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Original article

Prevalence and drug susceptibility of E.coli, Campylobacter, and Citrobacter from the eggshell surface of table and hatchable eggs in Lahore, Pakistan

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ABSTRACT

The poultry industry faces a significant and urgent challenge due to the increasing prevalence of bacterial infections such as E. coli, Campylobacter and Citrobacter. These bacteria can be transmitted through poultry and its by-products, potentially leading to outbreaks of colibacillosis and campylobacteriosis. A research study conducted in Lahore, Pakistan, aimed to detect and evaluate the presence and drug susceptibility of these bacteria on the surface of table and hatchable eggs. The study collected 630 egg samples from various sources, including small-scale farms, large commercial farms, and local markets. The bacterial contagions on the egg-shell surface were analysed using standard microbiological techniques followed by PCR. The results showed that all three bacteria, E. coli, Campylobacter and Citrobacter were present on the eggshell surface with varying degrees of prevalence. In table eggs E. coli, Campylobacter and Citrobacter were found to have prevalences ranging from 6% to 30%, 0% to 20% and 3% to 26% respectively. Meanwhile, in fertile eggs the prevalences of these bacteria ranged from 6% to 26%, 0% to 16% and 3% to 23%, respectively.
Moreover, the study brought to light a grave concern: the bacterial strains were found to be resistant to commonly used antibiotics. This discovery underscores the urgent need for more potent control measures to curb the spread of antibiotic-resistant bacteria, a burgeoning threat in the poultry industry. The implications of these findings are significant, serving as a crucial foundation for the development of effective strategies to mitigate the risk of bacterial infections caused by contaminated eggs.

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Introduction

Campylobacter, Citrobacter and E. coli gram-negative, motile bacteria associated with Enterobacteriaceae, pose a significant threat to the poultry industry. The consumption of poultry meat and its by-products can serve as potential sources of transmission of colibacillosis and campylobacteriosis in humans, while Citrobacter can cause gastrointestinal infections in poultry. Colibacillosis, a global disease affecting birds of all age groups, inflicts severe economic losses on the poultry production sector. Infected birds suffer from high mortality rates and reduced productivity, particularly during the peak egg production and late lay period [1]. These diseases, which are significant health concerns, cause zoonotic diseases that impact ruminants and non-ruminants, thereby adversely affecting the poultry industry's economy.

E. coli, a pathogen that can cause colibacillosis in poultry, leads to septicemia and eventual death. The disease manifests as lesions in various organs, including pericarditis, perihepatitis, and air sacculitis [1]. Broilers and layers are particularly susceptible to air sacculitis, which is caused by different serotypes of E.coli, including O78, O26, O1, O2, O157, and O111. E. coli in poultry can be transmitted through vertical, horizontal, and pseudo-vertical routes.

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Vertical transmission occurs when developing or freshly laid eggs are contaminated by E. coli from the hen's cloaca or reproductive tract. This route has led to the belief that newly laid eggs and day-old chicks are the primary sources of bacterial colonisation. Horizontal transmission occurs when E. coli is transmitted among chickens, and the environment can contaminate eggs. Pseudo-vertical transmission refers to the contamination of hatcheries by E. coli from the outer surfaces of contaminated eggs. E. coli typically colonises poultry's digestive tract within 24 hours of hatching. Factors that increase the likelihood of an outbreak of colibacillosis include the immune status of the birds, the breed of poultry, the virulence of APEC strains, the timing of exposure and the hygiene practices in the shed. Nutritional deficiencies and diseases that suppress the bird's immunity can also increase the likelihood of E. coli infection.

Campylobacter is a significant bacterial infection that negatively impacts commercial and wild birds, causing avian campylobacteriosis. The disease is caused by the bacteria of the genus Campylobacter with Campylobacter jejuni and Campylobacter coli being the most frequently isolated species [3]. Broiler flocks and carcasses have the highest occurrence of Campylobacter jejuni, approximately 45.9% and 43% respectively [4]. Horizontal transmission during nurture rather than vertical transmission from breeders, is responsible for the contamination of broiler flocks. Several factors predispose broiler chicken flocks to Campylobacter infection, including poor hygiene, insect and rodent infestation, the presence of other livestock and intermission of less than 14 days between flocks. Seasonal variation also affects the incidence of Campylobacter. During slaughtering of animals, the evisceration process can increase carcass contamination and transfer intestinal microflora. The de-feathering process can also lead to significant carcass contamination due to the escape of contaminated faeces from the cloacal opening [5].

Citrobacter species are rare opportunistic nosocomial bacteria that can cause gastrointestinal infections. Among Citrobacter species, Citrobacter

freundii is the primary cause of gastrointestinal infections and human food-borne diseases. Citrobacter can contaminate eggs through horizontal transmission from faeces and can be isolated from the surface of the eggshell. While, Citrobacter is a commensal and low-virulent bacteria present in the intestines of animals, certain predisposing factors can cause it to become pathogenic and cause disease in the host. The low-virulent nature of the bacteria allows it to survive in the host for an extended period, during which it can acquire antimicrobial resistance, further emphasising the need for disease prevention measures.

The increasing resistance to anti-microbial has become a significant public health concern globally. Its primary cause is the fraudulent use of antibiotics in pharmaceuticals and agriculture. The use of antibiotics in the poultry industry is mainly attributed to promoting growth, improving feed efficiency and preventing disease occurrence. This practice provides an opportunity for exposure to resistant bacteria through genetic or non-genetic mechanisms. Poultry becomes a significant reservoir of antimicrobial-resistant E.coli, Campylobacter, and Citrobacter due to the extensive use of medicated feeds in the broilers and layers.

