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Prevalence of Multiple Drug-resistant *E. coli and Salmonella* in Goat Meat Samples from South Andaman, India

Jai Sunder*, S.C.Mayuri, T.Sujatha, A.K.De, D. Bhattacharya& E. B. Chakurkar
Division of Animal Science, ICAR - Central Island Agricultural Research Institute, Port Blair, A & N Islands - 744105
**Corresponding author: jaisunder@rediffmail.com*

Abstract

The occurrence of food-borne microorganisms is one of the major global health problems. In recent years, the burden of food-borne infection has become more complicated due to antimicrobial resistance, posing a significant risk of treatment failure. Transmission of antimicrobial-resistant bacteria from livestock to the human food chain through contaminated food/meat and other products is widespread. The present study studied the prevalence of *E. coli* and *Salmonella* from goat meat samples. A cross-sectional survey of the prevalence and antibiotic susceptibility of *E.coli* and *Salmonella* isolates was carried out. Results showed that meat samples were contaminated with *E. coli* and *Salmonella* spp. An average of 12.75% of the *E. coli* isolates and 27.71% of *Salmonella* isolates were multiple antimicrobial-resistant. Rising levels of resistant *E. coli* and *Salmonella* spp. against multiple antimicrobials alarm the urgent need to control and monitor indiscriminate antimicrobial use in food-producing livestock in Andaman.

Key words: *Antibiotic resistance, goat meat, Salmonella, E.coli*

Introduction

The consumption of animal protein, *viz.* meat, milk, and the egg, has increased in the recent year and is expected to be doubled by 2050 due to the increasing demand of the burgeoning population. Food products, particularly meat-based products, are prone to contamination at different processing stages (Hemalata & Virupakshaiah, 2016). According to World Health Organisation (WHO), food-borne illnesses are infectious diseases usually caused by consuming contaminated food or water (Kadariya *et al*., 2014). In recent years, food-borne pathogens, particularly *Salmonella* and *E.coli*, have become a severe public health threat due to their impact on health and the economy (Akbar *et al*., 2011; Ejo *et al*., 2016). Diseases due to food borne have become a major problem in both developed as well as developing countries. According to WHO, approximately 30 % of the population suffers from food-borne illness yearly in developed countries, and up to 2 million die annually in developing countries (Abunna*et al*. 2016).Approximately 1.4 million cases are caused due to non-typhoidal *Salmonella*, and 270,000 patients are caused due to pathogenic *E.coli* (Bantawa *et al*., 2019).

Meat, including goat meat, is an essential protein source but can also serve as a potential source of food-borne pathogens (Eclonomou & Gousia, 2015). Nowadays, there has been increasing concern due to food-borne zoonotic infection. The most common pathogen associated with meat is *Salmonella, Staphylococcus, E.coli, Campylobacter, Listeria, Clostridium, Yersinia*, and *Aeromonas* (Zhao *et al*. 2001).In Andaman and Nicobar Islands, chicken is the most preferred and available meat, followed by *chevon*. The goats are usually slaughtered at retail shops with not much hygienic conditions. Due to the poor hygienic conditions of the retail meat shops, there are high chances of contamination of the carcass due to *Salmonella, E.coli*, and other pathogens. Several studies have been conducted worldwide to isolate and identify *E.coli, Salmonella*, and other pathogens from goat meat (Makwana *et al*., 2015; Dulo *et al*., 2015; Bhoomika *et al*., 2016; Burush *et al*., 2018; Ajulo *et al*. 2020). *Salmonella* species and *E. coli*have been identified as the highest number of pathogens causing food-borne illness in developed countries (Abouzeed *et al*. 2000).

The emergence of multidrug-resistant pathogens is increasing due to consuming contaminated food with resistant pathogens. For the past two to three decades,

antibiotic-resistant *Salmonella* spp and *E.coli* have become a severe health problem challenge around the globe. The frequent use of antimicrobial agents in food animal production and the routine practice of giving antimicrobial agents to domestic livestock to prevent and treat diseases has contributed to multi-drug-resistant pathogens. Very little study has been carried out on isolation, identification of antibiotic sensitivity, and drug-resistant pathogens from livestock and poultry of Andaman &Nicobar Islands (Bhowmick *et al*., 2020; Sunder *et al*., 2021). The present research has been carried out to study the antibiotic-resistant pathogens in the goat meat samples.

