Microbial safety of meat sold in Orathanadu region, Thanjavur

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Abstract
Microbial contamination of foods is a significant vital food safety issue throughout the world. Reducing the microbial contamination of meat will reduce the transmission of food-borne pathogens to consumers. Therefore, the study determined the prevalence and antimicrobial sensitivity pattern of food-borne pathogens in chevon and chicken samples sold at commercial markets in Orathanadu region, Tamil Nadu. A total of 50 meat samples were collected randomly from different regions of Orathanadu and analyzed for food-borne pathogens using selective medium. The characteristic Salmonella, E. coli and Staphylococcus aureus colonies isolated were subjected to biochemical tests and antibiotic resistance study. The level of Salmonella, E. coli and S. aureus in the chicken samples was observed as 53%, 37% and 27% respectively. Chevon samples had 65% Salmonella, 40% E. coli and 55% S. aureus. Higher prevalence of Salmonella followed by S. aureus and E. coli was observed in meat samples indicating the possible cross-contamination that occurred during slaughtering process may pose potential public health risk to consumers and meat handlers. S. aureus had showed higher resistance to antibiotics compared to E. coli and Salmonella. It is concluded that strict hygienic practices especially during slaughtering, appropriate use of use of antibiotics in animal husbandry practices; proper washing of meat before cooking and thorough cooking of meat could reduce and control the contamination of food-borne pathogens and the emergence of antimicrobial resistance in foods of animal origin.

Keywords: Chevon, chicken, microbial quality, antimicrobial resistance

Introduction
Microbial contamination is a significant food safety problem because microbial contamination with food-borne pathogens leads to a wide range of health problems [1]. Besides health problems, it can also lead to economic losses due to spoilage and meat recalls due to bacterial contamination. Meat-borne diseases are typically caused by bacteria, their metabolites, parasites, viruses and toxins. Contamination of meat can occur mainly due to inadequate hygienic conditions and handling in slaughter houses. During slaughter, various processes like use of contaminated water and evisceration can contaminate carcasses which can ultimately end in meat [2]. The contaminating microbes mainly come from faeces and hide of the animal. The food-borne pathogens associated with meat and meat products are Salmonella spp., Campylobacter, Yersinia enterocolitica, verotoxigenic Escherichia coli and Staphylococcus aureus [3-5]. Reducing the microbial contamination of meat will reduce the transmission of food-borne pathogens to consumers thereby reducing food-borne illness. Therefore, the objective of the study was to investigate the prevalence of food-borne pathogens in raw chevon and chicken meat samples sold in Orathanadu region. In addition, the antimicrobial sensitivity pattern of isolated food-borne pathogens was also studied.

Materials and Methods
Sample Collection
A total of 50 meat samples, including 30 raw poultry meat and 20 raw chevon were collected from different regions of Orathanadu. Meat samples of approximately 100 g were collected in sterile polythene bags and transported to the laboratory and processed within 2 h of sampling.

Microbiological analysis
A portion of 10 g of meat samples were aseptically transferred to sterilized mortar containing 90 ml of phosphate buffered saline (PBS) and homogenized using sterile pestle.
A volume of 1 ml of homogenate was added to 9 ml of selenite cysteine broth, Macconkey broth and Nutrient broth and incubated at 37 °C overnight for selective enrichment of Salmonella, E. coli and S. aureus. Following incubation, enriched cultures were streaked on Xylole Lysine Dextrose (XLD) agar, Sorbital Macconkey Agar (SMA) and Mannitol Salt Agar (MSA) for isolation of Salmonella, E. coli, E. coli O157:H7 and S. aureus. All the isolates were purified by subculturing in nutrient broth and further streaking on nutrient agar.

Biochemical characterization
The presumptive Salmonella, E. coli, and S. aureus colonies were subjected to Gram’s staining, catalase test, indole test, oxidase test, methyl red test, VP test, Simmons’ citrate utilization tests and triple sugar iron utilization tests.

Antimicrobial susceptibility testing.
All the isolated E. coli, S. aureus and Salmonella were tested for antimicrobial susceptibility using disc diffusion methods[6-7]. The agents used in this study were procured from Himedia, which include Methicillin (5 mcg), Vancomycin (30 mcg), Erythromycin (15 mcg), Streptomycin (10 mcg), Nalidixic acid (30 mcg), Gentamicin (120 mcg), Doxycycline (30 mcg), Tetracycline (30 mcg), Amikacin and Amoxycillin-clavulanic acid (20 and 10 mcg), Chloromphenicol (30 mcg), Trimethoprim (5 mcg), co-trimoxazole (25 mcg), norfloxacin (10 mcg), ampicillin (10 mcg), cefotaxime (30 mcg), cefpodoxime (10 mcg), ofloxacin (5 mcg), ciprofloxacin (5mcg) and ceftriazone (30 mcg). Pure bacterial cultures were enriched in brain–heart infusion broth at 37 °C for 6–8 h. The cultures were streaked on Mueller Hinton agar plates (Himedia, India) using a sterile cotton swab and the antibiotic discs were dispensed using a disc dispenser (Himedia, India) with sufficient space in between each disc to avoid overlapping. The agar plates were incubated at 37 °C for 16–18 h and the zones of inhibition for each antibiotic were measured.

