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Isolasion, Identification and Antimicrobial Resistance Patterns of *Salmonella* from Meat Products in Tehran

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Abstract: The present study was carried out from 400 samples in different slaughterhouses to report the isolation along with the serotypes and antibiogram pattern of *Salmonella* among products in Tehran. *Salmonella* was isolated from samples of chicken, beef; veal, mutton, roast beef and sausage fermentive meat collected at slaughterhouses. The isolates were characterized by serotyping and antimicrobial-susceptibility testing. Eighty isolates of *Salmonella enterica* belonging to 19 serotypes- *S. adelaide, S. agona S. abortus ovis*, *S. abortus bovis*, *S. derby*, *S. dublin*, *S. enteritidis*, *S. havana*, *S. heidelberg*, *S. indiana*, *S. infantis*, *S. kentucky*, *S. montevideo*, *S. newport*, *S. saint paul*, *S. senftenberg*, *S. typhimurium*, *S. thompson*, *S. worthington* were obtained with an overall prevalence of 20%. The most strains of *Salmonella enterica* were isolated from roast beef. *S. enterica* serotype *Thompson* and *S. enterica* serotype *typhimurium* were isolated most frequently. All of the isolates were resistant to at least one antibiotic and 94% were resistant to at least three antibiotics. Six% were resistant to ceftriaxone, the drug of choice for treating salmonellosis in children. One isolates of *S. enterica* serotype *infantis* had resistance to 15 antibiotics and the one isolate of serotype *Thompson* and one isolate of *Serotype havana* were resistant to 14 antibiotics. Norfloxacin, cefteriaxone and cefotaxime were most effective, whereas, erythromycin, tetracycline, nalidixic acid, furazolidone and nitrofurantoin were relatively less effective. Resistant strains of *Salmonella* are common in meat products. These finding provide support for adoption of guidelines for the prudent use of antibiotics in food animals and for a reduction in the number of pathogens present on farms and slaughterhouses. Thus, it is imperative that salmonellosis control measures adopted for humans should give adequate importance to its control in animals particularly their products.

Key words: Antibiogram, prevalence, *Salmonella*, Tehran, Iran

INTRODUCTION

Foodborne diseases caused by non-typhoid *Salmonella* represent an important public heath problem worldwide so nearly 1.4 million cases of salmonellosis occur each year in the United Stases (Angulo *et al.*, 2000). Intestinal salmonellosis typically resolves in five to seven days and does not require treatment with antibiotics. However, bacteremia occurs in 3 to 10% of reported, cultured-confirmed cases and is particularly common among patient at the extremes of age and those who are immunocompromised. When infection spreads beyond the intestinal tract, appropriate antimicrobial therapy (e.g., ciprofloxacin in adults and ceftriaxone in children) can be lifesaving (Glynn *et al.*, 1998; Hohmann, 2001). Non-typhoidal salmonellosis is an important enteric infection in humans, particularly in the neonates and younger children (Gupta and Vermo *et al.*, 1993). Most *salmonella* infections in humans result from the ingestion of contaminated poultry, beef, pork, eggs and milk (Gomez *et al.*, 1997). Lailler *et al.*, (2002) isolated *Salmonella* serotypes from birds, pork and beef in France. Esaki *et al.* (2004) isolated *Salmonella* serotypes from pork and beef in Japan. Nadine *et al.*, 2004 isolated different *Salmonella* serotypes from pork and slaughterhouse in Belgium. The use of antimicrobial agents in any environment creates pressures that favor the survival of antibiotic-resistant pathogens. According to the infectious-disease report that was released by the WHO (2000), such organisms have become increasingly prevalent worldwide. The routine practice of giving antimicrobial agents to domestic livestock as mean of preventing and treating disease, as well as promoting growth, is an important factor in the emergence of antibiotic-resistant bacteria that are subsequently transferred to humans through the food chain (Yu *et al.*,

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2004). Most infections with antimicrobial-resistant *Salmonella* are acquired by eating contaminated foods of animal origin (Angulo *et al*., 2000). Fey *et al* (2000) discovered a ceftriaxone-resistant *Salmonella* infection in a child that was acquired through exposure to cattle and the emergence of ceftriaxone-resistant *salmonella* infection are often unknown, but they most likely originate in contaminated food of animal origin.

Antibiotic resistance of *Salmonella* strains isolated from slaughtered and packed chicken meat in Tehran. All of isolates of *Salmonella* were resistant to streptomycin, penicillin and erythromycin.

We isolated and characterized *salmonella* strains from meat products obtained in slaughterhouses in Tehran with the use of methods described by Feingold and Baron (1990) and determined the antimicrobial-resistance phenotypes of the isolates with the use of disc method described by NCCLS. (2001).