Materials and Methods

Sample Collection

A total of 50 numbers of meat samples were collected from goat meat retail shops located at Bathubasti (11°37'23.46"N; 92°42'58.1"E), Junglighat (11°39'20.97"N; 92°43'31.93"E) and Mohanpura (11°40'14.42"N; 92°44'22.83"E) market of South Andaman district. About 50 g of the meat samples were collected in clean, dry, and sterile polythene bags and transported to the laboratory for microbiological analysis within one hour or refrigerated at 4°C for further research.

Processing of the Sample

The samples were aseptically cut into smaller pieces using a sterile knife, and small amounts of meat were inoculated in test tubes with 5ml of nutrient broth. The testtubes were incubated at 37°C overnight.

Direct Plating of the Culture

Growth was observed in the testtubes, and the culture was streaked on selective media for further isolation. For isolation of *E.coli*, the culture was streaked on Eosin Methylene Blue Agar, and for isolation of Salmonella, the culture was streaked on Rajhans media. The plates were kept in the incubator for overnight growth.

Microscopy and Colony morphology

Characterizing and identifying the bacteria was done by visualizing the colonial morphology followed by Grams staining. The appearances of the colony, size, elevation, form, edge, consistency, color, etc., were noted. Gram's staining from the colonies provided a preliminary identification of the pathogenic bacteria.

Biochemical Characterization

Biochemical characterization of the bacteria was done by performing specific tests such as indole, methyl red, voges proskauer, citrate,triple sugar iron (TSI), glucose, adonitol, arabinose, sorbitol, mannitol, rhamnose, and sucrose.

Disc method for phenotypic screening of antibacterial sensitivity

Antibacterial sensitivity tests were performed by the single-disk diffusion method (Bauer *et al.* 1966) in accordance with National Committee for Clinical Laboratory Standards (NCCLS,2002). A total of nine antibiotic discs such as gentamicin (10µg), tetracycline (30µg), ampicillin (10µg), chloramphenicol (30µg), erythromycin (15µg), sulphafurazole(300µg), trimethoprim(5µg), streptomycin(10µg) and Amoxycillin/ cloxacillin (10µg) were assayed. The diameter of the zones of inhibition was measured. The percentage of the isolate's resistance to antimicrobials was expressed. Multi-drug resistance was defined as resistance to three or more antimicrobials (Tricia *et al.* 2006).

DNA extraction and PCR amplification of antibiotic resistance genes

The bacterial cultures were grown into Nutrient agar (Himedia, India) at 37^0C overnight. Genomic DNA was extracted from *E. coli* using a Genomic DNA purification kit according to the manufacturer's instructions (GCC, India). The purified DNA was checked and run in agarose gel electrophoresis (1.5%) and kept at -20^0C for further use. The isolates were screened for the presence of antibiotic resistance genes *viz*, tetracycline (*tet A*), extended-spectrum beta-lactamase -*CTX-M* (Cefotaximase-Munich), and aminoglycoside acetyltransferase (AAC(3)-IV) gene. PCR was done by the method described by Fode-Vaughan *et al.* (2003). The product was then electrophoresed in 1.5 % agarose gel and visualized using

a Gel documentation system (Labmate Asia Pvt Ltd). A 100 bp DNA ladder was used as the standard to determine the size of the product. Except for annealing temperatures for the genes, the PCR running conditions were 95°C for 5 min; 35 cycles of 95°C for 60s, 72°C for 60s; 72°C for 5 min. The PCR Taq2X Master mix was used in this study.

Statistical analysis

The prevalence of *E.coli* was quantified and was compared among the different sources of origins. Similarly, the pattern of antimicrobial resistance was also quantified and compared. Data were analyzed as per the Snedeccor & Cochran (1994).

Table 1. *Escherichia coli* and *Salmonella* antimicrobial-resistant genes and primer sequences used for PCR identification

Antimicrobial Agent	Resistance Gene	Sequence	Size	Annealing temperature (°C)	References
Tetracycline	*tet A*	5'-GTAATTCTGAGCACTGTCGC-3 5'-CTGCCTGGACAACATTGCTT-3	500 bp	57	Miranda *et al.* 2003
Gentamicin	AAC(3)-IV	5'-AGTTGACCCAGGGCTGTCGC-3 5'-GTG TGC TGC TGG TCC ACA GC-3	300 bp	63	Brau *et al.* 1984
ESBL gene	CTX-M	5'- CCATGGTTAAAAAATCACTGCG-3' 5'-GGGTRAARTARGTSACCAGAAYSAGCGG-3	836 bp	66	Cao *et al.*, 2002

