Microbes and Environments
Vol. 15, No. 3, 173 – 181, 2000

The Incidence, Antibiotic Resistance and Survival of *Salmonella* and *Escherichia coli* Isolated from Broiler Chicken Retail Outlets

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(Received December 20, 1999—Accepted June 19, 2000)

The incidence of *Salmonella* and *Escherichia coli* in chicken retail outlets in a residential area of Coimbatore, Tamil Nadu, India., was studied with the view that accessories may be a source of cross-contamination. Accessories like cages, knives, chopping boards, weighing balance trays and the hands of the butcher were examined. A total of 14 *Salmonella* as well as 31 *E. coli* strains were isolated from different sources. Strains of which 13 were *S. enteritidis* and 1 was *S. cerro*. The incidence of *E. coli* was higher than that of *Salmonella*. The highest incidence of *Salmonella* was found in chopping boards and the maximum level of *E. coli* was detected in cages. *Salmonella* and *E. coli* isolates were able to survive on different types of wood and metal surfaces for up to 24 hours at room temperature ($28 \pm 2°C$) without any nutrients. This showed that viable cells of both the bacteria could remain on the surface of the chopping boards, knives and weighing balance trays and cause cross contamination. All the strains of *Salmonella* and *E. coli* isolated were examined for resistance against 10 antibiotics. All *Salmonella* strains were resistant to neomycin, polymyxin-B and tetracycline and more than 90% were resistant to ampicillin. *E. coli* strains (100%) were found to be resistant to ampicillin, neomycin, polymyxin-B, sulphamethoxazole and tetracycline. Multiple antibiotic resistance indexing of both the strains revealed that they originated from high-risk sources of contamination, where antibiotics were often used. In conclusion, these organisms persist in the outlet for long periods and prevention of cross contamination of chicken meat will be needed.

Key words: *Salmonella*, *E. coli*, cross contamination, antibiotic resistance, meat, broiler chicken outlets

Minimization of bacterial contamination is a major concern among poultry processors and food safety researchers, because of the link between poultry products and *Salmonella*[5,18]. Shell eggs[16] and poultry meat products[23] are regarded as an important source of human Salmonellosis. It has been reported that raw meat, particularly poultry meat, is important in the dissemination of potential human pathogens including *Salmonella* on kitchen utensils, work surfaces and hands[17].

If the surface on which a foodstuff is processed is not free of microorganisms, cross contamination can occur. A concern with cutting boards in the home is

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that bacteria of animal origin may cause cross contamination[2]. Fluid ("juice") from raw meat or poultry remaining on the work surface might transfer pathogenic agents to other foods not to be cooked further before being eaten. It was reported that the bacteria of greatest concern as cross contaminants on kitchen cutting boards were of animal origin, being significant causes of human infectious diseases transmitted via foods[12].

In the processing of raw chicken in retail shops, the cutting board, knife, hands of the butcher, and weighing balance tray are the principal surfaces to which chicken meat comes into contact and any remaining food might enhance the growth of microorganisms and cause more frequent cross contamination. To keep contamination to a minimum and obtain good quality meat, all equipment coming into contact with the food should be adequately cleaned, sanitized and tested. It is also important, however, to take measures to avoid cross contamination through employees. This factor reflects not only in the food but also in the health of the employees themselves. *S. enteritidis* PT4 was recovered from the fingers following the breaking of intact shell eggs artificially contaminated with bacterium[17]. It was observed that the kitchen utensils were used to mix egg dishes were *Salmonella* positive, some times even after washing.

The present study was designed to determine the incidence of pathogens such as *Salmonella* and *E. coli* on various utensils and appliances in chicken retail outlets and to characterize their survival capacities on different types of wood and metal surfaces. The prevalence of antibiotic resistance among these strains was also investigated.

Materials and Methods

Collection of Sample

The study was carried out in all the 15 chicken retail markets in a residential area of Coimbatore City. In the retail outlets, chopping boards, knives, weighing balance trays, cage floor and mesh, and hands of butchers were examined. The samples were collected in the morning, between 7 and 9 am without prior nontification of the butcher or owner.

Swabs were used to sample the surface of chopping boards, the metal part of knives used for cutting meat, the inner side of the trays of the weighing balance, the cage mesh and the butcher's hands. For sampling the cage floors, 25 g of dust/droppings was collected[11]. Twenty five grams of meat was also collected from each outlet.