MATERIALS AND METHODS

Four hundred samples of meat (100 samples of chicken, 100 of beef, veal, mutton, 100 of roast beef and 100 of sausage fermentive meat) were collected at slaughterhouses in Tehran between September and August 2005: 06. Selenite F Broth (SFB) was used as a selective enrichment broth for primary isolation of *Salmonella*. Brilliant Green Agar (BGA) and *Salmonella-Shigella* Agar (SSA) were used as selective medium for primary isolation and MacConkey's Lactose Agar (MLA) was used for purification of suspected colonies. Characterization and preliminary identification of suspected *Salmonella* cultures were made on the basis of morphology, characteristics and biochemical reactions (Glynn *et al*., 1998). The isolated strains of *Salmonella* were serotyped at Razi Vaccine and Serum Research Institute in Iran. In order to understanding of Antibiotic sensitivity, In vitro susceptibility of the organisms to various antimicrobial agents was determined by the disk diffusion technique. The antimicrobial agents (concentration in μg) used were:amikacin (AN, 30), ampicillin (AM, 10), cefalexin (CN, 30), cefotaxime (CTX, 30), ceftriaxone (CRO , 30), chloramphenicol (C, 30), ciprofloxacin (CP, 5), erythromycin (E, 15) furazolidone (FR, 100), gentamicin (GM, 10), kanamicin (K, 30) nalidixic acid (NA, 30), nitrofurantoin (FM, 300), norfloxacin (NOR, 10), streptomycin (S, 10)tetracycline (TE, 30), trimethoprim-sulfamethoxazole (SXT, 23.75, 1.25).

RESULTS

Serotypes: *Salmonella* were recovered from 80 of 400 samples of meat (20%). *Salmonella* was isolated more frequently from chicken (29%) than from roast beef (25%), from veal (19%), from beef(12%), from sausage fermentive meat (12%) or mutton (4%).

Ninetheen serotypes were identified among the 80 *Salmonella* isolated (Table 1). *S. enterica* serotype *thompson* (15%) and *S. enterica* serotype *typhimurium* (14%) were isolated most frequently. All of isolates of *S. enterica* serotype *Thompson* were recovered from chicken. In contrast, *S. enterica* serotype *typhimurium* was isolated from roast beef. Nine of the ten isolates of *S. enterica* serotype *enteritidis* were from chicken and one was from mutton. All four isolates of *S. enterica* serotype *Heideleberg* were recovered from sausage fermentive meat. Six of the eight isolates of *S. enterica* serotype *Infantis* were from veal and two were from roast beef. Two of the three isolates of *S. enterica* serotype *adelaide* were from veal and one was from beef. One of the two isolates of *S. enterica* serotype *agona* was from beef and another one was from veal. *S. enterica* serotype *Abortus ovis* was isolated from mutton and *S. enterica* serotype *Abortus bovis* was isolated from beef. *S. enterica* serotype *Derby* and *S. enterica* serotype *Dublin* were recovered from beef. Two of the four isolates of *S. enterica* serotype *Havana* were from veal and two were from roast beef. One of the two isolates of *S. enterica serotype ind*iana was recovered from roast meat and one was from sausage fermentive meat. Two isolates of *S. enterica* serotype *Kentucky* were recovered from chicken and veal and two isolates of *S. enterica* serotype *Montevideo* were recovered from beef and roast

Table 1: Number and percentage of *Salmonella* isolates from various meat products

Serotype	No. of isolates	No. positive for *Salmonella* (%)	Group
S. adelaide	3	4	O
S. agona	2	3	B
S. abortus ovis	2	3	B
S. abortus bovis	2	3	B
S. derby	1	1	B
S. dublin	2	3	D
S. enteritidis	10	11	D
S. havana	4	5	G
S. heidelberg	4	5	B
S. indiana	2	3	B
S. infantis	8	10	C
S. kentucky	2	3	C
S. montevideo	2	3	C
S. new port	3	4	C
S. saint paul	3	4	B
S. senftenberg	4	5	E
S. typhimurium	12	14	B
S. thompson	13	15	C1
S. worthington	1	1	G

Table 2: Isolation of *Salmonella* from meat products

Source Serotype	Chicken meat	Meat: Beef	Meat: Veal	Meat: Mutton	Roast beef	Sausage fermantive meat
S. adelaide		1	2			
S. agona		1	1			
S. abortus ovis				2		
S. abortus bovis		2				
S. derby		1				
S. dublin		2				
S. enteritidis	9			1		
S. havana			2		2	
S. heidelberg						4
S. indiana					1	1
S. infantis			6		2	
S. kentucky	1		1			
S. montevideo			1		1	
S. new port		2	1			
S. saint paul		1			2	
S. senftenberg						4
S. typhimurium					12	
S. thompson	13					
S. worthington			1			
Total	100		100		100	100

Table 3: Antibiotic resistance pattern of *Salmonella* isolates from various sources