The incidence of resistant strain infections is increasing daily, reducing the effectiveness of available antibiotics. Poultry and poultry products become the primary reservoir for transmitting resistant bacteria from animals to humans, posing potential health hazards. The developed countries imply various ways to reduce the spread of resistant bacteria, including the controlled use of antibiotics followed by the surveillance of antibiotic resistance patterns in the population and the routine assessment of the microbial load on table eggs before their retail marketing. Developing countries like Pakistan lack the strategies to control the spread of antibiotic resistance pathogens where the antibiotics are easily accessible to people in the local drug store without the consideration of a prescription from the doctor [6].

The research is focused on examining the presence of these microorganisms on the exterior and interior of eggs and determining their prevalence in the region. The goal is to evaluate the potential risks associated with consuming eggs that may carry these microorganisms and to inform appropriate interventions to mitigate these risks. Results from this study will provide valuable insights into the safety of egg consumption in the study area and could inform policy decisions and best practices for food safety.

MATERIALS AND METHODS:

Study Design:

The samples were obtained from both table and fertile eggs. Fertile eggs were collected from multiple hatcheries in different areas of Lahore, including Yazdani Road, Aziz Bhatti Town, and Gulshan-e-Ravi. On the other hand, table eggs were collected from retail outlets in Raiwind Road, Multan Road, Shadman, Gulshan-e-Ravi, and Township areas of Lahore, Punjab. The table and fertile eggs were classified into three categories: clean, mild, and dirty. Eggs which were visibly clean without any foreign material, stains, or discolouration were considered clean. Eggs with light stains or spots and dirt were included in the mild category. Finally, eggs with visible contamination of foreign material or dirt adhered to them, covering 1/8th of the eggshell surface, were considered dirty.

Collection of eggs:

Six hundred and thirty eggs were collected from different locations in Lahore for a research study. Each location provided 180 eggs, divided into 90 table eggs and 90 fertile eggs from two hatcheries. The table and fertile eggs were further divided into groups of 30 clean, 30 mildly dirty, and 30 dirty eggs each. The eggs were collected using latex gloves and marked CE, ME and DE to prevent contamination. The table eggs were then packed in an insulated foam box with ice to maintain a temperature of 4-6°C and transported to the microbiology laboratory of Ottoman Pharma for bacteriological analysis. The table eggs were stored at 4°C to ensure their freshness and quality before analysis. Fertile eggs were collected from two commercial poultry farms at Yazdani Road, Aziz Bhatti Town (44km from Ottoman Pharma), and Gulshan e Ravi (24km away from Ottoman Pharma). Latex gloves were used to collect the eggs, which were then placed with the blunt side facing upwards in filler trays labelled as CEF, MEF, or DEF. The eggs were transported to the microbiology section of Ottoman Pharma at 33°C for bacteriological analysis. To prevent bacterial growth and embryonic development, they were stored below 21°C. Proper padding was used during transportation to avoid damage [7].

ISOLATION AND GROWTH OF BACTERIA:

Media Preparation:

Tryptic Soy Agar (TSA), MacConkey Agar (MA), Salmonella-Shigella Agar (SSA), Xylose Lysine Deoxycholate Agar (XLD), Eosin Methylene Blue Agar (EMB) and Blood Agar (BA) media were used to isolate and identify bacteria from harvested eggs. All media were prepared according to the manufacturer's instructions (TECHLAB solutions-UK).

SAMPLE PROCESSING:

Shell Wash:

we followed the methodology of Gentry for sample collection [8]. First, sterile forceps were used to pick up the eggs, which were then placed in a sterile bag containing phosphate buffer saline (PBS). To detach the bacteria from the entire surface of the eggshell, we rubbed the eggs for 20 seconds. The bag was held so that the egg and diluent were in the corner, allowing for effective rubbing. The serial dilution of the suspension was performed using 10-fold serial dilution up to 10-5 using an injection of 1ml of egg rinsate to the 9ml PBS. Each dilution was further processed by dispensing 100ul to the TSA plates to enumerate bacteria in the sample. The plates were incubated at 37°C for 24 hours. For the selective isolation, 100ul of 10-1 dilution was spread on the different media. Calculation of the total viable count of the isolated bacteria was done by counting the colony-forming units per eggshell (CFU/eggshell).

Internal Egg Content:

To prevent cross-contamination from the eggshell to the egg contents, the surface of the eggshell was disinfected by immersing the egg in 70% ethanol for 5 seconds [9]. The eggs shell were cut with the help of sterile sccicor and their contents were harvested with sterile syringes. A drop of the 0.1ml mixture of egg yolk and egg content was placed on a media plate and spread using a sterile cotton swab. The plates were then incubated at 37°C for 24 hours [10,11].

Isolation and characterisation of bacteria:

Various media such as SS agar, EMB agar, MacConkey agar, and XLD agar were used to isolate gram-negative bacteria. The microorganism's colony characteristics were observed in different media. Morphological techniques were used to identify the isolated bacteria, including morphological features and staining techniques [12]. The gram staining technique separated the bacteria into gram-positive and gram-negative categories. The stained organisms were viewed using cedarwood oil under a compound microscope at 100X. The biochemical profile of the isolated organism from the sample was established using Rapid One Pannel (Remel-Thermo Fisher Scientific, USA), according to the manufacturer's instructions.

DNA extraction:

DNA was extracted using the QIAamp DNA Mini kit according to the manufacturer's protocol.