Bacteriological Method

A modified method of Hatha and Lakshmanaperumalsamy[15] was used with the replacement of lactose broth (pre-incubation medium) with buffered peptone water (BPW) (Hi media laboratories, Mumbai, India), for the isolation of *Salmonella*. The swabs were pre-enriched in 10 ml of buffered peptone water at 37°C for 24 hours. The cage dust/droppings were pre-enriched in 225 ml of BPW. The meat (25 g) was homogenized with 225 ml of BPW in a sterile blender for 5 min. Both cultures were incubated at 37°C for 24 h. One millilitre of the pre-enriched cultures was then transferred to 10 ml of tetrathionate broth (Hi media) and selenite broth (Hi media) and incubated at 37°C for 24 hours for selective enrichment. After selective enrichment, a loopful of the culture was streaked onto xylose lysine deoxycholate agar (Hi media), brilliant green agar (Hi media) and hektoen enteric agar (Hi media) and incubated at 37°C for 24–48 hours. Typical colonies were removed, purified and subjected to a preliminary biochemical screening, which involved hydrogen sulphide production in triple sugar iron agar (Hi media) and lysine iron agar (Hi media), indole production in tryptone broth (Hi media) and urea splitting on Christiansen's urea agar (Hi media). Cultures that matched typical reactions of *Salmonella* in the preliminary screening were further tested for carbohydrate utilization involving lactose, sucrose, dulcitol and salicin and the results confirmed by slide agglutination test using polyvalent O sera (Wellcome Laboratories, Dartford, England). The confirmed cultures were then sent to the National *Salmonella* and *Escherichia* Centre, Central Research Institute, Kasauli and serotyped.

For isolating *E. coli*, a loopful of pre-enriched culture was streaked onto MacConkey agar (Hi media)

and incubated at 37°C for 24 hr. Two representative colonies were selected from each dish, purified further and confirmed by Gram reaction, oxidase, indole, methyl red, voges proskauer and citrate (IMViC) tests[9].

Survival capacity of isolates

Three different types of dry hard wood commonly used as chopping boards in retail outlets, were selected for the survival study. The woods tested were mango, teak and tamarind. Small rectangular wooden blocks were made with a total surface area of 20 cm^2. Similarly, 20 cm^2 steel, stainless steel and nickel plates were selected for the experiment since the knives used were made of steel or stainless steel and the trays of the weighing balance were made of stainless steel or nickel-coated plates.

All 14 strains of *Salmonella* and 31 strains of *E. coli* isolated from various sources were examined for their capacity to survive on different types of wood and metal surfaces. The strains were inoculated in nutrient broth and incubated for 18 h at 37°C. After incubation, the culture broth was centrifuged at 2500 × g for 20 min in a refrigerated centrifuge. The cells were transferred to 0.85% NaCl and diluted to 10^5 cfu ml^{-1}.

Autoclaved wooden and metal blocks were used for the survival study, and 0.5 ml of the inoculum containing 10^5 cfu ml^{-1} of *Salmonella* and *E. coli* was spread over the entire surface of blocks and kept at room temperature for 24 hours. One set of the contaminated wooden and metal pieces was removed at 1, 2, 3, 4, 5, 6, 8, 10, 12, 14, 16, 20 and 24 h and was directly dipped into test tubes containing sterilized brilliant green broth for *Salmonella* and MacConkey broth for *E. coli*. The tubes were then incubated at 37°C for 24 h. Triplicates were maintained for all the experiments and the growth was examined. Sterilized wooden and metal blocks and pure cultures of both bacteria inoculated into the broth media were maintained as control.

Testing susceptibility to antibiotics

Disk diffusion assays for antibiotic susceptibility were conducted for all *Salmonella* and *E. coli* strains, as described by Bauer *et al.*,[8]. The bacterial strains were tested against antibiotic discs (Hi-media) of ampicillin (AP), 10 μg; chloramphenicol (CP), 30 μg; ciprofloxacin (CI), 5 μg; gentamicin (GE), 10 μg; kanamycin (KM), 30 μg; nalidix acid (NA), 30 μg; neomycin (NE), 30 μg; polymixin B (PL), 300 units; sulphamethizole (SMT), 300 units; and tertracycline (TC), 30 μg. After enrichment in tryptic soy broth (Hi media) for 6–8 h at 37°C, the cultures were streaked on Mueller Hinton agar (Hi media) plates using a sterile cotton swab. The antibiotic discs were placed on the agar surfaces sufficiently separated from each other so as to prevent over-lapping of the inhibition zones. After 30 minutes, the plates were inverted and incubated at 37°C for 16–18 h. Results were recorded by measuring the inhibition zones, comparing with the interpretive chart of the Kirby-Bauer sensitivity test method modified in July 1969 (Scherring Corporation, New Jersey, USA).

