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Original article

Cross-sectional study of antibiotic residues and antimicrobial-resistant pathogens from raw meat in and around Kanchipuram Tamil Nadu

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Abstract:
In the present study conducted in Kanchipuram, Tamil Nadu, India, significant findings emerged regarding antibiotic residues and antibiotic-resistant bacterial pathogens in chicken and goat meat from commercial vendors. The microbial analysis of 120 meat samples collected from 20 different shops revealed the widespread presence of various bacterial pathogens, with notable prevalence of Staphylococcus spp. and E. coli in both meat types. Notably, Salmonella spp. was more prevalent in chicken meat, highlighting the importance of stringent food safety measures in poultry products. In the realm of antibiotic susceptibility, Gram-negative pathogens exhibited variable resistance to different antibiotics, with ampicillin and penicillin showing high resistance rates, while imipenem demonstrated exceptional sensitivity. Among Gram-positive pathogens, linezolid and oxacillin proved highly effective, but ampicillin and penicillin faced significant resistance. Importantly, the Minimum Inhibitory Concentration (MIC) tests underscored antibiotic resistance issues among both Gram-positive and Gram-negative pathogens. These findings accentuate the need for judicious antibiotic use in meat production and the urgency for further research to counter antibiotic-resistant bacterial infections effectively. This research contributes significantly to our understanding of the complex interplay between antibiotic residues, antibiotic resistance, and meat product safety, offering critical insights for informed decision-making, healthcare practices, and potential policy interventions to ensure safer meat production and mitigate the global challenge of antibiotic resistance.

Keywords: *Antibiotic; Meat and meat products; E. coli; Salmonella spp; Antimicrobial resistance; ESBL*

Introduction:

Food animals, comprising species like cattle, pigs, sheep, goats, poultry, and fish, play a vital role in the worldwide food system, serving as a primary source of essential nutrients for human consumption. The Food and Agriculture Organization of the United Nations (FAO) highlighted in its recent report that global meat production achieved a record-breaking 346 million tons in 2020, with poultry emerging as the most extensively produced meat on a global scale[(1)]. Beyond their role in providing sustenance, food animals are utilized for various purposes, including the production of skins and hides, contributions to medical research, and recreational activities. The contemporary livestock industry heavily relies on antibiotics to enhance animal health and bolster production yields. However, this indiscriminate use of antibiotics raises substantial concerns regarding antibiotic residues in meat products and the concomitant emergence of antibiotic-resistant bacterial pathogens[(2)].

The transfer of antimicrobial resistance (AMR) agents from animals to humans is a compelling dimension of this multifaceted issue. The consumption of contaminated food products, such as meat and milk, has been recognized as a potential source of AMR infections in humans. This is particularly relevant in India, where livestock plays a pivotal role in the agricultural economy,

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contributing to a significant public health challenge related to zoonotic bacterial AMR. Studies conducted in the country reveal alarming resistance rates in common zoonotic bacteria, such as Escherichia coli, isolated from poultry, dairy cows, and human clinical samples[3,4]. Urgent action is imperative to address the spread of AMR in zoonotic bacteria, necessitating improvements in animal husbandry practices, enhanced regulatory policies, and responsible use of antimicrobial agents to mitigate the risk of transmission to humans. Surveillance and monitoring efforts in both animal and human populations are integral to understanding the epidemiology of AMR and formulating effective intervention strategies.

The complex process involves multiple factors, including antimicrobial agent use, consumption of contaminated food products, and environmental contamination. Addressing this issue mandates a comprehensive approach involving reductions in antimicrobial use in animal production, responsible use of antimicrobial agents in human medicine, improvements in food safety and hygiene, and effective environmental management practices. The transfer of AMR bacteria and genes from food animals to humans through the food chain poses a substantial risk to human health, necessitating ongoing surveillance efforts to identify emerging threats and inform public health strategies. The identification of various AMR genes in food animals worldwide underscores the urgency of a comprehensive approach, involving effective regulatory policies, responsible use of antimicrobial agents, and the development of alternative therapies and management practices. The continued surveillance of AMR in food animals and humans is crucial for proactively addressing this global health challenge[1].

Anticipated outcomes of this research include vital information on the types and concentrations of antimicrobials employed in meat production, the identification of antibiotic-resistant pathogens, and heightened awareness regarding the implications of antibiotic use in commercial meat products. Additionally, This research endeavours to serve as a significant stride in ensuring the safety of meat products and promoting judicious antibiotic usage within the livestock industry, underpinned by comprehensive insights into the extent of antibiotic residues and antibiotic resistance in meat. It also provides a foundation for informed decision-making and potential policy interventions. This study aims to meticulously investigate the prevalence of antibiotic residues and antibiotic-resistant bacterial pathogens in chicken and goat meat from commercial vendors in and around Kanchipuram, Tamil Nadu, India.

