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Microbiological Aspects and Recovery of *Salmonella* in Retailed Foods

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Abstract Microbiological analysis of 148 various food samples over one year period revealed that the microbial quality of raw meat and seafood samples was generally high except that of chicken. More than half of the chicken samples were found to contain *Salmonella* with four different serotypes. Repeated isolation of same serotype in same retail shop suggested the existence of secondary contamination. *Salmonella* and fecal coliforms and or fecal streptococci seems to vary independently. However, fecal coliform counts of the *Salmonella* positive samples (59%) exceeded 10^4 MPN/100 g. Antibiotic resistance of 50 *Salmonella* isolates revealed the persistence of drug resistance in most of the strains. *Salmonella* serotypes exhibited resistance to all the antibacterials tested with colistin (30 μg) as most common one. *S. paratyphi* B was resistant to 5 different antibiotics and all *Salmonella* serotypes isolated from food samples were found to show multi drug resistance.

Salmonellosis is a long-standing, persistent problem and the most important source of infection is food of animal origin. There is extensive movement of food, feeding stuff[1] animals and man around the world[2]. Foods can become contaminated during production either on farms or in water courses, during processing and preparation in food service establishments and home. Studies on the microbial quality of meat and meat products[3], edible offals of various animals[4] and seafoods[5] are documented. Mutiple antibiotic resistance in salmonellae is a serious global public health problem. The resistance conferring plasmids may also carry factors relating to increased virulence and invasiveness[2]. Antibiotic resistance of *Salmonella* and the transmission of resistant strains between animals and human is studied[6]. The present study is an attempt to know the microbial quality and recovery of *Salmonella* in chicken, porcine, bovine meats and in seafoods sold in retail at Fukuyama City, Japan as well as the nature of relationship between bacterial indicators of pollution and the isolation of salmonellae. The antibiotic resistant pattern in *Salmonella* isolated from various sources is also carried out.

MATERIALS AND METHODS

148 samples (Table 1) consists of chicken, pork and beef of both sliced and minced meat in addition to seafood samples were purchased from open and super markets of Fukuyama City, Japan during September 1986 to August 1987.

Preparation of Samples for MPN Analysis

40 g of samples was aseptically weighed and transferred to a homogenizer containing 100 mℓ

of sterile lactose broth (LB) and homogenized for 1 min at 8,000 g followed by blending with additional quantitiy of LB making the final volume to 400 mℓ. From the above mixture suitable aliquots were transferred to selective media for various bacteriological analysis. 10 mℓ of this mixture represents 1 g of sample.

Bacteriological Analysis

Appropriate decimal dilution of the samples were serially prepared by employing 0.1% peptone water as diluent. 0.1 mℓ of diluted samples were spread plated in surface dried Trypto Soy Agar in duplicate. Total viable counts (TVC) were counted after 48 h of incubation at 25°C. Standard 5 tubes most probable number (MPN) procedure—LB, BGLB, EC broth combination—was followed for the enumeration of total (TC) and fecal coliforms (FC) as described elsewhere[7]. For the enumeration of fecal streptococci (FS), the samples were inoculated in Azide Citrate (AC) broth (Nissui, Japan) and incubated for 48 h at 37°C. Tubes showing traces of growth were transferred into fresh AC broth and incubated at 45°C and growth after 48 h was treated as positive for FS and MPN was computed[8].

Remaining portion of LB were then placed at 37°C in a water bath for an overnight incubation as preenrichment for the isolation of *Salmonella*. 1:100 for Rappaport-Vassiliadis 10 broth and 1:10 for Hajna Tetrathionate broth as sample: enrichment media ratio[9] were maintained and incubated at 42.5°C. After 18-24 h, the enriched samples were streaked onto Xylose Lysine Brilliant Green, Novobiocin Glucose Brilliant Green and Sulphamandelate Brilliant Green agars and incubated at 37°C for 24-48 h. Typical *Salmonella* like colonies were picked onto Lysine Iron agar and subjected to various biochemical tests. Isolates which resembled *Salmonella* biochemically were further screened with a slide agglutination test with polyvalent 0 antisera. Confirmation of the isolates as being *Salmonella* spp. was performed by the National Salmonella and Escherichia Centre, Kasauli, India.

