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Microbiological analysis: Prevalence of salmonella, staphylococcus, and proteus on the surface of table and hatchable eggs, along with their antibiotic sensitivity profile

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Abstract
Eggs, a staple food consumed globally, are at risk of contamination, posing a severe threat to their safety and quality. The bacterial load on the eggshell surface is crucial in predicting bacterial penetration and egg interior contamination. Exposure to nesting material and faecal matter can introduce egg-borne pathogens, some of which can lead to food-borne illnesses. The global scale of epidemics caused by egg-borne pathogens underscores the criticality of egg safety.
A comprehensive study was conducted in Punjab, Pakistan, to assess the potential risk of contamination. A total of 360 eggs from various breeds of hens were tested and categorized as unclean, soiled and clean. The bacteria *Salmonella*, *Proteus* and *Staphylococcus* were isolated from the eggs. The highest percentage of isolates were found in unclean eggs: *Salmonella* (26.7%), *Proteus* (24.5%) and *Staphylococcus* (33%). In soiled eggs, the highest percentage of isolates were *Salmonella* (22.6%), *Proteus* (17.6%) and *Staphylococcus* (10.9%). In cleaned eggs, *Proteus* showed the highest prevalence (15.5%), followed by *Salmonella* (10.3%) and *Staphylococcus* (9.4%).
The antibiotic susceptibility test (AST) results showed that all bacterial isolates were sensitive to the drugs Ofloxacin (5 µg/ml) and Cefotaxime (30 µg/ml). However, *Staphylococcus* and *Proteus* also showed sensitivity to Trimethoprim + Sulphamethoxazole (2.25/23.75 µg/ml).
The study aimed not only to raise awareness about the importance of egg safety and identify the most common pathogens found on eggshells but also to develop effective strategies to reduce the risk of contamination of eggs and egg products. Once implemented, these strategies will ensure the safety and quality of this essential food source, offering a promising solution to the current challenges.

Keywords: PCR, salmonella, staphylococcus, proteus, antibiotic sensitivity test, microbiological analysis, table eggs, hatchable eggs

1. Introduction
Eggs, a vital source of nutrition worldwide, are at risk of contamination that can compromise their quality and safety. This contamination, which can lead to egg spoilage and the penetration of pathogenic bacteria, is often a result of environmental exposure. Freshly laid eggs are typically free of organisms. However, their exposure to environments like nesting material and faecal matter can introduce numerous egg-borne pathogens, some of which can be highly zoonotic. The severity of this issue is not limited to specific regions, as it is evident in several global epidemics associated with egg consumption caused by egg-borne pathogens isolated from eggshells and their contents. Notably, food-borne pathogens such as *Salmonella*, *Proteus*, and some gram-positive bacteria, like *Staphylococcus* aureus, have been found in eggshells and egg contents [1].
Salmonella is a type of bacteria that belongs to the Enterobacteriaceae family and is shaped like a rod. It is responsible for causing salmonellosis, which can be transmitted to humans through contaminated food, especially poultry eggs [2]. *Salmonella* can also cause severe illness in the host and have negative economic impacts on the poultry industry. In poultry, *Salmonella* is commonly associated with symptoms such as depression, loss of appetite and diarrhoea, which often lead to high mortality rates in young birds.

However, the continuous shedding of bacteria in adult birds is a major concern as it can lead to zoonotic infections [3]. *Salmonella* can be transmitted horizontally between farm animals and vertically through the trans-ovarian route. The long-term persistence of typhoidal and non-typhoidal *Salmonella* serovars in the environment and antibiotic resistance is increasing gradually due to the disproportionate use of promoters and prophylactic measures. The first multi-drug resistant *Salmonella* serovar was Typhimurium strain, and the second was *Salmonella* serovar Enteritidis, which became apparent as a significant poultry and egg pathogen [4]. *Proteus* is a gram-negative bacillus that causes significant diseases in humans and poultry worldwide. The primary source of *Proteus* infection in humans and eggs is poultry. This bacterium can be transmitted horizontally through colonisation in the gut or contaminated faecal matter or vertically through the infected reproductive organs, even before the laying of eggs. The antimicrobial resistance of *Proteus* from antibiograms is a severe issue that has gradually increased, especially the resistance to carbapenem drugs, a recently identified challenge [5]. *Staphylococcus* spp. is a group of gram-positive cocci bacteria that can contaminate eggshell surfaces [6]. These bacteria can survive in dry and harsh environments, particularly dust, which increases the likelihood of eggshell contamination [7]. The disease caused by Staphylococci is a major foodborne illness associated with ingesting *Staphylococcus* enterotoxins and is ranked third in the major public health disease category [8]. The contamination is mainly associated with transmission through humans during handling, as Staphylococci persist as normal skin flora in humans and sometimes cause opportunistic infections. *Staphy*lococci may also penetrate the egg content through eggshell cracking.

