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[Antibiotic resistant bacteria in](https://www.frontiersin.org/articles/10.3389/fsufs.2024.1460350/full) [goat meat and hygienic practices](https://www.frontiersin.org/articles/10.3389/fsufs.2024.1460350/full) [among retail stores in Nashville,](https://www.frontiersin.org/articles/10.3389/fsufs.2024.1460350/full) [Tennessee](https://www.frontiersin.org/articles/10.3389/fsufs.2024.1460350/full)

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This study explores into the levels of coliform contamination, prevalence of antibiotic-resistant bacteria, and the hygienic practices in goat meat retail stores. Goat meat from 10 retail stores was analyzed for *E. coli*, *Salmonella*, and *S. aureus* using serological and PCR methods. Antimicrobial susceptibility testing was performed using the Kirby-Bauer disk diffusion method. Data on hygienic practices were collected through a structured observational questionnaire. Pearson's correlation analysis was also employed to establish the relationship between hygienic practices and coliform loads. The average coliform loads on goat meat ranged between 0.88–5.04 log10 cfu/g. Our results revealed that 52% of examined goat meat was deemed unacceptable (>3.30 log10 CFU/g). The overall level of good meat handling practices among meat handlers in our study was 45.75%. Further, the study establishes a significant correlation between the level of food safety practices and coliform load. Hence, stores with higher hygienic practice scores exhibited lower coliform loads. The prevalence of *S. aureus* (44%) in goat meat was significantly higher (*p*  <  0.05) as compared to *E. coli* (29%), and *Salmonella* spp. (20%). *E. coli* isolates displayed the highest resistance to penicillin (31.2%), *Salmonella* spp. to oxytetracycline (13.9%), and *S. aureus* to ampicillin (29.0%). Resistance was observed across selected antibiotic classes, particularly in beta-lactams and tetracyclines, with penicillin (78.5%) and oxytetracycline (64.5%) exhibiting notable resistance. Cephalosporin resistance was noted, with 48.4 and 33.3% of isolates showing resistance to cephalothin and cefpodoxime, respectively. Bacterial isolates also demonstrated resistance to phenicol antibiotics, including chloramphenicol (9.7%) and florfenicol (16.1%), respectively. Approximately 44.1% of bacterial isolates displayed multidrug resistance and MAR index ranged from 0.25 to 0.75. The study's findings reveal heightened levels of coliform contamination, the presence of pathogenic and multidrug-resistant bacteria in goat meat, and suboptimal meat handling practices in retail stores. The significance of improving food safety practices in retail settings is emphasized to ensure the safety of goat meat, a matter of increasing importance due to its growing demand globally.

KEYWORDS

antibiotic resistance, Salmonella, *S. aureus*, *E. coli*, goat meat, retail stores, hygienic practices

Introduction

Meat serves as a crucial source of high-quality protein and bioavailable vitamins, along with essential minerals like iron, zinc, and phosphorus ([Adesokan et al., 2015](#page-13-0)). Despite these nutritional benefits, meat poses a potential risk for spreading foodborne illnesses due to its elevated protein content and nearly neutral pH, creating an environment conducive to bacterial growth and survival [\(Ahmed and](#page-13-1) [Shimamoto, 2014](#page-13-1)). Coliforms, common in meats, serve as reliable indicators of fecal contamination and the potential presence of pathogens [\(Seo et al., 2019](#page-15-0); [Some et al., 2021](#page-15-1)). In goat and sheep production, notable pathogenic bacteria, including *Staphylococcus aureus*, *E. coli*, and *Salmonella* spp., pose a significant health risk ([Hanlon et al., 2018](#page-14-0); [Ariffin et al., 2019](#page-14-1)). The contamination of meat by *E. coli* is closely associated with fecal contamination. According to [Stein and Katz \(2017\)](#page-15-2), small ruminant meat is a source of *E. coli* infection in humans. *E. coli* being a common commensal intestinal bacterium in both animals and humans, serves as a significant zoonotic agent linked to infectious diseases in both domains [\(Getaneh,](#page-14-2) [2019](#page-14-2); [Sarowska et al., 2019](#page-15-3)). The prevalence of *E. coli* O157:H7 in goat feces at slaughter has been reported at 11.1%, posing a potential spillover to the carcass during slaughtering and subsequently contaminating fresh meat. *Salmonella* and *S. aureus* are also highlighted as pathogens of concern in goat and sheep production ([Hanlon et al., 2018;](#page-14-0) [Ariffin et al., 2019\)](#page-14-1). *Salmonella* spp. is a common pathogen reported to cause illnesses in both animals and humans ([Chlebicz and Śliżewska, 2018\)](#page-14-3), and numerous outbreaks have been linked to the consumption of contaminated goat products ([Robinson](#page-15-4) [et al., 2020](#page-15-4)). [Li et al. \(2021\)](#page-14-4) suggests that animal-derived foods, such as meat, can serve as a mode of transmission for *Salmonella* to humans. Additionally, the presence of *S. aureus* in goat products has been documented ([Angelidis et al., 2020\)](#page-13-2), posing a potential threat to public health, especially given its association with udder infections in goat farming ([Nelli et al., 2022\)](#page-15-5).

The global escalation of multidrug resistance (MDR) poses a significant public health threat ([Catalano et al., 2022](#page-14-5)). To mitigate the development and transmission of antimicrobial resistance between animals and humans, the judicious use of antimicrobial agents in food-producing animals is imperative [\(Lekshmi et al.,](#page-14-6) [2017\)](#page-14-6). Excessive antibiotic use in food-producing farms is identified as a primary contributor to the increasing prevalence of antimicrobial resistance [\(Ma et al., 2021\)](#page-14-7). The overuse and misuse of antibiotics in both medical and agricultural settings are expected to contribute to the proliferation of antibiotic-resistant bacteria ([Mancuso et al., 2021](#page-15-6)). Particularly worrisome is the antimicrobial resistance observed in *Escherichia coli*, the most common gramnegative pathogen in humans ([Jans et al., 2018\)](#page-14-8). Widespread resistance in *Escherichia coli* to fluoroquinolones, aminoglycosides, and cephalosporins; some of the most widely used antibiotics, adds to the gravity of the situation ([Jans et al., 2018\)](#page-14-8). Additionally, contaminated raw meat stands out as a primary source of antibioticresistant *S. enterica* infections in humans ([Jaja et al., 2019\)](#page-14-9). During slaughtering, antimicrobial-resistant bacteria may leak and contaminate meat, posing a potential route for transmission to humans through food [\(Mcewen and Fedorka-Cray, 2017\)](#page-15-7). Meats are identified as both a cradle and vehicle for the dispersion of antibioticresistant bacteria to humans, emphasizing the need for urgent attention and intervention [\(Bosilevac et al., 2015\)](#page-14-10).

