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Unveiling Hygiene Standards: Uncooked Chicken and Raw Milk in Light of Salmonella and Antibiotic-Resistant Bacteria

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Abstract

Food safety is an important part of public health, and it is crucial to recognize the hygiene requirements connected with widely ingested raw food products. Salmonella and antibiotic-resistant microbes are prevalent and pose serious health concerns to people. This research focuses on two high-risk commodities, raw milk and undercooked chicken. The research evaluates the variety, frequency, and antimicrobial-resistant profiles of bacteria found in raw milk and undercooked chicken using sophisticated experimental techniques and statistical analysis of chi-square test. The study analyses possible contamination paths, taking into account pre- and post-harvest elements that affect the microbial burden present in raw foods. The samples of raw milk and uncooked chicken were gathered at Udgir, Maharashtra. The analysis of the antibiotic susceptibility test showed that chicken meat and milk included various drug-resistant strains of Salmonella. The current research highlights the need to implement these hygienic standards because it shows a significant incidence of Salmonella in raw chicken meat and milk as a result of inadequate hygiene practices. Finally, the goal of this research is to raise public awareness and support the adoption of strict hygiene standards in the production, processing, and consumption of food to protect public health and stop the spread of food-borne (FB) diseases linked to raw milk and undercooked chicken.

Keywords: Food Safety, Chicken, Milk, Antibiotic, Salmonella, Disease

INTRODUCTION

The higher chance of contracting diseases of animal origins while ingesting more meat and poultry. Both processed and raw forms of these animal-driven foods are eaten. Consuming meat is linked to increased risks of several illnesses. Aside from that, high levels of production usually result in increased greenhouse gas emissions, animal waste, massive use of antibiotics, congestion on farms and the accidental introduction of genetic responsible for developing antibiotic resistance into both water and soil, which aids in the propagation of microorganisms resistant to medicines and possess resistant mutations and environmental degradation. Reducing meat consumption improves human health, lessens greenhouse gas emissions and environmental damage, and lessens the widespread suffering of creatures residing on industrial farms (1). Another microbial pathogenic organism most frequently responsible for these bowel movements disorders is salmonella. These pathogens can remain in food processing facilities and food items, such as uncooked foods or little processed meals like raw seafood or fresh-cut fruits and vegetables. These results demonstrate how crucial strong sanitization protocols are to the food sector. Although heat has a strong germicidal effect, thermal processing of food can cause unwanted organoleptic qualities as well as nutritional loss. The way that consumer's food safety has been linked to their dislike of chemical dangers, such as herbicides, food preservation agents, and medication residues. Consequently, chemical-free non-thermal sanitization procedures are required (2). Due to the significant danger of both rising treatment costs and a decline in the efficacious therapy for bacterial illnesses in both human and animal healthcare, antimicrobial resistant bacteria is recognized as the most important issues pertaining to world

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health. Furthermore, studies from all across the world show that bacterial infections brought by bacteria that have developed resistance genes kill 700,000 people annually. Furthermore, the World Health Organization (WHO) issued a warning that infectious illnesses brought by drug-resistant microbes might kill up to 10 million people annually by 2050 (3). Pathogen-related food poisoning is a serious global public health challenge that has numerous nations devoting substantial funds to combating. Both wealthy and underdeveloped nations are concerned about microbial dietary illnesses (4). In Europe, the most common causes of FB disease are Campylobacter and Salmonella. With 246,571 recorded instances of campylobacteriosis, according to the European Centre for Disease Prevention and Control, or ECDC, Salmonella is the pathogen that causes the greatest number of human infections in the EU in 2018, sickening 91,857 individuals. FB outbreaks were reported from EU member states (5). FB infections are caused by bacterial contamination. Additional indicators of risk for disease include readily ingested fresh vegetables, undercooked or raw meat, poultry, and eggs infected with Salmonella. There was a high prevalence of salmonella infection in meat, eggs, and meat products (6, 20). Nature has provided civilization with a wide variety of food. Microbes produce substances as metabolic byproducts as they feed on food to sustain themselves. These chemicals can affect the quality of food in both favorable and harmful ways. Several metabolites enhance the meal's organoleptic properties, though most enable food to degrade or become contaminated, hence lowering its level of quality (7, 19). The integration emphasizes the movement of food around the world, which raises concerns about food safety and spoiling poisoning, initially consequently, effective preservation techniques are vital. A range of methods have been used by industries to improve food preservation and quality (8). The goal is to address hygienic practices that are specially designed to mitigate the dangers related to eating raw milk and undercooked chicken.