Materials and Methods:

Collection of raw meat in and around Kanchipuram

Meat samples will be collected from retail shops in and around Kanchipuram, Tamil Nadu, and India. Kanchipuram and the sub-urban of Kanchipuram will be divided into 10 Areas, based on assembly. In each area, 10 meat samples will be collected from retail shops. A total of 120 samples of raw meat muscle, randomly like a liver, and kidney will be collected. The date, organ and type of meat, shop and zone name of the collection will be recorded. Then the samples will be packed in sterile polyethylene bags and transported in an icebox to Central Research Laboratory, Meenakshi Medical College Hospital and Research Institute, Kanchipuram.

Estimation of antimicrobial residues in meat by *HPLC*

According to Doyuk et al., 2023[6], Antimicrobial residues will be confirmed by High-performance liquid chromatography techniques. The presence of the various antibiotic residues in the meat samples will be detected using methods previously reported in the literature by various authors with minor modifications.

The recovery test for ciprofloxacin, sulphanilamide, streptomycin and tetracycline will be performed in triplicate by standards at three levels into different blank meat samples. Two grams of each sample of the homogenized meat will be spiked with 100 mL of the mixed standard. The meat samples and blank samples without standards were then analysed by HPLC. Recovery will be calculated by comparing the analysed concentrations with spiked concentrations by the formula:

$$Recovery\ \% \frac{\text{Amount of residue obtained after spiking sample}}{Spiked\ Concentration} X100$$

The standard antibiotic stock solution will be prepared for each antibiotic detection[6]

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Isolation and identification of microbial pathogens from raw meat

Sample preparation

All samples will be examined for the presence of *E. coli*, *S. aureus*, *Enterococcus spp*., *Salmonella spp.*, *Yersinia spp*., *Listeria spp.* and *Campylobacter spp*. 25g of each sample will be homogenized and added to 225 mL of Buffered Peptone Water (REF: FSSAI, Govt. of India).

Identification:

Standard FSSAI recommended procedures will be followed for isolation, enumeration and identification of bacteria and mould (Ref: **F.No.1-110(2)/SP (Biological Hazards)/FSSAI/2010)**[7],

- Aerobic Plate Count: -IS 5402
- Yeast and Mould Count- IS 5403
- *Staphylococcus aureus* and Enterococcus- IS 5887
- *Escherichia coli*: IS 5887
- *Salmonella spp.*- IS 5887
- *Listeria monocytogenes*- IS 14988

*IS- represents the Bureau of Indian Standard

Antimicrobial susceptibility pattern of identified microbial pathogens in meet

Antimicrobial susceptibility test will be performed according to Clinical and Laboratory Standards Institute, 2023[8]. recommendations for the following antibiotics: penicillin, ampicillin, ampicillin-sulbactam, amoxicillin-clavulanic acid, methicillin, carbenicillin, ticarcillin, ticarcillin-clavulanic acid, cefuroxime, ceftriaxone, imipenem, ciprofloxacin, nitrofurantoin, gentamicin, amikacin, tetracycline, erythromycin, clindamycin, colistin, and vancomycin. The minimum inhibitory concentration (MIC) test will be performed by antimicrobial gradient strip method. Isolates found resistant to a particular antibiotic by the disc diffusion method will be tested for MIC to quantify the level of resistance[9].

The results of antimicrobial susceptibility testing will be stored and analysed using WHONET 5.6 software (World Health Organisation, Geneva, Switzerland). Each bacterial pathogen will be analysed for the multiple drug resistance (MDR) index as the ratio between the number of antibiotics at which the isolate was resistant over the number of antibiotics tested for that isolate. Comparative analyses of continuous variables were made using the Student's t-test for two groups and one-way analysis of variance (ANOVA) for more than two groups. A value of $P<0.05$ will be considered for statistical significance.

Antimicrobial Resistance -Real time PCR:

Real-time PCR amplifications were carried out in 25 mL reaction volumes. Each reaction consisted of 12.5 mL of Qiagen one-step master mix from Qiagen, 1 mL of forward and reverse primers (10 pmol each), 0.1 mL of the TEM TaqMan probe (5 pmol), 0.2 mL of each of the other four TaqMan probes (10 pmol each), 0.6 mL of sterile water, and 1 mL of the DNA mixture. DNA extraction was performed using the Qiagen DNA/RNA extraction kit, and the resulting DNA samples were stored at -20 degrees Celsius. To ensure the real-time PCR assay's specificity and optimize its conditions, all five primer/probe pairs were tested using strains that contained only one specific resistance gene for each primer/probe combination. This set of tests included five positive control strains (P1-5), along with one negative control strain (N1) known to lack the tested resistance genes. Furthermore, a "Negative Control" (NC) was included in each real-time run. The QIAGEN Roto gene q 5HRM RTPCR machine was utilized to detect CTX, TEM, SHV, and qnr, and the PCR was conducted using the QIAGEN OneStep RT-PCR Kit (Cat. No. 210212). Reference information for the primer/probe sequences can be found in Table 1.