During production, processing, transportation, and retailing, meats are frequently contaminated with pathogenic organisms ([Koutsoumanis et al., 2021\)](#page-14-11). Several factors may contribute to the contamination of carcasses during slaughter such as animals' skin and dung, equipment including cutting tools, an unhygienic environment, non-compliance with proper slaughter processes, and a lack of personal hygiene [\(Wardhana, 2019\)](#page-16-0). According to [Kamala and Kumar](#page-14-12) [\(2018\)](#page-14-12) and [Sánchez-Aldana et al. \(2020\)](#page-15-8), microbiological quality of meat depends mostly on the slaughter process, sanitation during processing and packaging, maintenance of adequate cold chain storage from the processing to the retail level, and finally, sanitation during handling at the retail stores.

In recent years, there has been a notable surge in the demand for goat meat in the U.S., as evidenced by increased imports ([Nakai, 2018\)](#page-15-9) and a rise in meat goat inventory from 591,543 in 1990 to 2,075,000 in 2018 ([Ibrahim et al., 2020](#page-14-13); [Mazhangara et al., 2019](#page-15-10)). This heightened demand can be attributed to factors such as the growing immigrant population, which traditionally favors goat meat, and the shifting consumer preferences towards healthier food choices like low-fat, low-cholesterol, and low-carb diets [\(Degala et al., 2018\)](#page-14-14). The inclination of immigrants towards goat meat, highlighted by [Ryan](#page-15-11) [et al. \(2021\)](#page-15-11), is coupled with its perceived nutritional benefits compared to other red meats ([Silva et al., 2022](#page-15-12)). This has positioned goat and sheep meat as the fourth most consumed meat in the U.S., following beef, pork, and poultry [\(Aravani et al., 2022\)](#page-14-15). Specifically in Tennessee, the demand for goat meat has experienced a significant upswing over the past two decades, driven by a growing ethnic population that increased from 159,004 in 2000 to 304,801 in 2013 ([Ekanem et al., 2016](#page-14-16)). This surge in demand has propelled the state of Tennessee to become the second-largest producer of goat meat in the country, trailing only behind Texas ([Sang, 2016\)](#page-15-13). The increasing demand for goat meat in Nashville, emphasizes the importance of addressing food safety practices in retail stores to safeguard consumer well-being. The main goal of this study was to assess coliform contamination levels, antibiotic resistance patterns of bacteria, and the relationship between coliform contamination and hygienic practices in retail establishments selling goat meat throughout the city. This research is vital to ensure the safety and quality of goat meat, particularly considering its growing popularity and production.

Materials and methods

Selection of ethnic retail stores, sample collection, and preparation

The study was approved by the Tennessee State University Institutional Review Board (HS-2021-4597). A total of one hundred $(n=100)$ goat meat samples were acquired from ten $(n=10)$ ethnic retail stores in Nashville, Davidson County, spanning a period of 7 months, from March 2022 to September 2022. Fresh meat was procured in duplicate from each selected store and discreetly labelled with letters A to J to ensure confidentiality. To prevent multiple samplings from the same shipments, meat samples were obtained at a biweekly interval from various stores. The quantity of meat samples obtained on each collection day depended on the availability of meat in the stores. All collected meat samples were labelled with store identification letters and collection dates.

Promptly after collection, the meat samples were transported to the Tennessee State University Food Safety and Microbiology Laboratory in a cooler filled with ice. Microbial analysis was conducted within 2 h upon arrival at the laboratory. Each meat sample yielded three sub-samples, each weighing fifty grams (50 g), which were aseptically placed in sterile stomacher bags (Fisher Scientific, Pittsburgh, PA, United States). Subsequently, 450 mL of 0.1% sterile buffered peptone water (BPW) (Oxoid, Solon, OH, United States) was added, and the samples were homogenized for ² min at 230 rpm using a 400 Circulator Stomacher® (Seaward, Norfolk, United Kingdom). The investigation of each homogenized sample included determining the total coliform count and prevalence of generic *E. coli*, *Salmonella* spp., and *S. aureus* in retail goat meat. Coliforms and *E. coli* levels were determined using the plating method, and the prevalence of bacteria was evaluated based on the number of positive samples.

Determination of coliform load and *Escherichia coli*

The homogenized meat samples underwent serial dilution with sterile peptone water (Oxoid, Basingstoke, United Kingdom). Using the serially diluted homogenate, 1mL from each sample was pipetted from 10⁻¹ to 10⁻⁵ dilutions, and each was spread-plated onto two petri dishes containing selective media Harlequin *E. coli* /Coliform Agar (Neogen, Lansing, Michigan, United States) which enables simultaneous enumeration of *E. coli* and coliforms in food and environmental samples. The plated samples were then incubated at 35°C for 24h ([Georgopoulou et al., 2020\)](#page-14-17). After incubation, plates displaying 25–250 colonies were counted. The total coliform count per gram of meat was calculated as:

$$
N = \sum C / (1^* n!) + (0.1^* n 2)^* (d),
$$

where *N* represents the number of colonies per mL/g of product, ∑*C* is the sum of all colonies on all counted plates, *n*1 is the number of plates in the first dilution counted, *n*2 is the number of plates in the second dilution counted, and *d* is the dilution from which the initial counts were obtained.

