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Antimicrobial Sensitivity of Bacterial Pathogens Isolated from Poultry Feed, Water, and Eggshell

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Abstract

Poultry is the most important source of proteins forconsumption. Egg shell and drinking water contributes significantly to the spread of viral, bacterial, and protozoan infections in poultry. A total of 150 samples from poultry feed (feeders n=25, feed bags n=25), water (water tanks n=25, drinkers n=25), and eggshell (n=50) were collected from ten layer farms of district Tando Allahyar. Overall results showed that higher 52% bacterial infection was recorded from the poultry feed, 42% was recorded from water samples while the lowest prevalence recorded from the eggshell 32%. The isolated bacterial species were *E. coli*, *Salmonellatyphi, Shigella sonnei, Klebsiella pneumonia, Proteus mirabilis,* and *Staphylococcus aureus.* In poultry feed, a higher prevalence percentage of *E. coli*(16%) was recorded, followed by *S. sonnei* (14%), *S.typhi*(8%), *S. aureus* (8%),*P. mirabilis* (6%), and *K. pneumonia* (4%). In water, a higher prevalence of *E. coli*(12%) was recorded, followed by *S. aureus* (14%),*S.typhi*(11%), *S. sonnei* (11%), *P. mirabilis (4%),* and *K. pneumonia* (2%). In eggshells, a higher prevalence of *E. coli*(10%) was recorded followed by *S. typhi*(6%), *S. sonnei* (6%), *S. aureus* (6%), *K. pneumonia* (2%), and *P. mirabilis* (2%). *E. coli* species were susceptible to Tetracycline and Streptomycin. *Salmonella typhi* was susceptible to Ciprofloxacin and Tetracycline. *Shigella sonnei* was susceptible to Ciprofloxacin, and Tetracycline.*Klebsiella pneumonia* was susceptible to Streptomycin and Gentamicin.*Proteus mirabilis* was susceptible to Streptomycin and Gentamicin.*Staphylococcus aureus* was sensitive to Gentamicin and Erythromycin. In conclusion higher (52%) bacterial infection was recorded from the poultry feed,(42%) was recorded from water samples and the lowest prevalence was recorded from the eggshell (32%). It is concluded from the present study that bacterial contamination of *E. coli*, *S. typhi*, *S. sonnei*, *K. pneumonia*, *P. mirabilis, S. aureus* is common in poultry feed, water, and eggshell.