Results and Discussion

Twenty eight *E.coli* and 43*Salmonella*spp were isolated and identified from 50 meat samples. The antibiotic sensitivity results (Table 2) revealed that the isolates showed resistance to almost all tested antimicrobial agents at various rates. An average of 12.75% of the *E.coli*isolates and 27.71% of Salmonella isolates were multiple antimicrobial-resistant. Salmonella isolates showed higher resistance levels than *E.coli* isolates. Percentage of resistance rates in *E.coli* isolates were 0.00%, 10.71%, 7.14, 21.43%, 21.43, 17.85, 10.71 % and for Salmonella isolates were 2.00, 70.00, 14.00, 42.00, 32.00, 18.00 & 16.00% respectively for aminoglycosides & penicillin, phenicols, tetracyclines, trimethoprim, macrolides and sulphafurazol antibiotics.

Table 2: Percentages of *E. coli* and *Salmonella* isolates from goat meat resistant to antimicrobial agents by NCCL disc diffusion method

Class of antimicrobial agents (µg)	E. coli isolates (%) (n=28)	Salmonella spp (%) (n=50)
Gentamicin	0.00	2.00
Ampicillin	10.71	70.00
Chloramphenicol	7.14	14.00
Tetracycline	21.43	42.00
Trimethoprim	21.43	32.00
Erythromycin	17.85	18.00
Sulphafurazole	10.71	16.00

Of the 28*E. coli*isolates tested, *CTX*-M, *tet* A, and *AAC(3)-IV*genes (Table 3) were identified in 18.51, 29.63, and 10.60%. While, out of 43 *Salmonella*isolates tested, *CTX*-M, *tet* A, and *AAC(3)-IV* genes (Table 3 and Fig 1& 2) were identified in 16.28, 39.53, and 13.95%.

In the present study, the meat samples showed the contamination of *E. coli* and *Salmonella* spp. This means that the contamination might have happened during the slaughtering of the carcass. Meat is also considered one of the essential vehicles for the transfer of antibiotic-

resistant microorganisms.*Salmonella* species have been reported as the highest documented cases of meat poisoning in a developed country (Tietjen & Fung, 1995). The high isolation rate of these bacteria poses a threat of food-borne infection. There is documentation of *E. coli* and *Salmonella* contamination of the goat meat with cases of food poisoning in humans (Okoli *et al*. 2006; Duffy*et al*. 2009). Ajulo *et al*. 2020 reported isolation of 100 % *E.coli* and 38 % *salmonella* from the meat sample of goats in Nigeria.

Table 3: Presence of antibiotic resistance gene in % of isolates

Resistance genes	E. coli isolates (%)	Salmonella spp (%)
CTX-M	18.51	16.28
tet-A	29.63	39.53
AAC(3)-IV	10.60	13.95

Fig 1 & 2

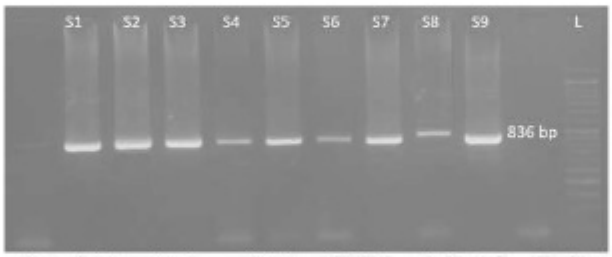

Fig 1 : Gel photo showing amplification of *CTX-M* gene in *E.coli.* Lane No: S1 to S9 (*E. coli*) Lane L : Ladder (100bp)

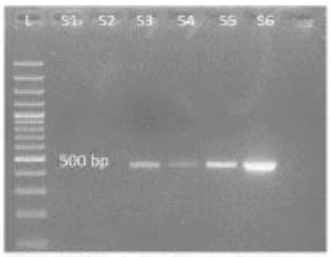

Fig 2 : Gel photo showing amplification of *tet-A* gene in *Salmonella* spp. Lane No: S1 to S6 (Samples) Lane L : Ladder (100bp)