The number of coliform colony forming units per gram (CFU/g) of goat meat samples was categorized as acceptable (≤100CFU/g or ≤2 log10 CFU/g), marginally acceptable (100–2000CFU/g or 2 log10– 3.30 log10 CFU/g), or unacceptable $(\geq 2000 \text{ CFU/g or } \geq 3.30 \text{ log}10$ CFU/g) based on the International Commission on Microbiological Specifications for Food (ICMSF) guidelines. For the isolation of *E. coli*, characteristic colony morphology on Harlequin *E. coli*/Coliform Agar, such as dark blue, blue-green, or purple colonies, was considered presumptive *E. coli*. These colonies underwent further identification using *E. coli* latex agglutination test kits (Prolex, Round Rock, TX, United States), with results interpreted following the manufacturer's instructions. Presumptive colonies were then inoculated into tryptic soy broth (TSB; Oxoid, Basingstoke, United Kingdom), incubated overnight at 37°C, and preserved in 50% glycerol at −80°C for subsequent confirmation through polymerase chain reaction (PCR). Coliforms, which possess the ß-galactosidase enzyme produced rosepink colonies.

Isolation of *Salmonella* spp

To isolate *Salmonella* spp., a 25-gram portion of each meat sample was placed in a sterile stomacher bag. Subsequently, 225mL of sterile lactose broth was added, and the mixture was homogenized for 2min at 230rpm using a 400 Circulator Stomacher® (Seaward, Norfolk, United Kingdom). The homogenized sample was then transferred into a sterile screw-cap jar, securely capped, and allowed to stand for 60min at room temperature. Then, the pH of each sample was adjusted to 6.8, thoroughly mixed, and incubated overnight at 35°C. After overnight incubation, 0.1mL of the mixture was transferred to 10mL Rappaport-Vassiliadis (RV) medium and incubated at 42°C for 24h. Subsequently, ten microliters of RV broth were streaked in duplicates on Xylose Lysine Deoxycholate (XLD) agar plates, which were then incubated at 35°C for 24h. Post-incubation, three colonies exhibiting typical *Salmonella* spp. characteristics on XLD agar, appearing as red or red with a black center, were selected as presumptive colonies. These presumptive *Salmonella* spp. colonies underwent further identification using latex agglutination kits (Wellcolex, Santa Fe, KS, United States), and the results were interpreted following the manufacturer's instructions. The identified colonies were inoculated into tryptic soy broth (TSB; Oxoid, Basingstoke, United Kingdom), incubated overnight at 37°C, and preserved in 50% glycerol at −80°C for subsequent confirmation.

Isolation of *Staphylococcus aureus*

To isolate, *S. aureus*, a 1mL aliquot from each homogenate was transferred to 9mL of brain heart infusion broth (BHI) (Oxoid, Basingstoke, United Kingdom) and incubated at 37°C for 24h. Subsequently, 1mL aliquots from BHI were transferred to 9mL of sterile peptone water (PW) and incubated as previously described. Loops of broth containing ten microliters of the organism were then streaked in duplicates on mannitol salt agar and incubated aerobically at 35°C for 24h. Yellowish colonies observed on mannitol salt agar were chosen as presumptive *S. aureus* [\(Raji et al., 2016\)](#page-15-14). These presumptive *S. aureus* colonies underwent further identification using latex agglutination kits (Bacistaph, Santa Fe, KS, United States), and the results were interpreted following the manufacturer's instructions. Presumptive colonies were subsequently inoculated into tryptic soy broth (TSB; Oxoid, Basingstoke, United Kingdom), incubated overnight at 37°C, and preserved in 50% glycerol at −80°C for further confirmation.

Bacterial DNA extraction and PCR confirmation of presumptive isolates

Presumptive isolates of *E. coli*, *Salmonella* spp., and *S. aureus* were cultured overnight at 37°C in TSB. DNA extraction from the overnight cultures (>5×106 cells) was performed using the DNeasy® UltraClean® Microbial Kit (QIAGEN, Hilden, Germany) following the manufacturer's instructions. DNA concentrations and integrity were determined by using a NanoDrop 2000 (Thermo Scientific, Pittsburgh, PA, United States) and agarose gel electrophoresis, respectively. Confirmation of presumptive isolates of *E. coli*, *Salmonella* spp., and *S. aureus* was carried out through PCR. The Hotstar Taq Plus Master Mix (Qiagen, Hilden, Germany) was Owusu et al. [10.3389/fsufs.2024.1460350](https://doi.org/10.3389/fsufs.2024.1460350)

employed in this study. The PCR mixture (20μL) consisted of 0.4μL each of forward and reverse primers, 2μL of coral load concentrate 10× (dye), 5.2μL of Rnase-free water (RH20), 2μL of DNA template, and a 10μL solution of Taq PCR Master Mix polymerase. The working concentrations for the primers were 10ng/μL and 100ng/μL for the DNA template. The sequences of primer pair used for *E. coli* target gene (16Sr RNA) was 5′-AGAGTTTGATCATGGCTCAG-3′ and 5′-GGACTACCAGGGTATCTAAT-3′ [\(Mamun et al., 2016](#page-15-15)), whereas the primer pair used for targeting *Salmonella* spp. (*sdi*A) was 5′-CGGTGGTTTTAAGCGTACTCTT-3′ and 5′-CGAATATGCT CCACAAGGTTA-3′ [\(Paião et al., 2013\)](#page-15-16). *S. aureus* (16Sr RNA) primer pair was 5′-CCTATAAGACTGGGATAACTTCGGG-3′ and 5′-CTTTGAGTTTCAACCTTGCGGTCG-3′ ([Mason et al., 2001](#page-15-17)). PCR amplification products (20μL aliquots) were subjected to electrophoresis on a 1.5% agarose gel (FMC Bioproducts, Rockland, Maine) in 1X TAE buffer (Tris-acetate-EDTA). Ethidium bromide (Fisher, Fair Lawn, United States) was used for enhanced visualization. Gel electrophoresis was conducted for 1h and 30min at 70 volts in 1X TAE buffer, and the results were visualized using a Bio-Rad Gel Doc Imager (735 BR EZ).