Results and discussion
The study evaluated the prevalence of food-borne pathogens viz, E. coli, S. aureus and Salmonella in chicken meat and chevon sold in the local markets of Orathanadu region based on colony morphology, Gram’s staining and biochemical characterization. The isolated Salmonella had characteristic red colonies with black centre in XLD agar, Grams negative rod with Gram’s staining, positive for Methyl red and citrate utilization, negative for Indole and Voges-Proskaur test and alkaline slant, acidic but with H₂S production in TSI utilization test (Figure 1). The isolated E. coli had pink colonies in SMA, Grams negative rod in Gram’s staining, positive for Indole and Methyl red tests and negative for Voges-Proskaur tests and acidic butt and slant acid in TSI utilization test (Figure 2). S. aureus isolated from meat samples had characteristic yellow colonies in MSA, Gram positive cocci, positive for methyl red, Voges-Proskaur and Citrate utilization tests and negative for Indole test (Figure 3). The prevalence of E. coli, S. aureus and Salmonella in chicken and chevon is given in the Figure 4. Chevon samples had 65% Salmonella, 55% S. aureus and 40% E. coli whereas Chicken samples had 53% Salmonella, 37% E. coli and 27% S. aureus. Both the meat samples had higher level of Salmonella.

Previous studies on prevalence of food-borne pathogens like Salmonella, E. coli and S. aureus in chicken and chevon meat samples were reported both nationally and internationally by several researches[8-12]. For example, Sharma et al.[11] analyzed 100 chickens and 100 mutton samples sold in open markets of Kolkata and found level of E. coli (98%), Enterococcus faecalis (90%), Staphylococcus aureus (20%), Staphylococcus epidermidis (20%), Pseudomonas spp. (10%), Salmonella spp. (2%) and Bordetella (1%). Another study[12] analyzed 33 chicken, 27 pork, 13 buffalo and 10 goat meat samples sold in Nepal and observed prevalence of Staphylococcus aureus, Escherichia coli, Salmonella, Shigella, and Vibrio were 68%, 53%, 35%, 6%, and 6% respectively. Ruban et al.[13] studied the prevalence of food borne pathogens in market samples of chicken meat sold in Bangalore under different processing condition. Results revealed higher prevalence of Salmonella in the range of 25 to 71%, Staphylococcus aureus in the range of 85 to 100% and E. coli with a range between 42 to 88%.

In addition, presence of antimicrobial resistance in food-borne pathogens is a huge public health concern. Antibiotic resistance enables the microorganism to escape from being killed by antibiotics. Therefore, resistance to antimicrobial agents has been considered one of the greatest threats to both human and veterinary medicine[14-15]. Meat also plays an important role in the transfer of genes of antibiotics resistance in term of antibiotic residues. Hence, the antimicrobial susceptibility test of isolated organism was determined. The results revealed that all S. aureus were sensitive to Amoxicillin, Cefotaxime, Gentamicin, Ampicillin, Ofloxacin, Ceftriaxone and Ciprofloxacin and resistant to Methicillin (100%), Vancomycin (100%), Trimethoprim (65%), Nalidixic acid (58%), Streptomycin (33%), Doxycycline (25%), Erythromycin (25%), Co-trimoxazole (8%) and Cefpodoxime (8%) (figure 5). The isolated E. coli was resistant to Methicillin (100%) and Trimethoprim (100%) whereas Salmonella were resistant to Methicillin (100%), Trimethoprim (100%), Cephalothin (20%) and Vancomycin (80%). The presence of variation in the levels of antibiotic resistance observed in the Figure 5 could be due to variation in antibiotic uses among different places at different times. The presence of antibiotic resistance in food-borne pathogens could be attributed to indiscriminate use of common antibiotics at sub-optimal level to improve animal productivity. The consumption of antibiotic resistance food-borne pathogens can lead to transfer of antibiotic resistance genes to other potential pathogens and normal commensal microorganisms present in both humans and animals[16, 17].

Fig 1: Salmonella on XLD agar (red colonies with black centre), IMViC (+/-/-/+) and TSI utilization (alkaline slant and acidic butt with H₂S production)
The level of microbes could be due to poor hygiene conditions prevailing during slaughtering process. Therefore, hygienic practices during slaughtering and its environment with good personal hygiene are required to improve the microbial quality of meat for public health. In addition, training and awareness programme on hygienic meat handling and personal hygiene need to be conducted to slaughterhouse workers and livestock farmers to reduce the microbial contamination and irrational antibiotics usage in poultry and livestock.

### References


13. Ruban SW, Prabhu NK, Naveen Kumar GS. Prevalence of food borne pathogens in market samples of chicken

### Conclusion

In conclusion, the results of study demonstrated high prevalence of Salmonella, E. coli and S. aureus in raw chicken and chevon meat samples sold in the Orathanadu region. The high level of microbes could be due to poor hygienic conditions prevailing during slaughtering process. Therefore, hygienic practices during slaughtering and its


