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Molecular Prevalence and Antimicrobial Resistance of *Streptococcus pneumoniae* Isolated from Raw Meat in Punjab, Pakistan

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Abstract

Streptococcus pneumoniae is a bacterium that can cause foodborne illnesses in humans through ingesting contaminated food, including meat and dairy products. Meat, an essential component of the human diet, provides high-quality proteins and essential amino acids. However, poor hygienic conditions at meat shops and slaughterhouses contribute to microbial contamination, increasing the risk of foodborne infections. This study aimed to identify S. pneumoniae *contamination in meat samples collected from various regions of Punjab, Pakistan. A total of 100 raw meat samples were analyzed for microbial contamination using culture-based methods and isolates were tested for antibiotic susceptibility using the disk diffusion method. For molecular identification, bacterial DNA was extracted and subjected to Polymerase chain reaction (PCR) was performed to amplify the* 16S rRNA *gene, yielding a product size of 1.5 kb.*

The results showed that bacterial growth was present in both liquid and solid culture media, with high contamination levels observed in samples from densely populated urban areas. Antibiotic susceptibility testing revealed that S. pneumoniae isolates were highly sensitive to ciprofloxacin, moderately sensitive to cefixime, and resistant to amoxicillin. PCR analysis confirmed the presence of S. pneumoniae in selected samples, with the expected 1.5 kb amplicon observed.

The findings highlight the need for stringent hygiene measures throughout the meat supply chain, including slaughterhouses, processing, storage, packaging, and transportation, to minimize the risk of S. pneumoniae contamination and ensure food safety.

Introduction

Food safety is a critical public health concern, particularly concerning bacterial contamination in meat and dairy products (Seyoum et al., 2024). Streptococcus pneumoniae, commonly known as pneumococcus, is a Gram-positive bacterial pathogen responsible for both non-invasive infections (otitis media, sinusitis) and life-threatening invasive diseases such as pneumonia, meningitis, and septicemia (Hiller and Orihuela, 2024). Moreover, its ability to survive and persist in food products raises concerns regarding its transmission through contaminated meat (Faiz et al., 2024). The polysaccharide capsule of *S. pneumoniae* is a key virulence factor, and its absence is linked to

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increased invasiveness in humans (Ecarnot and Maggi, 2025).

Meat is a fundamental component of the human diet, providing high-quality proteins, essential amino acids, and vital nutrients necessary for growth and development (Qureshi et al., 2024). However, poor hygienic conditions during slaughter, processing, storage, and transportation can introduce or exacerbate bacterial contamination, posing significant health risks (Rebezov et al., 2024). The presence of *S. pneumoniae* in meat products suggests an additional pathway for infection, particularly in immunocompromised individuals, children, and the elderly (Dhawale et al., 2025). Despite advances in vaccination and antibiotic therapy, antimicrobial resistance is emerging as a significant challenge. The indiscriminate use of antibiotics has contributed to the development of drug-resistant *S. pneumoniae* strains worldwide (Tiwari et al., 2025).

Despite increasing concerns, limited studies have investigated the presence of *S. pneumoniae* in raw meat. Previous research has primarily focused on respiratory and invasive infections, overlooking its potential as a foodborne pathogen (Henriques-Normark and Tuomanen, 2013). The lack of comprehensive data on its prevalence, contamination sources, and resistance patterns in meat highlights a significant research gap. Addressing this gap is crucial for understanding the transmission dynamics of *S. pneumoniae* and developing effective mitigation strategies to enhance food safety (Grudlewska-Buda et al., 2023).

Several studies in Pakistan have reported bacterial contamination in meat, emphasizing the presence of various pathogens. A study conducted in Karachi found that 84% of raw meat samples were contaminated with bacteria, including *Klebsiella*, *Enterobacter*, *Staphylococcus aureus*, and *Bacillus subtilis* (Ali et al., 2010). Another study identified *Salmonella typhimurium*, *Escherichia coli*, *Streptococcus pneumoniae*, and *Staphylococcus aureus* in meat samples collected from retail butcher shops, highlighting the prevalence of foodborne pathogens in Pakistan's meat supply chain (Hassan et al., 2021). Furthermore, research assessing poultry meat and meat contact surfaces found significant antibiotic resistance in *S. pneumoniae* and *Staphylococcus aureus*, with high resistance rates to amoxicillin (Tagar and Qambrani, 2023). Contaminated meat products may serve as zoonotic reservoirs, especially in regions like Punjab, Pakistan, where informal slaughterhouses and unregulated markets dominate (Ali and Alsayeqh, 2022).

