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Screening of raw meat samples for *E. coli* and *Salmonella* spp. and their antibiotic resistant patterns

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Abstract
Meat is not only highly susceptible to spoilage, but also frequently implicated in the spread of food borne illness. Contaminated raw meat is one of the main sources of forborne illnesses caused by *Salmonella* spp., *Campylobacter jejuni coli, Yersinia enterocolitica*, verotoxigenic *Escherichia coli* and, to some extent, *Listeria monocytogenes*etc.By keeping in view of the significance of food borne pathogens a small work was conducted to identify their prevalence and antimicrobial resistance patterns in raw meat samples of Proddatur town, YSR Kadapa District, Andhra pradesh. A total of 100 raw meat samples were collected from retail outlets and transferred to the laboratory under hygienic conditions. Nutrient broth was used as preenrichment media. *E. coli* and *Salmonella* spp. were selectively isolated and identified. Antimicrobial resistance patterns were identified by Disk Diffusion Assay. Out of 100 samples 47% of the samples were contaminated with *E. coli* and 58% were contaminated with *Salmonella* spp. All the isolates were highly resistant for lincomycin& ampicillin and sensitive for 5 other antibiotics. The extent and composition of antibiotic resistant microflora reflect the standard of hygiene and the above results indicated a sort of contamination of raw meat from different sources. Hence by taking proper hygienic measures at different levels of production and processing, the quality of meat can be conserved.

Keywords: Antibiotic resistance, *E.coli*, raw meat, *Salmonella* spp.

Introduction
Poultry meat is one of the most widely consumed meats in the world. It is rich in protein, essential amino acids that are requisite for human health. Meat has a low shelf life due to spoilage by bacterial contamination which in turn results in Food borne diseases. Major proportion of the chicken meat consumed in India comes from unorganised retail meat shops (Osano, O *et al.*, 1999) [1]. All the edible tissues of meat are prone to contamination by number of sources within and outside the carcass during slaughter/processing (Bhandare SG *et al.*, 2007) [2]. Unhygienic tools and equipment, poor hygiene of slaughter house are some of the external factors that contribute to contamination of raw meat. The meat, available at retail outlets comes through a long chain of slaughtering and transportation, where each step may pose a risk of microbial contamination. The sanitary conditions of abattoirs and its surrounding environment are major factors contributing in bacterial contamination of meat (Gill *et al.*,20`00) [3].

A great diversity of microbes inhabit fresh meat generally, but different types may become dominant depending on pH, composition, textures, storage temperature and transportation means of raw meat (Adu Gyamfi *et al.*, 2012) [4]. Meat and meat products are the main sources of Zoonoses with a variety of food borne pathogens such as *E. coli*, *Salmonella*, *Campylobacter Jejuni* etc. Among these *Salmonella* and *E. coli* are the two major pathogens having great public health significance and high morbidity. Improper cooking of eggs, chicken, meat products are the main sources of salmonellosis in humans. The indiscriminate use of antimicrobial drugs in food animals may result in transfer of resistance to human, it is unlikely that the so called reverse-antimicrobial drug will be restricted to use in human medicine (Schwartz and Chaslus Dancla, 2001) [5]. Hence, the present day study was aimed to study the prevalence and antibiotic resistance patterns of *Salmonella* spp and *E. coli* in chicken meat samples.

Materials and Methods
Collection of meat samples
A total of 100 raw meat samples were procured from different retail poultry shops and local street vendors of Proddatur, YSR Kadapa district, Andhra Pradesh, India.

All the samples were collected in sterile vials containing transport medium (Nutrient broth) and were transported to lab on ice. Samples were processed for further analysis within 4 hrs.

Microbiological Analysis

Suspension of meat samples were enriched in nutrient broth by incubating the tubes at 37 ºC for 24 hrs. For isolation of *E coli* and *Salmonella* spp. A loopful of the inoculum was taken and streaked on EMB agar and Hecktoen enteric agar respectively. For further confirmation biochemical tests like IMVIC tests were conducted.

Antibiotic Sensitivity Test

The antibiotic susceptibility of *E. coli* and *Salmonella* spp. isolates were determined using the standard disk diffusion assay (Kirby–Bauer, 1997) method. Overnight grown cultures were used for testing against the antibiotics like Ampicillin (10 µg), Amikacin (10 µg), Ceftriaxone (30 µg), Enrofloxacin (10 µg), Gentamycin (10 µg), Lincomycin (2 µg), sulphadiazine (10 µg). The discs were placed 24mmapart by using disc dispenser and gently pressed down on to the agar surface to provide uniform contact. The inoculated plates were incubated at 37 ºC for 24 hrs. After incubation each plate was examined and measured the diameter of zone of inhibition upto the nearest whole millimetre with ruler in non-reflecting background.

Results and Discussion

Out of 100 samples 47 samples were contaminated with *E. coli* and 58 samples were contaminated with *Salmonella spp*. All the isolates were highly resistant for lincomycin & ampicillin and showed sensitivity for 5 other antibiotics.