PCR Amplification:

The primers used in this study for E. coli were eco F (GACCTCGGTTAGTTCACAGA) and eco R (CACACGCTGACGCTGACCA) while for Campylobacter were 16s rRNA F (CATAGGATGAGCTACTTAAAAT) and 16s rRNA R(TTCTTCCATTATACGCTT) and for Citrobacter were 27 F (AGAGATTGATCMTCGCTCAG) and 1492 R (TACCGYTACCTTGTTACGTCTT). The total volume of the reaction mixture was 25µl consisting of 5µl of DNA template, 12µl of PCR master mix, 1µl of each forward and reverse primer, and finally, 7µl of the nuclease-free water Amplification was performed by the initial denaturation at 95°C for 5 minutes followed by the final denaturation at 94°C. The annealing of primers was done at 58°C for 45 seconds. The extension was performed at 72°C for 1 minute, followed by the final extension at 72°C for 5 minutes. The 30 cycles of the PCR were run this way, and the obtained PCR results were resolved by 2% agarose gel electrophoresis, followed by the visualisation under UV light [13].

Antibiotic Sensitivity Test:

The Kirby-Bauer method, as standard method is used to determine the sensitivity of bacteria to specific antibiotics [14]. First, we prepared a lawn culture of the organism being tested. Then, a sterile cotton swab was dipped into a well-mixed saline culture whose turbidity was compared to a 0.5 McFarland standard and used to swab the culture. Antibiotic disks were then placed on the culture with sterile forceps and gently pressed down to ensure proper adherence. The plates were then inverted and placed in an incubator at 37°C for 24 hours. After incubation, we measured the zone of inhibition, which indicates the organism's activity against the

antibiotics. The results were compared to the Clinical and Laboratory Standards Institute guidelines and the study by [15,16].

RESULTS:

Several techniques including morphological characteristics, biochemical analysis, and molecular characterization were used to confirm the bacterial isolates from both table and fertile eggs. The results obtained show the presence of 3 confirmed isolates of E. coli, Campylobacter, and Citrobacter.

Total Viable Count (TVC) and Morphological Analysis:

The plates with 30-300 cfu/ml were considered significant and processed for further identification. Morphological analysis of E. coli shows pink colonies on MacConkey, yellow on XLD and characteristic convex green metallic sheen colonies on EMB agar. Citrobacter shows pink rough colonies on MacConkey agar, colourless colonies with black or grey centre on SS agar, and yellow convex colonies on XLD agar. Whereas, few isolates of Campylobacter shows greyish irregular colonies on Blood agar. The gram staining of the isolates depicts that all were pink and had rod shapes as shown in Table 01.

TABLE 1: COLONY CHARACTERISTICS OF ISOLATES

Bacteria	Colour	Shape	Margin	Elevation	Texture	Media used	Gram staining
E.coli	Pink	Circular	Entire	Flat	Smooth	MacConkey	Gram-negative, rods
	Green metallic sheen	Circular	Entire	Convex	Smooth	EMB agar	
	Slightly pink	Circular	Entire	Flat	Smooth	SS agar	
	Yellow	Circular	Entire	Flat	Smooth	XLD agar	
Citrobacter	Pink	Mucoid or rough	Entire	Convex	Glossy	McConkey agar	Gram-negative, rods
	Colourless colonies with black or gray centre	Small and circular	Entire	Flat	Pale	SS agar	
	Yellow	Round	Entire	Convex	Smooth	XLD agar	
Campylobacter	Greyish	Irregular	Entire	Flat	Glistening	Blood agar	Gram-negative, rods

FIG. 01: Growth of isolates on selective and differential media

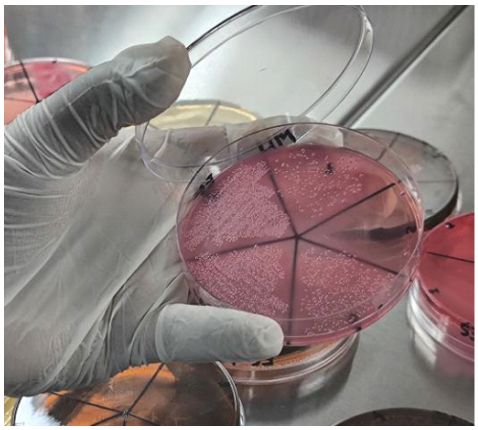


FIG. A: GROWTH OF ISOLATES ON SS AGAR



FIG. B: BACTERIAL GROWTH ON EMB AGAR

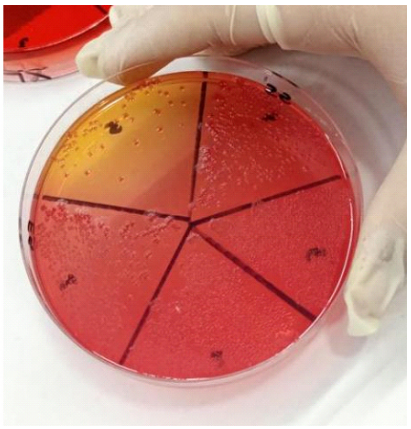


FIG. C: BACTERIAL GROWTH ON XLD AGAR

Biochemical Analysis:

The suspected E. coli isolates were lactose-fermenting colonies that shows positive results for ornithine, lysine, sorbitol, beta glucuronide, β galatosidase and indole. The Campylobacter suspected isolates were ornithine, β glucosaminide, indole and oxidase positive. The suspected Citrobacter isolates were sugar aldehyde, sorbitol, β glucuronide, β glucosaminide positive as shown in .

