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A Sangeetha
Department of Veterinary Public Health and Epidemiology Veterinary College and Research Institute, Orathanadu Tamil Nadu Veterinary and Animal Sciences University, Tamil Nadu, India

S Balakrishnan
Department of Veterinary Public Health and Epidemiology Veterinary College and Research Institute, Orathanadu Tamil Nadu Veterinary and Animal Sciences University, Tamil Nadu, India

P Kowsalya
Undergraduate student, Veterinary College and Research Institute, Orathanadu, Tamil Nadu, India

K Manimaran
Department of Veterinary Public Health and Epidemiology Veterinary College and Research Institute, Orathanadu Tamil Nadu Veterinary and Animal Sciences University, Tamil Nadu, India

M Dhanalakshmi
Department of Veterinary Public Health and Epidemiology Veterinary College and Research Institute, Orathanadu Tamil Nadu Veterinary and Animal Sciences University, Tamil Nadu, India

T Sivakumar
The Dean, Veterinary College and Research Institute, Orathanadu, Tamil Nadu, India

Corresponding Author:
A Sangeetha
Department of Veterinary Public Health and Epidemiology Veterinary College and Research Institute, Orathanadu Tamil Nadu Veterinary and Animal Sciences University, Tamil Nadu, India

Microbial safety of meat sold in Orathanadu region, Thanjavur

A Sangeetha, S Balakrishnan, P Kowsalya, K Manimaran, M Dhanalakshmi and T Sivakumar

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 containing90 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 Xylose Lysine Dextrose (XLD) agar, Sorbital MacConkey Agar (SMA) and Mannital Salt Agar (MSA) for isolation of *Salmonella, E. coli, E. coli O157:H7* and *S. aureus*. All the isolates were purified by sub-culturing 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, Simmon's 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), Nalidixc acid (30 mcg), Gentamicin (120 mcg), Doxycycline (30 mcg), Tetracycline (30 mcg), Amikacin and Amoxycilin-clavulinic acid (20 and 10 mcg), Chlormphenicol (30 mcg), Trimethoprim (5 mcg), co-trimoxozole (25 mcg), norfloxacin (10 mcg), ampicillin (10 mcg), cefotaxime (30 mcg), cefpodoxine (10 mcg), oflaxacin (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 characteristics 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 H_2S 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 acidic slant 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 [8-13]. 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%), *Staphylocoocus 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].

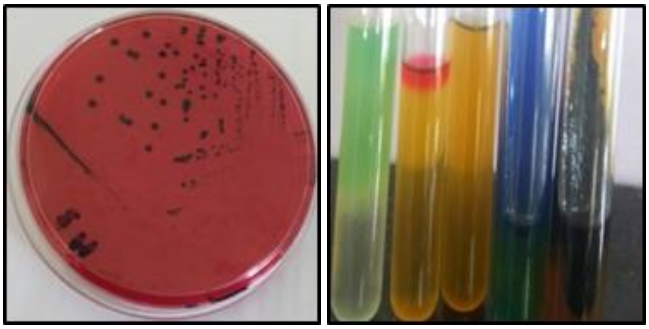

Fig 1: *Salmonella* on XLD agar (red colonies with black centre), IMViC (-/+/-/+) and TSI utilization (alkaline slant and acidic butt with H_2S production)

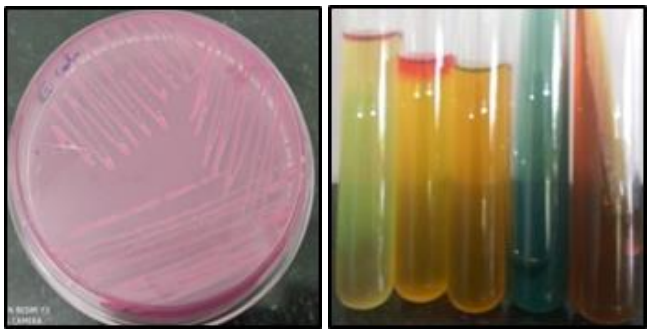

Fig 2: *E. coli* on SMA agar (pink colonies), IMViC (+/+/-/-) and TSI utilization (acidic slant and acidic butt)

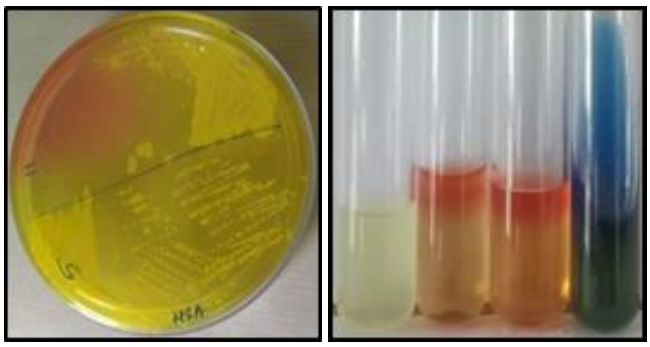

Fig 3: *S. aureus* on MSA agar (yellow colonies), IMViC (-/+/+/+)

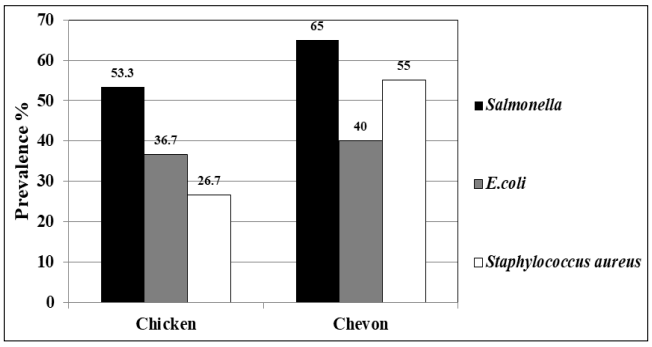

Fig 4: Prevalence of *Salmonella*, *E. coli* and *S. aureus* in chicken and chevon meat samples

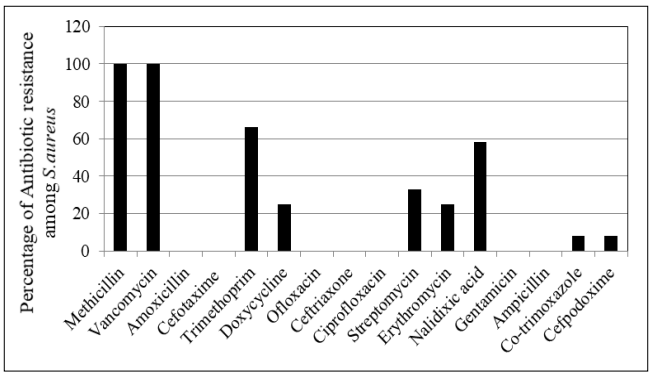

Fig 5: Percentage of antibiotic resistance among the *S. aureus* isolated from the study

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 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.

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