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Salmonella Cross-contamination in Retail Chicken Outlets and the Efficacy of Spice Extracts on *Salmonella enteritidis* Growth Inhibition on Various Surfaces

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The source of *Salmonella* cross-contamination in 15 retail chicken outlets in a residential area of Coimbatore City, southern India, was studied. Chopping boards (18.75%) and the butcher's hands (14.29%) were predominant for *Salmonella* followed by knives and the weighing balance tray. Serotyping of the *Salmonella* strains revealed that all the strains were *Salmonella enteritis*, except one, which was found to be *Salmonella cerro*. The antibacterial activity of commonly used spices, such as garlic, onion, ginger, turmeric, clove, cinnamon and dry ginger against *S. enteritidis*, was evaluated. While garlic showed very good antibacterial activity even at very low concentrations, onion and ginger showed only moderate levels of inhibitory effect. The efficacy of the spice extracts in controlling the growth of *S. enteritidis* on various surfaces such as wood, plastic and stainless steel was also studied. The results indicated that garlic extract was very efficient in removing the organism from different contaminated surfaces at various concentrations.

Key words: *Salmonella*, chicken, cross-contamination, spices, antibacterial activity

Salmonellosis is a leading food-borne outbreak worldwide and chicken is considered the most important causative food of animal origin. The extensive movement of food, live, processed or packaged, in addition to animals and people, around the world results in the easy dispersal of pathogens throughout the world. Food can become contaminated during production, either on farms, or during the processing and preparation in food service establishments and homes. Contaminated surfaces are unlikely to be a direct hazard. For an outbreak to occur, *Salmonella* must be in foodstuffs placed on such surfaces and must be able to multiply. Cross-contamination during processing frequently results from the rupture of digestive organs during evisceration and the subsequent immersion of carcasses in a common chill bath[16]. Raw meat, particularly poultry, is reported to be an important means of disseminating potential human pathogens, including *Salmonella*, to kitchen utensils, work surfaces and hands[15].

During processing in retail shops, the cutting board, knife, hands of the butcher and weighing balance tray are the principal surfaces with which chicken meat comes in contact. Remnants of food particles on these surfaces might enhance the growth of microorganisms and are perhaps the sources of frequent cross-contamination of *Salmonella* which can survive on metal[14], wood and plastic surfaces[1,2]. *Salmonella* is less sensitive to drying and could be isolated from artificially contaminated surfaces up to 6 h after the surface had dried[8]. Meat remnants, such as chicken liquor, blood and fat are found to provide a protective cover for

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Salmonella during drying[10].

As poultry products such as shell-on eggs and meat are frequently incriminated as contaminated with *Salmonella*, the minimization of bacterial contamination is of major interest among poultry processors and food safety researchers. The potential of various plant extracts such as antimicrobial extracts is of particular relevance[9,22], and it has been widely reported that many spice extracts possess some antimicrobial properties. Probably for this reason, spices such as garlic, onion, ginger, dry ginger, pepper, turmeric, clove and cinnamon have always been common in Indian meat dishes.

Considering the above points, we aimed to identify the routes of cross-contamination in retail chicken-processing outlets and the efficacy of spices extracts to control the growth of *S. enteritidis* on various surfaces, such as wood, plastic and stainless steel. *S. enteritidis* was chosen as this is the predominant *Salmonella* serotype encountered in the study area.

Materials and Methods

Isolation and characterisation of Salmonella

Fifteen retail chicken outlets in a residential area of Coimbatore City were sampled for this study. In these outlets, chopping boards, knives, weighing balance trays, the cage floor and the hands of butchers were examined. The samples were collected in the morning, between 7 and 9 am without prior notification to the butcher or owner. The swab technique was used to sample the surface of chopping boards, metal parts of knifes used for cutting meat, the inner side of the weighing balance trays, and the butcher's hands. For the isolation of *Salmonella*, a modified method[12] was used.

The samples were pre-enriched in buffered peptone water in a screw cap bottle at 37°C for 24 hours. One ml of pre-enriched culture was then transferred into 10 ml of selenite cystine broth (SCB) and tetrathionate broth (TTB) and incubated at 37°C for 24 hours for selective enrichment. After selective enrichment, a loopful of culture from both the selenite broth and tetrathionate broth was streaked onto selective media such as brilliant green agar (BGA), hektoen enteric agar (HEA) and xylose lysine deoxycholate agar (XLD) plates and incubated for 24 to 48 hours at 37°C. The BGA, HEA and XLD plates were observed after 24 hours and 48 hours for typical *Salmonella*-like colonies. Two typical colonies were picked up from positive plates, re-streaked to ensure purity and maintained on brain heart infusion agar (BHI) slants at room temperature.