Keywords:Antimicrobial sensitivity; Poultry;Feed;Water;Eggshell

Introduction

Poultry eggs are high in vitamins K, E, D, A, B9, B2, B1, B12, selenium, choline, as well as minerals like iron and phosphorus. The nutrients in eggs create an ideal habitat for microbial development and growth. The eggs could be contaminated with many microbes, for example, *Escherichia coli*,*Proteus species*, *Staphylococcus* species, *Listeria monocytogenes*, *Salmonella species*, *Bacillus* species, and *Streptococcus sp*ecies (Lee *et al*., 2016). Eggs are easily contaminated and are a good source of microbial development. Contamination of eggs occurs as a result of litter material in deep litter rearing methods or during care by laborers, egg boxes, and so on. A small number of organisms can invade eggshells and shell membranes, causing the transmission of waste and food-derived microorganisms (Vinayananda *et al*.,2017). Poultry feed is a fundamental piece of the poultry industry and records approximately 60-70 % of the complete production costs (Sherazi *et al*., 2015). Poultry feed is designed to meet the complex nutritional needs of birds. Because of the small digestive tract of birds and the intestinal flora's limited commitment to food digestion, poultry feed must be full (essential nutrients for proper development and egg production) and effectively digested (Matthew *et al*., 2017s). Eggshell protects against the mechanical effect, dehydration, and contamination of microorganisms. A cuticle that covers the exterior eggshell surface and prevents the entry into the shell pores regulates the porousness of the eggshell (Rodríguez *et al*., 2023; Ghazi, & Amer., 2013). Studied the contamination of egg contents and eggshell in broken and unbroken eggs in Yemen. They discovered *Klebsiella spp., E. coli*, *Proteus spp.*, *Campylobacter spp.*, *Pseudomonas spp.*, *Staphylococcus aureus,* and *Streptococcus* in the eggs.Water is an important and necessary nutrient for all birds. Factors such as physiochemical, heavy metals, and the microbiological burden should be evaluated to assess water sources and ensure that their levels are within an acceptable range (Maharjan *et al*., 2017)**.** The health of birds is affected directly or indirectly by various substances or microbiological contaminants present in drinking water (Ashraf *et al*., 2019). *E. coli* and *Salmonella spp*. were found in 62.50 % and 49.91 % of poultry farms, respectively. The prevalence of *E. coli* in the feed sample was 37.50 %, while it was 87.50 % in the litter sample. *Salmonella* was found in 66.66 % of the litter samples and 29.16 % of the feed samples (Islam *et al*., 2014). A total of 20 bacterial microorganisms were identified from 45 feed samples, including *E. coli* (13.33 percent), *Staphylococcus spp.* (13.3 percent), *Salmonella serovar* (2.2 percent), and *Bacillus* species (15.5 percent) (Parveen *et al*., 2017). A total of 118 samples of chicken feed were collected and examined for *E. coli* isolation. 44 (37.29%) of them (a total of 63 strains) tested positive for APEC (Djoman *et al*., 2020). A total of 50 samples of water were obtained from 3 distinct broiler farms' water tanks and drinkers of Dera Ismail Khan, the city of the province of Khyber Pakhtunkhwa, Pakistan, and examined to identify the presence of *E. coli* and *S. typhi*. When compared to *S. typhi*, the total pervasiveness of *E. coli* identified was essentially high (72%) (28 %). Furthermore, 44 % of *E. coli* bacteria were recovered from water users. Similarly, the prevalence of *S. typhi* in drinkers (19%) was higher than in the tanks (9%) of 3 chicken farms, indicating that they were infected only with bacteria (Zaman *et al*., 2012). The occurrence of pathogenic microbial isolates from the water supply in the examined poultry farm presented that the spreading of positive bacterial isolates was 41/60; 68.3% in completely explored water samples all through the study duration. Additionally, the most main bacterial isolates were *Shigella* spp. (10/41; 24.4%) trailed by *E. coli* (9/41; 21.9%), *S. kentucky* (8/41; 19.5%), *K. pneumonia* (8/41; 19.5%), *S. garoli* (6/41; 14.6%) (Mohammed *et al*., 2020)**[13]**.Antimicrobial resistance has turned into an expanding general medical problem around the world. Antimicrobial resistance, particularly of pathogenic microorganisms, has been incompletely attributed to the abuse of antimicrobial agents in medication and agriculture (Michael *et al*., 2014). Continuous use of antibiotics in poultry causes the evolution of antibiotic-resistant pathogens especially *Salmonella* spp. and *E. coli* (Davies & Wales., 2019).

Material and Methods

Sample collection: The study was conducted in different areas of the Tando Allahyar district. A total of one hundred fifty samples from poultry feed (feeders n=25, feed bags n=25), water (water tanks n=25, drinkers n=25), and eggshell (n=50) were collected from ten layer farms of district Tando Allahyar. The egg samples were packed aseptically in sterile bags, poultry feed and drinking water samples were collected in sterilized universal bottles. The samples were properly labeled and transported within 3 hours under refrigeration (4 °C) to the Central Veterinary Diagnostic Laboratory Tandojam for further processing. Samples of feed and egg shell were grinded and cultured in nutrient broth at 37ºC over night in incubator. After that samples were cultured and sub cultured on different media like Nutrient agar, Maconkeys agar, Blood agar and Salmonella Shigella agar etc. After culture bacterial species were identified by Grams staining and Biochemical tests like Catalase, Oxidase. Indole, urease, Triple sugar iron tests.

