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Isolation and Identification of Pathogenic Bacterial Contaminants associated with Poultry

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A B S T R A C T

The study was conducted aiming at the isolation and identification of *Salmonella* and *Escherichia coli (E. coli)* from different poultry farm in Padra Village, Gujarat, India. Drinking water samples were collected from feeding operation of two different poultry farms and were analysed for the presence of *Salmonella spp.* and *Escherichia coli* using microbiological analysis. The samples were analysed by culturing in different media such as nutrient broth (NB), nutrient Agar (NA), BGA (brilliant-green Agar), MCA (MacConkey), EMB (eosin methylene blue), and LuriaBertani media. *Salmonella spp.* and *Escherichia coli* were tested against 5 anti- microbial agents: Gentamicin, Streptomycin, Ampicillin, Penicillin, Chloramphenicol using the Kirby-Bauer disc diffusion method. The result of this research showed the presence of aerobic bacteria and also the resistant of bacteria to certain antibiotics in water samples. Strict hygienic practices and biosecurity measures should be instituted and maintained both at feed mills and poultry farms so as to eliminate contamination associated with poultry. The widespread occurrence of *Salmonella spp.* and *E. coli* in poultry feeds reinforces the need for effective control measures, hygiene in processing and handling of feeds.

Introduction

The intense international trade of animals and animal product facilitates the spread of *Salmonella* spp. making gastrointestinal disorder a worldwide public-health subject, accountable for serious economic losses to the poultry trade and governments (10). Contaminated poultry products are identified as the principal sources of *Salmonella* resulting in food borne illness in humans. Handling of raw poultry carcasses and products and consumption of undercooked poultry meat are the most causes of infection (20). *Salmonella spp.* and *E. coli* cause food borne sickness that transmitted through poultry meat (6). The genus Salmonella consists of quite 2300 serologically

distinguishable variants (20). Though humans will become infected by Salmonella spp. through an outsized range of foodstuff, poultry meat and eggs are among the foremost often involved sources of human Salmonella outbreaks (10). Salmonellosis are major bacterial diseases in poultry trade worldwide (9).

The US annual salmonellosis burden was recently estimated to be in the order of 1.5 million cases (including over 580 deaths), 95% of those cases were attributed to food borne infection (20).

Divided into two distinct serovar groups, Typhoidal and non-Typhoidal, based on the form of infection, non-Typhoidal *Salmonella* is one among the leading causes of food borne illnesses worldwide with an estimated 93.8 million cases and 155,000 deaths annually thanks to complications from gastrointestinal infections (21). Most of these cases occur as a results of consuming contaminated foods, significantly foods of animal origin (3).

Throughout the past twenty years, severe outbreaks of gastrointestinal sickness have occurred by food borne infective *E. coli*, particularly *0157:H7*. It had been reported that in India around 1.8 million people died from diarrheal diseases mostly due to contaminated food and water within the year 2005.

Among the diseases some are typically severe and generally fatal infections like communicable disease, endocarditis, tract infection, septicemia, epidemic diarrhea of adults and youngsters and yolk sac infection, omphalitis, cellulitis, swollen head syndrome, coligranuloma and colibacillosisn (18).

Enteritis caused by E. coli (colibacilliosis) could even be a vital sickness inside the poultry trade as a results of increased mortality and diminished performance. E. coli might even be variety one reason behind acute kidney disease in children (12). The ultimate goal of controlling food borne hazards is to cut back the danger of disease to consumers, and reduce the economic burden associated with food borne illness (20). This study was therefore designed to attain isolation, identification and antimicrobial susceptibility of E. coli and Salmonella spp. related to poultry in Vadodara, Gujarat (7).

Materials and Methods

Study area

The study was conducted in two different commercial poultry farm close to Padra village in Vadodara, Gujarat, India.

Sample collection

Sample of unpurified water from feeding operation was collected from two commercial poultry farm. Samples were obtained in sterilized baggage, glass bottles and properly labeled.

Chemicals used

Peptone, Luria Bertani broth, Brilliant green agar, Eosin methylene blue agar, nutrient agar, Nutrient broth, Nutrient agar, Crystal violet, Iodine, Alcohol, Safranin, Antibiotics (Gentamicin, Streptomycin, Chloramphenicol, Ampicillin and Penicillin), Tryptophan broth, Urease broth, Triple sugar iron agar, Simmon citrate agar, Kovac's reagent, Methyl Red Voges Proskauer broth, Methyl red reagent, Hydrogen peroxide, Distilled water.