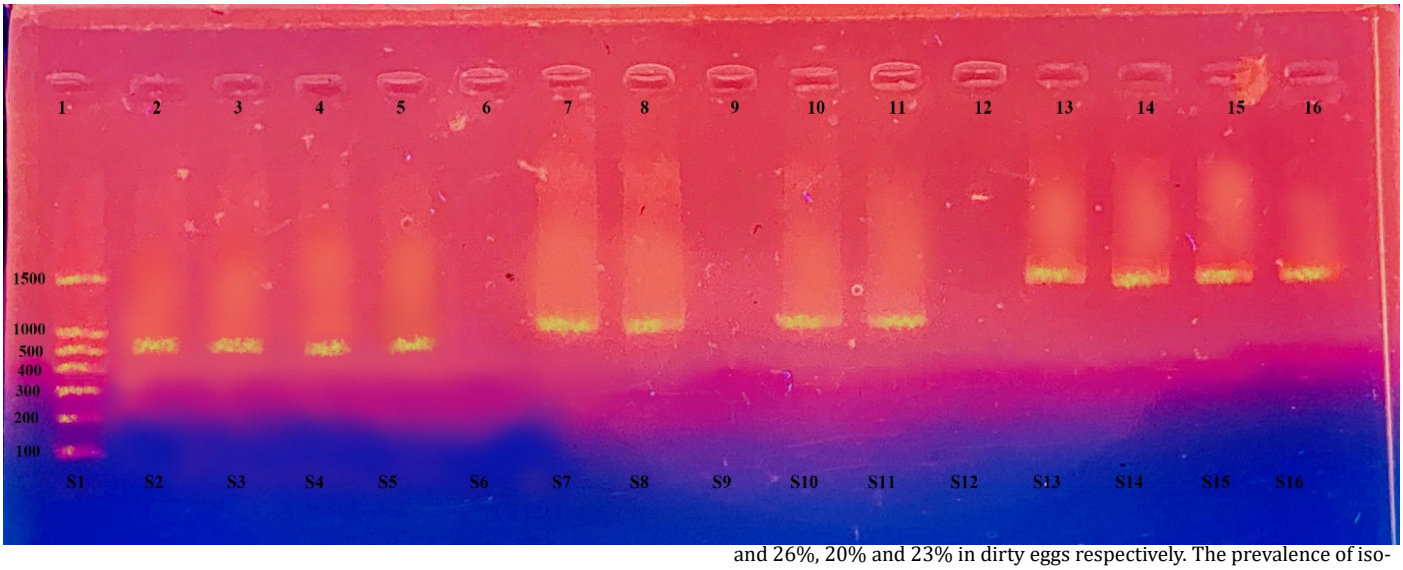
TABLE 02: BIOCHEMICAL IDENTIFICATION OF ISOLATES

Bacteria	URE	ADH	ODC	LDC	TET	LIP	KSF	SBL	GUR	ONPG	βGLU	βXYL	NAG	MAL	PRO	GGT	PYR	ADON	IND	OXI
Escherichia coli	-	-	+	+	-	-	-	+	+	+	-	-	-	-	-	-	-	-	+	-
Campylobacter	-	-	+	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	+	+
Citrobacter	-	-	-	-	+	-	+	+	-	+	-	-	+	-	-	+	+	-	-	-

Molecular Characterization:
Morphologically and biochemically confirmed isolates were subjected to further characterization through PCR and direct sequence analysis. The primers used amplified the partial sequence of an eco gene and 16s rRNA gene of E. coli, Campylobacter, and Citrobacter, resulting in the production of PCR products of 585bp, 1062bp, and 1500bp, respectively. The amplified PCR products of E. coli, Campylobacter and Citrobacter were analysed with a basic local alignment search tool (BLAST) at the National Center for Biotechnology Information (NCBI), as shown in and NCBI bankit accession numbers in . The NCBI bankit generated the accession no. Of PCR confirmed isolates of E.coli codes OP1(OR493517), OP2(OR423060), OP3(OR452423) and OP4(OR712913), Campylobacter OP1(OR40133), OP2(OR395096), OP3(OR395121), OP4(OR395171) and Citrobacter OP1(OR381571), OP2(OR394182), OP3(OR396891) and OP4(OR396930) respectively.

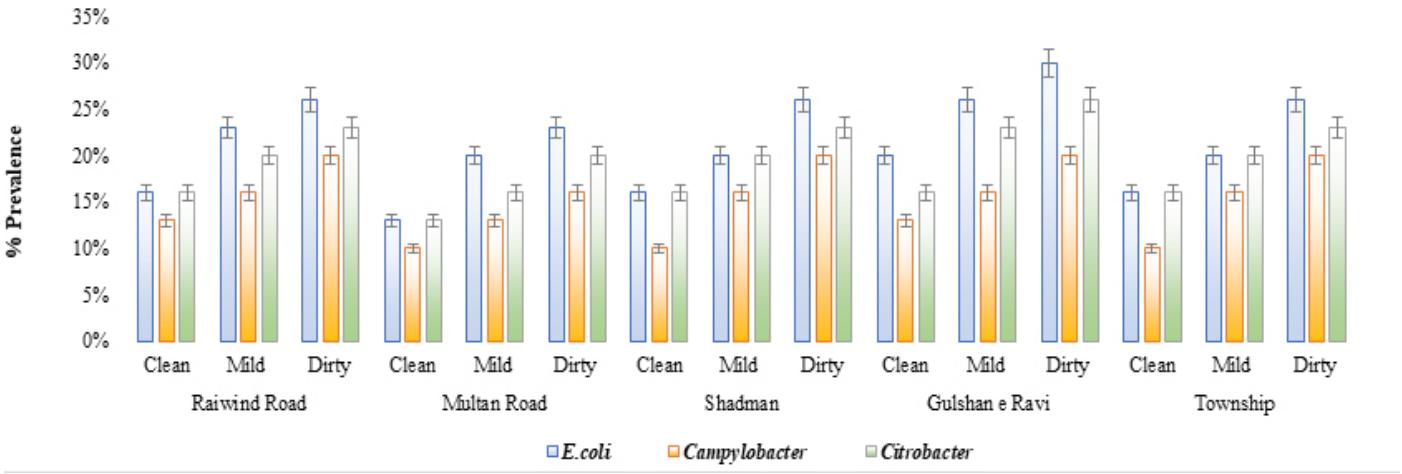
FIG. 2: PCR amplified product of 585 bp, 1062 bp, 1500bp from ECO gene of E. coli, 16s rRNA gene of Campylobacter and Citrobacter. Legends: Lane 1= DNA Ladder 1500bp, Lane 2-4= E.coli , Lane 7, 8, 10, 11= Campylobacter, Lane 13-16= Citrobacter isolates while, Lane 6, 9, 12= Negative control