Antimicrobial resistance: The antimicrobial sensitivity of bacterial isolates from eggshells, poultry feed, and water was assessed using an agar disc diffusion assay and disc diffusion method from the Clinical and Laboratory Standards Institute (CLSI) (CLSI, 2011). Ten antibiotics from various classes were examined i.e. Ciprofloxacin, Doxycycline, erythromycin, gentamicin, oxacillin, Ampicillin, Cephalothin, streptomycin, tetracycline, and nalidixic acid. A sterilized cotton swab was used to pick up the pure colony, which was then spread across the whole surface of the Muller Hinton Agar and left to dry for 3-5 minutes. Using sterile forceps, antibiotic discs were inserted into the plates. To establish complete contact with the agar surface, each disc was lightly squeezed. The discs were distributed evenly and a minimum distance of 24 mm was maintained from center to center (Four discs were placed in each petri dish). The plates were inverted and incubated at 37°C after 15 minutes of disc application. The widths of the zones were measured after 24 hours of incubation and classified as susceptible, intermediate, or resistant.

Results

Prevalence of bacterial species from poultry feed, water, and eggshell: This graph shows the prevalence percentage of bacteria in different sources of poultry collected from the different poultry forms of the TandoAllahyar. Higher (52%) bacterial infection was recorded from the poultry feed,whereas (42%) was recorded from water samples andthe lowest prevalence was recorded from the eggshell (32%).

Prevalence percentage of bacteria isolated from a different source of poultry: Total of 150 samples (50 samples from each source (poultry feed, water, and eggshell) were collected from the different sources of poultry farms located in Tando Allahyar. The isolated bacterial species were *E. coli, Salmonellatyphi, Shigella sonnei, Klebsiella pneumonia, Proteus mirabilis,* and *Staphylococcus aureus.* In poultry feed higher prevalence percentage of *E. coli* (16%) was recorded, followed by *Shigella sonnei* (14%), *Salmonellatyphi*(8%), *Staphylococcus aureus* (8%), *Proteus mirabilis* (6%), and *Klebsiella pneumonia* (4%). In water higher prevalence of *E. coli* (12%), was recorded, followed by *Staphylococcus aureus* (14%),*Salmonellatyphi*(11%), *Shigella sonnei* (11%), *Proteus mirabilis (4%), and Klebsiella pneumonia* (2%).In Eggshells, the prevalence of *E. coli* was greater (10%), followed by *Salmonellatyphi*(6%), *Shigella sonnei* (6%), *Staphylococcus aureus* (6%), *Klebsiella pneumonia* (2%), and *Proteus mirabilis* (2%).