Antimicrobial susceptibility and multiple antibiotic resistance

The Kirby Bauer disk diffusion method was used for antimicrobial susceptibility test. *E. coli*, *Salmonella* spp., and *S. aureus* isolates were subjected twelve antibiotics (Oxoid, Hampshire, United Kingdom) categorized into six classes with corresponding strength in parentheses: (class 1) *β*-lactam: ampicillin (AMP; 10μg), and penicillin (P; 10iu); (class 2) β-lactam/β-lactamase inhibitor combinations: amoxicillin/ clavulanic acid (AMC; 20/10μg); (class 3) cephalosporins: cefpodoxime (CPD; 10μg), cephalothin (KF; 30μ g), and ceftiofur (XNL; 30μ g); (4 class) phenicol: chloramphenicol (C; 30μg), and florfenicol (FFC; 30μg); (class 5) tetracyclines: doxycycline (DO; 30μg), tetracycline (TE; 30μg), and oxytetracycline (T; 30μg); and (class 6) aminoglycosides: neomycin (N; 30μg). Bacterial colonies were inoculated in TSB and incubated at 37°C for 18h. The turbidity of the broth was adjusted to 0.5 McFarland and spread-plated on Müeller Hinton Agar. On each Müeller Hinton Agar plate [\(Åhman et al., 2019](#page-13-3)), four antibiotic disks were positioned to avoid overlapping of inhibition zones. The plates were then incubated for 24h at 37°C. Following incubation, the zones of inhibition were measured, and results were categorized as resistant, intermediate, or susceptible using the Clinical Laboratory Standard Institute [\(CLSI](#page-14-18) [Clinical and Laboratory Standards Institute, 2020](#page-14-18)) guidelines. The diameter of inhibition zone (DIZ) was considered as a measure of the antibacterial activity. To identify multidrug-resistant (MDR) strains, the number of antibiotics to which each bacterium exhibited resistance was noted. MDR isolates were defined as those showing intermediate susceptibility or resistance to drugs in three or more antibiotic classes ([Wang et al., 2021\)](#page-16-1).

The multiple antibiotic resistance (MAR) indexes were calculated using the formula:

$$
MAR\ index = a/b.
$$

where "*a*" represents the number of antibiotics to which an isolate was resistant, and "*b*" represents the overall number of antibiotics

tested, according to [Titilawo et al. \(2015\)](#page-15-18) and interpreted as per [Igere](#page-14-19) [et al. \(2020\)](#page-14-19).

Investigation of food safety practices among retail goat meat handlers

The food safety practices of goat meat handlers were examined while they were involved in the handling and sale of goat meat to consumers in ten retail stores in Nashville, Tennessee. Each store had approximately 2 to 3 employed workers on-site at the time of the study. The study took place between March and September 2022, and data collection involved direct personal observation using a structured questionnaire survey, which was designed based on previous studies [\(Yenealem et al., 2020;](#page-16-2) [Al](#page-13-4) [Banna et al., 2021](#page-13-4); [Sarma et al., 2022\)](#page-15-19). The study focused on food safety practices outlined in Table 1, with each correctly executed practice earning one (1) point. For evaluation purposes, a score of $\geq 70\%$ indicated that meat handlers implemented seven or more out of the 10 hygienic practices specified in the observational questions. Stores achieving this score were categorized as demonstrating "good" food hygiene practices, while those scoring below 70% were classified as having "poor" food hygiene practices, following the criteria established by [Akabanda et al. \(2017\)](#page-13-5). The meat handling practice score from each store was then compared with its average total coliform colony-forming units per gram (cfu/g) of goat meat to explore potential correlations between hygienic practices and coliform contamination levels.

Statistical analysis

Descriptive statistics were utilized to present the mean log and standard deviation (SD) of coliform counts in goat meat from various stores. Coliform counts were further categorized into percentages of acceptable, marginally acceptable, and unacceptable, following the guidelines outlined by ICMSF. Plate counts between 25 and 250cfu/ plate were considered countable and log transformed. Microsoft Excel version 2020 (Microsoft, Redmond, WA, United States) was employed for data analysis. Variations in antimicrobial resistance patterns among bacteria isolated from goat meat in retail stores were determined using the chi-square test. To explore potential correlations, Pearson's correlation was applied to assess the relationship between hygienic practices and coliform contamination levels. A *p*-value of <0.05 was considered statistically significant in all analyses.

Results

Coliform levels in retail goat meat

The lowest (0.88 log10 cfu/g) and highest (5.04 log10 cfu/g) average coliform loads in meat were observed in retail stores F and E, respectively ([Figure 1\)](#page-4-1). The mean coliform load in meat from store E was significantly $(p<0.05)$ higher compared to all other stores. Following the guidelines established by ICMSF, the distribution of coliform levels in the goat meat samples revealed that 52% were unacceptable (>2000 cfu/g or 3.30 log10 cfu/g), 21% marginally acceptable $(100-2000 \text{ c} \text{fu/g or } 2-3.30 \text{ log} 10$ cfu/g), and 27% acceptable (\leq 100 cfu/g or \leq 2 log10 cfu/g) as indicated in [Figure 2](#page-5-0).

TABLE 1 Prevalence (%) of bacteria isolates from goat meat resistant to antimicrobial agents.

Mean (%) within first column and last row with different superscript differ significantly ($p < 0.05$).

ABacteria: species isolated from goat meat.

B Antibiotics: Amoxicillin/Clavulanic acid (AMC); Ampicillin (AMP); Penicillin (P); Ceftiofur (XNL); Cefpodoxime (CPD); Cephalothin (KF); Chloramphenicol (C); Florfenicol (FFL); Doxycycline (DO); Tetracycline (TE); Oxytetracycline (T30); Neomycin (N).

Recovery of *E. coli* and *Salmonella* spp., and *S. aureus* in fresh goat meat

From the presumptive isolates identified by cultural methods and latex agglutination tests, bacteria were confirmed by the amplification of specific target genes 16S rRNA, *sdiA*, and 16S rRNA for *E. coli*, *Salmonella* spp., and *S. aureus*, respectively ([Figures 3](#page-5-1)[–5](#page-6-0)). The prevalence of *E. coli* and *Salmonella* spp., and *S. aureus*, both combined are summarized as indicated in [Figure 6.](#page-7-0) The highest prevalence of bacteria isolates was recorded from meats in store A at 21.5% (20/93), followed by C and D at 18.3% (17/93), B at 12.9% (12/93), while G and H samples recorded the least at a rate of 2.3% (2/93), and 1.1% (1/93), respectively.