Antibiotic resistance has been considered one of the greatest threats to medicine. In the present study, all isolates of *Salmonella* spp. developed resistance against amoxicillin (100%), followed by tetracycline (42.86%), erythromycin (36.84%), and streptomycin (28.57%). Similarly, *E. coli* isolates also showed a high degree of resistance against amoxicillin (92.86%), erythromycin (65.52%), tetracycline (18.52%), and gentamicin (12.5%). Akbar *et al*., 2011 also reported absolute resistance to amoxicillin (100%) and a high degree of resistance against tetracycline (93%). However, there were also variations in the percentage of resistance; this could be due to the difference in antibiotics usage along with place and season of research (Bantawa *et al*. 2019).Adzitey *et al*. (2020) reported a high prevalence of isolates and antibiotic resistance. They reported an 88.89% prevalence of *E. coli* in meat samples from Ghana. *E.coli* was high resistance to erythromycin (85.00%), tetracycline (73.33%), and ampicillin (71.67%).Sharma & Bisi (2010) also reported high antibiotic resistance in *E. coli* isolated from raw meat samples of goats from the Mathura region and found to be multiple resistance to trimethoprim (28%), kanamycin (26.67%), co-trimoxazole (25.33%), bacitracin (24%), and nitrofurantoin (22.67%). The high prevalence of antibiotic resistance is a severe concern for livestock and human health due to the overuse of antibiotics in livestock health and production (Pornsukarom *et al*., 2018).

The distribution of three gene determinants targeting tetracycline resistance (*tet*), extended-spectrum beta-lactamases (bla_{CTX}), and gentamicin resistance (*aac(3)-IV*) were selected based on the reported incremented resistance to such antibiotics infood-borne bacteria. The *tet A* gene was present in 39 percent of *Salmonella* isolates and 29 percent of *E.coli* isolates. Presence of *the aac(3)-IV* gene was found to be 10 % in *E.coli* and 14 % in *Salmonella* spp. The prevalence of *the tetA* gene was found to be higher than other antibiotic-resistant genes.Similar to our findings, Lerma *et al*. (2014) and Ndegwa *etal*. (2019) also reported a high prevalence of the *tet A* gene. Resistance to

tetracycline is the most commonly detected antibiotics in food animals, commonly used as a growth promoter. The *tet* genes are often associated with plasmids, transposons, and conjugative transposons, which maycarry other antibiotic and heavy metal resistance genes. It is possible that bacteria isolates harboring tetracycline resistance existed or persisted in the soil or the gut of some older animals and colonized the study animals during grazing (Jernberg *et al*., 2007; Jakobsson *et al*., 2010).

Aminoglycosides are broad-spectrum antibiotics combined with other antibiotics such as β-lactams for various bacterial infections. Inactivation of aminoglycoside antibiotics by aminoglycoside modifying enzymes (AME), such as aminoglycoside phosphotransferase (APH), acetyl-transferases (AAC), and nucleotidyltransferase (ANT) enzymes, is the most common mechanism of aminoglycoside resistance. In the present study, the prevalence of the gentamicin-resistant gene was higher than in the earlier study conducted by Ndegwa *et al*. (2019)& Srinivasan*et al*. (2008).

CTX-M, a common and significant extended-spectrum beta-lactamase (ESBL) gene, has been reported from *Escherichia coli* and *Salmonella* worldwide. Extended-spectrum beta-lactamases (ESBLs) are one of the most important mechanisms of resistance to oxy-iminocephalosporin antibiotics in bacterial isolates (Pitout &Laupland, 2008). Among these enzymes, the *CTX-M* type ESBLs have emerged worldwide, progressively replacing the *TEM* and *SHV* families (Bonnet, 2004). These genetic markers in the present study indicate the potential risks of antibiotic resistance microbial contamination in a slaughterhouse on these Islands.

These antibiotic resistance gene determinants create great concern, considering the potential risks associated with the spread of antibiotic resistance genes throughout meat chain production to end products. Various studies have been conducted globally to understand the antimicrobial resistance profile of food-borne pathogens, especially *E. coli* and *Salmonella* (Helke *et al*., 2017). Antimicrobial resistance has been considered a global health issue. The isolation of antibiotic-resistant *E. coli* and *Salmonella* could also spread antibiotic-resistant genes to other gut pathogenic or commensal bacteria and the environment in these Islands. Reports revealed that specific antibiotic-resistance genes identified in the bacteria of animal food products have also been identified in humans (Sunder *et al*., 2021; Founou *et al*., 2016).

Goat slaughtering in A & N Islands is primarily traditional and is done on wooden /concrete floors without standard and proper hygienic facilities. Therefore, controlling microbial contamination during slaughtering is a complex and significant challenge. The study suggests that resistance might have disseminated throughout slaughterhouse places by carry-over contamination of meat products. So, hygienic handling and processing of meat in a slaughterhouse would be essential to reduce transmission risks and avoid food-safety problems.

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