Serotype	No. of *Salmonella* isolates resistant to various antibiotics: No.	FM	CTX	SXT	FR	AN	GM	NOR	CRO	CP	NA	K	AM	CN	S	E	C	TE
S. adelaide	3	2	0	2	2	1	0	1	0	1	2	2	1	0	1	3	1	2
S. agona	2	2	0	1	2	1	0	0	2	1	2	2	2	1	2	2	0	2
S. abortus	2	2	1	2	2	1	1	0	0	1	2	1	2	0	2	2	1	2
S. abortus bovis	2	2	0	2	2	1	1	1	0	1	2	1	2	0	2	2	0	2
S. derby	1	1	0	1	1	1	1	0	0	0	1	0	0	1	1	1	0	1
S. dublin	2	2	0	2	2	1	1	0	0	0	2	2	0	0	2	2	0	2
S. enteritidis	10	9	0	9	9	1	3	1	1	5	8	3	5	0	7	10	2	9
S. havana	4	3	1	3	3	3	3	1	0	3	3	3	3	0	4	4	1	4
S. heidelberg	4	3	1	4	4	0	0	0	1	1	3	3	3	1	4	4	2	4
S. indiana	2	2	0	2	2	0	0	0	0	0	2	2	2	0	2	2	0	2
S. infantis	8	4	2	5	8	1	2	0	0	3	8	6	7	2	6	8	6	8
S. kentucky	2	0	0	1	1	1	1	0	0	0	1	1	1	0	1	2	1	2
S. montevideo	2	1	0	1	1	0	0	0	0	0	2	0	1	0	2	2	0	2
S. new port	3	2	0	2	2	2	2	0	0	1	3	3	0	0	3	3	2	3
S. saint paul	3	2	0	2	3	2	3	0	0	1	2	3	2	0	2	3	2	2
S. senftenberg	4	3	0	2	3	1	1	0	0	0	4	1	1	0	2	4	1	4
S. typhimurium	12	10	1	7	9	0	0	0	1	2	8	6	5	4	9	10	3	9
S. thompson	13	13	0	13	11	3	4	1	0	3	13	5	10	1	9	13	7	13
S. worthington	1	1	1	1	1	0	0	0	0	0	1	1	1	1	1	1	1	1
Total	80	67	7	62	6	20	23	5	5	2	69	4	48	11	6	7	3	6
(%)	100	84	9	77	85	25	29	6	6	29	86	56	60	14	78	97	38	93

AN, amikacin; AM, ampicillin; CN, cefalexin; CTX, cefotaxime; CRO, ceftriaxone; C, chloamphenicol; CP, ciprofloxacin; E, erythromycin; FR, furazolidone; GM, gentamicin; K, kanamicin; NA, nalidixic acid; FM, nitrofurantoin; NOR, norfloxacin; S, streptomycin; TE, tetracycline; SXT, trimethoprim-sulfamethoxazole

beef. Two of the three isolates of *S. enterica* serotype *newport* were from beef and one was from veal. Two of the three isolates of *S. enterica* serotype *saint paul* were recovered from roast beef and one was from beef. *S. enterica* serotype *Worthington* was isolated from veal (Table 2).

Antimicrobial resistance: All the isolates (100%)were resistant to at least one of the 17 antibiotics tested and 96% (77 out of 80 samples) were resistant to at least three antibiotics. This could be due to the wide and varied use of different antibiotics with simultaneous evolution of newer antibiotics that have precipitated into pathogens of multiple drug resistance. Moreover, the presence of antibiotic residues in foods of animal origin may result in increased drug resistance amongst human isolates (2). Among multidrug- resistance isolates, resistance to erythromycin, tetracycline and nalidixic acid was most often observed. In the present study, isolates showed resistance against erythromycin (79%), tetracycline (67%), nalidixic acid (69.8%) furazolidone (68.8%), nitrofurantoin (67.8%), streptomycin (62.7%), trimethoprim-sulfamethoxazole (62.7%) ampicillin (48.6%), kanamicin (45.5%), chloramphenicol (30.3%), gentamicin (23.2%), ciprofloxacin (23.2%), amikacin (20.2%), cefalexin (11%), cefotaxime (7.9%), ceftriaxone (5.6%), norfloxacin (5.6%) (Table 3).

S. enterica serotype Worthington exhibited resistance to 12 antibiotics and one of the eight isolates of *S. enterica serotype infantis* exhibited resistance to 15 antibiotics.

All of the isolates of *S. enterica* serotypes *agona, abortus ovis, abortus bovis, derby, dublin, havana, heidelberg, indiana, infantis, kentucky, montevideo, new port, senftenberg, thompson* and *Worthington* were resistant to erythromycin and tetracycline. Thirteen isolates of *S. enterica* serotype *typhimurium* displayed resistance to at least five antibiotics, including nitrofurantoin, trimethoprim-sulfamethoxazole, nalidixic acid, erythromycin and tetracycline. Most of the strains of *Salmonella* were susceptible to norfloxacin, ceftriaxone and cefotaxime (Table 3).

