

Metallo- β -Lactamase Producing Gram-Negative Bacteria Isolated from Chicken Meat in Bharatpur, Chitwan [†]

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Abstract: This study addresses the global public health concern of the rapid dissemination of acquired metallo-beta-lactamases (MBLs) in major Gram-negative pathogens. It focused on isolating MBL-producing Gram-negative bacteria in chicken meat from Bharatpur. Conducted from April to June 2023 at Balkumari College's Microbiology laboratory in Bharatpur, Chitwan, the study analyzed 40 samples. The results revealed that *E. coli*, *Citrobacter*, *Salmonella*, *Proteus*, *Shigella*, and *Klebsiella* were prevalent, with varying degrees of multidrug resistance and MBL positivity. Notably, a significant proportion of the isolates exhibited MBL production, highlighting the need for vigilance and containment strategies in the face of this emerging threat.

Keywords: *E. coli*; prevalence; multidrug resistance; MBL

1. Introduction

Poultry farming plays a pivotal role in Nepal's economy, particularly in regions like Chitwan, serving as a significant income source and a crucial protein supply for the population. This industry, concentrated in Chitwan, produces millions of chickens daily, with a substantial portion being broilers, to meet the escalating demand driven by population growth, urbanization, and evolving dietary preferences. However, the poultry meat production process is highly susceptible to microbial contamination, which presents a substantial public health concern, as it can lead to foodborne diseases [1]. The contamination of poultry meat remains a pressing issue, given its vulnerability to foodborne pathogens, posing health risks. This underscores the need for heightened vigilance and control measures to ensure food safety in poultry production [2–4].

Notably, the overuse of antibiotics in the poultry industry has contributed to the emergence of multidrug-resistant (MDR) bacteria, which are responsible for foodborne diseases and resistant to multiple antibiotics. This resistance presents a significant threat to public health, not only in Nepal but worldwide. The escalating levels of antibiotic resistance jeopardize the treatment of common infections, including pneumonia, tuberculosis, and foodborne diseases, intensifying the need for responsible antibiotic use in poultry farming [5].

Understanding the intricate relationship between bacteria, antimicrobial agents, hosts, and consumers is essential when designing effective drug administration plans for the poultry industry [6]. This study focuses on the prevalence of multidrug-resistant and metallo-beta-lactamase (MBL)-producing Gram-negative bacteria in chicken meat, which potentially endangers consumer health. These bacteria can be transmitted through the consumption of contaminated meat, leading to infections that are challenging to treat. Given the pivotal role of the poultry industry in Nepal's economy and diet, addressing these concerns becomes imperative for the safety and well-being of the public.



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2. Methods

The study was carried out at Balkumari College's Microbiology laboratory in Bharatpur, Chitwan, from April to June 2023, utilizing 40 collected samples of chicken meat. Samples were obtained from meat shops, sealed in sterile bags, and quickly transported for analysis. Sample processing involved peptone water enrichment, streaking on MacConkey agar, and further incubation for 24 h at 37 °C.

Isolated Gram-negative bacteria were identified through Gram staining and biochemical tests, including IMViC, TSI, urease, catalase and oxidative and fermentative tests. Antibiotic susceptibility testing employed the Kirby–Bauer method with Ciprofloxacin, Gentamicin, Cotrimoxazole, Doxycycline, Azithromycin, and Amoxicillin [7,8].

A specific MBL test was performed using Imipenem and Imipenem EDTA disks on MHA agar for multidrug-resistant isolated strains [9]. Quality control was maintained throughout, ensuring standard procedures for sample handling, media preparation, and sensitivity testing. Plate quality was verified via incubation.

3. Results and Discussion

3.1. Distribution of Bacteria in Chicken Meat Sample

Among the 40 collected samples, as depicted in Figure 1, 30% of the isolates were *E. coli*, 25% of the isolates were *Citrobacter*, 20% of the isolates were *Salmonella* spp., 10% of the isolates were *Proteus* spp., 10% of the isolates were *Shigella* spp. and 5% of the isolates were *Klebsiella* spp.

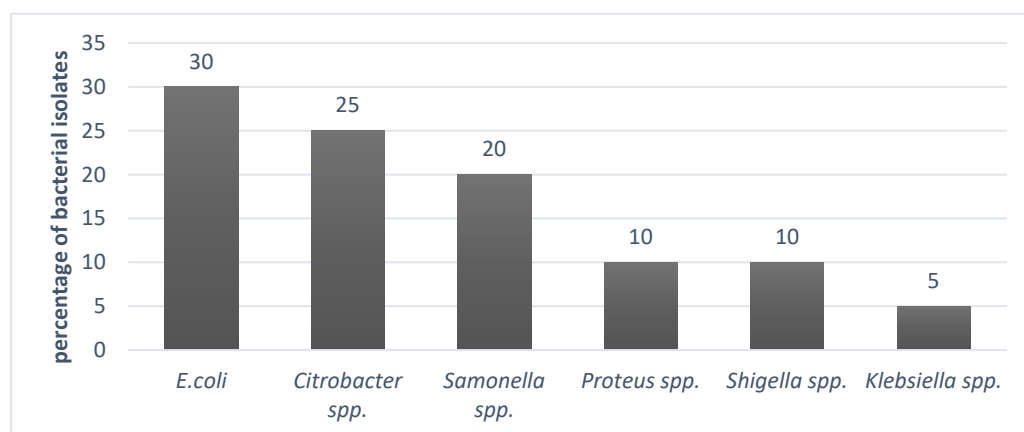


Figure 1. Distribution of bacteria in chicken meat sample.