The limited research on *S. pneumoniae* contamination in meat products necessitates further investigation. Unlike extensively studied foodborne pathogens such as *Salmonella* and *Escherichia coli*, pneumococcal contamination in meat remains largely unexplored (Ali and Alsayeqh, 2022). This study aims to detect *S. pneumoniae* in meat samples using both culture-based and molecular techniques from various regions of Punjab, Pakistan. This study will provide critical insights into *Streptococcus pneumoniae* contamination in meat, highlighting its potential as a foodborne pathogen and emphasizing the need for improved hygiene and regulatory measures to mitigate public health risks.

Materials and Methods

Sample Collection

The sample selection was based on random sampling to ensure diversity in geographic distribution and contamination sources (Ali et al., 2010). A total of 100 raw meat samples, including mutton, beef, and chicken, were collected from butcher shops, slaughterhouses, and farms in different urban and rural regions of Punjab, Pakistan. Each sample was aseptically collected using sterile forceps and stored in sterile plastic containers. The samples were then transported to the Microbiology Laboratory at the Virtual University of Pakistan for further analysis.

Microbial Analysis

All collected meat samples were subjected to microbiological analysis. For bacterial isolation, samples were cultured on Luria-Bertani (LB) agar and nutrient broth. Briefly, 0.5 g of nutrient broth was dissolved in 10 mL of distilled water, autoclaved, and inoculated with 1000 µL of sample solution. The broth cultures were incubated at 37°C for 24 hours under aerobic conditions. After incubation, bacterial cultures were streaked onto LB agar plates and incubated overnight. Colony morphology, hemolysis patterns, and Gram staining were performed for preliminary bacterial identification. Suspected *S. pneumoniae* colonies were further characterized using biochemical tests, including catalase and optochin sensitivity tests (Murray et al., 2022).

Antibiotic Susceptibility Testing

The susceptibility testing was conducted using the disk diffusion method following Clinical and Laboratory Standards Institute (CLSI) guidelines (CLSI, 2021). The zones of inhibition were measured to determine the susceptibility or resistance of the isolates to the tested antibiotics.

DNA Extraction and Molecular Identification

Genomic DNA was extracted from suspected *S. pneumoniae* isolates using the phenol-chloroform method and the purified DNA was dissolved in TE buffer and quantified using a NanoDrop spectrophotometer (Sambrook and Russell, 2006)(Sambrook & Russell, 2001). PCR was performed to confirm the presence of *S. pneumoniae* in the isolates. The 16S rRNA gene was targeted using specific

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primers: **Forward primer (9F):** 5'-GAGTTTGATCCTGGCTCAG-3' and **Reverse primer (1510R):** 5'-GGTTACCTTGTTACGACTT-3'. PCR was performed, and amplified products were analyzed as described by (El Aila et al., 2010).

Statistical analysis

Data obtained from culture-based methods, antibiotic susceptibility tests, and PCR analysis were recorded and analyzed using SPSS statistical software. The prevalence of *S. pneumoniae* contamination was calculated as a percentage, and antibiotic resistance patterns were compared using chi-square tests.

Results

Bacterial Isolation and Identification

Bacterial growth was observed in all cultured meat samples on LB agar and nutrient broth. Colonies displaying morphological characteristics of *S. pneumoniae* were further examined using Gram staining, revealing Gram-positive, cocci-shaped bacteria. Biochemical testing confirmed the identity of *S. pneumoniae* based on positive optochin sensitivity and catalase-negative results.

Antibiotic Susceptibility Testing

Antibiotic susceptibility profiling showed distinct resistance patterns among *S. pneumoniae* isolates. Ciprofloxacin showed the highest efficacy, with inhibition zones ≥22 mm indicating strong sensitivity, and only isolates ≤16 mm was classified as resistant. Cefixime demonstrated an intermediate response, with isolates ≥20 mm considered sensitive and those ≤16 mm resistant, reflecting moderate overall susceptibility. In contrast, amoxicillin exhibited the poorest performance, as most isolates fell within the resistant range (≤17 mm), while only those with inhibition zones ≥19 mm were categorized as sensitive Table 1.

Table 1. Antibiotic susceptibility categories for *Streptococcus pneumoniae* based on zone-of-inhibition

Antibiotics	Sensitive (S)	Moderately sensitive (MS)	Resistant(R)
Amoxicillin	≥19	15-18	≤17
Cefixime	≥20	17-22	≤16
Ciproflaxcin	≥22	17-21	≤16

Molecular Detection of *S. pneumoniae*

PCR analysis successfully amplified the 16S rRNA gene in positive isolates, confirming the presence of *S. pneumoniae* (Fig. 1). Among the 100 samples analyzed, the overall prevalence was 22.6% (n=34) Table 2.

