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Prevalence and antimicrobial resistance of *Salmonella* serovars isolated from poultry meat in Hyderabad, Pakistan

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Abstract: This study was conducted to investigate the prevalence of *Salmonella* in broiler chicken meat sold in Hyderabad, Pakistan. A total of 100 samples were randomly collected from poultry meat retail markets and examined for the presence of *Salmonella*. The prevalence rate recorded was 38%. The most prevalent serogroups were *S. enteritidis*, *S. typhi, S. pullorum*, and *S. typhimurium*. All the *Salmonella* isolates showed resistance to ampicillin, and sensitivity to tetracycline, streptomycin, cefotaxime, ceftazidime, gentamicin, tobramycin, ciprofloxacin, ofloxacin, and chloramphenicol. The findings highlighted the magnitude of *Salmonella* contamination in chicken meat sold in the city, and the antibiotic resistance of *Salmonella* isolates is an indication of indiscriminate and continuous use of antibiotics in poultry feed as well as in the broiler flocks. The results showed the possible significance of chicken meat as a source of antimicrobial-resistant *Salmonella* for human infections and suggested the need for further detailed epidemiological studies.

Key words: *Salmonella,* prevalence, antimicrobial resistance, poultry meat

The poultry industry has made great strides in the last few decades in Pakistan and has increased at the rate of 20% to 25% per annum producing 0.652 million tons of meat, which is 23% of the total meat production in the country (1). The broiler meat is largely consumed throughout the country in order to meet the nutritional requirements in the form of animal protein. However, the industry is facing major problems such as lack of disease control programs mainly associated with poor handling of raw material from production to marketing facilities. Increase in demand for meat without the infrastructure for

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proper sanitary handling may lead to transfer of pathogenic microorganisms from animals to the consumer. These bacterial hazards are of major concern in the hygienic production of food of animal origin.

Among the food-borne pathogens the genus *Salmonella* is one of the most common causes of food-borne infections worldwide (2). A characteristic feature of this organism is its wide host range, which comprises most animal species including mammals, birds and cold-blooded animals in addition to humans. It has been reported that *Salmonella* is one of the most important pathogens responsible for human food poisoning in the developed world, and chicken products are widely acknowledged to be a significant reservoir for *Salmonella*. Therefore, this organism has been isolated from a range of foods in almost every country in which it has been investigated (3). During transportation broiler flocks are thought to be in stress and that leads to shedding of *Salmonella* from *Salmonella* carriers, hence contaminating other birds. The level of contamination dramatically increases during the containment in holding cages before slaughter. Besides this the increasing incidence of salmonellosis is due to a number of technical practices, including traditional slaughtering, handling with different levels of hygiene, and transportation distances. After slaughter, the subsequent dressing of meats increases the spread of *Salmonellae* on meat surfaces and, by the time the meat is in retail outlets, contamination levels may have increased by up to 20% (4). Such practices have made salmonellosis a major economic problem in the food industry and a public health hazard for many countries. In addition to its pathogenicity, there has been concern about antimicrobial resistance in *Salmonella*, which has lead to failure of treatment for *Salmonella* and other bacterial pathogens (5). Since foods of animal origin are a major source of *Salmonella* spp., it has been suggested that antimicrobial use in food animal production may contribute to the presence of antimicrobial resistance in *Salmonella* spp. that infects humans. The purpose of the present study was to evaluate the prevalence of *Salmonella* isolated from poultry meat and to estimate the resistance profile of the isolates against commonly used antibiotics.

Sample collection: A total of 100 broiler carcasses were randomly collected from sale points located at different fresh poultry meat retailers of Hyderabad market. The samples were then brought to the Microbiology Section, Central Veterinary Diagnostic Laboratory, Tandojam, in sterile wide-mouth screw-capped bottles under refrigeration and then analysed for the presence of *Salmonella*.