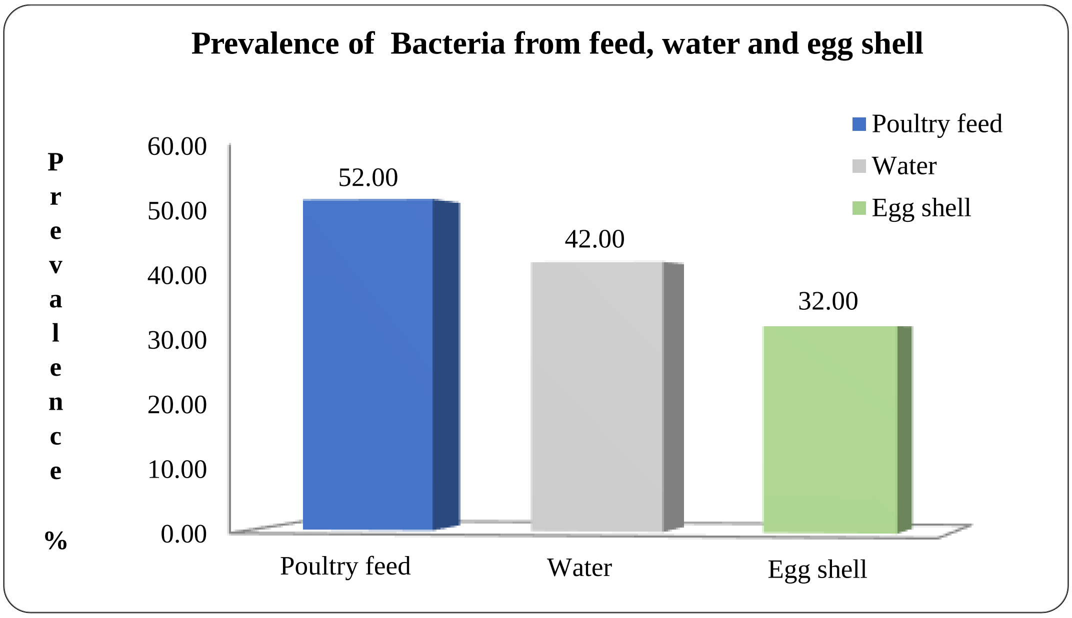

Figure 4.1Prevalence percentage of bacteria isolated from feed, water, and eggshell