The potential transmission of bacterial infections from poultry requires evaluating their prevalence, as it is a significant concern to public health. Therefore, a study was conducted in Punjab, Pakistan, to isolate, confirm, and assess the prevalence of *Salmonella, Proteus*, and *Staphylococcus* in the table and fertile eggs of selected breeds of hens. The study aimed to detect and assess the potential risk of contamination of eggs and egg products and to raise awareness about the importance of egg safety. We hope that the study results will help develop strategies to reduce the risk of contamination of eggs and egg products, indirectly ensuring the safety and quality of this essential food source.

2. Materials and Methods

2.1. Collection of Eggs

A study was conducted for bacterial enumeration of table and fertile eggs. Three hundred sixty tables and fertile eggs were collected from four different breeds of hens in Kasur, Kamalia, Sheikhupura and Gujranwala of Punjab, Pakistan. White Leghorn and Foyomi crossed Misri hens were selected for table eggs, whereas broiler and layer breeders were included for fertile eggs. Only one parent breeder farm from each city was chosen to collect eggs, with a sample size of 90 eggs from each farm. Sterilized polythene bags were used to collect eggs to prevent extraneous contamination. The eggs collected from each site were categorized as clean, unclean, and soiled based on the presence of stains or other contaminants, as listed in Table 1. Clean eggs had no visible stains, unclean eggs had visible stains of soil and faecal matter and soiled eggs had faint lines of nest wires. Table eggs were collected from the grocery store of the respective city and the freshly laid fertile eggs were collected from the hatchery sites. Clean, unclean and soiled table eggs were transported to the microbiology laboratory immediately using an ice box. The fertile eggs were packed and padded in a closed space to prevent shaking and cracking. The fertile eggs were transported in mid-afternoon at 34 ℃. All the samples were processed within four hours after collection to ensure the accuracy of the results [9].

Three hundred fifty-three eggs were sent to the microbiology laboratory for testing after removing the broken ones. These tests aimed to examine the prevalence of targeted bacteria in either the shell or the egg content. The collected samples were categorized and labelled according to their cleanliness status (clean, uncleaned or soiled) and breed numbering. For instance, samples taken from the same area with different categories were labelled as C.1.1, UC.1.1, and S.1.1 for sample 1. Samples taken from another were labelled C.2.1, UC.2.1 and S.2.1 for sample 1. All other samples were labelled chronologically, as displayed.

Table 1: Sampling of eggs from different locations

Area	Breed	Egg Type	Categorized		
			Clean	Unclean	Soiled
Gujranwala	Misri Hens (Fayomi breeds)	Table eggs	27*	30	30
Kasur	Broiler breeder	Fertile eggs	30	28*	29*
Kamalia	Layer breeder	Fertile eggs	30	30	30
Sheikhupura	White leg horn	Table eggs	29*	30	30

2.2. Media Preparation

In the study, four different types of bacterial growth media were used, including tryptic soya agar (TSA) from Oxoid, Germany; Mannitol Salt agar (MSA) from Oxoid, Germany; *Salmonella* Shigella Agar (SS) from Oxoid, Germany; MacConkey agar (MA) from Oxoid, Germany; and Xylose Lysine Deoxycholate agar (XLD) from Oxoid; Germany. The media were prepared according to the manufacturer's instructions and autoclaved for 15-20 minutes at 121 °C. Each petri plate was inoculated with a loop full of culture from every sample and incubated for 24 hours at 37 °C as shown in Fig. 8 [10].

2.3. Egg Shell Wash

Total bacterial contamination on eggshells was measured by the method of R.F. Gentry [11]. The categorized eggs were added into separate polythene bags with 10 ml of sterile phosphate-buffered saline (P.B.S.) (pH 7.2). The rinsate was collected effectively, by holding the bags in a slanted position and by rubbing the eggs for 10 seconds to get the suspension of maximum surface material into the PBS. The egg was then removed aseptically through forceps, as shown in Fig.7. 9 ml of PBS was added into the falcon tube with the markings 10^{-1} and 1 ml of PBS-washing was added in the first tube. Furthermore, 0.2ml of the PBS-washing was added in a tube labelled 10^{-2} with 1.8ml of PBS and the process was carried out until the 10^{-6} serial dilution. 1ml of dilutions were then transferred to Petri dishes. After slightly rotating it, we added approximately 20ml of tryptose soy agar and allowed it to solidify. Incubating it at 37°C for 48 hrs. The total bacterial concentration was determined by colony counts (30-300). The same procedure was applied to the remaining 352 eggshell samples.