The PCR protocol involved 30 cycles, each comprising the following steps: 95°C for 15 seconds, 50°C for 15 seconds, and 70°C for 20 seconds. Fluorescence signals were captured in four distinct channels: Green (465–510 nm)/6FAM, Orange (533–610 nm)/ROX, Red (618–660

nm)/Cy5, and Yellow (533–580 nm)/Yakima Yellow. the completion of the run, a cycle threshold (Ct) was determined by identifying the point at which the fluorescence surpassed a predefined threshold level. This threshold was manually established for each detection channel and individual experiment. Samples displaying a fluorescence signal above this established threshold were classified as positive.

Table-1 Primer and Probe Sequences for Real-time PCR Targeting Antibiotic Resistance Genes

Real-time PCR: Target	Primer/probe -Sequence (5'-3')	Reference
blaTEM	F:GCATCTTACGGATGGCATGA	10, 11
	R: GTCCTCCGATCGTTGTCAGAA	
	6-Fam-CAGTGCTGCCATAACCATGAGTGA-BHQ-1	
blaCMY	F:GGCAAACAGTGGCAGGGTAT	12, 13
	R: AATGCGGCTTTATCCCTAACG	
	ROX-CCTACCGCTGCAGATCCCCGATG-BHQ-2	
blaSHV	F: TCCCATGATGAGCACCTTTAAA	12, 13
	R: TCCTGCTGGCGATAGTGGAT	
	Cy5-TGCCGGTGACGAACAGCTGGAG-BBQ-650	
blaCTX-M	F:CGGGCRATGGCGCARAC	12, 13
	R: TGCRCCGGTSGTATTGCC	
	Yakima Yellow-CCARCGGGCGCAGYTGGTGAC-BHQ1	
qnrA	F: ATTTCTCACGCCAGGATTTG	
	R: GATCGGCAAAGGTTAGGTCA	

Results:

Sample Collection

A total of 120 meat samples were collected in and around Kanchipuram. These samples were obtained from 20 different shops (Table-2), with each shop providing three samples of both goat and chicken meat. This comprehensive dataset allowed for a thorough examination of the microbial isolates, antibiotic residues, and other parameters related to the quality and safety of meat products in the region. The samples were meticulously collected to ensure representative sampling and covered a diverse range of meat sources. The subsequent analyses and findings, which will be discussed in detail, shed light on the prevalence of various microbial pathogens, antibiotic residues, and other factors that are essential for assessing the quality and safety of meat products available in the study area. The results obtained from this extensive sampling effort will be discussed comprehensively in the following sections, providing valuable insights into the microbial and chemical profiles of chicken and goat meat in Kanchipuram and its surrounding areas.

Table 2: Sample collected in and around Kanchipuram

Shops	Samples		Total Samples
	Goat (3 sample/shop)	Chicken (3 sample/shop)	
20 shops	**60**	**60**	**120**

The microbial isolation and identification from chicken and goat meat samples in and around Kanchipuram has showed crucial insights into the prevalence of various bacterial pathogens. As presented in Table-3, the data from this study highlights the microbial isolates detected in these meat samples. *Staphylococcus spp.* was detected in both chicken and goat samples, with 18 and 19 samples testing positive, respectively. The combined total bacterial isolates of *Staphylococcus spp.* across both types of meat amounted to 37. Pseudomonas aeruginosa, another notable bacterium, was found in 6 chicken samples and 9 goat samples, resulting in a combined total

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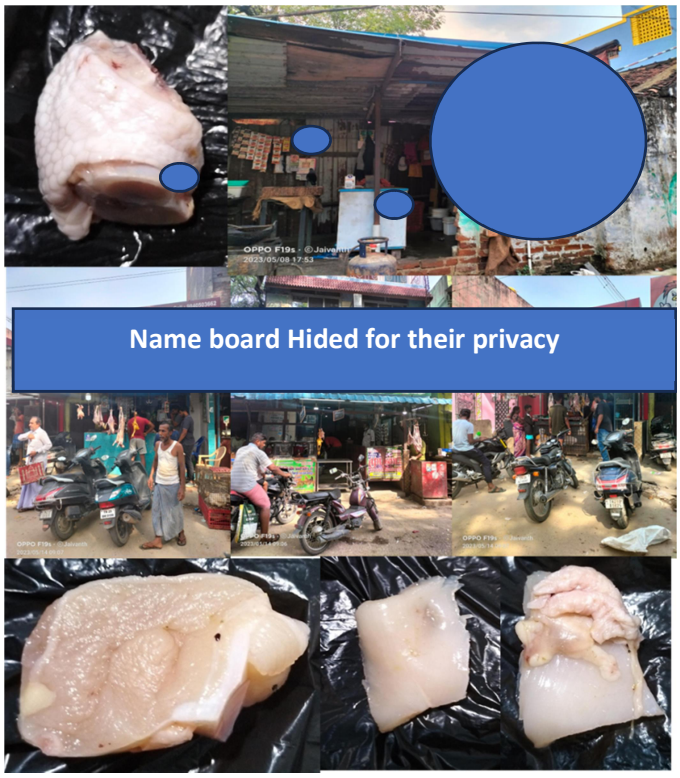
bacterial isolate of 15. A concerning discovery was made regarding *Salmonella spp.* In this case, 25 samples from chicken meat tested positive for this pathogen, while only 1 sample from goat meat was positive. The total bacterial isolates of *Salmonella spp.* in the study amounted to 26. *E. coli* was a prevalent bacterium in both chicken and goat meat, with 32 samples testing positive in chicken meat and 29 in goat meat. The combined total bacterial isolates of *E. coli* across both types of meat were 61.