Prevalence of isolates:



Data was collected from various retail outlets to determine the percentage prevalence of isolates present in the shells of table eggs that were categorised as clean, mild or dirty. The findings indicate that E. coli levels were elevated, followed by Citrobacter and Campylobacter. The percentage prevalence of E. coli, Campylobacter and Citrobacter from Raiwind Road were 16%, 13% and 16% in clean eggs 23%, 16% and 20% in mild, and 26%, 20% and 23% in dirty eggs respectively. The prevalence of isolates from Multan Road was 13%, 10% and 13% in clean eggs, 20%, 13% and 16% in mild and 23%, 16% and 20% in dirty eggs respectively. The prevalence of isolates from Shadman was 16%, 10% and 16% in clean eggs 20%, 16% and 20% in mild and 26%, 20% and 23% in dirty eggs as shown in Fig. 3

FIG. 3: Prevalence of bacterial isolates on the surface of table eggs



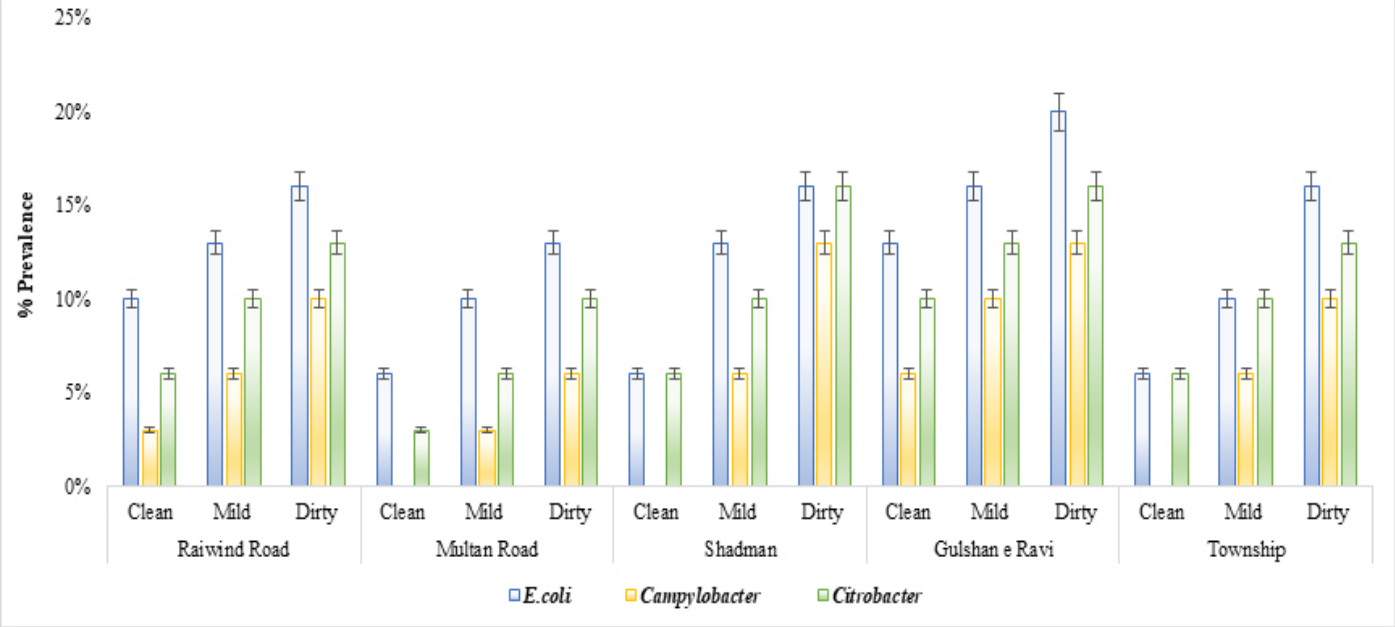
The prevalence of bacterial isolates in table eggs is classified as clean, mild or dirty and is obtained from various retail outlets. The results indicate elevated levels of E. coli, followed by Citrobacter and Campylobacter.

The percentage prevalence of E. coli, Campylobacter and Citrobacter in table eggs obtained from Raiwind Road was 10%, 3% and 6% in clean eggs 13%, 6% and 10% in mild eggs and 16%, 10% and 13% in dirty eggs respectively. In Multan Road, the percentage prevalence was 6%, 0%, 3% in clean eggs, 10%, 3% and 6% in mild eggs and 13%, 6% and 10% in dirty eggs respectively.

In the Shadman, the percentage prevalence was 6%, 0%, 6% in clean eggs, 13%, 6% and 10% in mild eggs and 16%, 13% and 16% in dirty eggs respectively. In Gulshan e Ravi, the percentage prevalence was 13%, 6%, 10% in clean eggs, 16%, 10%, 13% in mild eggs and 20%, 13% and 16% in dirty eggs respectively.

