Antimicrobial Resistance Profile of *Salmonella* Serovars Isolated from Chicken Meat

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ABSTRACT

The study was designed to investigate the prevalence and antibiotic resistance profile of *Salmonella spp.* in broiler chicken meat sold in the Karachi City-Pakistan. A total of 160 meat samples were randomly collected from different retail markets and examined for the presence of various species of salmonella. The prevalence rate recorded was 48.75%. Different species of salmonella detected were *S. enteritidis*, *S. typhi*, *S. pullorum* and *S. typhimurium* (48.71, 20.51, 20.51 and 10.25% respectively). All the isolates were resistant to ampicillin, streptomycin, cefotaxime, kanamycin, neomycin, nalidixic acid, tetracycline, bacitracin, erythromycin, novobiocin, and spectinomycin. However, the isolates showed sensitivity to ceftazidime, gentamicin, tobramycin, ciprofloxacin, ofloxacin and chloramphenicol. In conclusion, the chicken meat may be a source of multiple antimicrobial-resistant salmonella for human infections.

***Key Words*:** Salmonella; prevalence; antimicrobial resistance; poultry meat

INTRODUCTION

Poultry is one of the largest industries of Pakistan that has increased at the rate of 20 to 25% per annum for last few decades and producing 19% (0.60 million tons) of the total meat production in the country (Anonymous, 2008). The industry has been facing devastating hazards; lack of disease control programs being one of them. Salmonellosis is a food borne disease of primary concern in developed and developing countries. It is one of the major public health problems in terms of socio-economic impact (Gracia and Finlay, 1994). A wide array of animal reservoir and commercial distribution of both animals and food products favor the spread of the disease.

Poultry birds have frequently been incriminated as a mean of salmonella contamination and consequently act as major source of the pathogen in humans (Baeumler et al., 2000). This organism has been isolated from a range of foods in almost every country (Rumeu et al., 1997). The level of contamination dramatically increases during the containment of the animals in holding pens before slaughter (D'Aoust, 1994). Besides this, the increasing incidence of salmonellosis is due to a number of technical practices (Kent et al., 1981). After slaughter, the subsequent dressing of meat increases the spread of salmonella on meat surfaces, and by the time the meat is in retail outlets, contamination levels may be increased by 20% (Forsythe and Hayes, 1998). Such practices have made salmonellosis a major economic and public health problem in many countries (Rumeu et al, 1997).

Most salmonella infections in humans result from the ingestion of contaminated poultry, beef, pork, eggs, and milk (Gomez et al.,

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1997). When infection spreads beyond the intestinal tract, appropriate antimicrobial therapy can be lifesaving (Hohmann, 2001; Glynn et al., 1998).

Use of antimicrobials in any environment creates selection pressures that favor the survival of antibiotic-resistant pathogens. The routine practice of giving antimicrobials to domestic livestock for growth promotion and prophylaxis is an important factor in the emergence of antibiotic-resistant bacteria in the food chain (Tollefson et al., 1997; Witte, 1998). Most antimicrobial-resistant salmonella infections are acquired by eating contaminated foods of animal origin (Angulo et al., 2000; Fey et al., 2000). The present study planned to estimate the prevalence of *Salmonella* serotypes in broiler meat and evaluate resistance profile of these isolates against commonly used antibiotics.

MATERIALS AND METHODS

Sample Collection

A total of 160 broiler carcasses were randomly collected from different fresh meat markets of the City Karachi. Forty samples from each of four localities designated as groups A, B, C and D were collected. The samples were analyzed for the presence of salmonella and also to assess the antimicrobial resistance profile.

Isolation and Identification of Salmonella

Approximately 10 g of meat sample was minced and placed in 10 mL of buffered peptone water (Oxoid, Basingstoke, UK) as a pre-enrichment media and incubated at 37°C for 18 hours. Aliquots from pre-enrichment were inoculated into selective enrichment liquid media at a ratio of 1:10 in Selenite-Cysteine broth. A loop full of broth was streaked on plates of Brilliant Green agar, MacConkey agar, and Salmonella-Shigella agar (Oxoid, Basingstoke, UK). The plates were incubated at 37 ºC for 24 hours. Suspected colonies of salmonella from each plate were collected for presumptive identification based on their morphological characteristics and various biochemical tests that included catalase, oxidase, motility, triple sugar iron agar (TSI), indole, methyl red, Voges-Proskauer and citrate utilization test. The colonies identified on the basis of biochemical tests were subjected for serological tests using polyvalent serum against O and H salmonella antigens (Difco, Detroit, USA). The colonies that agglutinated during the period of one to two minutes were considered as positive for salmonella, and were preserved in Nutrient agar at 4°C. Suspected colonies (maximum five) were randomly selected from each plate and confirmed by further biochemical tests including fermentation of glucose, lactose and sucrose, hydrogen sulfide production, urease activity, phenylalanine deamination, and lysine decarboxylation tests.