2.4. Egg Contents

The egg's outer surface was disinfected to collect egg content by swabbing it with 70% ethanol. An opening was created in

the egg using a hard object and the content was mixed thoroughly. A sterile needle was used to extract 0.1ml of the egg yolk and egg white mixture from the table egg along with some fluid from the embryonated egg. This mixture was then spread onto different agar mediums using a swab. Finally, the agar mediums were incubated for 24 hours at 37 °C according to the method described by Salihu [12].

2.5. Identification and isolation of microorganisms

Microorganisms were identified using the method of Harvey and Green-Wood [13]. The colonies morphological features, such as shape, size, edge, elevation, consistency, and colour changes, were observed on different selective media, as described in Table 2. The isolated organisms were identified using standard microscopy and macroscopy techniques, including morphology and staining. The stained organisms were examined for microscopic characteristics using a compound microscope at 100x magnification with oil immersion. Biochemical analysis used Rapid One Panel (Remel-Thermo, USA) and API *STAPHYLOCOCCUS*. IDENT System Tests [14].

2.6. Isolation of Genomic DNA from Bacteria

After confirmation by culture characteristics and biochemical profile, genomic DNA was isolated from pure colonies of isolates with a Genomic DNA purification kit. A commercial QIAamp DNA Mini Kit was used to prepare the genomic DNA [15].

2.7. Polymerase Chain Reaction (PCR)

The extracted DNA of isolates was amplified using PCR to isolate the genes using the following primers shown in Table 2. The PCR tubes were incubated in a thermocycler after adding taq DNA polymerase, PCR buffer, forward and reverse primers, DNTPs mixture, and $MgCl_2$ to the reaction mixture, followed by nuclease-free water and extracted DNA. The initial denaturation started at 94°C for 2 minutes, followed by 30 cycles of denaturation at 94°C for 30 seconds, then was done for 30 seconds at 58°C and extension was done at 72 °C for 30 seconds. Lastly, the final extension was done at 72 °C for 5 minutes [15].

Table 2: Primer Sequences for PCR Amplification

Genes	Primers	Sequences	Amplicon size
Salmonella genus	flhB F	ATC GCT GAC ATA TGC AAT CC	379bp
	flhB R	GGG GTT GCG TTA TAG GTC TG	
Proteus mirabillis	ure R F	CTGGTGGCTCATTCATGT	509bp
	ure R R	ACAGTTAGGGGGTGGTTT	
Staphylococcus aureus	16S rRNA F	AACTCTCTTATTAGGGAAGAA	756bp
	R	CCACCTTCGTCCGGTTTGTCAC	

2.8. Antibiotic susceptibility test

All bacterial isolates were tested for culture sensitivity using Kirby-Bauer's disk diffusion method, as described by Bauer *et al*., 1966. The procedures were carried out according to the recommendations of the Clinical and Laboratory Standard Institute (CLSI) in 2016 [16]. A 0.5 McFarland solution was prepared and compared with the bacterial suspension of the isolates. The bacterial suspension was then inoculated on Muller Hinton agar using a swab and incubated for 18-24 hours as shown in Figure 5. The culture sensitivity of the isolates was then tested against the following antibiotics: OFX, Ofloxacin 5µ/ml); CFX, Cefixime 5µg/ml; DOX, Doxycycline 30µg/ml; TET, Tetracycline 30µg/ml; AMX, Amoxicillin 20/10µg/ml; GEN, Gentamycin 10 µg/ml; CTX, Cefotaxime 30 µg/ml; AMP, Ampicillin 5 µg/ml; CFX, Ciprofloxacin 5 µg/ml; K, Kanamycin 30 µg/ml; DA, Clindamycin 2 µg/ml, SXT, Trimethoprime+ Sulphamethexole 2.25/23.75 µg/ml. (Oxoid Ltd. UK). Finally, the plates were incubated at 37°C for 24 hours and the zone was measured and compared to CLSI standards.

3. Results

3.1. Isolation and morphological characterization

Only the plates that showed a significant colony count ranging from 30-300 were selected for identification. The morphological characterization of the isolated bacteria from eggshells and egg content of three categories of eggs (clean, unclean and soiled) was carried out on different agar media, including SS agar, MacConkey agar, XLD agar and MSA. The results of the characterization are presented in the Table 3 and Figure 6. The results of biochemical profiling of the presumptive isolates are shown in Table 4 and Table 5.