The Study (9) exposed to chicken meat was approximately three logs smaller than other FB and waterborne dissemination pathways. The frequency and concentration of contaminated inputs were shown to have the greatest impact on the model's output, indicating that these are crucial areas that require the collection of additional and higher-quality data. When inputs were represented in a non-log-linear manner, the exposure to chicken flesh did not alter. The study (10) focused on evaluating connections among genotype and personal treatments, indicators, and socioeconomic characteristics multilayered logistic regression approaches were applied to verified instances of S. Heidelberg and S. Typhimurium that occurred sporadically in Ontario in 2015. Those infected with S. Typhimurium and S. Heidelberg had different signs of disease and illness risk factors. The study (11) surface-exposed proteins found in the bacterial cell wall that are covalently attached and assembled by the group of transpeptidase inhibitors. Sortases play an important function in gut persistence and immune stimulation in probiotic bacteria in addition to exhibiting latency and pathogenicity to host cells. The study (12) explained many manufacturers worldwide continue to struggle with controlling Salmonella in their chicken processing facilities, particularly as consumer appetite for poultry rises and processing speeds increase. Inorganic antibacterial agents, such as substances including chlorine and organic acids, are among the techniques employed. Nevertheless, the growing incidence of Salmonella resistance, the difficulty of maintaining the meat's flavor, and the stricter application of antibiotics are making these existing techniques less common. The study (13) examined salmonella infection has been a problem for the milk business, but the hazards have significantly grown because all bacteria are resistant to a broad spectrum of market medications. The purpose of the work was to identify a bacteriophage that may be employed versus Salmonella in milk and dairy products as a sterilizing drug. Here, the phage ZCSE6 was identified using molecular and chemical characterization from a raw milk sample. The study (14) explained the research was done to find out which *Salmonella spp*. isolates from milk have virulence genes to identify. Next, using chromosomal PCR, the existence of six virulence factors in the Salmonella serotype was verified. The study (15) focused on guaranteeing food safety, trace Salmonella must be found quickly. To identify at least one proliferating Salmonella cell in 25 g of food, described a unique preparation technique that utilizes a two-step enriched cultivation and immune magnetic (IM) extraction paired with a chemiluminescence microparticle antigen. IM beads of different sizes were tested for their ability to capture Salmonella. The study (16) assessed the incidence of Salmonella in retail raw chickens. The findings demonstrated that hens examined in summer and springtime had a higher prevalence of Salmonella than those collected in the winter months. The study (17) examined how

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public safety is seriously threatened by Salmonella Enteritidis (*S. Enteritidis*), one of the most common FB diseases in the world. The objective of the research was to identify the specific nanobodies that target S. Enteritidis to create a better sandwich ELISA based on nanobody-horseradish peroxidize that can identify *S. Enteritidis* in real-world samples. The study (18) evolved ready to eat food items have grown in popularity recently because of their effectiveness, cost-effectiveness, and ease of use. The purpose of the study was to identify FB pathogens, several techniques were employed, such as growth on particular conditions, bacterial counts using numerical concentrations of homogenization specimens.

METHODOLOGY

Dataset

A total of 370 samples were collected in the Maharashtra city of Udgir. This collection included 188 fresh chicken and 112 raw milk. Samples are taken from chicken sheds, markets, retailers, and vendors. Every sample had a label that accurately identified its kind, spot, date, and duration. These samples were sent to the lab right away, with a cold chain in place to ensure biological separation.

Salmonella isolation and confirmation

Salmonella spp. was isolated and identified using standard techniques. Before initiating bacterial culture, several bacteriological culture media were produced aseptically in a laminar air flow environment by the manufacturer's instructions. Each time, four to five millilitres of the disintegrated meat solution were added to five millilitres of nutritional liquid in each test tube. The nutritional agar was then let to rest at 370C for a full day. After the alleged colony was injected, it endured incubation at 370C for 24±2 hours for isolation. The tube was infused with *Xylose-lysine Deoxycholate (XLD) agar* and *Salmonella-shigella agar*. Figure (1) shows the schematic protocol outlining the specific steps for isolating Salmonella, identifying it, and testing for antibiotic resistance throughout the investigation. Multidrug resistance (MDR) to create a pure colony, culture-positive specimens were cultured many times. Each test tube was inoculated with a likely obtained microbial population before being incubated for 24 hours at 370C with 5 milliliters of triple sugar iron phosphate agar (TSI) agar medium. Gram staining and biochemical assays were then performed. The milk from the specimen tube was poured into 5 ml of nutritional broth, and the mixture underwent incubation at 370C for a whole day. Then, using a loop for inoculation, it was injected from turbid nutritional broth into several selective agars, such as SS agar and XLD agar for Salmonella spp. Separate packets of the infected medium were underwent incubation at 370 C for 24±2 hours. As per the described protocols, colony-positive specimens were cultured many times in specific conditions to achieve pure culture.