Table-3: Microbial Isolates in Chicken and Goat Meat Samples Collected in and Around Kanchipuram

Microbial Pathogen	Chicken sample	Goat sample	Total Bacterial isolates
Staphylococcus spp.	18	19	37
Pseudomonas aeruginosa	6	9	15
Salmonella typhi.	25	1	26
Escherichia coli	32	29	61
Enterococcus faecalis.	5	18	23
total	86	76	162



Picture-1: Meat Samples and Collection Locations in and Around Kanchipuram

The prevalence of Enterococcus faecalis showed variation, with 5 chicken samples and 18 goat samples testing positive. The total bacterial isolates of Enterococcus faecalis was 23. The study involved the analysis of 162 meat samples, collected from 20 different shops in the vicinity of Kanchipuram. These results provide valuable insights into the microbial landscape within the region's meat products. Notably, the presence of *Salmonella spp.* in a significant number of chicken samples underscores the importance of stringent food safety measures, particularly in poultry products. To prevent bacterial contamination, it is crucial to emphasize proper hygiene, storage conditions, and handling practices throughout the production and supply chain. This results contribute to our understanding of food safety and the associated potential risks, and they form the basis

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for further research, guiding the development of strategies to enhance the safety and quality of meat products in Kanchipuram and its surroundings. Salmonella is a group of bacteria that can cause foodborne illnesses in humans. In the context of this study, the detection of *Salmonella spp.* in chicken meat is a cause for concern, as it indicates potential contamination with a pathogen that can lead to food poisoning. Preventing Salmonella contamination involves thorough cooking of poultry products, maintaining proper hygiene during food preparation, and ensuring that meat is stored at appropriate temperatures to prevent bacterial growth.

Antibiotic susceptibility testing:

The antimicrobial susceptibility testing of Gram-negative bacterial pathogens isolated from chicken and meat products revealed significant findings. .

The data presented in the table demonstrates the percentage of resistance and sensitivity for various antibiotics among these pathogens. Notably, some antibiotics exhibited high resistance levels, such as ampicillin with 72.55% resistance and penicillin with 73.53% resistance. In contrast, imipenem showed an impressive 99.02% sensitivity, indicating its effectiveness against these bacterial pathogens. Other antibiotics, including aztreonam, cefepime, ceftazidime, and cefotaxime, displayed substantial sensitivity rates, ranging from 80.39% to 84.31%. These results highlight the importance of carefully selecting antibiotics for the treatment of infections caused by Gram-negative bacterial pathogens.

It also underscores the necessity for prudent antibiotic use to prevent the development of further resistance.

Table 4: Antimicrobial susceptibility testing by disk diffusion Gram-Negative Bacterial pathogen (102 no.)

Antibiotics	Percentage of Resistance(no.)	Percentage of Intermediate Resistance(no.)	Percentage of Sensitivity(no.)
Amikacin	14.71% (15)	14.71% (15)	70.59% (72)
Amoxcillin	49.02% (50)	21.57% (22)	29.41% (30)
Ampicillin	72.55% (74)	12.75% (13)	14.71% (15)
Aztreonam	13.73% (14)	2.94% (3)	83.33% (85)
Cefepime	4.90% (5)	14.71% (15)	80.39% (82)
Ceftazidime	5.88% (6)	9.80% (10)	84.31% (86)
Cephotaxime	24.51% (25)	13.73% (14)	61.76% (63)
Ciprofloxacin	54.90% (56)	27.45% (28)	17.65% (18)
Gentamicin	51.96% (53)	16.67% (17)	31.37% (32)
Imipenem	0% (0)	0.98% (1)	99.02% (101)
Penicillin	73.53% (75)	11.76% (12)	14.71% (15)
Piperacillin/tazobactam	0% (0)	10.78% (11)	89.22% (91)
Tetracycline	87.25% (89)	4.90% (5)	7.84% (8)
ticaracillin	0% (0)	3.92% (4)	96.08% (98)
Tobramycin	0% (0)	13.73% (14)	86.27% (88)
Trimethoprim/sulfamethoxazole	15.69% (16)	5.88% (6)	78.43% (80)