[Figure 7](#page-7-1) shows the levels of specific bacteria found in each store. Of the total 93 bacteria isolates analyzed, *S. aureus* showed the highest prevalence at 47.3%, significantly surpassing *E. coli* (31.2%) and *Salmonella* spp. (21.5%) with a *p*-value <0.05. Store D exhibited the highest occurrence of *S. aureus* at 10.7%, followed by C at 8.6%, and B and E both at 6.5%. Stores F and J had a prevalence of 2.2%, and stores G and H showed no presence of *S. aureus*. On the other hand, *Salmonella* spp. isolates were found in varying levels in different stores as: 5.4% for A and C, 3.2% for E, 2.2% for B and D, 1.1% for F, I and J and H recorded no *Salmonella*. Regarding *E. coli* isolates recovered from goat meat, store A exhibited the highest prevalence at 8.6%, followed by D (5.4%), B (4.3%), and C (4.3%). *E. coli* in goat meat from store A was significantly higher (p < 0.05) compared to stores F, H, and J. Notably, *E. coli* was not recovered from store E.

Resistance of *E. coli, Salmonella* spp., and *S. aureus* to specific antibiotics

The increasing trend of foodborne bacterial pathogens developing antibiotic resistance is adding to the rising challenge of foodborne

FIGURE 2

Total coliform load in retail goat meat. Unacceptable: >2000  cfu/g or 3.30 log10 cfu/g, marginally acceptable: 100–2000  cfu/g or 2–3.30 log10 cfu/g, and acceptable: ≤100  cfu/g or ≤2 log10 cfu/g.

infections, leading to infections that are challenging to treat and pose greater complications for consumers. [Table 1](#page-4-0) displays antibiotic resistance to selected antibiotics. AMR analysis demonstrated that the *S. aureus* isolates were resistant to all evaluated antibiotics. Particularly, noteworthy is its significant resistance $(p < 0.05)$ to penicillin (35.5%; 33/93), oxytetracycline (34.4%; 32/93), ampicillin (29.0%; 27/93), and cefpodoxime (25.8%; 24/93) compared to other antibiotics. *Salmonella* spp. displayed notable resistance, with the highest rates observed for oxytetracycline (13.9%; 13/93) and penicillin (11.8%; 11/93), significantly differing (*p*<0.05) from ceftiofur (0%; 0/93) and chloramphenicol (1.1%; 1/93). Notably, *Salmonella* spp. exhibited resistance to at least one of the twelve antibiotics, except for ceftiofur. *E. coli* isolates demonstrated significant resistance (p <0.05) to penicillin (31.2%; 29/93) when compared to cephalothin (22.6%; 21/93), tetracycline and oxytetracycline (16.1%; 15/93), and doxycycline (15.1%; 14/93). All *E. coli* and *Salmonella* spp. isolates were sensitive to ceftiofur (100%).

Multidrug-resistant patterns and MAR index

In this study, multidrug-resistant (MDR) of *E. coli*, *Salmonella* spp., and *S. aureus* was identified. The antimicrobial susceptibility test

DNA amplification of the sdiA gene in *Salmonella* spp. Lane 1: 1  kb ladder, lane 2: positive control *salmonella typyimurium* ATTC 13311, lane 3: negative control, lane 4–10: positive samples.

indicated that 29.3% of *E. coli*, 19.5% of *Salmonella* spp., and 51.2% of *S. aureus* isolates displayed multidrug resistance, as detailed in [Table 2.](#page-8-0) MAR index ranged from 0.25 to 0.75. Resistance patterns of AMP-C-CPD-DO-FFL-KF-P-TE-T, AMP-C-CPD-DO-FFL-KF-P-TE-T, and AMC-AMP-CPD-DO-KF-N-P-T-XNL were observed in EC53, EC68, and SA13 isolates, respectively [\(Table 3](#page-9-0)). *E. coli* isolates from various stores exhibited diverse drug resistance patterns, such as AMC-DO-FFL-KF-P-TE-T, AMP-C-CPD-DO-FFL-KF-P-TE-T, and AMC-AMP-CPD-FFL-N-P-T in store A, AMC-AMP-C-DO-FFL-P-TE-T and C-CPD-DO-FFL-KF-P-TE-T in store B, and CPD-DO-KF-P-TE-T and AMP-CPD-DO-KF-P-TE-T in store D. Despite being the least recovered in this study, *Salmonella* spp. isolates demonstrated a diverse array of multiple drug resistance patterns. Distinct resistance patterns observed among *Salmonella* spp. isolates from different

stores, included AMC-AMP-CPD-FFL-N-P-T and AMC-AMP-C-CPD-KF-FFL-P-T in store A, AMC-AMP-CPD-FFL-P-T and P-T-N in store D, and AMC-AMP-CPD-FFL-KF-PT, and AMC-AMP-KF-FFL-P-TE-T in store E. *S. aureus* exhibited a noteworthy display of twenty-one multi-drug resistance patterns, emphasizing the complexity of drug resistance ([Table 4\)](#page-9-1).

Resistance of bacteria to antibiotic classes

Notably, beta-lactams, such as penicillin (78.5%) and ampicillin (40.9%), exhibited significant resistance. Tetracyclines also displayed resistance, with oxytetracycline (64.5%), tetracycline (39.8%), and doxycycline (35.5%) showing notable levels. Cephalosporin resistance

TABLE 2 Multi-drug resistance of *E. coli* spp., *Salmonella* spp. and *S. aureus* to various antimicrobial agents in goat meat.

a Antibiotic agent used in the study. P, penicillin; A, ampicillin; AMC, amoxicillin/clavulanic acid; XNL, ceftiofur; CPD, cefpodoxime; KF, cephalothin; C, chloramphenicol; FFL, florfenicol; DO, doxycycline; TE, tetracycline; T, oxytetracycline; N, neomycin.

was observed at rates of 48.4, 33.3, and 17.2% for cephalothin, cefpodoxime, and ceftiofur, respectively. Neomycin, an aminoglycoside, demonstrated a resistance of 19.4%. Among the antibiotic classes, phenicol showed the least resistance, with chloramphenicol (9.7%) and florfenicol (16.1%) exhibiting lower resistance to bacterial isolates [\(Figure 8\)](#page-11-0).