Isolation and identification of Salmonella: Approximately 25 g of meat was excised from each collected sample, minced and placed in 225 mL of buffered peptone water (Oxoid, Basingstoke, UK) as pre-enrichment media, and incubated at 37 °C for 18 h. Aliquots from pre-enrichment were inoculated into selective enrichment liquid media at a ratio of 1:10 in Selenite-Cysteine broth. A loopful of broth was streaked on plates of Brilliant Green agar, MacConkey agar, and *Salmonella-Shigella* agar (Oxoid, Basingstoke, UK). The temperature and the period of incubation were standardised at 37 °C for 24 h, respectively. Suspected colonies of *Salmonella* from each plate were collected for presumptive identification by their morphological characteristics and biochemical tests. The primary tests included Gram's stain, catalase, oxidase, motility, Triple Sugar Iron agar (TSI), indol, methyl red, Voges-Proskauer, and citrate utilisation test. Colonies with biochemistry profile of *Salmonella* were submitted to serological tests by using polyvalent serum against O and H *Salmonella* antigens (Difco, Detroit, MI, USA). The colonies that agglutinated during the period of 1 to 2 min were considered as positive for *Salmonella*, and were preserved in nutrient agar at 4 °C. Suspected colonies (maximum 5) were randomly selected from each plate and confirmed by biochemical tests including fermentation of glucose, lactose and sucrose, hydrogen sulphide production, urease activity, phenylalanine deamination, lysine decarboxylation, citrate, methyl red, and indole tests.

Antimicrobial sensitivity test: The behaviour of all *Salmonella* isolates was checked for their sensitivity against the antimicrobial agents. The isolates were submitted to sensitivity tests according to the Bauer-Kirby method (6). Each isolate was inoculated in BHI (brain heart infusion) broth (Oxoid, Basingstoke, UK), following the 24 h of incubation at 37 °C, the broth was streaked by using sterile swabs on Mueller-

Hinton agar (Oxoid, Basingstoke, UK) plates. Plates were kept at room temperature for 5 min, and then diffusion disks with antimicrobial drugs were placed on the plates and incubated for 24 h at 37 °C. The antibiotics (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), and trimethoprim–sulfamethoxazole (25 µg). Results were interpreted by measuring inhibition zones with a millimetre scale.

Out of 100 samples tested 38 (38%) were found positive for various species of *Salmonella*. *S. enteritidis* was found in 16 (42.10%) samples, *S. typhi* in 11 (28.94%), *S. pullorum* in 7 (18.4%), and *S. typhimurium* in 4 (10.52%) (Table 1).

The different *Salmonella* serotypes and their rates of isolation from poultry meat are shown in Table 1. The most common serogroups found in poultry meat samples were from groups B and D. The majority of the *Salmonella* serotypes isolated from all the sources are known to be pathogenic to humans.

As seen from Table 2, all isolates showed sensitivity to cefotaxime, ceftazidime, gentamicin, tobramycin, ciprofloxacin, ofloxacin, and chloramphenicol, whereas resistance to streptomycin, tetracycline, and ampicillin was also detected. A lower proportion of isolates were resistant to kanamycin and trimethoprim–sulphametoxazole.

Poultry are the most important animal reservoir for *Salmonella*; the incidence in chicken carcasses in most countries ranged from 20% to 70% (7). The incidence 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 in agreement with those observed in other countries: 23%-34% in Belgium (8), 25% in the United Kingdom (9), and 36% in Malaysia (10).

The high prevalence (42.10%) of *S. enteritidis* observed in the present study is comparable to the situation described in most countries in recent years (11). It reflects the presence of this serovar in the intestinal tract of live broilers, contaminating carcasses during slaughter and processing. The

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

Sampling group	Samples collected (no.)	No. of samples positive	Percentage	Serovar isolated	(no.)
A	25	12	48	*S. pullorum*	(3)
				S. typhi	(3)
				S. enteritidis	(6)
B	25	10	40	*S. pullorum*	(2)
				S. typhimurium	(2)
				S. typhi	(3)
				S. enteritidis	(3)
C	25	9	36	*S. pullorum*	(2)
				S. typhi	(3)
				S. enteritidis	(4)
D	25	7	28	*S. typhi*	(2)
				S. enteritidis	(3)
				S. typhimurium	(2)
Total	100	38	38		

Table 2. Antibiotic resistance profile of *Salmonella* serovars isolated from broiler meat.

Antibiotics (µg/ disc)	***S. enteritidis*** (n = 16)	***S. typhi*** (n = 11)	***S. pullorum*** (n = 7)	***S. typhimurium*** (n = 4)
Ampicillin (10)	16	11	7	4
Cefotaxime (30)	-	-	-	-
Ceftazidime (30)	-	-	-	-
Streptomycin (10)	15	10	6	4
Gentamicin (10)	-	-	-	-
Kanamycin (30)	-	-	-	1
Tobramycin (10)	-	-	-	-
Neomycin (30)	4	2	4	2
Nalidixic acid (30)	10	6	5	2
Ciprofloxacin (5)	-	-	-	-
Ofloxacin (5)	-	-	-	-
Chloramphenicol (30)	-	-	-	-
Tetracycline (30)	15	10	7	4
Trimethoprim–sulfamethoxazole (25)	1	2	2	1

presence of *S. typhi* and *S. typhimurium* in poultry is of considerable importance from the standpoint of public health,whereas *S. pullorum* isolated in the present study indicates the faecal contamination of carcass as reported earlier (12).