Table 3: Colony characteristics of isolates

Bacteria	Color	Shape	Margin	Elevation	Texture	Media used	Staining
Proteus	Black centered, Colorless	Round	Entire	Convex	Smooth	SS agar	Gram –ve rods
	Pale colorless colonies.	Circular	Entire	Low convex	Smooth	MacConkey	
Salmonella	Por colorless colonies	Circular	Irregular	Low Convex	Smooth	MacConkey	Gram –ve rods
	Red with black center	Circular	Entire	Convex	Smooth	XLD	
	Colorless with black centered	Large and round	Entire	Convex	Translucent	SS agar	
Staph. aureus	Golden yellow color	Round	Entire	Convex	Smooth shiny	MacConkey	Gram +ve cocci
	Yellow	Circular	Entire	Convex	Smooth	MSA	

Table 3. Colony characterization of Salmonella, Proteus, Staph. aureus on SS agar, MacConkey agar, XLD agar and MSA. Round black-centred colonies (Salmonella, Proteus) were observed on SS agar, Circular pale colorless colonies (Salmonella, Proteus) were observed on MacConkey agar, red with black-centred (Salmonella) on XLD, and yellow colonies (Staph. Aureus) were observed on MSA agar. On gram staining, two Gram-ve rods (Salmonella, Proteus) were observed under the microscope, by picking round black-centred colonies from SS agar, and gram+ve cocci (Staph. Aureus) were observed when gram staining was performed for yellow colonies on MSA agar.

Table 4: Biochemical profile of *Salmonella* and *Proteus* by Rapid One kit

	Rapid One Kit Tests																				
Bac.	**URE**	**ADH**	**ODC**	**LDC**	**TET**	**LIP**	**KSF**	**KSF**	**GUR**	**OPNG**	**βGLU**	**βXYL**	**NAG**	**MAL**	**PRO**	**PRO**	**GGT**	**PYR**	**PYR**	**ADON**	**IND**
Sl.	+	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Pr.	+	v	-	-	+	-	-	-	-	-	v	+	+	+	+	+	+	v	-	-	-

Abbreviation: Urea(URE), Arginine(ADH), Ornithine (ODC), Lysine(LDC), Aliphatic thiol(TET), Fatty acid ester(LIP), Sugar aldehyde(KSF), Sorbitol(SBL), p-Nitrophenyl-β, D-glucuronide(GUR), o-Nitrophenyl-β, D-galactoside(OPNG), p-Nitrophenyl-β, D-glucoside(βGLU), p-Nitrophenyl-β, D-xyloside (βXYL), p-Nitrophenyl-N-acetyl-β, D-glucosaminide (NAG), Malonate(MAL), Proline-β-naphthylamide(PRO), γ-Glutamyl-β-naphthylamide(GGT), Pyrrolidonyl-β-naphthylamide (PYR), Adonitol (ADON), Tryptophane(IND).

Table 5: API STAPH.IDENT System Tests.

	API STAPH.IDENT System Tests										Add. Tests
Bac.	**Phs**	**Ure**	**GIs**	**Mne**	**Man**	**Tre**	**Sal**	**Glc**	**Arg**	**Ngp**	**Coag.**
Staph.	+	+	+	+	+	+	-	-	+	-	+

Abbreviations: Phs, alkaline phosphatase; Ure, urease; GIs,-glucosidase; Mne, acid formation from mannose; Man, acid formation from mannitol; Tre, acid formation from trehalose; Sal, acid formation from salicin; Glc-glucuronidase; Arg, utilization of arginine; Ngp, 3-galactosidase; coag, coagulase

3.2. PCR Amplification

PCR amplification was performed for each DNA-extracted isolate based on their confirmed morphological and biochemical profiles. The primers amplified partial sequences of the APC, ureR, and 16S rRNA genes of *Salmonella*, *Proteus*, and *Staphylococcus* aureus, resulting in PCR products of 204bp, 509bp and 756bp, respectively, as shown in the Figure 1. The amplified PCR products of *Salmonella*, *Proteus*, and *Staphylococcus* aureus were analysed using the Basic Local Alignment Search Tool (BLAST) at the National Center for Biotechnology Information (NCBI). As a result, the NCBI generated the following accession numbers for the confirmed PCR isolates of *Salmonella* (PP511204, PP713041, PP511207, PP516532), *Proteus* (PP418879) and *Staphylococcus* aureus (OR232960).