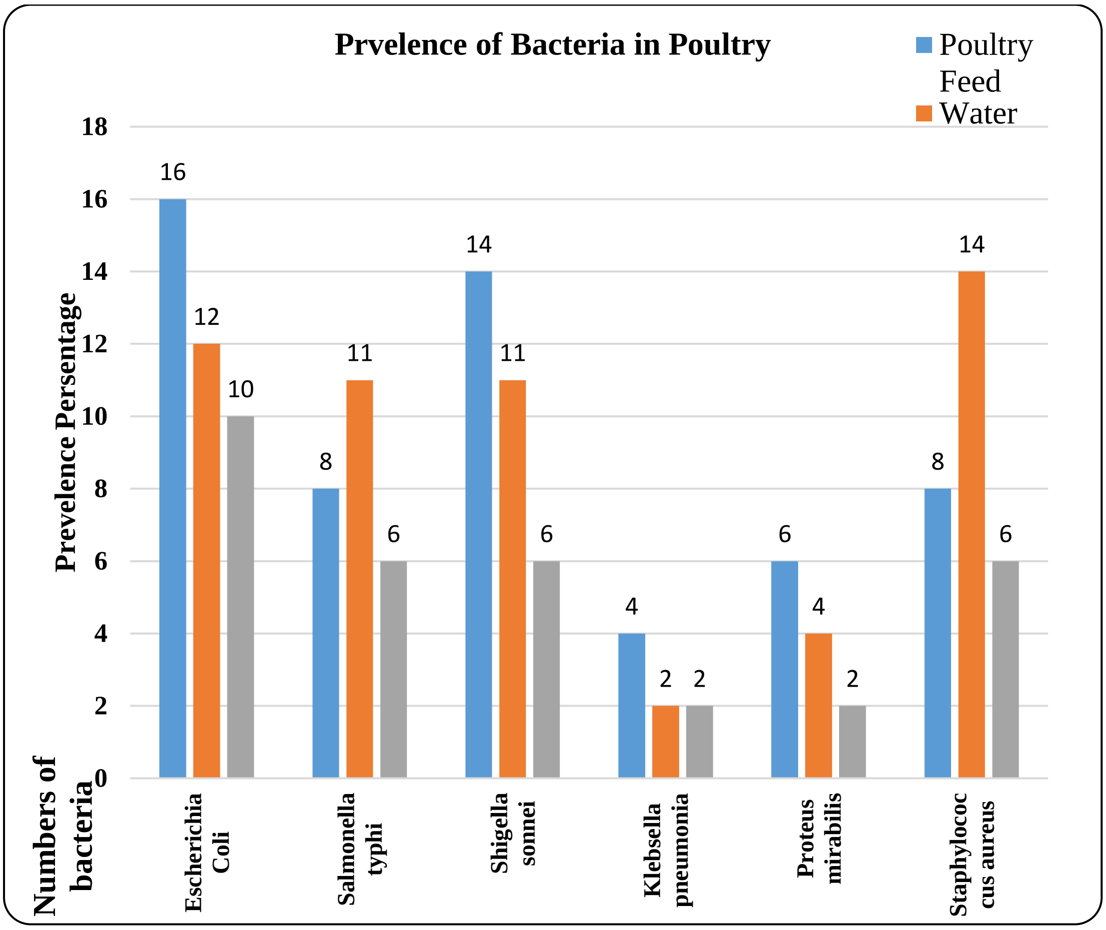

Figure 4.2 Prevalence percentage of bacteria isolated from a different source of poultry

Table. Biochemical tests conducted after the isolation of bacterial pathogens for the confirmation of identification

Biochemical test	***E. coli***	***Salmonella typhi***	***Shigella sonnei***	***Klebsiella pneumonia***	***Proteus mirabilis***	***Staphylococcus aureus***
Catalase	+ve	+ve	+ve	+ve	+ve	-ve
Oxidase	-ve	-ve	-ve	-ve	-ve	-ve
Indole	+ve	-ve	-ve	-ve	-ve	-ve
Methyl red test	+ve	+ve	+ve	-ve	+ve	+ve
Voges-Proskauer	-ve	-ve	-ve	+ve	-ve	-ve

Antimicrobial sensitivity test for various bacterial pathogens isolated from poultry feed, water and egg shell.: Isolated bacteria*E. coli*was observed for the antimicrobial-resistant to the ten antibiotics. The results indicated that *E. coli* was highly resistant to Ciprofloxacin, Ampicillin, Cephalothin, Nalidixic acid, Erythromycin, Oxacillin, Gentamycin, and Doxycycline. While *E. coli* species were sensitive to Tetracycline, and Streptomycin.Isolated *Salmonellatyphi* was observed for the antimicrobial-resistant to ten antibiotics. The results of resistance indicated that *Salmonellatyphi* was highly resistant to Ampicillin, Cephalothin, Erythromycin, Oxacillin, Gentamicin, and Doxycycline. While *Salmonella*typhi have shown intermediate sensitivity to the Nalidixic acid. While *Salmonellatyphi* wassensitive to Ciprofloxacin, Tetracycline, and Streptomycin. Isolated*Shigella sonnei* was observed for the antimicrobial-resistant toten antibiotics. The results of resistance indicated that *Shigella sonnei* was highly resistant to Ampicillin, Cephalothin, Erythromycin, Oxacillin, While *Shigella sonnei* has shown intermediate sensitivity to the Nalidixic acid. While *Shigella sonnei* wassensitive to Ciprofloxacin, Tetracycline, Streptomycin, Gentamicin, and Doxycycline.Isolated *Klebsiella pneumonia*was observed for the antimicrobial-resistant to ten antibiotics. The results of resistance indicated that *Klebsiella pneumonia* was highly resistant to Tetracycline, Ampicillin, Cephalothin, Erythromycin, Oxacillin. Whileit has shown intermediate sensitivity to Doxycycline and Ciprofloxacin.While it was alsosensitive to Streptomycin, Gentamicin, and Nalidixic acid.

Isolated *Proteus mirabilis* was observed for the antimicrobial-resistant to ten antibiotics. The results of resistance indicated that *Proteus mirabilis* was highly resistant to Tetracycline, Ampicillin, Cephalothin, Nalidixic acid, Erythromycin, Oxacillin.While*Proteusmirabilis* has shown intermediate sensitivity to the Doxycycline. While *Proteus* mirabilis wassensitive to, Streptomycin, Gentamicin, and Ciprofloxacin.Isolated *Staphylococcus aureus* was observed for the antimicrobial resistance to ten antibiotics. The results of resistance indicated that *Staphylococcus aureus* was highly resistant to Tetracycline, Ampicillin, Cephalothin, Streptomycin, Ciprofloxacin, Nalidixic acid, and Oxacillin, While *Staphylococcus aureus*has shown intermediate sensitivity to the Doxycycline. While *Staphylococcus aureus* wassensitive to Gentamicin and Erythromycin.