Broiler meat is an important source of protein and a valuable commodity for the local consumers in the city of Hyderabad, Pakistan. This study on poultry meat sale points revealed that most of the shops do not operate in a safe and clean environment, and rarely practice the appropriate covering for displayed carcass. Moreover, the practice of using the same cutting knives for the uninfected and infected carcass, results in a further chance of cross-contamination. The processing of carcasses as per consumer demand further spreads contamination by exposing carcass surface and susceptible fleshy parts to the contaminants by using the same cutting tables. In addition, the water used for washing of carcasses is from the same container and it could be contaminated with *Salmonella* from faeces or from the butcher's hands during washing. For the comparison of our findings with other studies several factors should be considered, such as differences in origin, time period, and age of the samples; sampling procedure; contamination level of animals; slaughterhouse sanitation; cross-contamination of the products; and differences in methodology applied for detection of pathogens (8).

It is estimated that nearly 90% use of all antibiotic agents is in food animals, given either at sub-therapeutic concentrations prophylactically or in order to promote growth. The 40% of antibiotic production in the USA was for use in stock feeds, which included 55%-60% of penicillin and tetracycline (13).

Ampicillin resistance was observed in all the isolated serotype, which is in agreement with the findings of Suresh et al. (14); they also observed a higher proportion of ampicillin-resistant salmonella strains isolated from eggs. The resistance to tetracycline was observed in 94.73% of the isolates, which is higher than that reported in different studies: 46.6% in Senegal (15) and 36% in Portugal (16). Tetracycline has been one of the most commonly used antibiotics for production animals; from day-old chicks to broiler chickens, they are exposed to antimicrobial drugs during their growth phase. Therefore, resistance to drugs such as tetracycline could be expected since the members of this class (chlortetracycline and oxytetracycline) are

approved for use in broiler feeds for the purpose of growth promotion (17). Resistance to streptomycin (92.10%) was also higher and is in conformity with other findings (18). This resistance to tetracycline and streptomycin commonly observed among the *Salmonella* isolates has been frequently reported; this elevated resistance may be explained by the possible diffusion of the tet (A) resistance gene observed in an epidemiological study with *Salmonella* strains isolated from animals (19). The *Salmonellae* revealed resistance to nalidixic acid (60.52%). Recently, some authors have reported an increase in quinolone resistance in *Salmonella* (20). This is a worrying finding because quinolone resistance is chromosomally mediated, thus allowing an increase of *Salmonella* quinolone resistant in humans or animals (19). On the other hand, no resistance to ciprofloxacin was observed, which is in accordance with Cardoso et al. (18). Ciprofloxacin is a fluoroquinolone antimicrobial that is increasingly and successfully used for the treatment of septicaemic salmonellosis in humans. Our findings regarding kanamycin resistance (2.6%) are almost in agreement with the 2.8% found in *Salmonella enteritidis* isolated from a poultry slaughterhouse in Spain (21).

The uncontrolled use of the antimicrobial agents in food animals may have contributed to the development of the pattern of resistance observed. The lack of stringent regulations and monitoring in dispensing of antibiotics in veterinary establishments as well as the mass inoculation of herds of animals by some farmers has been raised as a contributory factor in the increase in antibiotic resistance. Therefore, vigilance against the rise in resistance of *Salmonella*e to antibiotics is essential.

The present study underlines the need for adequate consumer protection against *Salmonella*. In order to prevent zoonotic *Salmonella* serovars from entering the food chain, bacteriological monitoring of broiler flocks and separation of infected flocks from food production together with introduction of good manufacturing practices and hygiene control must be implemented.

The present study gives a clear perspective on the extent of *Salmonella* contamination in broiler chicken meat marketed in Hyderabad, Pakistan. 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 are needed on a long-term basis.