The isolates were most likely to be resistant to erythromycin, tetracycline, nalidixic acid, furazolidone, nitrofurantoin and to a lesser extent, streptomycin.

DISCUSSION

Our observations show that the incidence of *Salmonella* serotypes isolated from meat products in Tehran during (2005-6) in contrast with other studies in this field, was a high rate (20%). Table 1 shows nineteen serotypes with their percentage and groups isolated from meat products. Resistance to two or more drugs was more commonly observed than resistance to a single drug.

Glynn MK studies of infections with *S. enterica* serotype *typhimurium* by the centers for disease control and prevention revealed that the percentage of isolates that were resistant to ampicillin, chloramphenicol, streptomycin, sulfonamides and tetracycline increased from 0.6% during the period from 1979 to 1980 to 34% 1996 (Glynn *et al.*, 1998). Resistance to some of the aminoglycosides such as kanamycin is of great concern since it has been a major drug of choice in the treatment of infections (Threlfull *et al.*, 1986).

Lailler *et al.* (2002) announced that 48% of *S. enterica* serotype *typhimurium* isolated from birds, pork and beef in France displayed resistance to ampicillin, streptomycin, chloramphenicol, tetracycline and sulfonamids. Nanna *et al.* (2002) determined antimicrobial resistance pattern for *S. enterica* serotype *agona* from animals, food and human in Finland. All of the isolates displayed high resistance to tetracycline and streptomycin.

Nadine *et al.* (2004) determined antimicrobial resistance of *Salmonella* serotypes isolated from pork and slaughterhouses in Belgium. All the isolates displayed high resistance to tetracycline, sulphadiazine, ampicillin, chloramphenicol and streptomycin.

As observed in other studies and this study, isolated strains displayed a high multi-drug resistance. However, the contamination of meat products with resistant *Salmonella* mainly reflects carriage of the organism by the livestock; intervention strategies should therefore focus principally on reducing the number of pathogens present on farms and slaughterhouses. Although *S. enterica* serotype *agona* is less commonly associated with disease in human than is *S. enterica* serotype *typhimurium*. It has been the cause of several foodborne outbreaks in recent years (Synnott *et al.*, 1998; Taylor *et al.*, 1998).

Present study identified one isolate that was resistant to 15 antibiotics and was recovered from roast beef and 14 isolates that were resistant to twelve antibiotics and were recovered from different types of meat (except chicken). The dissemination of *Salmonella* that is resistant to multiple drugs, including cephalosporins, through food has important public health implications. The ability of bacteria to acquire antibiotic-resistance genes and subsequently spread them to many different bacterial species is well known (Hall, 1997). Although we have no corresponding culture data from humans, our data provide support for the theory that the food supply is a major source of antimicrobial-resistant *Salmonella*. The high prevalence of multidrug-resistant *Salmonella* in meat reflects a reservoir of resistance in animals that can be transmitted to humans. The prevalence of *Salmonella* resistant to multiple drugs is quite a serious problem from the standpoint of epidemiology and public health as resistance is transferred to pathogenic enteric organisms both directly and by way of non-pathogenic enteric bacteria such as *E. coli* and *Klebsiella* (Centers for Disease Control and Prevention (CDC), 1998). Furthermore, the problem of multiple drug resistance is not limited to *Salmonella* and *Shigella*, but is also a problem with all *Entrobacteriaceae* (Quendnau *et al.*, 1998). Apart from this medical importance, multidrug resistance offers interesting material for bacterial genetics. The resistance transfer factor is a new episome (extra chromosomal elements). Eighty strains of *S. enterica* isolated in Tehran were resistant to erythromycin, tetracycline and nalidixic acid (92%) and furazolidone (85%). This high percentage of resistance to antibiotics observed in our country may be related to the extensive usage of these drugs during the past decade as feed additives for growth promotion (Ranjbar *et al.*, 2004). Such development of multiple antibiotic-resistance strains in poultry could be due to the use of antibiotics as feed additives for growth promotion and prevention of disease, as confirmed by the present study (Geornaras *et al.*, 2001; Panigrahy and Ling, 1990, Rafiiei and Nasirian, 2004). Efforts are needed to reduce the prevalence of resistant *Salmonella* in food, including the adoption of guidelines for the prudent use of antimicrobial agents in animals used for food, the passage of new foodsafty regulations and a reduction in the number of pathogens present on farms and in slaughterhouses. In addition surveillance program focusing on the identification and molecular subtyping of zoonotic foodborne pathogens that are present in foods should be established.

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