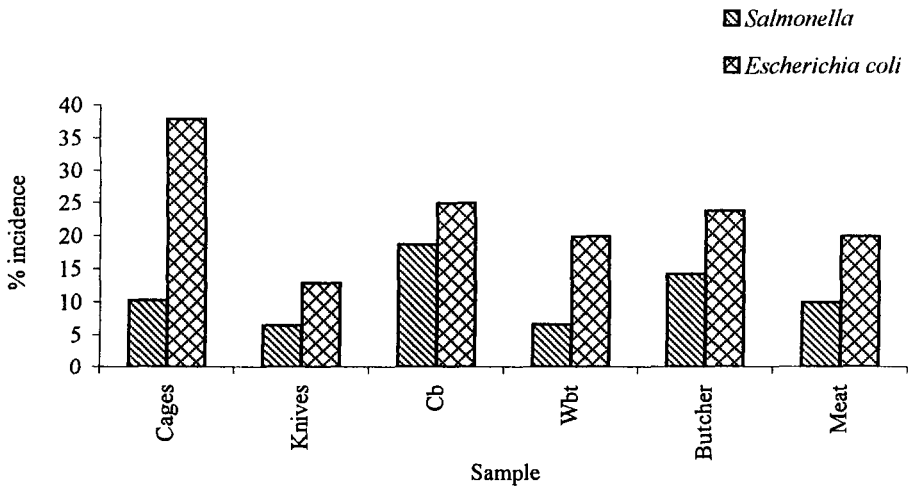
Multiple antibiotic resistance indexing of isolates

The multiple antibiotic resistance (MAR) index is defined as a/b where 'a' represents the number of antibiotics to which the particular isolate is resistant and 'b' the number of antibiotics to which the isolate is exposed[20]. MAR index values higher than 0.2 are considered to have originated from high risk sources where antibiotics are often used. MAR index values of less than or equal to 0.2 indicate a strain originated from sources where antibiotics are seldom or never used.

Results

Salmonella was isolated from 3 of 16 (19%) chopping boards, and the hands of 3 of 21 (14%) butchers in 15 broiler chicken retail outlets (Fig. 1). *Salmonella* contaminated 3 of 29 (10%) cages. Two of 20 (10%) meat samples collected from the outlets were positive for *Salmonella*. Two of 31 (6.5%) knives and 1 of 15 (6.7%) weighing balance trays were positive for *Salmonella* in the 15 retail outlets.

The areas most often contaminated by *E. coli* were the cages, 11 of 29 (38%). Among chopping boards, 4 of 16 (25%) samples were positive for *E. coli*. The



Cb - Chopping boards
Wbt - Weighing balance tray

Fig. 1. Percent incidence of *Salmonella* and *Escherichia coli*.

Table 1. Source and antibiotic resistance pattern of *Salmonella*

Shop No.	Strain No. & serotype	Source	MAR index	Antibiotic resistance									
				AP	CP	CI	GE	KN	NA	NE	PL	SMT	TC
2	S1 – *S. enteritidis*	Knife	0.4	+	–	–	–	–	–	+	+	–	+
5	S2 – *S. enteritidis*	Butcher	0.5	+	–	–	–	–	+	+	+	–	+
7	S3 – *S. enteritidis*	Cage	0.5	+	+	–	–	–	–	+	+	–	+
	S4 – *S. enteritidis*	Cb	0.5	+	–	–	–	–	+	+	+	–	+
	S5 – *S. enteritidis*	Butcher	0.5	+	–	–	–	–	+	+	+	–	+
	S6 – *S. enteritidis*	Meat	0.5	+	–	–	–	–	+	+	+	–	+
8	S7 – *S. enteritidis*	Wbt	0.5	+	–	–	–	–	+	+	+	–	+
9	S8 – *S. enteritidis*	Cage	0.4	+	–	–	–	–	–	+	+	–	+
	S9 – *S. enteritidis*	Cb	0.4	–	–	–	–	–	+	+	+	–	+
11	S10 – *S. enteritidis*	Cb	0.5	+	–	–	–	–	+	+	+	–	+
	S11 – *S. enteritidis*	Butcher	0.5	+	–	–	–	–	+	+	+	–	+
	S12 – *S. enteritidis*	Meat	0.5	+	–	–	–	–	+	+	+	–	+
12	S13 – *S. cerro*	Knife	0.5	+	–	–	–	–	+	+	+	–	+
13	S14 – *S. enteritidis*	Cage	0.4	+	–	–	–	–	–	+	+	–	+