Table 2. Prevalence of *S. pneumoniae* across different species

Specie	Sample Number (N)	*S. pneumoniae* Positive	P-Value
Chicken	36	12 (38%)	0.002 **
Beef	34	15 (16%)	
Mutton	30	03 (14%)	
Total	100	34 (22.6%)	

P <0.05 = Significant

Species-specific analysis revealed significant variation (P = 0.002), with the highest contamination rate observed in chicken (38%), followed by beef (16%), and mutton (14%). The highest prevalence was detected in meat samples collected from urban butcher shops compared to rural slaughterhouses.

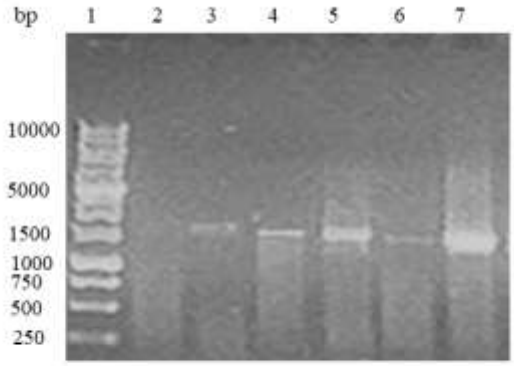

Figure 1. PCR-Amplification of *Streptococcus pneumoniae* 16S rRNA Gene. **Lane 1**: 1 kb DNA ladder. **Lane 2**: Negative control. **Lanes 3–7**: Positive samples

Discussion

S. pneumoniae is primarily known for causing pneumonia, meningitis, and septicemia in humans; however, its presence in food products raises serious public health concerns (Faiz et al., 2024). The ability of this bacterium to persist in meat products highlights the need for enhanced food safety measures to prevent transmission and mitigate associated health risks. The findings of this study confirm the presence

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of *Streptococcus pneumoniae* contamination in raw meat samples from various regions of Punjab, Pakistan. The detection of *S. pneumoniae* in 22.6 % of tested samples highlights the significance of this pathogen as a potential foodborne contaminant, suggesting immediate attention from public health authorities. The results are in agreement with previous studies that have documented bacterial contamination in raw meat and the potential role of such pathogens in foodborne illnesses (Ali et al., 2021). The high prevalence observed in urban butcher shops compared to rural slaughterhouses suggests that factors such as poor hygiene, inadequate refrigeration, and high population density contribute to increased contamination risks (Ali et al., 2010). The variation in contamination rates among different meat types, with chicken showing the highest prevalence (38%), followed by beef (16%) and mutton (14%), may be attributed to differences in meat handling, processing, and storage practices. Poultry meat has been frequently reported to harbor a wide range of bacterial contaminants, likely due to intensive farming practices, slaughtering methods, and cross-contamination in retail settings (Rouger et al., 2017).

Antibiotic susceptibility testing revealed a concerning trend of antimicrobial resistance. While ciprofloxacin exhibited the highest efficacy, resistance to amoxicillin was alarmingly high. This finding is consistent with previous research indicating rising antibiotic resistance among foodborne pathogens, which poses a serious public health challenge (Ali et al., 2021). The overuse and misuse of antibiotics in both human medicine and livestock farming may be contributing to this resistance pattern (Ahmed et al., 2024). The moderate sensitivity of *S. pneumoniae* to cefixime further emphasizes the need for continuous surveillance and the development of targeted intervention strategies to mitigate antibiotic resistance.

The molecular identification of *S. pneumoniae* through PCR amplification of the 16S rRNA gene provides a robust confirmation of its presence in the examined meat samples. The observed 1.5 kb amplicon aligns with previous molecular studies, reinforcing the reliability of PCR as a precise diagnostic tool for bacterial detection in food matrices (El Aila et al., 2010). This molecular approach enhances the sensitivity and specificity of pathogen detection, surpassing traditional culture-based methods in terms of accuracy and efficiency (Sambrook and Russell, 2006).

The presence of *S. pneumoniae* in meat raises concerns about its potential role as a zoonotic pathogen, particularly in immunocompromised individuals, children, and the elderly (Dhawale et al., 2025).

Conclusion

The study highlights the significant prevalence of *Streptococcus pneumoniae* contamination in raw meat, emphasizing its potential as a foodborne pathogen. The high resistance to amoxicillin underscores the urgent need for improved antimicrobial stewardship and strict hygiene protocols in the meat supply chain. Future research should focus on large-scale surveillance, improved detection methods, and alternative antimicrobial strategies to enhance food safety and public health.

Contributions

AM designed the study. KA and AM collected the samples; AM performed the lab experiments; KA analyzed the data and AM prepared the manuscript.

Source of funding

N/A

Conflict of interest

The authors affirm that they have no conflicts of interest.

Ethical statement

All animal handling procedures were approved by the Department of Biological Sciences, Virtual University, Bahawalpur, Punjab, Pakistan.

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