Antibiotic Sensitivity Tests

Sensitivity to various antibacterials was determined by disc diffusion method as described[10]. Isolates which had been purified and identified from environmental, food and animal sources were subcultured into Trypto Soy broth and incubated for 8 h at 37°C. The broth culture was then diluted with sterile saline water and satisfactory dilution was spread plated over sensitivity test agar (Eiken, Japan). After 30 min of absorption time at 37°C, Kirby-Bauer discs (Eiken, Japan) of six different antibacterials were transferred onto the plates and incubated at 37°C overnight. The antibiotics used were (μg/disc): nalidixic acid, NA (30); tetracycline, TC (30); chloramphenicol, CP (30); kanamycin, KM (30); colistin, CL (30) and ampicillin, ABP (10). The diameter of the inhibition zone were measured to estimate the sensitivity according to the manufacturers instruction and the strains were coded as resistant or sensitive, with intermidiate strains being included in the resistant class.

RESULTS

Microbial Quality of Food Samples

148 of various samples were collected either from super markets or open markets. The quality of food obtained from super markets is high when compared to open market. Table 1 shows the microbial quality of various food samples. The bacterial quality of meat samples showed high TVC population in pork as well as beef compared to chicken. The bacterial in-

Table 1. Microbial quality of food samples[*1]

Food items	No. of samples examined	No. of *Salmonella* positive (%)	*Salmonella* serotype	TVC[*2] ×10⁵	Coliforms[*3] ×10⁴	Fecal[*3] coliforms ×10⁴	Fecal[*3] streptococci ×10⁴
Chicken meat	36	18(50)	*S. hadar*	2.4−55.5	1.1−240	0.5−92	0.2−17
			S. II:4, 12:b:-	(16.7)	(66.6)	(14.3)	(3.7)
			S. typhimurium				
Chicken minced	24	16(66.6)	*S. hadar*	3−160	0.3−1600	0.1−900	1.7−16
			S. typhimurium	(62)	(330)	(166)	(8.8)
			S. paratyphi B				
Chicken liver	12	8(66.6)	S. II:4, 12:b:-	15−35	1.7−9	1.7−5	0.1−2.4
			S. typhimurium	(26)	(5.2)	(3.8)	(1.3)
Pork meat	18	0	−[*4]	0.2−299	0.2−240	<2−3.5	<2−0.3
				(57.8)	(31.5)	(0.8)	(0.2)
Pork minced	18	0	−	46−1300	1.2−240	<2−35	0.7−240
				(342)	(72)	(7)	(60)
Beef meat	10	0	−	26−115	0.2−1.7	<2−1.4	N. D.[*5]
				(61)	(1)	(1)	
Beef minced	10	0	−	13−564	1.1−1.7	<2−0.4	N. D.
				(191)	(1.4)	(0.2)	
Pork+Beef minced	4	0	−	10−375	1.1−17	0.2−4	<2−0.1
				(192)	(9)	(2)	(0.04)
Little neck clam (*Tapes* spp.)	4	0	−	11−331	<2−9	<2−2	N. D.
				(171)	(4.5)	(1)	
Ear shell (*Haliotes* spp.)	4	0	−	10−111	11−17	<2−2	N. D.
				(41)	(14)	(1)	
Oyster	4	0	−	72−93	14−17	<2−12	N. D.
				(82.5)	(15.5)	(6)	
Shrimp	4	0	−	17−517	11−14	<2	N. D.
				(267)	(12.5)	(<2)	

[*1] Values expressed are minimun−maximum; parentheses denotes mean,

[*2] CFU/g, [*3] MPN/100 g, [*4] Absent, [*5] Not detected.