Table-5: Antimicrobial susceptibility testing by disk diffusion Gram-Positive Bacterial pathogen (60 no.)			
Antibiotic	**Percentage of Resistance(no.)**	**Percentage of Intermediate Resistance(no.)**	**Percentage of Sensitivity(no.)**
Ampicillin	58.33% (35)	11.67% (7)	30% (18)
Chloramphenicol	56.67% (34)	5% (3)	38.33% (23)
Ciprofloxacin	20% (12)	13.33% (8)	66.67% (40)
Clindamycin	23.33% (14)	3.33% (2)	73.33% (44)
Co-trimoxazole	20% (12)	6.67% (4)	73.33% (44)
Erythromycin	63.33% (38)	11.67% (7)	25% (15)
Gentamycin	38.33% (23)	20% (12)	41.67% (25)
Linezolid	0% (0)	0% (0)	100% (60)
Oxacillin	8.33% (5)	6.67% (4)	85% (51)
Penicillin	71.67% (43)	11.67% (7)	16.67% (10)
Streptomycin	68.33% (41)	11.67% (7)	20% (12)
Tetracycline	55% (33)	8.33% (5)	36.67% (22)
Vancomycin	0% (0)	1.67% (1)	98.33% (59)

In Table 4, the antimicrobial susceptibility testing results for Gram-positive bacterial pathogens are presented. The table reveals the percentages of resistance, intermediate resistance, and sensitivity to various antibiotics, along with the corresponding sample counts in parentheses. Significant percentages of resistance were observed for some antibiotics, indicating the reduced effectiveness of these drugs against the tested Gram-Positive Bacterial pathogens. Ampicillin exhibited a notable resistance of 58.33% (35 no.), while Erythromycin showed a high resistance percentage of 63.33% (38 no.). Penicillin displayed a significant resistance percentage of 71.67% (43 no.), suggesting its limited efficacy.

Conversely, some antibiotics displayed high percentages of sensitivity. Linezolid, a powerful antibiotic, demonstrated 100% sensitivity, indicating its effectiveness in combating Gram-positive bacterial pathogens. Oxacillin exhibited a substantial sensitivity percentage of 85% (51 no.), while Ciprofloxacin showed a promising sensitivity of 66.67% (40 no.). These findings highlight the varying degrees of antibiotic resistance and sensitivity among Gram-positive bacterial pathogens, emphasizing the importance of selecting appropriate antibiotics for effective treatment. Healthcare professionals must consider these results when determining the most suitable antibiotic therapies for bacterial infections caused by Gram-positive pathogens.

Minimum Inhibitory Concentration test:

Table 6 provides a detailed account of the outcomes of antimicrobial susceptibility tests conducted via Minimum Inhibitory Concentration (MIC) for a set of 12 distinct antibiotics, encompassing a range of Gram-Positive and Gram-Negative pathogens. This dataset delivers a comprehensive perspective on the prevalent resistance and susceptibility profiles detected within these microbial strains. Among Gram-positive pathogens, several antibiotics were tested to determine their effectiveness. Ampicillin showed a mixed result, with 36 pathogens being resistant, 3 displaying intermediate resistance, and 21 showing susceptibility. Chloramphenicol performed more favourably, as 35 pathogens exhibited resistance, but none displayed intermediate resistance, and 25 were susceptible to this antibiotic. Gentamicin, on the other hand, offered a balanced outcome, with 28 pathogens resistant, 4 with intermediate resistance, and 28 susceptible to the treatment. Penicillin faced substantial challenges, with 44 pathogens demonstrating resistance, 5 displaying intermediate resistance, and only 11 pathogens remaining susceptible. Finally, Streptomycin showed a similar pattern, with 42 pathogens being resistant, 5 revealing intermediate resistance, and 13 showing susceptibility. The Gram-negative pathogens also underwent testing with the same antibiotics. Amoxicillin faced significant resistance, as 55 pathogens were resistant, 10 had intermediate