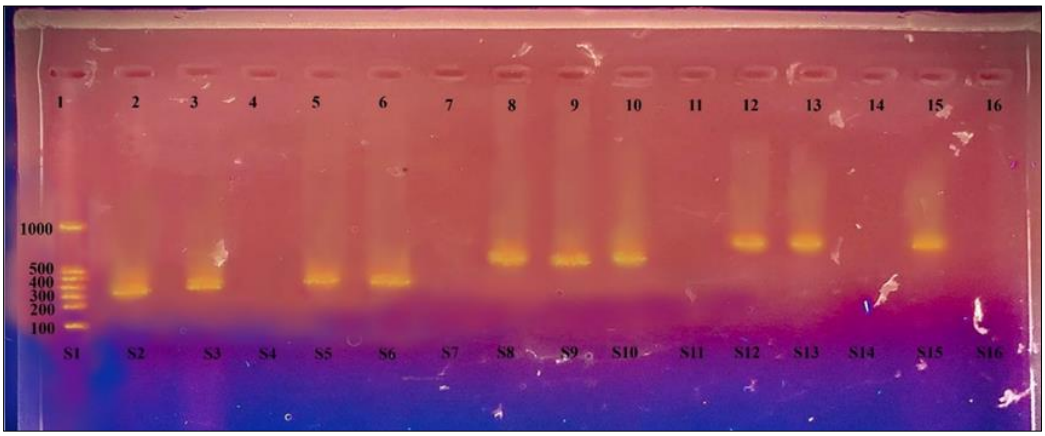

Fig 1: Gel electrophoresis outcome: Lane 1: DNA ladder 1500bp, Lanes 2,3,5,6 were *Salmonella*, Lanes 8,9,10, *Proteus*; Lanes 12,13 and 15 were *Staphylococcus*, while Lanes 4, 7, 11 and 16 were negative controls.

3.3. Prevalence of isolates

The study found that out of 353 samples, 118 were unclean, 119 were clean, and 116 were soiled, showing a significant count of bacteria in both eggshells and egg whites of the unclean category. *Salmonella* was isolated from the eggshells and egg content of four different breeds at the following rates: Fiyomi crossed misri hens-eggshell (20%), egg content (30%); Broiler Breeder-eggshell (10%), egg content (10%); Layer Breeder-eggshell (10%), egg content (9%); White Leg Horn-eggshell (9%), egg content (7%). *Proteus* showed the highest prevalence in Fiyomi crossed misri hens: eggshell (24%), egg content (15%), compared to other breeds: Broiler Breeder-eggshell (15%), egg content (7%); Layer Breeder-eggshell (10%), egg content (7%); and White Leg Horn-eggshell (16%), egg content (4%). *Staphylococcus* was isolated at the following rates: Fiyomi crossed misri hens-eggshell (26%), egg content (14%); Broiler Breeder-eggshell (16%), egg content (5%); Layer Breeder-eggshell (21%), egg content (9%); and White Leg Horn-eggshell (19%), egg content (7%). The cumulative prevalence is shown in Figure 2.

In contaminated eggs, the following order of *Salmonella* isolation was observed: Fiyomi crossed Misri hens-eggshell (20%), egg content (11%); Broiler Breeder-eggshell (25%), egg content (22%); Layer Breeder-eggshell (3%), egg content (5%); and White Leg Horn-eggshell (6%), egg content (4%). *Proteus* was isolated in the following order: Fiyomi crossed Misri hens-eggshell (17%), egg content (8%); Broiler Breeder-eggshell (22%), egg content (12%); Layer Breeder-eggshell (4%), egg content (3%); and White Leg Horn-eggshell (2%), egg content (2%). Additionally, Fiyomi-crossed Misri hens showed the highest prevalence of *Staphylococcus*, while no bacteria were isolated from broiler breeder and layer breeder. The cumulative prevalence of the respective isolates is shown in Figure 3.

For clean eggs, the prevalence of *Salmonella* and *Proteus* follows a specific order across different breeds. *Staphylococcus* was only isolated from two breeds. The cumulative prevalence of the respective isolates is shown in Figure 4.