Table 2. Relationship between fecal coliforms and *Salmonella* in chicken samples

Chicken samples	Level of fecal coliforms MPN/100 g				Total
	10^4 and below	10^4-10^5	10^5-10^6	10^6 and above	
Sliced meat	4[*1]	24	8	−	36
	4(100)[*2]	8(33.3)	6(75)		18(50)
Minced meat	4	12	4	4	24
	0	8(66.6)	4(100)	4(100)	16(66.6)
Liver	−	12	−	−	12
		8(66.6)			8(66.6)

[*1] Number of fecal coliforms incidence,

[*2] Number of *Salmonella* positive; parentheses denotes percentage of *Salmonella* positive with fecal coliforms.

dicators of pollution was much higher in chicken than the other samples. The mean TC of chicken sliced meat, minced meat and liver were 66.6, 330 and 5.2×10^4 MPN/100 g respectively and FC counts were 14.3, 166 and 3.8×10^4 MPN/100 g respectively. FS counts showed high population in pork than chicken. Comparing the quality of minced meat to sliced meat the

Table 3. Antibiotic resistance pattern in *Salmonella*

Pattern	Number of resistance displayed	Resistance comprising pattern*1	Number of isolates	*Salmonella* serotype*2
A	5	NA TC CP KM CL	1	*S. paratyphi* B
B	3	TC KM CL	2	*S. typhimurium*
				S. hadar
C	2	KM CL	2	*S. derby*
				S. typhimurium (A)
D	2	TC ABP	2	*S. thompson (A)*
				S. agona (B)
E	2	TC CP	1	*S. thphimurium (B)*
F	2	TC KM	2	*S. hadar* (2, A)*3
G	2	TC CL	1	S. II:4, 12:b:- (A)
H	1	CL	10	*S. montevideo* (2)
				S. thompson (2)
				S. agona
				S. typhimurium
				S. litchfield
				S. schwarzengrund
				S. I rough
				S. meleagridis
I	1	KM	1	*S. senftenberg*
J	1	TC	2	S. II:4, 12:b:- (2)
K	1	ABP	1	*S. infantis*
L	1	NA	1	*S. heidelberg*

*1 Refer text for expansion,

*2 Isolated from food (A), feces (B) and water (no marking),

*3 Parentheses denotes number of strains.

former have contaminated heavily than the latter in all the bacteiral population examined. Microbial quality of seafood samples was much better than that of other food items as evidenced by very low FC densities ranging from <2 to 6 MPN/100 g.

Salmonella spp. were isolated only from chicken among the various food tested. 50%, 66.6% of the total samples are *Salmonella* positive in sliced meat and minced as well as liver of chicken respectively. This showed that this pathogen is widely distributed in poultry. Four serotypes namely *S. hadar, S. typhimurium, S. paratyphi* B and *S.* II:4, 12:b:- were found to be observed with the repeated isolation of same serotype in the same retail shop. Table 2 describes the relationship between FC and *Salmonella* in chicken samples. The results suggested that chicken minced and liver samples had 66.6% of *Salmonella* incidence when the level of FC densities were 10^4–10^5 MPN/100 g and increased to 100% when the range rose to 10^5–10^6 MPN/100 g and above. But the chicken sliced meat showed irregular pattern and the *Salmonella* incidence did not correspond to the increase in FC densities.

Antibiotic Resistance Pattern in *Salmonella*

50 strains comprising 26 salmonellae serotypes used in this study were isolated in this laboratory from environment, food and animal feces. Table 3 shows 12 patterns of resistance exhibited by different serotypes of salmonellae. Among the salmonellae tested, 26 strains of 15

serotypes were susceptible to all the six antibacterials tested and 52% were resistant to one or more antibiotics. The environmental, food and animal feces isolates contributed 9, 4 and 2 patterns respectively with an overall 12 different antibiotic resistant patterns during the present study. *S. paratyphi* B was resistant to as high as 5 antibacterials followed by 3 in *S. typhimurium* and *S. hadar*. In contrast, the most common pattern (H), i. e. resistant to colistin, was shared by 8 serotypes. Several seroytypes exhibited different resistant patterns. Isolates of *S. typhimurium* displayed 4 different patterns (B, C, E, H) while 2 different patterns were noticed in isolates of *S. hadar* (B, F), *S. thompson* (D, H) and *S.* II:4, 12:b:- (G, J). It is a point of fact to state that all strains isolated from food are resistant to more than one antibacterial.

DISCUSSION

Salmonellae are very important causative agents of zoonotic and foodborne disease. Meat and meat products especially those that are undercooked are frequently implicated.