resistance, and 37 remained susceptible to treatment. Ampicillin, too, showed high resistance, with 73 pathogens being resistant, 4 displaying intermediate resistance, and only 25 pathogens being susceptible. Ciprofloxacin had a substantial challenge, with 63 pathogens revealing resistance, 14 demonstrating intermediate resistance, and just 25 proving susceptible to the treatment. Gentamicin offered a relatively balanced outcome, with 55 pathogens exhibiting resistance, 14 revealing intermediate resistance, and 33 being susceptible. Penicillin showed a notable pattern of resistance, with 70 pathogens being resistant, 5 displaying intermediate resistance, and 27 remaining susceptible. Tetracycline presented high resistance among Gram-Negative pathogens, with 75 pathogens resistant, 4 with intermediate resistance, and 23 exhibiting susceptibilities to the antibiotic. The results from these MIC tests underscore the prevailing issue of antibiotic resistance among both Gram-positive and Gram-negative pathogens. While some antibiotics retain their effectiveness in specific cases, a substantial prevalence of resistance and intermediate resistance is evident. This emphasizes the importance of utilizing antibiotics judiciously and investing in research and development to counter bacterial infections effectively. Physicians and healthcare providers should carefully consider these results when selecting antibiotic treatments to optimize both patient outcomes and the broader public health perspective.

Table 6: Antimicrobial susceptibility testing by Minimum Inhibitory Concentration

Antibiotic	Resistant	Intermediate Resistance	Susceptibility
Gram-Positive Pathogens (60 no.)			
Ampicillin	36	3	21
Chloramphenicol	35	0	25
Gentamycin	28	4	28
Penicillin	44	5	11
Streptomycin	42	5	13
Gram-Negative Pathogens (102 no.)			
Amoxicillin	55	10	37
Ampicillin	73	4	25
Ciprofloxacin	63	14	25
Gentamicin	55	14	33
Penicillin	70	5	27
Tetracycline	75	4	23
Cefepime	24	3	75
Ceftazidime	20	4	78
Cephotaxime	25	5	72

RT-PCR:

These gene combinations are pivotal markers of antibiotic resistance, shedding light on the intricate mechanisms of resistance inherent to these bacterial species. The significance of these findings can be tabulated in Table-7. 10 *E. coli*, 4 *Salmonella serovar Typhi*, and 6 *P. aeruginosa* isolates were positive to blaCTX-M, the presence of blaCTX-M signifies resistance to extended-spectrum cephalosporins, a crucial class of antibiotics. Its widespread prevalence underscores the expanding scope of this resistance mechanism among these bacteria. blaTEM: Prevalent in 14 *E. coli*, 3 *Salmonella serovar Typhi*, and 3 *P. aeruginosa* isolates, the blaTEM gene imparts resistance to beta-lactam antibiotics, representing one of the most commonly encountered beta-lactamase genes. Its prevalence among numerous isolates is an unsettling revelation. blaCMY: Detected in 9 *E. coli*, 6 *Salmonella serovar Typhi*, and 5 *P. aeruginosa* isolates, blaCMY is linked to resistance against cephalosporin antibiotics, signifying resistance within these isolates against this antibiotic class. blaSHV: Among *E. coli*, 15 isolates

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carry blaSHV, while 3 and 2 isolates within *Salmonella serovar Typhi* and P. aeruginosa, respectively, possess this resistance gene. Similarly, it confers resistance to beta-lactam antibiotics, and its high prevalence in *E. coli* merits attention. qnr: Present in 15 *E. coli*, 10 *Salmonella serovar Typhi*, and 1 *P. aeruginosa* isolate, qnr genes are associated with quinolone antibiotic resistance. The substantial prevalence among *E. coli* and *Salmonella serovar Typhi* isolates signifies resistance to this specific class of antibiotics.

Intriguingly, the table also unveils combinations of resistance genes. For instance, "blaTEM + blaCMY" is evident in one isolate for each bacterium. These combinations potentially lead to multidrug resistance, complicating treatment modalities. Particularly disquieting is the high prevalence of blaSHV in *E. coli*, alongside the occurrence of multiple resistance genes within certain isolates. These revelations indicate an elevated level of antibiotic resistance within these bacteria, constraining the array of available treatment options. These results accentuate the pressing requirement for the perpetual surveillance of antibiotic resistance in the specified bacterial species. The comprehension of the prevalence of distinct resistance genes and their combinations can provide invaluable guidance for therapeutic strategies and the formulation of endeavors aimed at curtailing the dissemination of resistance. Table 7 showed a valuable repository of data concerning the distribution of resistance genes and gene combinations among clinical isolates of *E. coli*, *Salmonella serovar Typhi*, and P. aeruginosa. These findings underscore the indispensability of judicious antibiotic employment and the perpetual necessity for research initiatives and interventions to combat antibiotic resistance effectively.