Antimicrobial Sensitivity Test

The isolates were subjected to sensitivity tests as described earlier (Bauer et al., 1996). Each isolate was inoculated in brain heart infusion broth (BHI) separately and incubated for 24 hours at 37ºC. The broth were streaked using sterile cotton swabs on Mueller-Hinton agar plates. The diffusion discs with antimicrobial drugs were placed on the plates and incubated for 24 hours at 37ºC. The antibiotics discs (Oxoid, Basingstoke, UK) used were: ampicillin (10 µg), cefotaxime (30 µg), ceftazidime (30 µg), streptomycin (10 µg), gentamicin (10 µg), kanamycin (30 µg), tobramycin (10 µg), neomycin (30 µg), nalidixic acid (30 µg), ciprofloxacin (5 µg), ofloxacin (5 µg), chloramphenicol (30 µg), tetracycline (30 µg), bacitracin (10 µg), erythromycin (10 µg), novobiocin (30 µg) and spectinomycin (10 µg).

RESULTS AND DISCUSSION

Of 160 samples tested, 78 (48.75%) were found positive for various species of

salmonella (Table 1). Out of the positive samples, *S. enteritidis* was found in 38 (48.71%), *S. typhi* in 16 (20.51%), *S. pullorum* in 16 (20.51%) and *S. typhimurium* in 8 (10.25%) samples. The distribution of various species of salmonella in all four localities is mentioned in Table 2.

Poultry are the most important reservoir for salmonella, with prevalence in chicken carcasses ranging from 20-70% in most countries (D'Aoust, 1989). The high prevalence of salmonella in chicken meat may be a result of cross-contamination from intestines during processing and cutting or from cages, floor and workers during retailing or marketing. The contamination rates observed in our results are not in agreement with those observed in other countries, 23-34% in Belgium (Uyttendaele et al., 1998), 25% in United Kingdom (Plummer et al., 1995), 26% in Ireland (Duffy et al., 1999) and 36% in Malaysia (Rusul et al., 1996). However, Beli et al. (2001) reported the low prevalence (8 %) of salmonella in poultry products in Albania. The difference in the prevalence rates may be due to socio-economic factors.

The high prevalence (48.71%) of *S. enteritidis* observed in this study is comparable to the situation described in most countries in recent years (Bailey et al., 2002). It appears that the presence of this serovar in the intestinal tract of broilers can contaminate carcasses during slaughter and processing. The presence of *S. typhi* and *S. typhimurium* in poultry is of considerable importance from the standpoint of public health. *S. pullorum* indicated the higher level of fecal contamination in the present study, as earlier reported by Orji et al. (2005).

Broiler meat is an important source of protein and a valuable commodity for the local consumers in the City Karachi, Pakistan. The study revealed that most shops may not operate in a safe and clean environment. The processing of carcass as per consumers demand further increases the chances of contamination. The water used for washing of carcasses is mostly from the same container and it could be contaminated with salmonella from feces or from the butcher's hands during washing. For the comparison of our findings with other studies, several factors may be considered,

Table 1 Species-wise prevalence of *Salmonella* serovars isolated from broiler meat

Sampling group	Positive samples (%)	Serovar(s) isolated	No. of Serovar out of positive samples (%)
A (n = 40)	21 (52.5)	*S. pullorum*	5 (23.8)
		S. typhi	9 (42.8)
		S. enteritidis	7 (33.3)
B (n = 40)	13 (32.5)	*S. typhimurium*	2 (15.3)
		S. typhi	4 (30.7)
		S. enteritidis	7(53.8)
C (n = 40)	27 (67.5)	*S. pullorum*	11 (40.7)
		S. enteritidis	16 (59.2)
D (n = 40)	17 (42.5)	*S. typhi*	3 (17.6)
		S. enteritidis	8 (47.0)
		S. typhimurium	6 (35.29)

such as differences in origin, time period, age of the samples, sampling procedure, contamination level of animals, slaughter house sanitation, cross-contamination of the products, and differences in methodology applied for detection of pathogen (Bryan and Doyle, 1995).