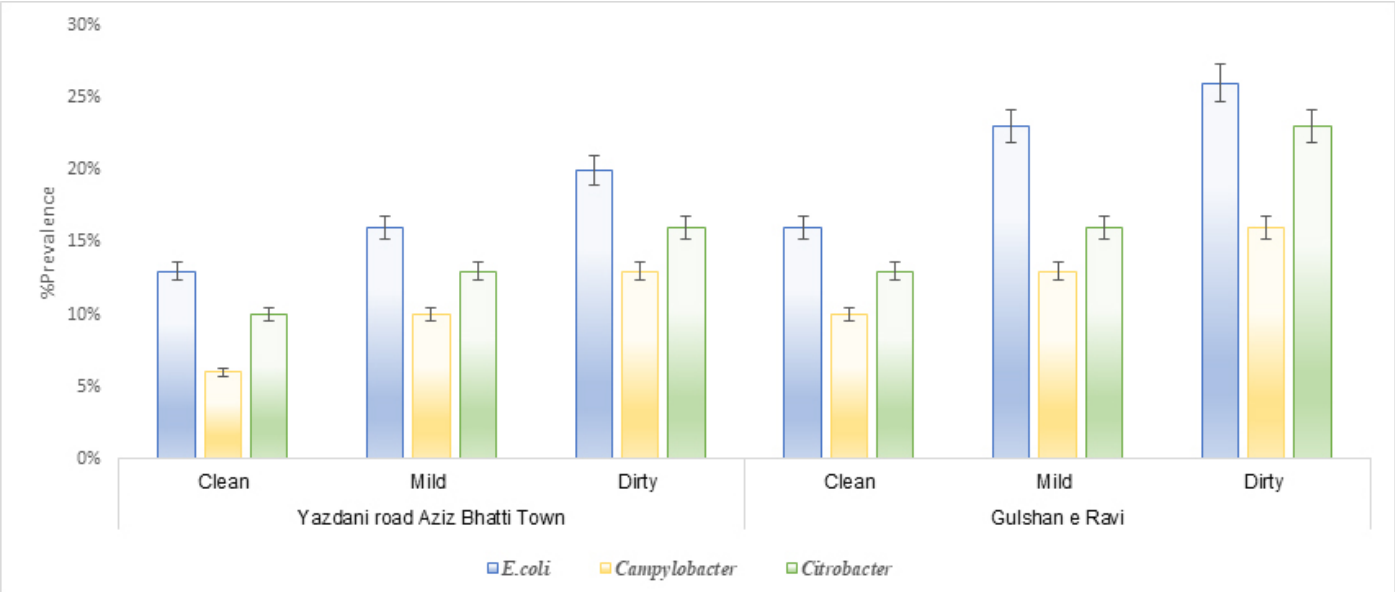
The percentage prevalence in Shamans was 6%, 0%, 6% in clean eggs 10%, 6% and 10% in mild eggs while, 16%, 10% and 13% in dirty eggs, respectively, as shown in Fig 4.

FIG. 4: Prevalence of bacterial isolates in the internal contentof table eggs



It has been found that E. coli, Campylobacter and Citrobacter are present in the eggshells of fertile eggs collected from various hatcheries situated in different locations of Lahore. The highest level of E. coli was found in fertile eggs collected from different sites, followed by Citrobacter and Campylobacter. The prevalence of E.coli, Campylobacter and Citrobacter in eggs collected from Yazdani Road Aziz Bhatti town was 13%, 6% and 10% in clean eggs, 16%, 10% and 13% in mildly dirty eggs and 20%, 13% and 16% in dirty eggs. In contrast, the prevalence of E.coli, Campylobacter and Citrobacter in eggs obtained from Gulshan e Ravi was 16%, 10% and 13% in clean eggs, 23%, 13% and 16% in mildly dirty eggs and 26%, 16% and 23% in dirty eggs, respectively as shown in Fig 5.

FIG. 5: Prevalence of bacterial isolates on the surface of hatchable eggs



Fertile eggs obtained from various hatcheries in different locations of Lahore including Yazdani Road Aziz Bhatti Town and Gulshan e Ravi were tested for the presence of E. coli, Campylobacter and Citrobacter. The eggs were categorised as clean, mild or dirty. The results showed that E. coli was found in higher levels in fertile eggs from the various hatcheries followed by Citrobacter and Campylobacter. In eggs from Yazdani Road Aziz Bhatti, the prevalence of E.coli, Campylobacter and Citrobacter was 6%, 0% and 3% in clean eggs 13%, 3% and 10% in mild eggs and 16%, 6% and 13% in dirty eggs. Eggs from Gulshan e Ravi had a prevalence of 10%, 3% and 16% in clean eggs 16%, 6% and 10% in mild eggs and 20%, 10% and 16% in dirty eggs. These findings are illustrated in Fig 6.

Antibiotic Susceptibility Test:

The antibiotic susceptibility pattern of PCR-confirmed E.coli, Campylobacter isolates and Citrobacter has been determined. E.coli is resistant to Amoxicillin, Doxycycline, Trimethoprim/ sulfamethoxazole, Ampicillin, Streptomycin, Nitrofurantoin, and Tetracycline. At the same time, Campylobacter shows resistance to Amoxicillin, Doxycycline, Gentamicin, Trimethoprim/ sulfamethoxazole, Nitrofurantoin, and Tetracycline. In contrast, Citrobacter is only resistant to Amoxicillin Norfloxacin, Streptomycin, and Tetracycline, as shown in . TABLE 04.