Retail goat meat handlers' food safety practices

The data presented pertains to the food safety practices observed among goat meat handlers in retail stores [\(Figure 9\)](#page-11-1). Hygienic practices score of goat meat handlers in retail stores are presented in [Table 3.](#page-9-0) The findings revealed that none of the stores met the criteria for "good hygienic practices," with none scoring 70% or more on the hygienic practices scale, as depicted in the study. The overall frequency of glove use was 60% and apron use was 40%. Strikingly, the absence of hairnet use was noted in all retail stores. A significant proportion of participants (92.5%) did not use the same knives, cutting boards, and utensils for different types of meats. However, 100% of retail stores used the same knife and cutting board for both muscle meat and offal (organ meat). Meat handlers (10%) handled money while working. Approximately 70% of retail stores lacked proper labels on packaged goat meat, including important information such as the sell-by date, cooking temperature, and consumer handling instructions. Pearson's correlation analysis showed that the coefficient for hygienic practices and the coliform count was −0.8913968 (*p*<0.05) suggesting that hygienic practices affected coliform contamination in the retail stores (see [Figure 10\)](#page-12-0).

Discussion

The aim of this study was to evaluate the prevalence of coliforms, antimicrobial resistance (AMR) of *E. coli*, *Salmonella* spp., and *S. aureus* on goat meat, along with assessing meat handling practices in retail stores. The results indicated that 96% of the tested meat samples were positive for coliform, surpassing the 75.6% reported. The mean coliform counts observed in our study was exceeded the findings of [Thapa \(2016\).](#page-15-20) Considering specifications, 52% of goat meat was deemed unacceptable (>2000 cfu/g). This aligns with the results of [Al-Mahmood \(2020\),](#page-13-6) reporting that 51.5% of retail meats from North Carolina, South Carolina, and Georgia exceeded total coliform counts of 3 log10 cfu/g. In our study, 21% of goat meat was marginally acceptable (100–2000 cfu/g), and 27% was acceptable (\leq 100 cfu/g). The presence of coliforms suggests potential unhygienic conditions during goat slaughter and meat portioning into different parts ([Laban](#page-14-20) [et al., 2021](#page-14-20)), indicating fecal contamination or unhygienic meat handling [\(Abuzaid et al., 2020](#page-13-7)). Studies have indicated that individuals involved in slaughtering and meat selling, lacking stringent hygienic practices, significantly contribute to coliform contamination of meat ([Rani et al., 2017](#page-15-21); [Abayneh et al., 2019](#page-13-8); [Sebsibe and Asfaw, 2020](#page-15-22)). Frequent handling, unhygienic practices, meat exposure to contaminated environment, equipment, and utensils increase the microbial contamination ([Augustin et al., 2020](#page-14-21); [Mallhi et al., 2019;](#page-15-23) [Das et al., 2019](#page-14-22); [Alimi et al., 2022](#page-13-9)). The microbial safety of retail meat products is crucial, given that raw meats are often associated with cases of foodborne diseases [\(Bantawa et al., 2018](#page-14-23)). Implementing effective measures, such as providing regular hands-on meat safety training for meat handlers, is essential to prevent coliform contamination. Coliforms serve as indicators of pathogenic bacteria, making proactive training crucial in maintaining food safety standards in the meat handling process.

Our study observed a prevalence of *E. coli* at 31.2%, contrary to the findings of [Wilson-Smith et al. \(2021\)](#page-16-3), who reported a higher prevalence (60%) in retail goat meat. Conversely, [Kim et al. \(2020\)](#page-14-24) reported a lower prevalence (16.7%) compared to our study. Also, our findings align with [Ajulo et al. \(2020\)](#page-13-10), supporting their report on contamination of goat meat with *E. coli*. Commensal bacteria such as *E. coli* inhabit the intestinal tract of food animals. During slaughter, processing, and meat handling, deficits in hygiene cause bacteria contamination of fresh meat [\(Ramos et al., 2020](#page-15-24)). According to [Stein](#page-15-2) [and Katz \(2017\),](#page-15-2) small ruminant meat serves as a reservoir for *E. coli*. Certain strains of *E. coli*, including *E. coli* O157:H7, have the potential to cause severe illnesses in both animals and humans [\(Yakubu et al.,](#page-16-4) [2020\)](#page-16-4). In the current study, the prevalence of *Salmonella* spp. (21.5%) exceeded the 9% contamination reported by [Naik et al. \(2015\)](#page-15-25) in goat meat but was lower than the higher prevalence of 65% reported by [Sangeetha et al. \(2020\)](#page-15-26). Additionally, *Salmonella* has been identified in 12% of goat meat samples in India [\(Das et al., 1990\)](#page-14-25) and 3.3% of goat meat in Kathmandu, Nepal [\(Maharjan et al., 2006](#page-14-26)). This presents TABLE 3 Observational questions and answers for assessment of food safety practices of meat handlers in retail stores.

a noteworthy risk of zoonotic transmission through the consumption of contaminated goat meat. In our study, the observed prevalence of *S. aureus* at 47.3% aligns with [Latha et al. \(2017\)](#page-14-27) findings of 40% but differs from [Torki Baghbaderani et al. \(2020\)](#page-15-27) lower prevalence of 20.4% in retail goat meat. The prevalence of *S. aureus* (non-MRSA) in raw, unprocessed red meat has been reported in Egypt ([Al-Amery](#page-13-11) [et al., 2019](#page-13-11)). The growth of *S. aureus* and the production of enterotoxins in food are attributed to improper handling and inadequate storage conditions that support the proliferation of this pathogen ([Umeda et al., 2017](#page-16-5)). Contaminated equipment can also serve as a means of transferring *S. aureus* to meats; this pathogen has been identified on all equipment used in abattoirs [\(Adugna et al.,](#page-13-12) [2018\)](#page-13-12). Following appropriate slaughter and food handling protocols

TABLE 4 Antibiotic resistance profile and multiple antibiotic resistance index.

(Continued)

TABLE 4 (Continued)

AEC-retail stores. *Escherichia coli*; S, *Salmonella* spp.; SA, *Staphylococcus aureus*. ^BAtibiotic resistance profiles: Amoxicillin/Clavulanic acid (AMC); Ampicillin (AMP); Penicillin (P); Ceftiofur (XNL); Cefpodoxime (CPD); Cephalothin (KF); Chloramphenicol (C); Florfenicol (FFL); Doxycycline (DO); Tetracycline (TE); Oxytetracycline (T30); Neomycin (N).