Table 7: Summary of Resistance Gene Combinations in *E. coli*, *Salmonella serovar Typhi*, and *P. aeruginosa* Isolates

Resistance Gene Combinations	*E. coli*	*Salmonella serovar Typhi*	*P. aeruginosa*
blaCTX-M	10	4	6
blaTEM	14	3	3
blaCMY	9	6	5
blaSHV	15	3	2
qnr	15	10	1
blaCMY + blaCTX-M	Nil	Nil	Nil
blaSHV + blaCTX-M	Nil	Nil	Nil
blaCTX-M + qnr	2	2	1
blaTEM + blaCMY	1	1	1
blaTEM + blaSHV	1	1	1
blaTEM + blaCTX-M	1	1	1
blaTEM + qnr	2	3	Nil
blaCMY + blaSHV	1	1	Nil

Antibiotic Residue Profiles in Poultry and Goat Meat through HPLC Analysis:

HPLC to scrutinize antibiotic residues in chicken and goat meat samples, addressing concerns regarding antibiotic contamination in meat products. A total of six antibiotics, including Ciprofloxacin, Tetracycline, Doxycycline, Gentamicin, Penicillin, and Chloramphenicol, were analyzed in the meat samples. Results showed the presence of various antibiotic residues. Such as, chicken samples exhibited Ciprofloxacin residues within a range of 30.81 to 55.6 ppb, while goat samples displayed levels from 6.64 to 10.54 ppb. Tetracycline residues in chicken samples were found within the range of 143.56 to 300.45 ppb. Goat samples showed Doxycycline residues at levels varying from 12.34 to 33.56 ppb. Gentamicin residues in chicken samples were detected within the range of 23.93 to 38.54 ppb, and Penicillin residues in chicken samples ranged from 34.25 to 56.22 ppb. Notably, Chloramphenicol residues in chicken samples displayed a broader range, spanning from 24.27 to 343.89 ppb. This HPLC analysis underscores the necessity for robust antibiotic residue monitoring in meat products to ensure consumer safety (Table-8). It highlights the

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importance of implementing stringent regulations and best practices in animal husbandry to minimize antibiotic residues in food products.

Table-8: Antibiotic Residue Levels in Chicken and Goat Meat Samples		
Name of the Antibiotic	**Type and number of samples**	**Antibiotic residues in Residue Level (ppb)**
Ciprofloxacin	Chicken (8)	30.81-55.6
Ciprofloxacin	Goat (3)	6.64-10.54
Tetracycline	Chicken (4)	143.56- 300.45
Doxycycline	Goat (4)	12.34-33.56
Gentamicin	Chicken (3)	23.93-38.54
Penicillin	Chicken (5)	34.25-56.22
Chloramphenicol	Chicken (4)	24.27 - 343.89

Discussion:

The study analysis of chicken and goat meat samples collected in and around Kanchipuram. The identified microbial pathogens, including *Staphylococcus spp., Pseudomonas aeruginosa, Salmonella spp., Escherichia coli, and Enterococcus faecalis*, highlight the potential risks associated with meat consumption. Notably, the prevalence of Salmonella spp. in chicken samples raises concerns about foodborne illnesses, emphasizing the critical need for stringent food safety measures.

The results, as presented in Table-2, provide a comprehensive overview of the microbial isolates. Staphylococcus spp. and *E. coli* were highly prevalent in both chicken and goat samples, with the total bacterial isolates of *E. coli* reaching 61. The differential prevalence of Salmonella spp. between chicken and goat samples underscores the importance of targeted interventions to mitigate the risks associated with specific meat sources.

The antibiotic susceptibility testing results, detailed in Tables 3 and 4, reveal significant resistance patterns among both Gram-negative and Gram-positive bacterial pathogens. High resistance percentages were observed for antibiotics like ampicillin, penicillin, and tetracycline, emphasizing the urgent need for prudent antibiotic use in animal farming. On the positive side, certain antibiotics, such as imipenem, linezolid, and vancomycin, demonstrated high sensitivity, providing potential alternatives for treatment.

The variations in antibiotic resistance among different bacterial species highlight the complexity of antimicrobial resistance and the necessity for tailored treatment approaches[16-17]. The observed resistance patterns should guide healthcare professionals in selecting appropriate antibiotics, taking into account the specific pathogens involved.

The MIC testing results, outlined in Table 6, offer detailed insights into the resistance and susceptibility profiles of Gram-positive and Gram-negative pathogens against a range of antibiotics. The prevalence of resistance genes, such as blaCTX-M, blaTEM, blaCMY, blaSHV[13, 14], and qnr, further underscores the challenges posed by multidrug resistance. The data emphasizes the importance of ongoing research and interventions to address the evolving landscape of antibiotic resistance.

A study from Muthu et al 2014[12], colleagues conducted a cross-sectional study in chennai, adopting a One Health approach to scrutinize multidrug-resistant (MDR) bacteria in raw meat, recognizing the interconnectedness of human health, animal health, and the environment. This parallels the holistic approach of the current study, emphasizing the importance of a unified strategy in comprehending antibiotic resistance dynamics associated with animal source foods. The prevalence of multidrug-resistant bacteria emerges as a shared concern, with both Baah et al. and the current study reporting a 14.9% prevalence of MDR. Escherichia coli stands out as a predominant pathogen in both studies, signifying its pervasive presence and potential as a reservoir for antibiotic resistance genes investigation of antimicrobial-resistant bacteria in raw meat-based dog diets (RMDDs) in the USA aligns with the public health risks associated with raw meat consumption, shedding light on the need for pathogen reduction strategies. The shared concerns between the current study and Hathcock et al., 2023[11] extend to the prevalence of resistance to various antimicrobials, including amoxicillin/clavulanic acid, ampicillin, cephalexin, and tetracycline.