FIG. 6: Prevalence of bacterial isolates in the internal content of hatchable eggs

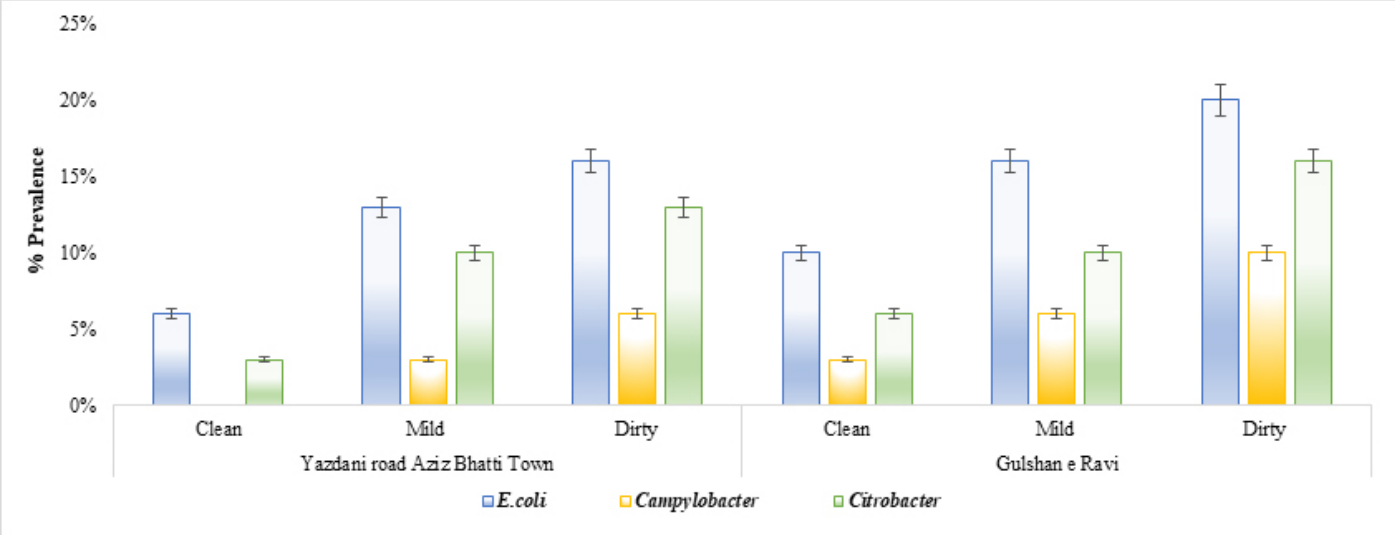
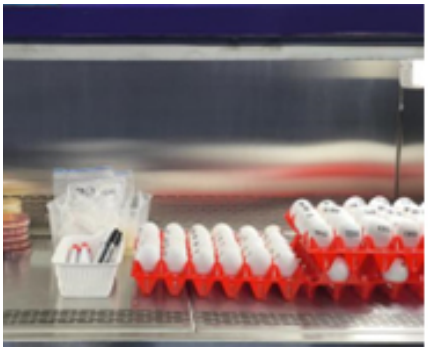


TABLE 04: ANTIBIOTIC SUSCEPTIBILITY OF ISOLATES

Antibiotic discs used	Zone of Inhibition (CLSI)			Zone of inhibition (mm)		
	Sensitive (mm)	Intermediate (mm)	Resistance (mm)	E.coli	Campylobacter	Citrobacter
Amoxicillin (10µg)	≤17	14-16	≤13	12	10	8
Fosfomycin (200 µg)	≥16	13-15	≤12	20*	18*	16*
Gentamycin (10µg)	≥15	13-14	≤12	17*	12	18*
Norfloxacin (10µg)	≥17	13-16	≤12	18*	17*	11
Doxycycline (30µg)	≥14	11-13	≤10	9	13	17*
Trimethoprim/sulfamethoxazole (1.25/ 23.75 µg)	≥16	11-15	≤12	11	10	17*
Ampicillin (10µg)	≥17	14-16	≤13	12	18*	20*
Streptomycin (10µg)	≥15	12-14	≤11	8	17*	10
Nitrofurantoin (300µg)	≥17	15-16	≤14	12	10	19*
Tetracycline (30 µg)	≥15	12-14	≤11	7	9	10
Ciprofloxacin (5µg)	≥26	22-25	≤21	30*	28*	26*

Note: * indicates the susceptibility of isolated bacteria against the antibiotic.

FIG. 7: Preparation and labelling of samples





DISCUSSION

Eggs are a common food item in the human diet due to their nutritional value. They are rich in vitamins such as A, D, E, K, and B-complex, as well as minerals like phosphorus and iron. However, studies have shown that eggs can be contaminated with harmful bacteria, which can cause foodborne illnesses. This contamination can impact the quality of eggs and negatively affect the poultry industry, particularly in international trade. Foodborne illnesses associated with eggs and egg products cause a significant concern for public health. In the US alone, they cause 48 million cases and 3,000 deaths each year. The most common cause of foodborne illness worldwide is colibacillosis, which affects the reproductive system of hens and breeders, leading to salpingitis, oophoritis, and peritonitis. This results in increased mortality rates and reduced egg production in flocks, having a significant economic impact on layer production. Contaminated eggshells are believed to be the primary cause of harmful bacteria on eggs. This can occur due to faeces, soil, dust, and poor hygiene. Pathogens can infect a hen's reproductive organs and contaminate the eggshell membrane, yolk, and albumin before the egg is laid. Bacteria can also grow on the eggshell and pores allow bacteria to enter the internal egg content.