Table. No Antimicrobial sensitivity test for different bacterial pathogens isolated from poultry feed, water, and eggshell

Bacterial Species	**Antibiotic disc used**	**The inhibitory zone around the disc**	**Interpretive categories**
	Ciprofloxacin-15	10mm	Resistant
	Tetracycline-30	20mm	Sensitive
	Ampicillin-10	0mm	Resistant
	Cephalothin-30	0mm	Resistant
E. coli	Nalidixic acid-30	0mm	Resistant
	Erythromycin-15	0mm	Resistant
	Oxacillin-1	0mm	Resistant
	Streptomycin-10	15mm	Sensitive
	Gentamicin-10	12mm	Resistant
	Doxycycline-30	9mm	Resistant
Salmonellatyphi	Ciprofloxacin-15	30mm	Sensitive
	Tetracycline-30	16mm	Sensitive
	Ampicillin-10	0mm	Resistant
	Cephalothin-30	0mm	Resistant
	Nalidixic acid-30	17mm	Intermediate
	Erythromycin-15	0mm	Resistant
	Oxacillin-1	0mm	Resistant
	Streptomycin-10	19mm	Sensitive
	Gentamicin-10	17mm	Resistant

	Doxycycline-30	15mm	Resistant
	Ciprofloxacin-15	35mm	Sensitive
	Tetracycline-30	20mm	Sensitive
	Ampicillin-10	0mm	Resistant
	Cephalothin-30	0mm	Resistant
Shigella sonnei	Nalidixic acid-30	21mm	Intermediate
	Erythromycin-15	0mm	Resistant
	Oxacillin-1	0mm	Resistant
	Streptomycin-10	21mm	Sensitive
	Gentamicin-10	20mm	Sensitive
	Doxycycline-30	17mm	Sensitive
	Ciprofloxacin-15	25mm	Intermediate
	Tetracycline-30	0mm	Resistant
	Ampicillin-10	0mm	Resistant
	Cephalothin-30	0mm	Resistant
Klebsiella pneumonia	Nalidixic acid-30	26mm	Sensitive
	Erythromycin-15	0mm	Resistant
	Oxacillin-1	0mm	Resistant
	Streptomycin-10	18mm	Sensitive
	Gentamicin-10	20mm	Sensitive
	Doxycycline-30	13mm	Intermediate
	Ciprofloxacin-15	28mm	Sensitive
	Tetracycline-30	0mm	Resistant
	Ampicillin-10	0mm	Resistant
	Cephalothin-30	0mm	Resistant
Proteus mirabilis	Nalidixic acid-30	0mm	Resistant
	Erythromycin-15	0mm	Resistant
	Oxacillin-1	0mm	Resistant
	Streptomycin-10	15mm	Sensitive
	Gentamicin-10	15mm	Sensitive
	Doxycycline-30	8mm	Intermediate
	Ciprofloxacin-15	0mm	Resistant
	Tetracycline-30	0mm	Resistant
	Ampicillin-10	0mm	Resistant
	Cephalothin-30	0mm	Resistant
Staphylococcus aureus	Nalidixic acid-30	10mm	Resistant
	Erythromycin-15	24mm	Sensitive
	Oxacillin-1	0mm	Resistant
	Streptomycin-10	2mm	Resistant
	Gentamicin-10	23mm	Sensitive
	Doxycycline-30	15mm	Intermediate