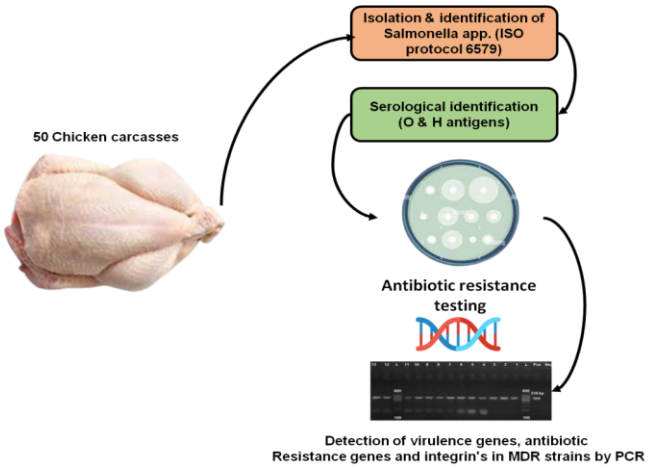

Figure (1). Schematic diagram for uncooked chicken (Source: Author)

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Antibiotic-resistant bacteria in uncooked chicken

An examined resistance to antibiotics were isolates and obtained from raw chicken flesh. It should be noted that market-based resistance to antibiotics was the main topic of most research. Salmonella was found in raw chicken flesh with a median frequency. The most common serotypes were *S. Enteritidis, S. infantis, S.newport, and S.gallinarum.* This is a result of the markets' proximity to human illnesses as the final link in the chicken breeding chain and in chicken flesh.

Pathogenic bacteria isolation and identification in raw milk

The untreated milk specimens were cultured for 24 hours Brilliant Green, and Phenol-Red agar (BGA) at 37 °C. After being moved to *Samonella-Shigella* agar plates, the suspicious cultures underwent incubation for 24 hours at 37 °C. Biochemical assays were performed on the presumed cultures on the supports using Christensen's Lysine Iron agar and Triple Sugar Iron Agar.

Multiplex-PCR Procedure

The multiplex-PCR experiment had the following components: 0.4 uM of each primer, 10 uM of deoxynucleotide triphosphates, 5 ul of template DNA, 2.5 U of Tag polymerase, 5 ul of 10-PCR buffer, 1.25 mM of MgCL (25 mM) and water to reach a final reaction volume of 50 UL. The PCR test was run in a thermal cycler to analyze the results. At 95°C, the DNA was denaturised for 5 minutes. The combination was set through thirty five cycles of primer heating at 50°C for one minute, primer expansion at 72°C for two minutes, and decomposition at 95°C for one minute. The extension lasted for ten minutes at 72°C. The results of the PCR were examined using an agarose gel electrophoresis and shown using ethidium bromide. The Gelpro Analyzer V4 was used to examine the data.

Antibiotic susceptibility testing

The analysis of every single isolate AMR pattern was conducted using the Kirby-Bauer disc diffusion method. Twelve antimicrobial agents, comprising seven distinct classes, were chosen for the panel. These included streptomycin (S) 10µg, ampicillin (AMP) 10µg, ciprofloxacin (CIP) 5µg, levofloxacin (LE) 5µg, nalidixic acid (NA) 30µg, cefotaxime (CTX) 30µg, cefoxitin (CX) 30µg, gatifloxacin (GAT) 5µg, sulfisoxazole (SF) 300µg, cefazolin (CZ) 30µg, erythromycin (E) 15µg and tetracycline (TE) 30µg. On tryptone soya agar (TSA) plates, confirmed Salmonella isolates were cultured overnight. Antibacterial platters were placed on MHA plates using sterile forceps, after that, the outsides were subjected to incubation at 37 °C for 24 hours. The presence of the no-zone or the zone of inhibition was examined on the support surfaces the following day. Infections that show immunity to three or more antibiotic families are classified as MDR isolates. MIC screening was performed utilizing E-strips on strains demonstrating resistance to both cephalosporin and quinolones. We adhered to the boundaries set out by the Clinical and Laboratory Standards Institute (CLSI).