Ensuring proper hygiene and safety measures is crucial during egg production, handling, and consumption to prevent the spread of harmful bacteria and foodborne illnesses. Colibacillosis is a severe zoonotic disease mainly caused by E. coli, although Campylobacter and Citrobacter can also be isolated. Both phenotypic and genotypic methods are used to confirm the presence of these bacteria. Phenotypic methods involve identifying the bacteria based on their physical characteristics and biochemical profile, while genotypic methods confirm the isolates using PCR. The antibiogram of the isolates is also discussed in this study. E. coli is known for lactose fermenting and producing pink-coloured colonies on MacConkey agar. On EMB agar, it appears as a green metallic sheen [16] . Campylobacter species found in poultry significantly cause food-borne transmission to humans. The bacteria can spread from contaminated faeces when the egg passes through the cloacal opening and colonises the eggshell and internal contents. Although Campylobacter outbreaks associated with eggs are rare because the bacteria can hardly be found in the internal contents, it can cause embryonic mortality in experimentally infected eggs. The horizontal transmission of Campylobacter is mainly due to its spread rather than vertical transmission [17]. The study revealed that the prevalence of Campylobacter in the shell of table eggs varied depending on whether they were classified as clean, mild, or dirty. The percentages ranged from 0% to 20%, with the highest prevalence in dirty eggs. The harvest of table eggs also showed varying prevalence rates, ranging from 0% to 13%. Fertile eggs had a lower prevalence of Campylobacter in their shells, with rates ranging from 6% to 16%. The study's results differ from those of other studies, such as one from Iran that found a prevalence of 31.6% and another that found a prevalence of 25.6%. However, the absence of Campylobacter in eggs from some farms in a previous study may have been due to the eggs exposure to dry conditions and ambient temperature before washing. E. coli is a bacteria that causes colibacillosis in poultry leading to symptoms such as colispeticemia, airsacculitis, pericarditis, perihepatitis and swollen head syndrome. This disease has a high mortality and morbidity rate, negatively impacting global poultry production and raising economic concerns [18]. A recent study investigated the prevalence of E. coli in table eggs, categorised as clean, mild and dirty. The results showed that the prevalence of E. coli was 16%, 13%, 20%, 16%, and 16% in clean eggs, 23%, 20%, 20%, 26%, and 20% in mild eggs, and 26%, 23%, 26%, 30%, and 26% in dirty eggs. The eggs were collected from five locations, including Gulshan e Ravi, Shadman, Multan Road, Township, and Raiwind Road. For fertile eggs, the incidence of E. coli was 13% and 16% in clean eggs, 16% and 23% in mild eggs, and 20% and 26% in dirty eggs. The harvest of fertile eggs showed a prevalence of 6% and 10% in clean eggs, 13% and 16% in mild eggs, and 16% and 20% in dirty eggs, collected from Yazdani Road Aziz Bhatti Town and Gulshan e Ravi, respectively. These results are from a previous study, one which reported a prevalence of 38.2% for E. coli in eggshells and 16.2% in egg contents. However, the results differ from those of a study conducted in Australia, which reported a much higher prevalence of E. coli in eggs at 60.3% [19].

Citrobacter is a bacteria commonly found in poultry and can cause food-borne illnesses. It mainly affects the gastrointestinal tract, leading to various health problems like diarrhoea, peritonitis, meningitis, brain abscess, bacteremia and urinary tract infections. Recent studies have shown that Citrobacter is widespread in the table, fertile egg shells, and internal content. These eggs are classified as clean, mild, or dirty, and the prevalence of Citrobacter varies between them. In different areas, the prevalence of Citrobacter in clean, mild, and dirty table eggs ranged from 3 to 16%, 6 to 23%, and 10 to 26%, respectively. In fertile eggs, the prevalence ranged from 3 to 13%, 10 to 16%, and 13 to 23% in clean, mild and dirty eggs respectively. Furthermore, the study revealed that the prevalence of Citrobacter was 20% in eggs.

Antibiotics are substances that can kill bacteria and prevent their growth. They are crucial for treating diseases in both humans and animals. However, their overuse has led to an increase in bacterial resistance to antibiotics. Due to concerns over antimicrobial resistance, the United States has banned antibiotics essential for humans. A recent study found that bacteria resisted Trimethoprim during in-vitro antibiotic susceptibility testing [20]. However, they were sensitive to fosfomycin, doxycycline, gentamicin and neomycin.

The issue of antimicrobial resistance is becoming increasingly concerning, particularly in developing countries. Several factors contribute to rise in antimicrobial resistance, including the misuse of antibiotics. According to recent research, E. coli is vulnerable to norfloxacin, gentamicin, ciprofloxacin and fosfomycin but resistant to trimethoprim/sulfamethoxazole, doxycycline, amoxicillin, streptomycin, nitrofurantoin, tetracycline and ampicillin. The resistance of E. coli to trimethoprim/sulfamethoxazole is due to the widespread use of antibiotics in clinical and veterinary practices in Pakistan without considering the selective nature of these drugs. The indiscriminate use of antibiotics in the livestock and poultry industries has developed resistance among bacteria in these animals and their by-products. The resistance to doxycycline is due to the unregulated use of antimicrobials as growth promoters, which are readily available to farmers. Additionally, the high cost of gentamicin has led to its reduced use by farmers in poultry production. The susceptibility of E. coli to norfloxacin may be attributed to the pattern of antibiotic use. Citrobacter was found to be resistant to amoxicillin, norfloxacin, streptomycin, and tetracycline, while it was susceptible to other antibiotics like gentamicin, fosfomycin, nitrofurantoin, and ampicillin, doxycycline, trimethoprim/sulfamethoxazole and ciprofloxacin. However, several studies have shown that eggs can be a source of antibiotic-resistant bacteria.

CONCLUSION

The study focuses on the prevalence of E.coli, Campylobacter, and Citrobacter on the surface of table and hatchable eggs in three specific areas of Lahore, Pakistan. The study highlights the challenges faced by the poultry industry due to bacterial infections and the fraudulent use of antibiotics. The study emphasises the importance of implementing proper hygiene practices, nutritional management, and disease prevention measures to prevent the spread of bacterial infections and the emergence of resistant strains. The study also recommends limiting the use of antibiotics in the poultry industry and promoting alternative methods to prevent and control bacterial infections, ensuring the safety of both animals and humans while maintaining the productivity and profitability of the poultry industry.

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