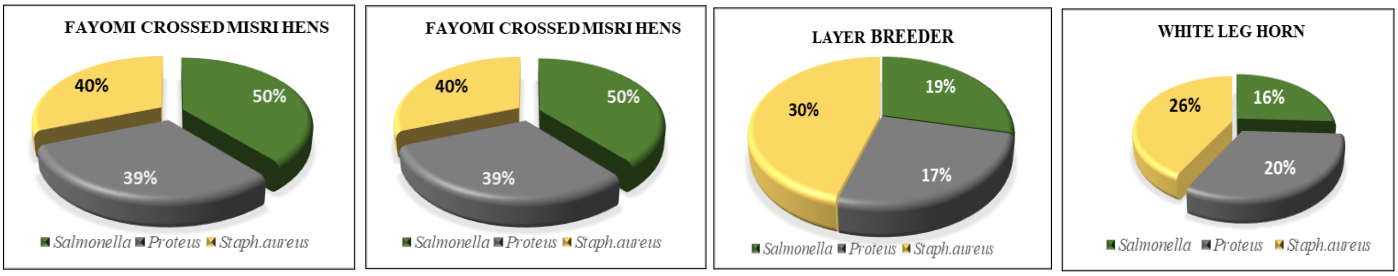

Fig 2: Percentage Prevalence Bacterial isolates in unclean eggs

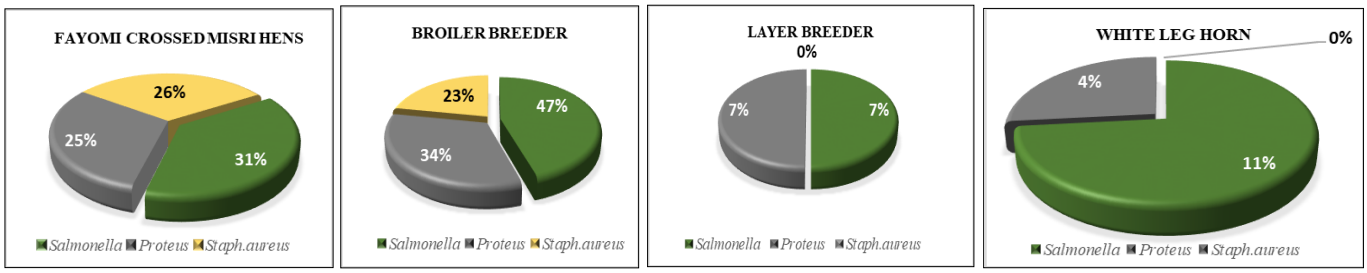

Fig 3: Percentage Prevalence bacterial isolates in soiled eggs

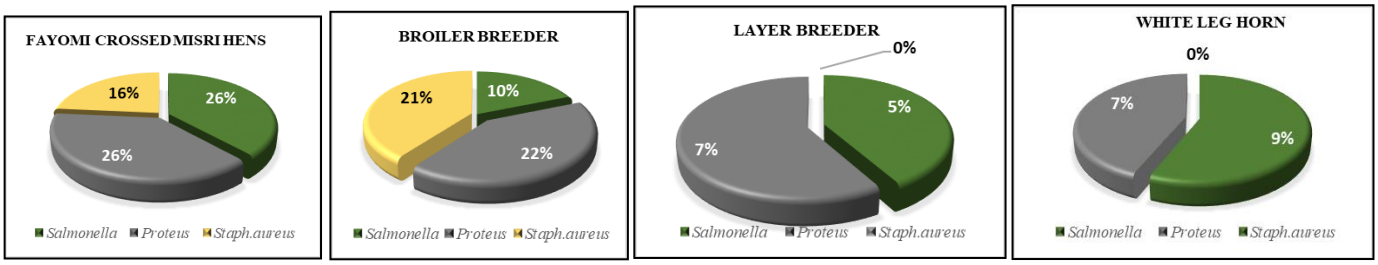

Fig 4: Percentage Prevalence bacterial isolates in clean eggs

3.4. Antibiotic susceptibility test

The susceptibility of *Salmonella*, *Proteus*, and *Staphylococcus* to the following antibiotics is represented by the following values: OFX, Ofloxacin 5µg/ml; CFX, Cefixime 5µg/ml; DOX, Doxycycline 30µg/ml; TET, Tetracycline 30µg/ml; AMX, Amoxicillin 20/10µg/ml; GEN, Gentamycin 10 µg/ml; CTX, Cefotaxime 30 µg/ml; AMP, Ampicillin 5 µg/ml; CFX, Ciprofloxacin 5 µg/ml; K, Kanamycin 30 µg/ml; DA, Clindamycin 2 µg/ml, SXT, Trimethoprim + Sulphamethoxazole 2.25/23.75 µg/ml. These values can be compared with the CLSI standards breakpoints. *Salmonella* was sensitive to the drugs with these zones * and was resistant to the remaining drugs. *Proteus* was sensitive to the drugs with these zones * and was resistant to remaining drugs. *Staphylococcus* was sensitive to only one drug with these zones *, showed intermediate effect with the drugs having these zones ^, and was resistant to remaining drugs.