3.2. Antibiotic Resistance Pattern of Different Bacterial Isolates

In Table 1, it is evident that a significant number of bacterial isolates exhibited notable resistance to Amoxicillin, with a striking 100% resistance among *E. coli*, *Citrobacter* spp., and *Klebsiella* spp. While resistance to other antibiotics varied across these isolates, they all showed some degree of resistance, emphasizing the challenge of antibiotic resistance in these bacterial strains. Notably, *E. coli* demonstrated the highest resistance to Doxycycline (66.67%) and the lowest to Ciprofloxacin (16.67%). *Citrobacter* spp. exhibited substantial resistance to Cotrimoxazole (70%) and Gentamicin (70%). *Salmonella* spp. displayed moderate resistance across the antibiotics, with 62.5% resistance to Doxycycline. *Proteus* spp. and *Shigella* spp. showed resistance to a subset of antibiotics, and *Proteus* spp. demonstrated the highest resistance to Cotrimoxazole (50%) and Doxycycline (50%). *Klebsiella* spp. displayed 100% resistance to Amoxicillin, Doxycycline, and Azithromycin, underlining the concerning trend of multidrug resistance among these bacterial isolates.

Table 1. Antibiotic resistance pattern of different bacterial isolates.

Bacteria	Ciprofloxacin	Gentamicin	Cotrimoxazole	Doxycycline	Azithromycin	Amoxicillin
<i>E. coli</i>	16.67	33.33	58.33	66.67	41.67	100
<i>Citrobacter</i> spp.	60	20	70	70	20	100
<i>Salmonella</i> spp.	25	12.5	37.5	62.5	37.5	50
<i>Proteus</i> spp.	25	25	50	25	50	75
<i>Shigella</i> spp.	25	25	25	50	25	25
<i>Klebsiella</i> spp.	0	0	0	50	50	100

3.3. Multidrug Resistance Pattern of Different Bacterial Isolates

Table 2 illustrates multidrug-resistant patterns in various bacterial isolates and the corresponding percentage of isolates with specific levels of drug resistance. Among the isolates, *E. coli* exhibited resistance to three drugs (Amoxicillin, Doxycycline and Cotrimoxazole) in 41.61% of cases, with increasing resistance percentages for additional drugs. *Citrobacter* spp. showed a range of resistance levels, with 20% resistant to three drugs and up to 20% resistant to five drugs. *Salmonella* spp. displayed resistance to three and four drugs in 50% and 12.5% of cases, respectively. *Proteus* spp. and *Shigella* spp. both showed resistance to three to five drugs. *Klebsiella* spp. demonstrated resistance to three drugs in 50% of isolates.

Table 2. Multidrug resistance pattern of different bacterial isolates.

Bacteria	3 Drugs	4 Drugs	5 Drugs	6 Drugs
<i>E. coli</i>	AMOX, DO, COT (41.61%)	AMOX, DO, COT, AZM (25%)	AMOX, DO, COT, AZM, GEN (16.6%)	-----
<i>Citrobacter</i> spp.	DO, AMOX, COT (20%)	AMOX, COT, DO, CIP (10%)	AMOX, COT, DO, CIP, AZM (20%)	AMOX, COT, DO, CIP, AZM, GEN (20%)
<i>Salmonella</i> spp.	AMOX, DO, COT (50%)	AMOX, DO, COT, AZM (12.5%)	AMOX, DO, COT, AZM, CIP (12.5%)	-----
<i>Proteus</i> spp.	AMOX, COT, AZM (25%)	-----	AMOX, AZM, COT, CIP, DO (25%)	-----
<i>Shigella</i> spp.	AMOX, DO, AZM (25%)	AMOX, DO, AZM, CIP (25%)	AMOX, DO, AZM, CIP, COT (25%)	-----
<i>Klebsiella</i> spp.	AZM, AMOX, DO (50%)	-----	-----	-----

3.4. Distribution Pattern of Metallo- β -Lactamase Producing Gram-Negative Bacteria

The results, as shown in Figure 2, revealed varying proportions of MBL-positive isolates among different bacterial species. *E. coli* showed 66.67% to be MBL positive, while *Citrobacter* spp. had 90% of their isolates testing positive. Among *Salmonella* spp., 62.5% of the isolates were MBL positive, *Proteus* spp. displayed 75% MBL positivity, and *Shigella* spp. and *Klebsiella* spp. had 50% and 100% of their isolates testing positive for MBL production, respectively. These findings highlight the prevalence of MBL-producing strains in these Gram-negative bacteria, which can have implications for antibiotic resistance and treatment strategies.