AP – Ampicillin
CP – Chloramphenicol
CI – Ciprofloxacin
GE – Gentamicin
KN – Kanamycin
NA – Nalidix acid
NE – Neomycin
PL – Polymixin-B
SMT – Sulphamethizole
TC – Tetracycline
Cb – Chopping board
Wbt – Weighing balance tray
(+) – Resistant
(–) – Sensitive

hands of butchers were *E. coli* positive in 5 of 21 (24%) cases, *E. coli* contamination was found in 3 of 15 (20%) weighing balance trays and 4 of 31 (13%) knives. *E. coli* contaminated 4 of 20 (20%) meat samples.

A total of 14 *Salmonella* (13 *S. enteritidis* and 1 *S. cerro* – S.c 13 from a knife in shop No. 12) strains from 8 broiler chicken retail outlets (Table 1) and 31 *E. coli* strains from 11 retail outlets were isolated (Table 2). The overall incidence rate of *E. coli* was found to be

Table 2. Source and antibiotic resistance pattern of *Escherichia coli*

Shop No.	Strain No.	Source	MAR index	Antibiotic resistance									
				AP	CP	CI	GE	KN	NA	NE	PL	SMT	TC
1	E1	Cage	0.5	+	–	–	–	–	–	+	+	+	+
	E2	Wbt	0.5	+	–	–	–	–	–	+	+	+	+
	E3	Butcher	0.5	+	–	–	–	–	–	+	+	+	+
	E4	Meat	0.5	+	–	–	–	–	–	+	+	+	+
2	E5	Knife	0.7	+	+	–	–	–	+	+	+	+	+
	E6	Butcher	0.5	+	–	–	–	–	–	+	+	+	+
3	E7	Cage	0.5	+	–	–	–	–	–	+	+	+	+
	E8	Cage	0.5	+	–	–	–	–	–	+	+	+	+
	E9	Cb	0.5	+	–	–	–	–	–	+	+	+	+
	E10	Meat	0.5	+	–	–	–	–	–	+	+	+	+
7	E11	Cage	0.6	+	–	–	–	–	+	+	+	+	+
	E12	Knife	0.5	+	–	–	–	–	–	+	+	+	+
	E13	Cb	0.5	+	–	–	–	–	–	+	+	+	+
	E14	Meat	0.6	+	–	–	–	–	+	+	+	+	+
8	E15	Cage	0.6	+	–	–	–	–	+	+	+	+	+
	E16	Butcher	0.6	+	+	–	–	–	–	+	+	+	+
9	E17	Cage	0.5	+	–	–	–	–	–	+	+	+	+
	E18	Cage	0.5	+	–	–	–	–	–	+	+	+	+
	E19	Knife	0.5	+	–	–	–	–	–	+	+	+	+
	E20	Wbt	0.5	+	–	–	–	–	–	+	+	+	+
10	E21	Cage	0.7	+	+	–	–	–	+	+	+	+	+
11	E22	Cb	0.5	+	–	–	–	–	–	+	+	+	+
	E23	Butcher	0.5	+	–	–	–	–	–	+	+	+	+
	E24	Butcher	0.5	+	–	–	–	–	–	+	+	+	+
	E25	Meat	0.5	+	–	–	–	–	–	+	+	+	+
12	E26	Cage	0.6	+	–	–	–	–	+	+	+	+	+
13	E27	Cage	0.7	+	+	–	–	–	–	+	+	+	+
	E28	Wbt	0.5	+	–	–	–	–	–	+	+	+	+
15	E29	Cage	0.6	+	–	–	–	–	+	+	+	+	+
	E30	Knife	0.7	+	+	–	–	–	+	+	+	+	+
	E31	Cb	0.6	+	–	–	–	–	+	+	+	+	+

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higher than *Salmonella* in all sample types (Fig. 1).