The identification of resistance gene combinations, as presented in Table 7, adds a layer of complexity to the understanding of antibiotic resistance. The co-occurrence of resistance genes, particularly in *E. coli*, suggests the potential for multidrug resistance, necessitating a comprehensive and targeted approach to treatment.

The HPLC analysis of antibiotic residues in poultry and goat meat samples reveals the presence of various antibiotics, including Ciprofloxacin, Tetracycline, Doxycycline, Gentamicin, Penicillin, and Chloramphenicol. The antibiotic residue levels in chicken and goat meat samples, as outlined in Table-8, reveal the presence of several antibiotics, each with specific concentration ranges. The study conducted by Doyuk et al., 2023 [6] provides crucial context to these findings, as it focused on the simultaneous determination of six antibiotics from four different classes in chicken meat using an HPLC/DAD method, with verification by LC-MS/MS. In the present study, the antibiotic residue levels are reported in ppb (parts per billion) for each antibiotic in chicken and goat meat samples. Ciprofloxacin, detected in both chicken and goat samples, exhibited levels ranging from 6.64 to 55.6 ppb in chicken and 30.81 to 55.6 ppb in goat meat. Tetracycline, found in chicken samples, displayed levels between 143.56 and 300.45 ppb. Doxycycline, identified in goat meat, showed levels ranging from 12.34 to 33.56 ppb. Gentamicin in chicken samples ranged from 23.93 to 38.54 ppb, Penicillin in chicken samples ranged from 34.25 to 56.22 ppb, and Chloramphenicol in chicken samples displayed levels between 24.27 and 343.89 ppb.

Doyuk and Dost's study aimed to develop an extraction method enabling the simultaneous extraction of six antibiotics from four different classes in chicken breast meat. The validation data confirmed the success of this hypothesis, with satisfactory average recoveries ranging from 75.68 to 101.3%. The limits of detection (LODs) for five antibiotics ranged from 0.6 to 2.7 µg kg-1, while the limits of quantification (LOQs) ranged from 2.0 to 8.9 µg kg-1. For penicillin G, the LOD was 0.16, and the LOQ was 0.52 mg kg-1. These analytical parameters underscore the robustness of the method used by Doyuk and Dost, providing confidence in the accuracy of antibiotic residue measurements in chicken and goat meat samples in the current study.

The incorporation of findings from external studies enhances the discussion by providing a broader context. The detection of antibiotic residues aligns with the challenges identified in a study employing a microbial inhibition Concentration assay (MIC) for antibiotic residue screening in chicken meat (reference). The presence of resistance genes, such as tetA, tetB, aadA1, QnrS, blaSHV-1, ermC, and aacC2, in various sources corroborates with the external study's focus on antibiotic resistance gene detection in diverse environments[17, 18].The external studies contribute valuable insights into the broader implications of antibiotic use, residue detection methods, and the prevalence of resistance genes across different sources, reinforcing the significance of the current study's findings.

Conclusion:

A comprehensive assessment of antibiotic residues and antibiotic-resistant bacterial pathogens in chicken and goat meat from commercial vendors has provided significant insights. The analysis of 120 meat samples collected from 20 different shops revealed a wide prevalence of various bacterial pathogens, with a notable presence of *Staphylococcus spp.* and *E. coli* in both meat types. *Salmonella spp.* was particularly prominent in chicken meat, underscoring the need for rigorous food safety measures in poultry products. The antibiotic susceptibility profiles of Gram-negative pathogens varied, with high resistance observed for ampicillin and penicillin, and exceptional sensitivity to imipenem. Among Gram-positive pathogens, linezolid and oxacillin demonstrated high effectiveness, while ampicillin and penicillin faced substantial resistance. Importantly, the Minimum Inhibitory Concentration (MIC) tests highlighted antibiotic resistance challenges among both Gram-positive and Gram-negative pathogens. These findings emphasize the imperative for prudent antibiotic use in meat production and the urgency for further research to effectively counter antibiotic-resistant bacterial infections. This study significantly contributes to our understanding of the intricate relationship between antibiotic residues, antibiotic resistance, and meat product safety, offering vital insights for informed decision-making, healthcare practices, and potential policy interventions to ensure safer meat production and mitigate the global challenge of antibiotic resistance. The research sets the stage for a safer and more sustainable approach to antibiotic use in

the meat industry, ultimately safeguarding both public health and the global ecosystem.

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