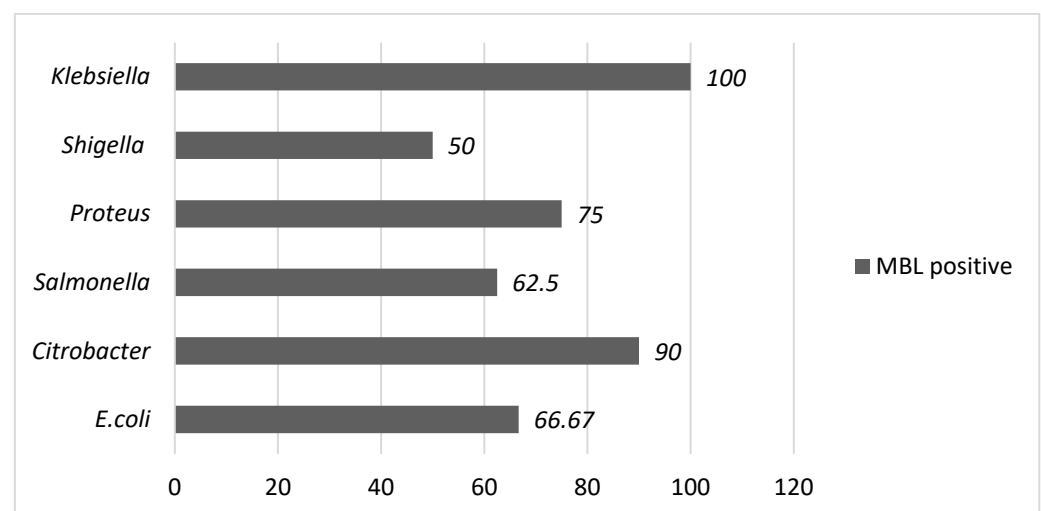


Figure 2. Distribution pattern of Metallo- β -lactamase producing Gram-negative bacteria.

4. Discussion

In this study, the prevalence of various bacteria in chicken meat samples was assessed, revealing that 30% of the samples were contaminated with *E. coli*, with *Citrobacter* spp. (25%), *Salmonella* spp. (20%), *Proteus* spp. (10%), *Shigella* spp. (10%), and *Klebsiella* spp. (5%) also present. Comparisons with similar studies in Bangladesh and Germany showed varying prevalence rates of *E. coli*, likely influenced by factors such as origin, sampling methods, farm hygiene, and detection techniques. The administration of antimicrobial agents in poultry creates selective pressure leading to the detection of antibiotic-resistant bacteria [10,11].

In this study, *E. coli* isolates displayed high levels of resistance to several antibiotics, contributing to a 79.6% prevalence of multidrug-resistant bacteria in chicken meat. Notably, high levels of antibiotic resistance were observed in many of these bacteria. For *Citrobacter* spp., 100% resistance to Amoxicillin, 70% resistance to Doxycycline, Cotrimazole, and Ciprofloxacin, 20% resistance to Azithromycin, and 20% resistance to Gentamicin were observed [12,13]. Similarly, *Salmonella* spp. showed resistance, with 50% resistance to Amoxicillin, 62.5% resistance to Doxycycline, and varying resistance to other antibiotics [14,15]. *Proteus* spp. exhibited 75% resistance to Amoxicillin and resistance to other antibiotics [16,17]. *Shigella* spp. and *Klebsiella* spp. also demonstrated resistance to multiple antibiotics, with 75% of *Shigella* isolates being multidrug-resistant and 100% of *Klebsiella* isolates showing resistance to Amoxicillin [18]. Additionally, a significant proportion of these bacteria were found to be positive for Metallo- β -lactamase (MBL), indicating the presence of enzymes that can hydrolyze a broad range of beta-lactam drugs. This study underscores the urgent need for effective antimicrobial stewardship to combat antibiotic resistance, particularly in the context of public health and food safety.

5. Conclusions

A study of 40 chicken meat samples revealed varying prevalence rates of different bacterial isolates, with *E. coli* being the most common (30%), followed by *Citrobacter* (25%), *Salmonella* spp. (20%), *Proteus* spp. and *Shigella* spp. (10% each), and *Klebsiella* spp. (5%). The study also uncovered concerning levels of antibiotic resistance, particularly multidrug resistance (MDR), among these isolates. Various bacteria, including *E. coli*, *Citrobacter*, *Salmonella* spp., *Proteus* spp., *Shigella* spp. and *Klebsiella* spp., displayed different degrees of MDR. Additionally, Metallo-beta-lactamase (MBL) positivity was detected in some isolates. Antibiotic susceptibility testing indicated that Gentamicin was the most effective antibiotic against Gram-negative bacteria, while Amoxicillin was the least effective. Notably, Amoxicillin, Doxycycline, and Cotrimoxazole exhibited high resistance, while Gentamicin, Ciprofloxacin, and Azithromycin displayed lower resistance levels. These

findings underscore the importance of responsible antibiotic use in poultry farming and highlight the necessity of enhanced food safety measures to reduce the transmission of antibiotic-resistant bacteria to consumers through poultry products.

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