Table 6: Antibiotic susceptibility testing of Isolates

Antibiotics	Isolates			Zone of inhibition (CLSI.2020)		
	Staph (mm)	Proteous (mm)	Samonella (mm)	S	I	R
OFX	21*	18*	18*	≥16	13-15	≤12
CFX	19^	19*	12	≥19	17-19	16-18
DOX	2	6	10	≥14	11-13	11-13
TET	10	10	0	≥15	12-14	12-14
AMX	6	20*	18*	≥18	14-17	14-17
GEN	15^	18*	0	≥15	13-14	13-14
CTX	27*	27*	26*	≥26	23-25	≤22
AMP	10	17*	10	≥17	14-16	≤13
CFX	17	29*	19	≥26	22-25	≤21
K	16^	15^	19*	≥18	14-17	≤13
DA	22*	12	13	≥21	15-20	≤14
SXT	17*	18*	12^	≥16	11-15	≤10

Abbreviations: OFX, Ofloxacin (5µ/ml); CFX, Cefixime 5µg/ml; DOX, Doxycycline 30µg/ml; TET, Tetracycline 30µg/ml; AMX, Amoxicillin 20/10µg/ml; GEN, Gentamycin 10 µg/ml; CTX, Cefotaxime 30 µg/ml; AMP, Ampicillin 5 µg/ml; CFX, Ciprofloxacin 5 µg/ml; K, Kanamycin 30 µg/ml; DA, Clindamycin 2 µg/ml, SXT, Trimethoprime+ Sulphamethexole 2.25/23.75 µg/ml

Fig 5: Antibiotics sensitivity & measuring the zone of Inhibition

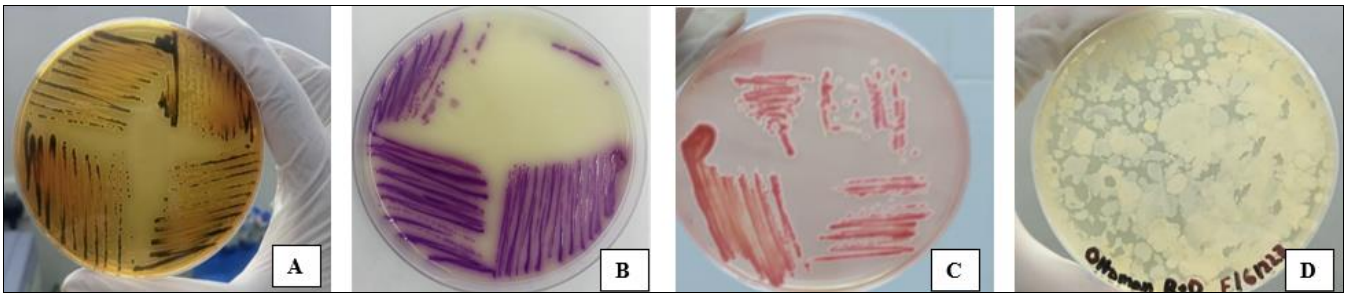

Fig 6: Growth of isolated bacteria on Selected Media *A: Sal on SSA, B: Sal on CA, C: Proteous on SSA & D: Staph on TSA*

Fig 7: Sample Labelling, Handling and Processing

4. Discussion

Eggs are a popular and affordable source of nutrition, but recent concerns have been raised regarding their contamination by harmful microorganisms. Consuming contaminated eggs can lead to foodborne illnesses and pose a health risk to consumers. Factors such as bedding material, air, humidity, and the chicken's gut health directly affect egg quality [17].

A study was conducted in different districts of Punjab, Pakistan, to assess the potential risk of harmful microorganisms in poultry. It was found that gram-negative bacteria belonging to the Enterobacteriaceae family were the most common contaminants found in table and fertile eggs, with a high percentage of *Staphylococcus* also present. These findings are consistent with a previous study that reported that the poultry industry's most dominant pathogenic bacteria also belonged to the Enterobacteriaceae family [12].

Microorganisms were discovered in both the eggshell and egg content samples collected from the eggs. This finding aligns with the USDA's [12] statement that microorganisms can be present in both the shell and the egg content due to the possibility of vertical and horizontal transmission. Fecal contamination can also seep through the egg's pores after it has been laid.