Discussion

The purpose of the study was to determine the prevalence and antibiotic sensitivity of bacterial pathogens isolated from poultry feed, water, and eggshell. In this study, the highest (52%) bacterial infection was recorded from the poultry feed, whereas (42%) was recorded from water samples and the lowest prevalence was recorded from the eggshell (32%). In poultry feed higher prevalence percentage of *E. coli* (16%) was recorded, followed by *Shigella sonnei* (14%), *Salmonella typhi* (8%), *Staphylococcus aureus (*8%), *Proteus mirabilis* (6%), and *Klebsiella pneumonia* (4%). In water, a higher prevalence of *E. coli* (12%), was recorded, followed by *Staphylococcus aureus* (14%), *Salmonella typhi (11%), Staphylococcus aureus (14%), Shigella sonnei (11%), Proteus mirabilis (4%), and Klebsiella pneumonia (2%).* Whereas, in Eggshells higher prevalence of *E. coli* (10%) was recorded, followed by *Salmonella typhi (6%), Shigella sonnei (6%), Staphylococcus aureus (6%), Klebsiella pneumonia (2%), and Proteus mirabilis (2%).*Our results are in agreement with (Parveen *et al*., 2017). who found the most bacterial pathogens in feed samples (44.44 %) as compared to eggshells (27.78 %). In poultry feed samples, the prevalence of *E. coli* and *Salmonella serovars* was (13.33%), (2.22%) respectively. *Salmonella serovars* were found to have the highest proportion of pathogenic bacteria in feed (15.55 %).

Bacterial pathogens such as *Escherchia coli* (11.1 %) and *Salmonella serovar* (2.7 %) were isolated from eggshell samples. *E. coli* had the highest percentage of bacteria in eggshells (11.11 %). This could be due to some circumstances, including contamination of foodborne pathogens during harvest and subsequent selling of feed bags and eggs, as well as contamination of the eggshell surface.In four poultry farms, the comprehensive pervasiveness of *E. coli* and *Salmonella sp.* was 62.50 %, and 49.91 %, respectively. *E. coli* was found in 37.50 % of the feed samples. *Salmonella* was found in 29.16 percent of the feed samples. The presence of *Salmonella* at the recorded rate in feed poses a substantial public health concern if basic sanitary measures are not maintained (Islam *et al*., 2014). Approximately fifty water samples were taken from water tank and drinker in three chicken farms in district D. I. Khan and studied for the occurrence of *S. typhi and Escherichia coli.* Prevalence of *E. coli* was higher seventy two percent as compare to *S. typhi* twenty eight percent. Furthermore, *E. coli* in water drinkers was forty percent. Similarly, prevalence of *S. typhi* was high at nineteen percent in drinkers while in a water tank was nine percent. It was seen that they were only contaminated with microorganisms (Zaman *et al*., 2012; Ifeanyichukwu *et al*., 2016) **[12]**. reported examining samples from eggshell (n = 15), drinking water (n = 14), and poultry feed (n = 12) for the isolation of *Salmonella* species. A total of 8 (16.3%) samples, 7 (14.3%), and 4 (8.2%) were positive for the isolation of*Salmonella*species from eggshell samples, poultry drinking water, and poultry feed, respectively. Approximately 74% of samples of egg (148 out of 200) tested positive for bacterial contamination. 100 shells of the samples were infected with bacteria of various genera; however, only 48 (48%) of the egg contents exhibited growths. *Pseudomona*s species (6.43%), *Staphylococcus species* (24.29%), Coagulase-positive *Salmonella sp.* (20.71%), Coagulase negative *Staphylococcus* (10%), *Bacillus sp.* (3.93%), and *E. coli* (34.64%), are most common contaminants. The isolated bacteria *Pseudomonas spp.*, Coagulase positive *Staphylococcus*,*E. coli*, and *Salmonella spp*. were susceptible to azithromycin, ciprofloxacin, and ceftriaxone while exhibiting resistance to tetracycline, amoxicillin, and ampicillin.