The chopping boards used in the retail outlets were cut from the main trunk of a tree with a radius not less than 60 cm and 90 to 120 cm in height. The chopping boards in all broiler chicken retail outlets were not well maintained. They were rough with knife markings and also found to have remnants of chicken meat on the surface. The retail outlets had poor sanitation. Periodic sanitation of the processing environment and workers' hands was not practiced. Also, the same knife was used to process many birds, without sanitising between the processing. The chopping boards are made of mango, tamarind or teakwood. These three types of wood species were used to test the survival of the isolates. It was found that all the strains of *Salmonella* and *E. coli* were able to survive on all the three types of wood and metal for 24 h. The control tubes with the sterile wooden blocks did not show any visible color change in the medium, which indicates that the woods do not contain any reactive agents which make visible color changes.

The results of the antibiotic sensitivity testing revealed that all of the 14 *Salmonella* strains were resistant to neomycin, polymyxin-B and tetracycline. Ninety three percent of strains were ampicillin resistant and 86% were resistant to nalidixic acid. Chloramphenicol resistant strains composed only 7%. All the *Salmonella* strains were resistant to four and five antibiotics and showed MAR indices of 0.4 and 0.5 (Table 1). Five different types of resistance patterns were recorded among the *Salmonella* strains encountered in the study.

All of the *E. coli* isolates were resistant to ampicillin, neomycin, polymyxin-B, sulphamethoxazole, and tetracycline. And 24% and 16% of strains showed resistance against nalidixic acid and chloramphenicol, respectively. All *E. coli* strains were resistant to 5 to 7 antibiotics and exhibited MAR indices of 0.5–0.7 (Table 2). *E. coli* strains showed four different resistance patterns.

Discussion

Salmonella are capable of prolonged survival outside the living host[10], in dry livestock housing[6,7]. Infection of humans with *S. enteritidis* has often been associated with the consumption of poultry meat and other poultry products contaminated with *S. enteritidis*[4,25,26]. In the present investigation *Salmonella* spp. and *E. coli* with multiple antibiotic resistance were readily isolated from cages, knives, chopping boards, weighing balance trays, hands of butchers and meat samples. In the broiler chicken retail outlets, live birds are processed. *Salmonella* and *E. coli* contamination of knives, cutting boards, weighing machines and workers' hands, suggests cross contamination during processing.

The birds' are sacrificed by cutting their throat. The head and leg portions below the knee were cut and removed. The birds are then defeathered and the gut contents removed. The meat is chopped on wooden blocks, which are generally in a poor sanitary condition with blood and meat on them. Periodical sanitizing of utensils, the processing table and workers' hand is also found to be lacking at retail outlets. These conditions are ideal for the multiplication of bacteria and for cross contamination.

The level of incidence for the chopping boards showed clearly that the organisms could survive in the presence of scraps of chicken meat. The rough surface on the chopping boards may provide ideal conditions. Wooden boards being porous, are supposed to be harder to clean and decontaminate than plastic[2]. Ak *et al.*,[1] reported that bacteria might even multiply between being deposited on the surface and contaminating other foods and identified *E. coli* 0157: H7, *Listeria monocytogenes* and *S. typhimurium* as potential cross contaminants on chopping boards.

The hands of the butcher are also a potential source of cross contamination. There were individuals positive for both *Salmonella* and *E. coli*. It was reported that *S. entritidis* PT4 was isolated from the hands after the processing of a contaminated egg[16]. The knives and the weighing balance trays also tested positive for *Salmonella* and *E. coli* in some shops. Shop number 2 was positive for both the bacteria. The results of our study also showed that *Salmonella* spp. and *E. coli* could withstand moisture loss on metal surfaces and survive for a long period. Recovery of *S. typhimurium* from stainless steel surfaces inoculated with a

ground pork slurry after storage for two weeks at 10°C and 50% relative humidity was also reported[13]. This is consistent with our study, which indicates that *Salmonella* and *E. coli* survive on metal surfaces and may cause cross contamination via the knives and weighing balance tray. In our investigation, the cages were also positive for *Salmonella* and *E. coli*. It was previously reported that *Salmonella* could survive on small pockets of litter and wild birds' droppings[10].