The targeted bacteria were identified using both phenotypic and genotypic techniques. For phenotypic characterization, the bacteria's specific "fingerprint" (specific colony characteristics and biochemical profile) was used for identification, and their biochemical profile was determined using commercially available API KIT and STAPHYLOCOCCUS-IDENT kit. Genotypic analysis was performed using the allied technique of PCR. Each technique has advantages and disadvantages for isolating and identifying bacteria regarding reliability, suitability, specificity/sensitivity, economic considerations, and availability of necessary resources [18]. A recent study identified Enterobacteriaceae, including *Salmonella* and *Proteus*, as gram-negative rods and *Staphylococcus* aureus as gram-positive cocci in the eggshell and egg content samples [18]. The PCR-based analysis of isolated bacteria *Salmonella*, *Proteus*, and *Staphylococcus* aureus targeted their respective genes flhB [19], Ure [20] and 16sRNA [21]. This led to the identification of 4 isolates of *Salmonella*, one isolate of *Proteus*, and one isolate of *Staphylococcus*. These isolates were further submitted to the NCBI database under the Accession numbers (PP511204, PP713041, PP511207, PP516532), (PP418879), and (OR232960) respectively.

Salmonella was found in unclean eggs, with a prevalence rate of 26.7% in unclean eggs, 22.6% in soiled eggs, and 10.3% in clean eggs. The prevalence is higher than the prevalence reported by Salihu [12]. This high prevalence rate may be attributed to the vertical transmission of *Salmonella* through infected reproductive organs. The author also reported that *Salmonella* is highly prevalent in eggs due to its ability to be transmitted vertically and horizontally from contaminated environments. They pose an alarming health issue in humans

and poultry [22]. The study revealed that *Salmonella* has developed resistance to major broad-spectrum drugs such as Tetracycline (30µg) and Gentamycin (10 µg), which is concerning. However, it is still susceptible to Amoxicillin (20/10µg/ml), Ofloxacin (5µg/ml), Cefotaxime (30 µg/ml), and Kanamycin (30 µg/ml). Another report indicates that *Salmonella* spp. is sensitive to drugs like levofloxacin, norfloxacin, ciprofloxacin, chloramphenicol, gentamycin, streptomycin, and doxycycline, while it is more resistant to ampicillin, erythromycin, and lincomycin [23].

The prevalence of *Proteus* was found to be 24.5% in unclean eggs, 17.6% in soiled eggs, and 15.5% in clean eggs. This rate is similar to that reported by Mojisola [20]. *Proteus* is a potentially disease-causing organism for humans. The prevalence of *Proteus* is still worrying some due to the enhanced probability of significant health issues threatening human and poultry life [23]. Data generated from the recent study is of great public health concern as it indicates the significant evidence that has highlighted the role of *Proteus* in causing diseases associated with humans and as a dominant contaminant in poultry and poultry products. Moreover, the prevalence of *Proteus* mainly indicates a poor hygiene environment due to uncontrolled biosecurity measures on the farm [24].

The antimicrobial susceptibility pattern showed that *Proteus* was resistant to tetracycline, similar to the findings of Dadheech, who reported 100% resistance to tetracyclines [25]. *Proteus* also showed resistance to Doxycycline. The most effective drugs against *Proteus* were Amoxicillin, Gentamycin, Ofloxacin, and Cefixime. Kuznetsova reported low resistance against Gentamycin [26].

Staphylococcus aureus bacteria were found at a rate of 33% in unclean eggs, 10.9% in soiled eggs, and 9.4% in clean eggs, but no *Staphylococcus* aureus was found in the egg contents. Another study isolated 28.45% of *Staphylococcus* aureus [27]. Egg contamination is often caused by cracked eggs, dirty nesting material, and contaminated eggshells. The invasion of *Staphylococcus* is mainly associated with fecal contamination, which can cause severe diarrheal problems in humans and poultry. *Staphylococcus* aureus has shown high sensitivity against Ofloxacin but was resistant to Doxycycline, Tetracycline, and Amoxicillin. A study showed 100% resistance to tetracycline and low resistance to Gentamycin and trimethoprime+ Trimethoprime+Sulphamethexole [27].

Conclusion

In conclusion, the microbiological analysis of *Salmonella*, *Staphylococcus*, and *Proteus* from the shell surface of the table and hatchable eggs revealed essential findings regarding these bacteria's prevalence and antibiotic susceptibility. The study highlighted the potential risk of contamination, with unclean and soiled eggs showing higher percentages of bacterial isolates than clean eggs. The antibiotic susceptibility test results indicated sensitivity to certain drugs, which can guide future strategies for mitigating the risk of egg contamination and ensuring egg safety and quality. This research contributes to raising awareness about the importance of egg safety and identifying common pathogens found on eggshells. Implementing effective strategies to reduce the risk of contamination in eggs and egg products is crucial for safeguarding this essential food source. The findings of this study emphasize the need for continued vigilance and proactive measures to address the challenges posed by bacterial contamination in eggs, ultimately contributing to public health and food safety.

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Conflict of Interest

Not available

Financial Support

Not available

6. References

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