STATISTICAL ANALYSIS

By utilizing Minitab v16.2.3 and the chi-square test, variations in the identification rates of Salmonella-positive specimens, types, and resistant to antibiotics isolates in various months were detected. $P< 0.05$ was designated as a significant distinction and $P< 0.01$ was the severe significant difference. Using unreliability analysis and CANOCO v5.0, the link between the Salmonella genotype and resistance to antibiotics, sample moment, spot, market type, and chicken kind was ascertained. Redundancy analysis was used to ascertain the association between antibiotic resistance and the previously listed parameters. With Graph Pad Prism v7.0, descriptive and comparative analyses of the overall serotype count and antimicrobial resistance characteristics in each specimen were carried out. Table (1) lists the primary antimicrobial resistance profile and multidrug susceptibility seen each month in samples of *Salmonella* that were acquired from retail raw chickens.

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Table (1). Observations of MDR in salmonella (Source: Author)

Period	Month	Proportion resistant to the listed antimicrobial classification			
		Less than three	Three to five	Six to seven	Total
2019	April	73.4	7.7	22.8	28.8
	May	5.4	6.5	90.5	96.8
	June	62.6	24.1	16.5	39.6
	July	4.0	52.4	45.8	97.2
	August	26.0	29.2	47.9	76.0
	September	58.5	11.7	32.9	42.7
	October	-	-	100	100
2020	January	-	99	-	100
	February	-	100	-	97
	March	17.0	83.0	3.0	85.0

S. Thompson, S. Typhimurium, S. infantis, S. enteritidis ,S. essen, and *S. risen* are the six major prevalent serotypes. The presence of *S.typhimurium* was limited to retail hens gathered between May and July of 2019. In May and June, *S.typhimurium* was found at higher frequencies (P<0.01) compared to July. Six months were needed to find S. Thompson, and in October, identification rates were greater. (P<0.05) than it was in the previous five months compared to the other months, March showed detectably higher (P<0.01) frequencies of *S.rissen, S.enteritidis* and *S.essen* . Compared to April or May, these subsequent period had a higher (P<0.01) recognition rate for *S. infantis* specimens, but there were no appreciable changes in these proportions between July and October.

RESULT AND DISCUSSION

Antibiotic susceptibility Testing

The biochemical characteristics, color, and social structure of *Salmonella spp.* were isolated and identified. Salmonella species produced turbid formation on nutritious broth, black centered colony on SS agar, pink spores on EMB agar and XLD agar, and colonies on nutritional agar that are smooth white eventually turn gray white and have a strange, putrid smell. Salmonella species were discovered to be solitary or paired, Gram-negative, and tiny rods after being stained with Gram stain. Only three of the five basic sugars mannitol, lactose, and maltose were fermented, producing gas and acid; the majority of isolates did not ferment lactose or sucrose. The result of antibiotic susceptibility testing is shown in Figure (2) and Table (2).

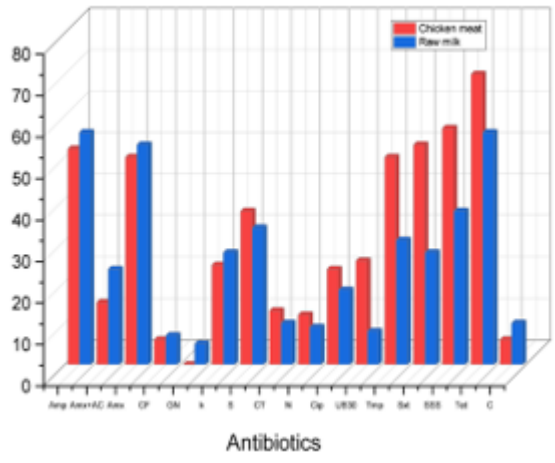

Figure (2). Antibiotic susceptibility (Source: Author)

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Table (2). Antibiotic susceptibility testing (Source: Author)

Antibiotic susceptibility		
	Chicken meat	Raw milk
Amp	52	56
Amx+AC	15	23
Amx	50	53
CF	6	7
GN	0	5
k	24	27
S	37	33
CT	13	10
N	12	9
Cip	23	18
UB30	25	8
Tmp	50	30
Sxt	53	27
SSS	57	37
Tet	70	56
C	6	10