^CMultiple antibiotic resistance index.

is crucial to minimize the risk of contamination with pathogenic microorganisms. The occurrence of *E. coli*, *Salmonella* spp., and *S. aureus* in goat meat is concerning, as they are the leading causes of foodborne diseases. Therefore, understanding and adhering to safety measures along the entire meat supply chain is crucial to safeguard consumers' health.

This study outlines the antimicrobial resistance profiles of *E. coli* spp., *Salmonella* spp., and *S. aureus* to commonly used antibiotics. The prevalence and potential for pathogenicity and antibiotic resistance gene acquisition in *E. coli*, *Salmonella* spp., and *S. aureus* is becoming a growing threat to human health ([Výrostková et al., 2021](#page-16-6)). According to this study, *E. coli* isolates were significantly ($p < 0.05$) resistant to penicillin as compared to cephalothin, tetracycline and oxytetracycline, and doxycycline. [Mwanyika et al. \(2016\)](#page-15-28) reported a notable level of antimicrobial resistance in *E. coli* isolated from goat meat. Our findings align with those of [Momtaz et al. \(2013\)](#page-15-29), who reported similar resistance patterns in *E. coli* isolates from goat meat, emphasizing the need for ongoing monitoring due to *E. coli*'s role as an indicator for antibiotic resistance in foods [\(EFSA Panel on](#page-14-11) [Biological Hazards \(BIOHAZ\) et al., 2021](#page-14-11)). Considering the potential health threat posed by antibiotic-resistant *E. coli* to consumers, it is imperative to implement measures aimed at reducing their occurrence in fresh goat meat ([Rega et al., 2022](#page-15-30)).

Our study reveals that *Salmonella* spp. exhibited resistance to all antibiotics except for ceftiofur. Notably, *Salmonella* significantly showed higher (p < 0.05) resistance to oxytetracycline and penicillin, compared to ceftiofur and chloramphenicol. The resistance of *Salmonella* to oxytetracycline may be linked to its extensive use in prophylaxis and disease treatment in food animal production, including goats, since its approval by the USFDA ([Mog et al., 2020\)](#page-15-31). Our findings underscore the potential of goat meat as a source of antibiotic-resistant *salmonella* spp. *S. aureus* demonstrated resistance to all antibiotics evaluated in our study, with the highest resistance observed against penicillin, followed by oxytetracycline, ampicillin and cefpodoxime, significantly differing (*p* < 0.05) from other antibiotics. These findings are consistent with those reported by [Mechesso et al. \(2021\),](#page-15-32) highlighting the extensive resistance of *S. aureus* isolates from goat carcasses to penicillin. The predominant resistance trait in goat meat isolates was displayed to penicillin, maybe attributed to its widespread use in animal production in the U.S., particularly in sheep and goat farming ([Susan, 2020](#page-15-33)). The resistance of *S. aureus* to multiple clinically important antibiotics raises concerns about potential dissemination to humans through frequent contact with infected animals and the food chain ([Chang](#page-14-28) [et al., 2015\)](#page-14-28). Antimicrobials are commonly used in livestock for prevention and control of diseases [\(Vanderhaeghen and Dewulf,](#page-16-7) [2017\)](#page-16-7), as well as for sustainable production. However, it is documented that antibiotics use in food animal production is a foremost cause of the evolving AMR in humans ([Manyi-Loh](#page-15-34) [et al., 2018\)](#page-15-34).

This study identified multidrug-resistant (MDR) bacterial isolates, defined as resistance to three or more antibiotic classes [\(Wang et al.,](#page-16-1) [2021\)](#page-16-1). The observed MAR index ranged from 0.25 to 0.75, with prevalent values at 0.58 and 0.67, indicating a high-risk source of contamination often associated with frequent antibiotic usage [\(Kahn](#page-14-29) [et al., 2019](#page-14-29)). Bacteria with a multiple antibiotic resistance index (MARI) greater than 0.2 are indicative of a high-risk source of contamination, where the use of multiple antibiotics is to a great degree [\(Mthembu et al., 2019\)](#page-15-35). *E. coli*, *Salmonella* spp. and *S. aureus* in our results was MDR. *E. coli* and *Salmonella* spp. exhibited resistance to eleven out of the twelve antimicrobials tested, raising serious concerns. Multidrug-resistant Enterobacteriaceae have been linked to higher mortality rates compared to other bacteria [\(Alqasim](#page-13-13) [et al., 2018;](#page-13-13) [Scheich et al., 2018](#page-15-36)). *S. aureus* displayed resistance to all twelve antibiotics, posing a significant public health concern due to its association with high mortality rates from systemic infections and food poisoning [\(Hong et al., 2018;](#page-14-30) [Vanamala et al., 2021\)](#page-16-8). The distinct drug resistance patterns identified in this study raise concerns for public health, considering the widespread use of these drugs for infection treatment and prevention in both animals and humans ([Geletu et al., 2022](#page-14-31)).

This study assessed the susceptibility of isolated bacteria from goat meat to various major classes of antibiotics. Notably, a high resistance to beta-lactams (penicillin) was shown for isolated bacteria. The presence of beta-lactam-resistant bacteria in goat meat is alarming due to the potential transmission to humans through the food chain, leading to treatment failures for infections caused by these bacteria [\(Chishimba et al., 2016](#page-14-32)). To mitigate this risk, it is imperative to reduce the use of beta-lactam antibiotics in farm animals to curb the development and spread of resistance through the food chain [\(World Health Organization, 2017\)](#page-16-9). Continuous surveillance of beta-lactam-resistant bacteria in meat is essential for detecting emerging patterns and guiding appropriate interventions. Additionally, the study found resistance among bacterial isolates to tetracyclines, including oxytetracycline and doxycycline. Tetracyclines are commonly employed in food-animal production,

their use has been linked to the emergence of tetracycline resistance in bacteria ([Roberts and Schwarz, 2016\)](#page-15-37). Furthermore, bacteria isolated from goat meat displayed resistance to cephalosporins, such as cephalothin, cefpodoxime, and ceftiofur. Given the frequent use of cephalosporins in animals, their association with cephalosporin resistance in bacteria is noteworthy ([Cameron-Veas et al., 2015](#page-14-33)). The lower resistance levels to ceftiofur may be attributed to differences in the mechanism of action between 3rd and 1st generation cephalosporins [\(Collineau et al., 2020\)](#page-14-34). Moreover, neomycin, classified as an aminoglycoside, exhibited a lower resistance rate. Neomycin is commonly used to treat susceptible bacterial infections in goats and sheep. Resistance to phenicol, including chloramphenicol and florfenicol, was also noted in the study. The relatively lower resistance levels may also be explained by the infrequent use of phenicol in livestock production.

