On the other hand, staphylococci isolated from chicken were susceptible to mupirocin (MIC_{90}, 2 μg/mL), but were resistant to gentamicin (MIC_{90}, 64 μg/mL) (Table 7). No apparent differences in MICs were noted between beef, chicken and pork for other antimicrobial agents tested.

All the new antimicrobial agents tested, inhibited most staphylococci isolates at concentrations between 0.5～2 μg/mL. Among the antimicrobials tested, tigecycline and ciprofloxacin showed the highest activity against all staphylococci isolates, which included methicillin-resistant staphylococci (MIC_{90}, ≤0.5 μg/mL) (Table 6, 7 and 8).

CONCLUSION

The results of this study indicated that the consumption of raw meat could represent notable hazards to the health of its consumers. Recently, the Ministry of Agriculture and Forestry in Korea enforced the application of HACCP (Hazard Analysis Critical Control Point) in slaughterhouses for the betterment of the hygienic administration level. It must be noted that the hygienic condition of raw meat is related to various factors, which include the slaughtering process as well as the distribution and storage of meat products (Hardin *et al.*, 1995; Gill, 1998; Lee *et al.*, 1999). Therefore it is essential that the environments under which meat products are processed/stored are improved in an attempt to limit contamination of the meat by pathogenic microorganisms. Other interventions on antibiotic usage in the animal field, such as the banning of virginiamycin, avoparcin and other antimicrobial growth promoters, may be required to prevent the spread of antimicrobial-resistance.

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