The stored cultures were subjected to preliminary biochemical screening, which included hydrogen sulphide production in triple sugar iron agar (TSI) slants, indole production in tryptone broth and urea splitting ability on Christiansen's urea agar. Cultures matching the typical reactions of *Salmonella* in preliminary biochemical screening were further subjected to lactose, sucrose, dulcitol and salicin utilization. All bacteriological media used in the study were purchased from Hi-Media Laboratories Ltd., Mumbai, India. The cultures matching the biochemical reactions of *Salmonella*, described in Bergey's Manual of Determinative Bacteriology[18], were further confirmed by slide agglutination test using polyvalent 0 sera (Wellcome Laboratories, Dartford, England). The confirmed cultures were sent to the National *Salmonella* and *Escherichia* Centre, Central Research Institute, Kasauli, Himachal Pradesh for serotyping.

Preparation of wooden plank, metal and plastic plates

Mango, teak and tamarind are the three types of hard wood commonly used as chopping boards in retail chicken outlets, and these were selected for the survival study. Small rectangular blocks of wood, plastic and stainless steel with a surface area of 20 cm^2 (5 cm×4 cm×1 cm) were made to imitate the surfaces of the cutting blocks, weighing balance tray and knives, respectively.

Selection of culture and preparation of inoculum

One strain of *S. enteritidis* (lps 32) isolated from a cutting board was used to examine their survival on wooden, plastic and metal surfaces. This strain is resistant to ampicillin, nalidixic acid, neomycin, polymyxin-B and tetracycline. The strain was inoculated in nutrient broth and incubated for 18 h at 37°C. After incubation, the cells were harvested by centrifugation at 3000 rpm for 10 min in a refrigerated centrifuge. The cells were washed twice in isotonic saline, re-suspended and diluted in isotonic saline to give an inoculum density of 10^5 cfu ml^{-1}.

Preparation of crude extracts of spices

Two types of spices were used in this experiment, fresh spices and dry spices. The fresh spices used were garlic, ginger and onion. The dry spices included in the study were pepper, turmeric, clove, cinnamon and dried ginger. To study the efficacy of these spices, aqueous extracts of various concentrations such as 10%, 25%, 50%, 75% and 100% were used. The extracts of the fresh and dry spices were prepared as described below.

After removing the dried outer scales of fresh garlic, onion and ginger, 100 g of each were homogenised in a blender. The homogenate was filtered through cheesecloth and

filter sterilised (0.45 μm). This extract of fresh spices were considered a 100% concentration, and other concentrations, such as 10%, 25%, 50% and 75% were prepared by adding the proportionate amount of sterile distilled water. To prepare a 10% concentration, 10 ml of 100% concentration spice extract were diluted with 90 ml of sterile distilled water, for 25% concentration, 25 ml of 100% concentration spice extract were diluted with 75 ml of sterile distilled water. The other concentrations were prepared similarly.

Dry spices (100 g) were powdered in a sterile blender with 100 ml of sterile distilled water and the resultant slurry was filtered through cheesecloth and then filter sterilised. This dried spice extract was considered as 100% concentration. Other concentrations were prepared by diluting the 100% concentration with appropriate volumes of sterile distilled water. Initially, the sensitivity of *S. enteritidis* strains against various concentrations of spices extract was determined using the agar-well method. To test the effectiveness of spice extracts in controlling the growth of *S. enteritidis* on various test surfaces, 0.5 ml inoculum containing 10^5 cfu ml^{-1} of *S. enteritidis* was spread over the entire surface of autoclaved wooden, metal and plastic blocks. Excess water on the surface of the blocks was removed using a desiccator, which was also used to quickly dry the test surfaces in order to replicate the conditions in the processing environment, where the high ambient temperature and low humidity air results in immediate drying of the processing tools. These treated plates were then dipped into spice extracts at various concentrations (100, 75, 50, 25 and 10%) maintained at room temperature (28±2°C). The blocks were removed at regular intervals (1, 2, 3, 4, 5, 6, 7, 8, 9 and 10 min), dipped directly into conical flasks containing sterile brilliant green broth (BGB) and then incubated at 37°C for 24 h. Respective controls of sterile and contaminated wooden and metal blocks were also maintained.