In the above table (3) describes the amount of Ampicillin (Amp), Amoxicillin plus Clavulanic Acid (Amx+AC), Amoxicillin (Amx), Cephalothin (CF), Gentamicin (Gn), Kanamycin (K), Streptomycin (S), Colistin (CT), Neomycin (N), Ciprofloxacin (Cip), Flumequine (UB30), Trimethoprim (TMP), Sulfamethoxazole-Trimethoprim (Sxt: Sulfamethoxazole-Trimethoprim), Sulfonamide (Tet: Tetracycline), and Chloramphenicol (C).

Phylogenetic groups

The study of evolutionary links between organisms is known as phylogenetics. The process includes examining genetic, morphological, and biochemical information to comprehend the evolutionary background and interspecies relationships. According to our findings, phylogenetic groups that have at least one infectiousness gene are also resistant to anti-microbes. Figure (3) shows the graphical representation of phylogenetic group categorization for raw milk and undercooked chicken and table (3) shows the number of categorizations of salmonella in milk and chicken.

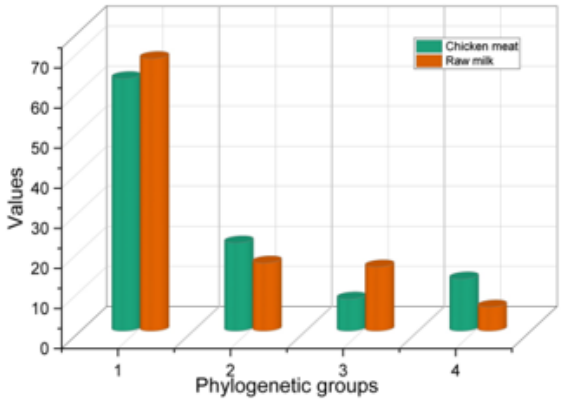

Figure (3). Phylogenetic groups (Source: Author)

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Table (3). Relation between Phylogenetic groups(Source: Author)

Phylogenetic groups	Values	
	Chicken meat	Raw milk
1	63	68
2	22	17
3	8	16
4	13	6

DISCUSSION

In this investigation, raw milk and undercooked chicken meat had greater Salmonella prevalence. Salmonella outbreaks have been linked to milk, poultry, a variety of meat products, and raw chicken flesh. If the meat processing wasn't done properly, the bacteria outbreak may occur sooner in the chopping stage. Handling raw chicken meat and other goods, including uncooked chicken flesh, became one of the most common methods to get the infection (21). However, they neglected to use safety gear like aprons and gloves, and they failed to use detergent to clean the floor after butchering. Acidity, preservatives, temperature, and water activity are some of the factors influencing the presence and proliferation of pathogens in raw milk (22). Antibiotic susceptibility test findings revealed that salmonella isolates had a high level of resistance, with both specimens showing considerable resistance to cephalothin, cefoxitin, clindamycin, and oxacillin. Their widespread usage in animal feed is shown in their substantial resistance ratio. The cephalosporin group exhibited varying degrees of resistance. The diverse range of results can be attributed to distinct generations of cephalosporins, which explains why the antimicrobial proportions in cefaxaine and ceftriaxone are mild in comparison to the significant rates in cephalothin and cefoxitin (23).

CONCLUSION

The results of the research indicate that to identify the contaminants stage and implement control measures at the appropriate stage to enhance the microbe quality of the raw milk and uncooked chicken and protect consumers from uncooked chicken and raw milk poisoning, it is necessary to evaluate a number of significant control points at different phases of the production of the raw milk and uncooked chicken until it reaches the consumers. The dynamics of MDR patterns in the food chain are characterized by significant perception of Salmonella. The existence of multidrug resistance (MDR) in bacterial isolates indicated a significant risk of FB infection by Salmonella in humans. When Salmonella spp. is found in food, it usually means that the food was contaminated by contaminated water, handled, processed, or stored improperly, or had inadequate personal hygiene and sanitation. Azithromycin and other regularly used medicinal products are resistant to Salmonella isolates from food animals and poultry. This suggests interspecies propagation of mutant genes, which might provide significant risks to the public's health. The antimicrobial profile of the samples will help to determine which antimicrobial agents are most effective in addressing diseases linked to *Salmonella spp.* in poultry and cattle.

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