The level of incidence of *Salmonella* and *E. coli* varied considerably. The results showed more frequent isolations of *E. coli* in the case of cages, where the cage dust contains mainly birds' droppings and feed, which allow the proliferation of bacteria. If inadequately cleaned, the cages can also be a source of cross contamination, as the birds are caged here before they are processed. The incidence of *Salmonella* and *E. coli* in the meat samples is a reflection of cross contamination of these microorganisms, which were found on the surface on which the meat was processed. In the present study, the overall incidence rate of *E. coli* was higher than that of *Salmonella* in all the retail outlets. The results of our present study showed that the bacteria on chopping boards and cage floors will cause cross contamination, while their growth may be enhanced by the presence of nutrient rich chicken meat on the chopping board and litter and spilled feed on the cage floor in humid tropical conditions.

Rough surfaced cutting boards with remnants of chicken meat and cages with birds droppings and feed spillage may be considered a high risk source of cross contamination. These surfaces can help bacteria to proliferate, which will be difficult to remove. The knife, balance tray and butcher's hands may play an intermediate role in bacterial contamination.

Little variation was found in antibiotic resistance of *Salmonella* isolated from different sources. Our results were similar to that of strains from broiler flocks in Canada[24], although their strains were sensitive to ciprofloxacin. The *Salmonella* strains encountered in our study were sensitive to ciprofloxacin. However, we could not find polymixin-B sensitive strains in the present investigation. It was reported that *S. typhi* isolated from two patients from Madras (Chennai) were resistant to ampicillin, trimethoprim, sulfamethizole, and chromphenicol, which was similar to our results and reduced susceptibility to ciprofloxacin was not noticed in our study[21].

The antibiotic resistance of the *E. coli* strains indicated that these organisms came from a high risk source. The antibiotic resistance pattern shown in both *Salmonella* and *E. coli* indicated some similarities. Both bacterial strains (100%) were resistant to neomycin, polymixin-B and tetracycline. For ampicillin, *E. coli* and *Salmonella* strains showed 100% and 93% resistance, respectively. A difference was found only in the resistance against sulphamethizole. While all the *E. coli* strains were resistant to sulphamethizole, none of the *Salmonella* strains were. In case of nalidixic acid, *E. coli* strains were marginally less resistant than *Salmonella* strains.

A logical interpretation of the results of the MAR index data is that all the *Salmonella* and *E. coli* strains from the retail chicken outlets have arisen from high risk sources of contamination (such as poultry, swine, cattle and human environments) where antibiotics are often used. Poultry, being one of the major reservoirs of *Salmonella* species, is considered to be a faecal contamination. There is a large body of literature[22] demonstrating that the subtherapeutic use of antibiotics in the mass production of poultry, eggs and pork has promoted the emergence and maintenance of MAR pathogenic bacteria in the faecal environment of these animals. In India, large scale production units of poultry are located in Tamil Nadu and Andhra Pradesh, which are also adopting such practices in order to obtain massive yields. A high frequency of antibiotic resistance to polymyxin-B, bacitracin and erythromycin, and low resistance to chloramphenicol and nalidixic acid were noticed among *Salmonella* strains isolated from veterinary sources in India[3].

The wide use and abuse of antibiotics in human therapy has produced MAR pathogenic microorganisms in the faeces of humans as well. There were 5 types of resistance patterns recorded for *Salmonella*, but most of the strains had a common pattern of antibiotic resistance. Similarly, the resistance pattern of *E. coli* strains was dominated by one type among 4 different patterns recorded. The antibiotic resistance pattern suggests that the origin of the organ-

isms is the same. It has also been suggested that isolates with an identical MAR index and the same resistance profile have a common origin[19]. The main reason why *Salmonella* and *E. coli* strains have a similar pattern of antibiotic resistance in some retail outlets may be due to the purchase of chicken from the same producer: the resistant bacteria in these chickens may be present on the farm and the bacteria shed by the broilers contaminate the retail outlets.

The results of the present study highlight the possibility of cross contamination from knives, cutting boards and workers' hands. As of now there are no standard operational procedures to sanitize these utensils. This poses a real health hazard to the consumers of chicken from these retail outlets. Legal measures should be implemented to install proper sanitation measures such as provision for chlorinated potable water, shower wash facilities, periodical sanitising schedules for the processing environment and utensils, maintenance of good personal hygiene and mandatory inspection of the retail outlets by officials of the food safety department in order to prevent a possible health hazard.

Acknowledgments

The authors wish to thank the Head, Department of Environmental Sciences, Bharathiar University, for providing necessary facilities and the owners and employees of the broiler chicken retail outlets for their cooperation.

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