Results and Discussion

Incidence of Salmonella in chicken retail outlets

The incidence results of *Salmonella* in the processing tools of broiler-chicken retail outlets are presented in Table 1. The incidence of *Salmonella* was most frequent on cutting boards, followed by the butcher's hands. The weighing balance trays and knives had 6.67% and 6.45% incidence of *Salmonella*, respectively. Fourteen *Salmonella* strains (13 *S. enteritidis* and 1 *S. cerro*) were isolated during this study.

Salmonella spp. was isolated from knives, chopping boards, weighing balance trays and the butcher's hands in nearly half of the retail chicken processing outlets sampled.

Table 1. Incidence of *Salmonella* in the processing tools at chicken retail outlets

Sample	No. of shops analysed	No. of samples analysed	% incidence of *Salmonella*
Knives	15	31	6.45 (2)*
Weighing balance tray	15	15	6.67 (1)
Cutting board	15	16	18.75 (3)
Cage floor	15	29	10.34 (3)
Butcher's hands	15	21	14.29 (3)

* Values in parentheses indicate the number of samples testing positive for *Salmonella*.

As the highest incidence was found on the chopping boards, this suggests that it can survive and multiply on chopping boards with a rough surface. As wooden boards are porous, they are thought to be harder to clean and decontaminate than plastic boards. Ak *et al.*[2] reported that chopping boards could facilitate *E. coli* 0157: H7, *L. monocytogenes* and *S. typhimurium* cross-contamination. The chopping boards examined during the study were not well maintained. They were rough with knife cuts, and remnants of chicken meat and blood were found on their surface. The peak trading time for the retail outlets is the early morning and hence we chose to sample during that time. The processing tools and utensils are washed on a daily basis, and the remnants found on them were from the same day as most of the shops had already served some customers. Suggestions and complaints to improve the hygiene in the processing environment were regularly received from customers but were largely ignored, and the customers were not aware of the transmission of human pathogens through cross-contaminations in retail outlets.

The butcher's hands were also a potential source of cross-contamination as evidenced by our results. It was observed that when the butcher's hands had *Salmonella* contamination, the processed meat from the retail outlets also tested positive for *Salmonella*[24]. The surface of knives and the weighing balance trays were also positive for *Salmonella*. Unlike large-scale meat processing plants, where periodical hand-dipping in 50 ppm chlorine water, wearing gloves and the periodical sanitation of utensils in 100 ppm chlorine water are usually practiced[13], no such standard sanitation operational procedures (SSOPs) are followed in the city's retail outlets, except for the occasional removal of meat and blood from the cutting board and knives. *Salmonella* is capable of prolonged survival outside the living host[7] and in a dry livestock housing arena[4,5]. Infection of humans with *S. enteritidis* has often been associated with the consumption of poul-

try products contaminated with *S. enteritidis*[3,21,26]. The easy isolation of *S. enteritidis* from knives, chopping boards, weighing balance trays, and butcher's hands in seven of the 15 meat processing outlets is implicit enough to suggest the possibility of Salmonellosis among the chicken-consuming population of Coimbatore. Furthermore, as has been shown by Enkiri and Alford[11], *Salmonella* may survive on metal surfaces and cause cross-contamination via knives and weighing balance trays with remaining chicken meat, thus supporting the growth of bacteria.

The serotyping of *Salmonella* isolates from various cross-contaminating sources revealed the persistence of *S. enteritidis* as the predominant serotype in the processing environment. Of the 14 *Salmonella* strains isolated, 13 were *S. enteritidis*. The only other serotype encountered was *S. cerro*. Qin *et al.*,[20] reported that, as *S. enteritidis* could survive in dry conditions for a longer time, the higher incidence of *S. enteritidis* might also be a hazard.

Antimicrobial activity of various spices

Prior to the treatment of various surfaces with spice extracts, the sensitivity of *S. enteritidis* to various concentrations of spices was tested (Table 2). The maximum inhibitory effect was with garlic extract, with *S. enteritidis* being sensitive at all concentrations (10–100%). Undiluted extract of onion was also found to be very effective against this organism. *S. enteritidis* were also sensitive to lower concentrations (75% and 50%) of onion extract. While ginger extract could inhibit *S. enteritidis*, at their highest concentration, other spices showed no inhibitory activity against *S. enteritidis*.

Garlic and onion are known for their antimicrobial properties[19,23,25] and the extensive application of garlic, onion and ginger in various therapeutic preparations has been described by Kapoor[17]. The antibacterial activity of garlic, onion and ginger against *S. enteritidis* is useful and suggests that retail outlets could use the extracts to treat their utensils.

