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Molecular detection of integrons in *Escherichia coli* isolates obtained from raw chicken meat

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Abstract

This study aimed to assess the presence of integron-producing *E. coli* in animal-derived foods, specifically raw chicken meat. Out of 63 isolates, 42 *E. coli* isolates tested positive for Class 1 integron integrase (*int11*). Class 1 integron are highly capable of lateral DNA transfer, allowing them to move into a variety of commensal and pathogenic bacteria and accumulate a diverse array of antibiotic resistance genes.

Keywords: Escherichia coli, isolates obtained, raw chicken meat

Introduction

Antibiotics have been extensively and indiscriminately used for therapeutic purposes for a long time. Beyond treatment, antibiotics are also employed to promote growth in food-producing animals. The indiscriminate use of antibiotics in livestock, especially poultry, is considered a major factor in the accumulation of antibiotic resistance genes (ARGs) in both commensal and environmental bacteria. This contributes to the emergence of antimicrobial resistance (AMR) in human bacterial pathogens. Several mechanisms facilitate the transfer of antimicrobial resistance genes among microorganisms. The genetic elements involved in these processes, which contribute to the development of antimicrobial resistance (AMR) in bacteria, include plasmids, transposons, and integrons (Wright, 2010)^[5]. Integrons function as a genetic toolkit for bacteria, enabling the rearrangement of smaller mobile elements known as gene cassettes. Essentially, integrons are genetic structures that can capture, integrate, and express gene cassettes linked to antimicrobial resistance, significantly contributing to the spread of this resistance (Fluit and Schmitz, 2004; Mazel, 2006)^[2, 3]. Integrons consist of an *intI* gene, which encodes an integrase (a site-specific recombinase), a recombination site (attI), and a common promoter (Pc) that facilitates the expression of captured gene cassettes. They play a crucial role in the evolution and transmission of antibiotic resistance genes (ARGs) in enteric bacteria. Gram-negative bacteria commonly harbor all classes of integrons, which are linked to the transfer of antimicrobial resistance genes from commensal to pathogenic microbes. This raises significant public health concerns, such as AMR (antimicrobial resistance) and MDR (multi-drug resistant) bacteria. Since the late 20th century, the development of new antibacterial drugs has been minimal, while resistance has emerged against many narrow-spectrum, broad-spectrum, and extended-spectrum antimicrobial drugs.

Materials and Methods

A total of 63 *E. coli* isolates from 50 chicken meat samples were analyzed for the presence of Class 1 integron integrase (Jhandai *et al.*, 2022)^[9]. Polymerase chain reaction was carried out on extracted DNA from *E. coli* isolates for the detection of Class 1 Integron integrase (*Int11*) gene (F 5"-ACGAGCGCAAGGTTTCGGT-3"; R 5"-GAAAGGTCTGGTCATACATG-3") as described by Gupta *et al.*, 2019 (Fig 1). The reaction mixture for PCR was prepared in 0.2 ml thin walled PCR tubes. PCR components included 12.5 μ l Sapphire fast PCR- hot start master mix (2X), 0.5 μ l of 10 μ M of each primer, ~200 ng of template DNA and nuclease free water to make 25.0 μ l. The PCR amplification was achieved with initial denaturation of DNA then followed by 35 cycles of denaturation, annealing and extension and final extension, finally the

PCR products were stored at 4 °C for further analysis.



Fig 1: Amplification condition optimised for IntII gene.

Results and Discussion

In the present study, out of total 63 *E. coli* isolates of meat origin, 42 (66.67%) were found positive for class 1 integron integrase by PCR (Fig 2). Class 1 integron, have properties that they are well equipped to move by lateral DNA transfer into a wide range of commensal and pathogenic bacteria, and accumulate diverse antibiotic resistance genes (Gillings *et al.*, 2015)^[4].

Similar results have been reported by Soufi *et al.*, 2009 ^[6] in Tunisia (54.5%), Altalhi *et al.*, 2010 ^[10] in Saudi Arabia (40.5%), Soufi *et al.*, 2011 ^[7] in Tunisia (50.6%) and Silva *et al.*, 2012 ^[8] Portugal (47%). In contrast to present study Jouini *et al.*, (2009) ^[12] in Tunisia and Koo and Woo, 2012 in Korea have reported much less carriage of *int11* ranging from 14%-16.4%. In one study *E. coli* isolates obtained from eviscerated chicken carcass 299/373 (78.28%) isolates were positive for class 1 integron (Wu *et al.*, 2015) ^[14] whereas Jiang *et al.*, (2017) ^[11] in China has reported carriage of class 1 integron in chicken product and found only 17.3% of isolates to be positive class 1 integron.



Lane M – 100 bp DNA ladder Lanes 1-4 – amplicons from *E. coli* isolates Lane 5 – negative control

Fig 2: Agarose gel showing intI1 gene (565 bp), amplified from *E. coli* isolates.

Conclusion

This study revealed a high prevalence of *E. coli* containing the integron integrase gene in foods of animal origin, highlighting the role of livestock and poultry as reservoirs for antibiotic resistance genes (ARGs). Among 63 *E. coli* isolates, 42 (66.67%) were found to carry the intI1 gene. This high prevalence poses a significant threat to both human and animal health. The indiscriminate use of antibiotics in livestock, particularly poultry, is implicated as a leading cause of ARG

accumulation in commensal and environmental bacteria, contributing to the emergence of antimicrobial resistance (AMR) in human bacterial pathogens.

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