Table 1: Antibiotic resistance of *E. coli* isolates by disk diffusion assay

S. No.	Antibiotic	Sensitive	Intermediate	Resistance
1.	Ampicillin	0%	0%	100%
2.	Amikacin	68%	5%	29%
3.	Ceftriaxone	24%	5%	71%
4.	Enrofloxacin	22%	11%	67%
5.	Gentamycin	53%	13%	34%
6.	Lincomycin	0%	0%	100%
7.	Sulphadiazine	19%	3%	78%

The *E. coli* isolates from the meat samples showed high resistance (100%) against Ampicillin in the present study, which is higher than that reported by (Ahmed *et al.*2013) [6] and (Laarem*et al.*, 2017) [7] which accounts to 80%. Resistance of 62% and 65.8% against ampicillin was reported by (Tadasse*et al.*, 2012) [8] and (Hayes, 2004) [9] respectively. Least resistance of 14.1% was reported by (Abraham *et al.*, 2019) [10]. In the present study *E. coli* isolates showed 100% resistance for Lincomycin, which is in contrary to the results observed by (Masse D *et al.*, 2000) [11]. Resistance against Sulphadiazine is 78% in the present study, whereas nearly similar percentage of resistance (66.7%) was observed by (Ahmed *et al.*, 2013) [6] and (Laarem*et al.*, 2017) [7] respectively. Least resistance of 8.7% was observed by (Abraham *et al.*, 2019) [10]. Resistance against Gentamycin is 34% in the present study whereas slightly higher resistance of 38% was reported by (Schroder *et al.*, 2004) [12] and (Saenz *et al.*, 2001) [13].

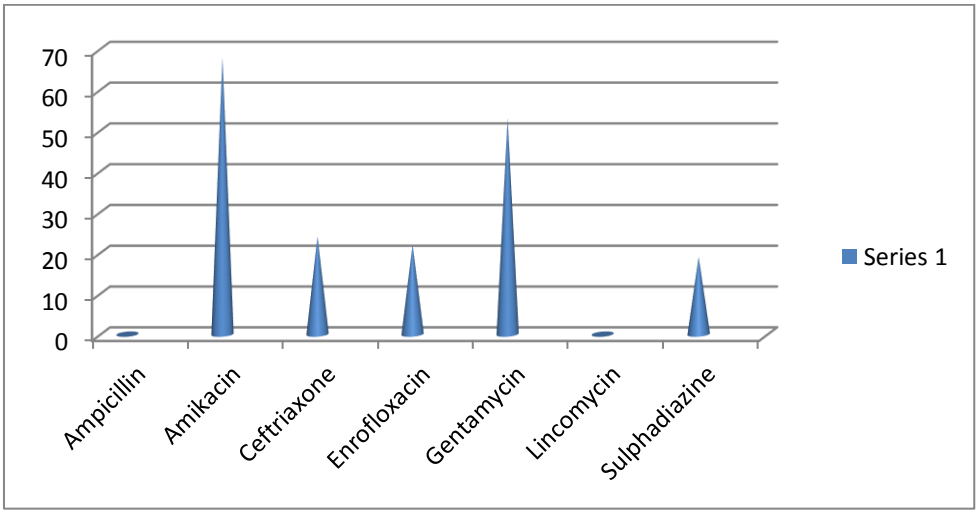

Fig1: Antibiotic sensitivity patterns for E.coli isolates

Table 2: Antibiotic resistance of *Salmonella spp.* isolates by diskdiffusion assay

S. No.	Antibiotic	Sensitivity	Intermediate	Resistance
1.	Ampicillin	0%	0%	100%
2.	Amikacin	54%	18%	28%
3.	Ceftriaxone	18%	13%	69%
4.	Enrofloxacin	74%	11%	15%
5.	Gentamycin	89%	7%	4%
6.	Lincomycin	0%	0%	100%
7.	Sulphadiazine	76%	12%	12%

In the present study, *Salmonella spp.* isolates from meat samples showed high resistance (100%) against Ampicillin, which is higher than that reported by (Larkin *et al.*, 2004) [14] (72.4%). Very low resistance of 3.8% was reported by (Abraham *et al.*, 2019) [10]. The resistance patterns of Ceftriaxone is 69% in the present study in contrast to 12.5% and 11.3% which was observed by (Yang *et al.*, 2014)[15] and (Abraham *et al.*, 2019) [10] respectively. Resistance of *salmonella* isolates against Enrofloxacin is 15% in the present study whereas 50% resistance against Enrofloxacin was reported by (Antunes*et al.*, 2003) [16]. In the present study resistance against Gentamycin (4%) and Sulphadiazine (12%) was noted, which is in contrary to the reports of (Trongit*et al.*, 2017) [17] (71%) and (Altalhi*et al.*, 2010) [18] (24.3%).

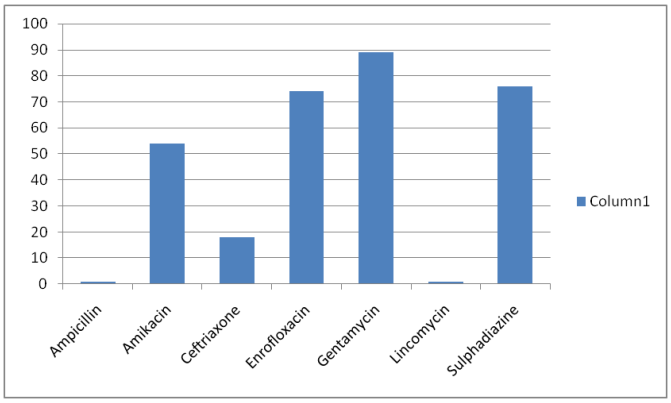

Fig 2: Antibiotic sensitivity patterns of *Salmonella spp*. isolates

Conclusion

This study indicated that food borne pathogens like *E.coli* and *Salmonella spp*. are quite common in raw meat samples. Hence proper care should be taken at all stages of slaughter, and also during entire chain of processing till it reaches the consumer.

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