More than half of the chicken samples examined were contaminated with salmonellae. In many countries poultry is often considered to be the most important source of salmonellae[11] and high incidence up to 90% have been reported in surveys carried out in Europe and elsewhere[12]. Absence of salmonellae in bovine as well as porcine meat might be due to lower infection rate in cattle than chicken[4]. Moreover, as these meats are generally handled seperately, cross contamination is rare. *S. typhimurium, S. hadar* and *S.* II:4, 12:b:-, serotypes responsible for salmonellosis in animals and poultry[13], indicated that the contamination would have originated from farmyard. On the other hand, existence of cross contamination could not be ruled out as because of the repeated isolation of same serotypes in same retail shop. However, isolation of *S. paratyphi* B in minced chicken suggested the need for higher sanitation. *Salmonella* and FC seems to be varied independently. Moreover, of 64 samples containing 10^4 MPN/100 g of FC, 38 (59%) had *Salmonella* whereas among the 8 samples with less than 10^4 MPN/100 g of FC, only 4 samples (50%) were positive for salmonellae. In addition to these, salmonellae were found to be undetectable in pork minced meat even though FC counts exceeded 10^4 MPN/100 g. This showed that incidence of salmonellae was not correlated with FC. The present study also revealed that FS had no relationship with the presence of salmonellae[4]. Among various food items tested, in general, the quality reaching the consumer was acceptable as evidenced by low counts of indicator bacteria excepting chicken samples. Although the seafood samples had high TVC ($41\text{-}267 \times 10^5$/g), these samples did not appear or smell spoiled indicating that the quality of seafood is not reflected by total bacterial population and the excess counts might be due to secondary contamination during handling.

In view of the association of waterborne or foodborne salmonellosis with human, the undesirable public health implications of carriage by environmental and food salmonellae with resistant to wide variety of drugs should be noted. Certainly the serotypes of salmonellae demonstrated to be drug resistant are commonly associated with human infections[14]. Resistant to different antibacterials 5 out of 6 tested in *S. paratyphi* B, and different pattern of resistance notice in *S. typhimurium, S. hadar, S. thompson, S.* II:4, 12:b:- pose more problem to the public health of view. NEU et al.[15] also reported that *S. typhimurium* was found to have a much higher prevalence of resistance plasmids. The resistance pattern exhibited by the examined salmonellae differ markedly from those most recently reported in which resistance to

chloramphenicol and nalidixic acid was not encountered[3]. But, resistance to chloramphenicol and nalidixic acid, the drug of choice in systematic salmonellosis, exhibited during the present study questioned the use of antibiotics in breeding, or maintenance of various animals possibly infected by *Salmonella*. In addition, resistance to one or more antibiotics shown by the food isolates warned to maintain high quality before being served to the consumer.

High rate of *Salmonella* contamination in chicken meat proposed a considerable public health hazard due to 1) consumption or direct contact with raw or inadequately cooked products, 2) secondary contamination due to handling and 3) feeding pet animals with raw chopped meat which then can infect humans. Hence, it is imperative that the retailers and consumers are made aware of this concern and take the appropriate measures to prevent microbiological health risks.

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市販食品の微生物学的品質とサルモネラ

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1年間にわたって小売店から購入した148例の生肉および水産食品について微生物学的な品質測定を行った結果，鶏肉を除いて一般に良好であった。また，鶏肉の半数以上がサルモネラ（4血清型）陽性であった。しかも，同じ店から同じ血清型のサルモネラが繰り返して分離されたことから，2次汚染が推定された。サルモネラと糞便大腸菌，あるいは糞便レンサ球菌の出現との間には関係が無いように見える。しかしながら，サルモネラ陽性例の59%は糞便大腸菌の MPN が 10^4/100 g 以上であった。

つぎに，われわれの研究室で分離，保存していた50株のサルモネラ（26血清型）について，6種類 (NA, TC, CP, KM, CL, ABP) の抗生物質耐性試験を行った結果，半数以上が耐性を示した。中でも Colistin (30 μg) 耐性を示す血清型のものが，もっとも多かった。*S. paratyphi* B は5剤耐性であり，また，食品から分離したサルモネラはすべて多剤耐性を示した。