These antimicrobial-resistant bacteria have the potential to transmit to people via infected eggs, which is a public health concern in terms of food safety (Islam *et al*., 2018) .Out of 180 chicken eggs, 120 poultry feed samples, and 120 poultry water samples, the frequency of isolation of *Salmonella spp*.they were 3.3%, 2.5% and 3.3%, respectively. Among the isolates, *Salmonella* was the predominant serotype. All isolates showed sensitivity to ampicillin, enrofloxacin, and colistin whereas they produced resistance against vancomycin, penicillin, oxacillin, and clindamycin. Thus the comparatively high resistance amongst the microbes found in the poultry, customers may face treatment and public health concerns as possible carriers of food-borne drug-resistant*Salmonella* (Singh *et al*., 2013). The prevalence of the sample (216) of eggshell (88), drinking water (64) poultry feed (64) from 4 poultry farms was 34.2 percent (74/216). A part of the eggshell swab samples, 23.86 % (21/88) was positive for the *Proteus*, whereas 43.7 % (28/64) and 39 % (25/64) of the water and feed were positive for *Proteus* species correspondingly. There was a hundred percent sensitivity for amikacin and 25.7% resistance for tetracycline (Owoseni *et al*., 2019). The pervasiveness of *Salmonella spp.*, *E. coli*, and *Staphylococcus spp.* was found at 28.3%, 35%, and 23.3%, correspondingly. *E. coli* were susceptible to ciprofloxacin (85.7%), amoxicillin (71.4%), and gentamicin (95.2%) and highly resistant to tetracycline (80.9%), penicillin (100%), erythromycin (85.7%) ampicillin (100%), and *Salmonella spp.* were susceptible for ciprofloxacin (70.58%), amoxicillin (76.47%), gentamicin (94.11%), and was highly resistant to penicillin (100%), erythromycin (82.35%) and tetracycline (82.35%). *Staphylococcus spp.* was sensitive to ciprofloxacin (85.71%), and gentamicin (92.85%) but was resistant to ampicillin (100%), erythromycin (78.5%), penicillin (100%), amoxicillin (100%), tetracycline (85.7%), and the higher occurrence of multidrug-resistant (MDR) bacteria can easily access the food chain and pose a public health risk (Haque *et al*., 2012) .*E. coli* were tested to identify the sensitivity rat from 10 antibiotic discs, including Ciprofloxacin, Gentamicin, Doxycycline, Erythromycin, Tetracycline, Chloramphenicol, Ampicillin, Amoxicillin, Meropenem, and Ceftriaxone. Their sensitivity zones were explained and measured according to the Clinical and Laboratory Standards Institute guidelines (CLSI 2017). *E. coli* showed 92.85% to 64.28% susceptibility to ceftriaxone, gentamycin, and chloramphenicol. The highest susceptibility was found 100% for meropenem. *E. coli* was resistant to erythromycin, ciprofloxacin, amoxicillin, ampicillin, and tetracycline, ranging from 50% to 71.4%. Careful use of antimicrobial drugs and increased awareness of public can aid to decrease the progress of antibiotic resistance (Hossain)

Conclusions

It is concluded from the present study that Bacterial contamination of *Escherichia coli, Salmonella typhi, Shigella sonnei, Klebsiella pneumonia*, *Proteus mirabilis*, *Staphylococcus aureus* is common in poultry feed, water, and eggshell.Higher bacterial infection was recorded from the poultry feed, followed by water samples and eggshells. A higher prevalence of *E.coli* was observed in poultry feed, water, and eggshell as compared to other isolates.Ciprofloxacin, Tetracycline, Streptomycin, Gentamicin, and Erythromycin were sensitive to *E. coli*, *Salmonella typhi, Shigella sonnei, Klebsiella pneumonia, Proteus mirabilis, and Staphylococcus aureus.*

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