References

1. Anonymous: Pakistan economic survey 2008-09. Government of Pakistan, Finance Division, Economic Advisor's Wing, Islamabad, 2008: 32-34.
2. Baird-Parker, A.C.: Foodborne Salmonellosis. Lancet, 1990; 336: 1231-1235.
3. Rajashekara, G., Harverly, E., Halvorson, D.A., Ferris, K.E., Lauer, D.C., Nagaraja, K.V.: Multidrug resistant *Salmonella typhimurium* DT104 in poultry. J. Food Prot., 2000; 63: 155-161.
4. Forsythe, S.J., Hayes, P.R.: Food hygiene, microbiology and HACCP. Gaithersburg: Aspen Publishers, Inc., 1998.
5. Butt, T., Ahmad, R., Mahmood, M., Zaidi, S.: Ciprofloxacin treatment failure in typhoid fever case, Pakistan. Emerg. Infec. Dis., 2003; 9: 1621-1622.
6. Bauer, A.W., Kirby, W.M., Sherris, J.C.: Antibiotic susceptibility testing by a standard single disc method. Amer. J. Clin. Pathol., 1996; 45: 493-496.
7. Mead, G. C.: Food poisoning *Salmonella* in the poultry meat industry. Br. Food J., 1990; 92: 32-36.
8. Uyttendaele, M., De Troy, P., Debevere, J.: Incidence of *Salmonella*, *Campylobacter jejuni, Campylobacter coli*, and *Listeria monocytogenes* in poultry carcasses and different types of poultry products for sale on the Belgian retail market. J. Food Prot., 1999; 62: 735-740.
9. Plummer, R.A.S., Blissett, S.J., Dodd, C.E.R.: *Salmonella* contamination of retail chicken products sold in the UK. J.Food Prot., 1995; 58: 843-846.
10. Rusul, G., Khair, J., Radu, S., Cheah, C.T., Yassin, R.M.: Prevalence of *Salmonella* in broilers at retail outlets, processing plants and farms in Malaysia. Inter. J. Food Microbiol., 1996; 33: 183-194.
11. Cardinale, E., Gros-Claude, J.D.P., Rivoal, K., Rose, V., Tall, F., Mead, G.C., Salvat, G.: Epidemiological analysis of Salmonella enterica spp. enterica serovars Hadar, Brancaster and Enteritidis from humans and broiler chickens in Senegal using pulsed-field gel electrophoresis and antibiotic susceptibility. J. Appl. Microbiol., 2005; 99: 968-977.

12. Orji, M. U., Onuigbo, H. C., Mbata, T.I.: Isolation of Salmonella from poultry droppings and other environmental sources in Awka, Nigeria. Inter. J. Infec. Dis., 2005; 9: 86-89.

13. Prescott, J.F.: Antibiotics: miracle drugs or pig food? Canad. Vet. J., 1997; 38: 763-766.

14. Suresh, T., Hatha, A.A.M., Sreenivasan, D., Sangeetha, N., Lashmanaperumalsamy, P.: Prevalence and antimicrobial resistance of *Salmonella enteritidis* and other salmonellas in the eggs and egg-storing trays from retails markets of Coimbatore, South India. Food Microbiol. 2006; 23: 294-299.

15. Bada-Alambedji, R., Fofana, A., Sedi, M., Akakpo, A.J.: Antimicrobial resistance of *Salmonella* isolated from poultry carcasses in Dakar (Senegal). Braz. J. Microbiol., 2006; 37: 510-515.

16. Antunes, P., Reu, C., Souza, J.C., Peixe, L., Pestana, N.: Incidence of *Salmonella* from poultry products and their susceptibility to antimicrobial agents. Int. J. Food Microbiol., 2003; 82: 97-103.

17. Jones, F.T., Ricke, S.C.: Observations on the history of the development of antimicrobials and their use in poultry feeds. Poul. Sci., 2003; 82: 613-617.

18. Cardoso, M.O., Ribeiro, A.R., Santos, L.R., Pilotto, F., Moraes, H.L.S., Salle, C.T.P., Rocha, S.L.S., Nascimento, V. P.: Antibiotic resistance in *Salmonella* Enteritidis isolated from broiler carcasses. Braz. J. Microbiol., 2006; 37: 368-371.

19. Pezzella, C., Ricci, A., Di Giannatale, E., Luzzi, I., Carattoli, A.: Tetracycline and streptomycin resistance genes, transposons, and plasmids in *Salmonella enterica* isolated from animals in Italy. Antimicrob. Agents Chemoth., 2004; 48: 903-908.

20. Molbak, K., Gerner-Smidt, P., Wegerner, H.C.: Increasing quinolone resistance in *Salmonella enterica* serotype *Enteritidis*. Emerg. Infec. Dis., 2002; 8: 514-515.

21. Carraminana, J.J., Rota, C., Agustin, I., Herrera, A.: High prevalence of multiple resistance to antibiotics in *Salmonella* serovars isolated from a poultry slaughterhouse in Spain. Vet. Microbiol., 2004; 104: 133-139.