The different salmonella serotypes and their rate of isolation from poultry meat are shown in Tables 1 and 2. Majority of the salmonella serotypes isolated from all the sources are known to be pathogenic to human. A total of 17 antibiotic sensitivity discs were used. Out of these salmonella was sensitive to six antibiotics and resistant to eleven (Table 3). All isolates showed resistance to ampicillin, cefotaxime,

Table 2 Total number of Salmonella serovars isolated from poultry meat

Serovar	No. of isolated samples	Isolated samples (%)
S. pullorum	16	20.51
S. typhi	16	20.51
S. enteritidis	38	48.71
S. typhimurium	08	10.25
	78	99.98

Table 3 Antibiotic resistance profile of *Salmonella* serovars isolated from broiler meat

Antibiotics	*S.enteritidis*	*S. typhi*	*S. pullorum*	*S. typhimurium*	*Mean*
Ampicillin (10)	78.9	87.5	100	87.5	88.4
Cefotaxime (30)	2.6	-	-	-	2.6
Ceftazidime (30)	-	-		-	-
Streptomycin (10)	31.5	68.7	81.2	75.5	64.2
Gentamycin (10)	-	-	-	-	-
Kanamycin (30)	7.8	6.2	-	-	3.5
Tobramycin (10)	-	-	-	-	-
Neomycin (30)	47.3	62.5	100	25.0	58.7
Nalidixic acid (30)	55.2	87.5	31.2	50.0	55.9
Ciprofloxacin (5)	-	-	-	-	-
Ofloxacin (5)	-	-	-	-	-
Chloramphenicol (30)	-	-	-	-	-
Tetracycline (30)	84.21	93.75	93.7	87.5	89.7
Bacitracin (10)	28.9	12.5	50.0	75.0	41.4
Erythromycin (10)	31.5	62.5	56.2	12.5	40.6
Novobiocin (30)	71.0	6.25	68.7	62.5	52.1
Spectinomycin (10)	-	12.5	6.2	-	4.6

streptomycin, kanamycin, neomycin, nalidixic acid, tetracycline, bacitracin, erythromycin and novobiocin. On the other hand, the isolates were sensitive to ceftazidime, gentamycin, tobramycin, ciprofloxacin, ofloxacin and chloramphenicol. Ampicillin resistance was observed in all the isolated serotype which is in agreement with the findings of Suresh et al. (2006). The resistance to tetracycline was also observed in 89.7% of the isolates that is higher than the reported studies (Bada-Alambedji et al., 2006; Antunes et al., 2003; Santos et al., 2003). Tetracycline has been one of the most commonly used growth promoters. Therefore, resistance to tetracycline could be expected since the members of this class (chlortetracycline and oxytetracycline) have been routinely used as antibiotic growth promoters (Jones and Ricke, 2003). Resistance to streptomycin (64.2 %) was also higher and is in

conformity with the findings of Cardoso et al. (2006). This elevated resistance may be explained by the possible diffusion of the *tet* (A) resistance gene, as observed in Italy by Pezzella et al. (2004).

The *Salmonellae* were also resistant to nalidixic acid (55.9 %). Recently, some authors have reported an increase in quinolone resistance in salmonella (Molbak et al., 2002). This is interesting as quinolone resistance is chromosomally mediated, thus allowing increased salmonella quinolone resistance in humans or animals (Pezzella et al., 2004). On the other hand, no resistance to ciprofloxacin was observed which is in accordance to Cardoso et al. (2006). Ciprofloxacin is a fluoroquinolone antimicrobial that is increasingly and successfully used for the treatment of septicemic salmonellosis in humans (Brown et al., 1994). Our findings regarding kanamycin resistance (3.5%) are similar to results of Carraminana et al. (2004).

A high level of resistance to erythromycin, bacitracin and novobiocin was observed in the present study (Table 3). Previous study on 86 strains of *S. enteritidis* by Singer et al. (1992) showed 100% resistance to bacitracin. Salmonella resistance at varying concentrations of penicillin, chloramphenicol, streptomycin, spectinomycin and erythromycin has also been reported by Sultana et al. (1992).

In conclusion, antibiotic resistance profile indicates the limited therapeutic value of ampicillin, streptomycin, kanamycin, neomycin, nalidixic acid, tetracycline, bacitracin, erythromycin, novobiocin and spectinomycin. There is a need for continued surveillance is emphasized to determine regular antimicrobial susceptibility data to identify the changing pattern of resistance. Keeping in view the several possibilities of salmonella contamination in the poultry industry, specific epidemiological studies on the spread of salmonella at various levels of production is needed on a long term basis.

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