Survival of S. enteritidis on wood, metal and plastic surfaces

After testing the sensitivity of *S. enteritidis* to various spice extracts, we selected aqueous extracts of garlic, onion and ginger for further treatment studies. The bactericidal activity results of these extracts against *S. enteritidis* on wooden, plastic and metal surfaces are shown in Table 3. It was found that garlic extract was very effective in controlling the growth of *S. enteritidis*. The 75% and 100% concentrations were effective from the first minute on all three types of examined surfaces. At 50% strength, bactericidal effect was observed within a minute for metal and plastic surfaces smeared with *Salmonella* cells, but contact of five minutes or more was needed for this concentration to be effective on wood surfaces. The extracts of onion and ginger showed no bactericidal activity on *S. enteritidis* spread on wooden, plastic and metal surfaces.

The bactericidal effect results after the application of extracts on contaminated wooden, plastic and metal surfaces were different from the results observed in the agar-well method, as spice extracts other than garlic showed no antibacterial effect, even at the highest concentration.

The results of this investigation revealed that there is a considerable degree of cross-contamination during the processing of chicken in many retail outlets. The isolation of *S. enteritidis* as the predominant serotype indicates that the source of cross-contamination is the chicken itself, as the association of this serotype with chicken and eggs is well documented[6,7]. The lack of adherence to SSOPs in retail outlets adds to the gravity of the problem. As chicken has been identified as the most frequently implicated vehicle of food-borne outbreaks, effective steps should be taken to prevent cross-contamination. Simple precautionary measures, such as wearing gloves, the periodical sanitation of the utensils and knives in 50 ppm chlorine water, are recommended. Although chlorine is considered a cheap and effective disinfectant, the pungent odour may be a deterrent to its

Table 2. Antimicrobial activity of some spices against *S. enteritidis*

Concentration of spice extracts (%)	Inhibition zone (mm diameter)							
	Garlic	Onion	Ginger	Pepper	Turmeric	Clove	Cinna-mon	Dry ginger
100	34.33	26.33	14.67	—	—	—	—	—
75	31.00	18.67	—	—	—	—	—	—
50	29.67	16.33	—	—	—	—	—	—
25	22.67	—	—	—	—	—	—	—
10	20.33	—	—	—	—	—	—	—

Table 3. Inhibitory effect of different concentrations of garlic, onion, and ginger extracts against *S. enteritidis* applied on different surfaces

Treatment Time (min)	Concentrations (wt/vol) of spices extracts																										
	50%									75%									100%								
	Garlic			Onion			Ginger			Garlic			Onion			Ginger			Garlic			Onion			Ginger		
	W	P	M	W	P	M	W	P	M	W	P	M	W	P	M	W	P	M	W	P	M	W	P	M	W	P	M
1	+	−	−	+	+	+	+	+	+	−	−	−	+	+	+	+	+	+	−	−	−	+	+	+	+	+	+
2	+	−	−	+	+	+	+	+	+	−	−	−	+	+	+	+	+	+	−	−	−	+	+	+	+	+	+
3	+	−	−	+	+	+	+	+	+	−	−	−	+	+	+	+	+	+	−	−	−	+	+	+	+	+	+
4	+	−	−	+	+	+	+	+	+	−	−	−	+	+	+	+	+	+	−	−	−	+	+	+	+	+	+
5	−	−	−	+	+	+	+	+	+	−	−	−	+	+	+	+	+	+	−	−	−	+	+	+	+	+	+
6	−	−	−	+	+	+	+	+	+	−	−	−	+	+	+	+	+	+	−	−	−	+	+	+	+	+	+
7	−	−	−	+	+	+	+	+	+	−	−	−	+	+	+	+	+	+	−	−	−	+	+	+	+	+	+
8	−	−	−	+	+	+	+	+	+	−	−	−	+	+	+	+	+	+	−	−	−	+	+	+	+	+	+
9	−	−	−	+	+	+	+	+	+	−	−	−	+	+	+	+	+	+	−	−	−	+	+	+	+	+	+
10	−	−	−	+	+	+	+	+	+	−	−	−	+	+	+	+	+	+	−	−	−	+	+	+	+	+	+

+ indicates growth. − indicates no growth.
W: Wooden surface, P: Plastic surface, M: Metal surface.

use. The results of our study also indicate the good antibacterial effect of garlic extract, sprays of which could also be used on cutting surfaces instead of chlorine water. The economics of using garlic extract to control pathogens in retail outlets suggest that $2 per day would be sufficient to dip the processing tools in garlic extract at 2-hour intervals.

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