Based on the outcomes of our study, it is evident that most of the retail stores investigated exhibited inadequate practices in handling meat. None of the stores met the criteria for "good hygienic practices," as none achieved hygienic practices score of 70% or higher. The overall level of good meat handling practices among meat handlers in our study was found to be 45.75%. However, previous studies have reported higher percentages of good meat handling practices, such as 66.4% [\(Yenealem et al., 2020\)](#page-16-2) and 86.8% [\(Tegegne and Phyo, 2017\)](#page-15-38). Examining specific practices, the overall frequency of glove and apron use was 60 and 40%, respectively, with no observed use of hairnets. This contrasts with findings from other studies, where hairnet usage was reported at 96.6% in Saudi Arabia [\(Al-Shabib et al.,](#page-13-14) [2016](#page-13-14)), apron usage at 95.9% in Iran [\(Ansari-Lari et al., 2010](#page-13-15)), and glove usage at 89.7% in Bangladesh [\(Al Banna et al., 2021\)](#page-13-4). Notably, a significant proportion of participants in this study did not use the same knives, cutting boards, and utensils for different types of meats. Nevertheless, all retail stores (100%) employed the same knife and cutting board for both muscle meat and offal, which is not a recommended practice. This approach poses the risk of transferring foodborne pathogens from offal meats to muscle meats. According to [Abdalrahman et al. \(2015\),](#page-13-16) edible offal is frequently contaminated with pathogenic bacteria. In terms of personal accessories, meat

handlers at eight stores (77.5%) in our study wore rings, necklaces, and watches, surpassing the 70% reported by [Kanko et al. \(2023\).](#page-14-35) The frequency of handling money while working was 10% in our study, lower than the 45.5% reported in Ethiopia ([Birhanu et al., 2017](#page-14-36)) and 87% in Kenya [\(Chepkemoi et al., 2015](#page-14-37)). All stores had refrigerated meat, with only three stores providing labels, sell-by dates, and consumer handling instructions. Consumer handling instructions provide guidance on storage conditions, cooking temperatures, and other essential information. This empowers consumers to handle and prepare food safely, minimizing the risk of contamination and foodborne illnesses.

Pearson's correlation analysis revealed a significant negative correlation (coefficient: −0.8913968, *p*<0.05) between hygienic practices and coliform count, suggesting that hygienic practices influenced coliform contamination in fresh goat meat. Stores with higher hygienic practices scores demonstrated lower coliform loads than those with lower scores. These findings align with [Mahato's](#page-14-38) [\(2019\)](#page-14-38) observation that meat from stores with unhygienic meat handling practices and poor sanitary conditions exhibited high microbial counts compared to those with hygienic handling practices and good sanitation. In our study, the presence of *E. coli* in goat meat serves as an indicator of fecal contamination [\(Anihouvi et al., 2020](#page-13-17)), suggesting potential exposure to human or animal feces due to inadequate hygienic and handling practices in slaughterhouses or retail outlets. *E. coli* is a good indicator of fecal contamination ([Anihouvi et al., 2020](#page-13-17)) and therefore, their detection in of goat meat samples suggested a contamination by human or animal feces through inappropriate hygienic and handling practices in slaughterhouses or

at retail outlets. The prevalence of multidrug-resistant (MDR) *E. coli*, *Salmonella* spp., and *S. aureus* underscore the potential safety concerns associated with goat meat purchased from retailers, emphasizing the necessity for enhanced hygienic practices. Furthermore, this study indicates that goat meat may pose a risk as a potential source of MDR bacteria. Unhygienic meat handling has specifically been associated with meat-borne diseases [\(Sulleyman et al., 2018](#page-15-39)). Other studies also attribute 97% of foodborne illness outbreaks to food handler behaviors or mistakes [\(Tauxe, 2019](#page-15-40); [Lin and Roberts, 2020](#page-14-39)). Promoting hygienic behaviors in food handling is considered a plausible and potentially effective strategy to safeguard consumers from foodborne illnesses and deaths ([Wambui et al., 2017](#page-16-10)).

In summary, this study establishes a significant correlation between hygienic practices and coliform load in retail stores selling fresh goat meat in Nashville, Tennessee. Stores with higher hygienic practice scores demonstrated lower coliform loads compared to those with lower scores. Our findings highlight the potential threat to consumer health posed by the presence of multidrug-resistant *E. coli*, *Salmonella* spp., and *S. aureus* in goat meat, particularly concerning the growing demand for meat products. The observed resistance in bacteria from goat meat spans major antibiotic classes, including betalactam, tetracycline, and cephalosporins. It is crucial to exercise caution during the slaughter process to prevent fecal contamination of goat meat, as this could introduce pathogenic bacteria, including multi-resistant strains. The results strongly advocate for the development of educational materials to enhance the food safety knowledge of goat meat handlers, addressing both meat safety and broader food safety concerns. Furthermore, it is advisable for

consumers to cook meats at recommended temperatures to effectively destroy foodborne pathogens.

Data availability statement

The original contributions presented in the study are included in the article/supplementary material, further inquiries can be directed to the corresponding author.

Ethics statement

The studies involving humans were approved by the Tennessee State University Institutional Review Board (HS-2021-4597). The studies were conducted in accordance with the local legislation and institutional requirements. The participants provided their written informed consent to participate in this study.

Author contributions

MO: Writing – review & editing, Writing – original draft, Validation, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. AK-N: Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Resources, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization. AB: Methodology, Data analysis, Data curation, Writing – review & editing.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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