Apparatus used

Autoclave, Laminar airflow, Incubator, Petri dishes, Conical flasks, Test tubes, glass slides, pipettes, Microscope, weigh machine

Isolation method

Non-selective pre-selective enrichment: The water sample obtained from feeding operation were pre-enriched in 225 ml buffered peptone water (BPW, Oxoid) and incubated at 37°C for 24 h.

Selective- enrichment: 10ml BPW pre-enrichment step transferred to 100ml Luria Bertani broth (LB broth) for selective enrichment, and incubated at 37°C for 24 h

Serial six-fold dilution in peptone water was prepared (10^{-1} to 10^{-6}) for test samples. All test tubes and pipette were autoclaved before their utilization and were corked properly, labeled.

Selective agar plating: 0.5 ml solution of 10^{-6} tube was taken on brilliant green Agar plate (BGA) and MacConkey agar plate. Spread out using glass spreader and Plates were labelled, inverted and incubated overnight (18–24 hours) at 37°C.

Sub-cultivation: a 10 µl wire loop was used to pick a suspect colony that caused the colour of the medium to vary from yellow to red/ pink were sub-cultivated unto nutrient agar and incubated overnight (18–24 hours) at 37°C.

On removal from the incubator, plates were stored in a refrigerator at 4°C, ready for biochemical analysis and gram staining (1).

Microbiological and biochemical characteristic of isolated bacteria

The Gram staining was performed to observe its cellular morphology and gram nature of the bacteria. The biochemical characterization of the strains were also performed. Test like Catalase test, sugar fermentation using Triple sugar iron test, Indole test, Citrate test, Urease test and Methyl red test were performed (1).

Antibiotic sensitivity test for isolates

Antibiotic sensitivity tests were applied on the various bacteria isolated. This was done using the disc diffusion technique as explained by *Kirby-Bauer*, the in-vitro antibiotic testing of bacterium was done on Nutrient agar, 0.1ml smeared on the surface of the medium before putting the antibiotic discs and incubating for 24 hours before taking the readings of the diameter zones of inhibition using a millimetre rule (7).

Results and Discussion

The water from the feeding operations for poultry is an excellent source of harmful bacteria. In this study we have studied bacterial population in water from feeding operations.

For characterized bacterial isolation gram staining and biochemical test were performed. Biochemical test were performed such as urease test, methyl red test, catalase test, Indole test, Citrate test, triple sugar iron (Table 1).

Antibiotic susceptibility test

Antibiotic susceptibility test (Sample 1)

The antibiotic sensitivity test for Salmonella in Sample 1, resulted that the bacteria is sensitive to Gentamicin, Streptomycin, Chloramphenicol with a sensitivity zone of 7mm, 6mm, 7mm respectively and resistance to Ampicillin and Kanamycin (no zone)

For E. coli in Sample 1, test resulted that the bacteria is sensitive to Gentamicin, Streptomycin, Chloramphenicol with a sensitivity zone of 7mm, 5mm, 2mm respectively and resistance to Ampicillin (no zone)

Antibiotic susceptibility test (Sample 2)

The antibiotic sensitivity test for Salmonella in Sample 2, resulted that the bacteria is sensitive to Gentamicin, Streptomycin, Chloramphenicol with a sensitivity zone of 8mm, 7mm, 5mm respectively and resistance to Ampicillin and Penicillin (no zone)

For E. coli in Sample 2, test resulted that the bacteria is sensitive to Gentamicin, Streptomycin, Chloramphenicol with a sensitivity zone of 7mm, 8mm, 3mm, respectively and resistance to Ampicillin and Penicillin (no zone)

The present study indicates that *Salmonella* and *E. coli* bacteria are present within the water samples collected from poultry farms almost Padra village, Vadodara, Gujarat, India. This could be due to the activities of humans, soiled contamination, and conjointly the ability of the organism to survive throughout a large selection of habitats. The water provided to chickens are involved in the spread of *Salmonella sp*. among poultry and potential transmission to humans. The presence of these microorganisms within the poultry feeds suggested poor sanitary.

The presence of these two organisms (*E. coli and Salmonella typhi*) demonstrated a possible health risk as a results of the organisms are infective and can cause ill health in humans and animals; though *S. Typhi* might be a strict human organism and has not been according to cause ill health in animals. The water samples for *Salmonella and E. coli revealed that*, the sensitivity zone was shaped for Gentamycin, streptomycin and chloramphenicol with resistance to ampicillin and penicillin. The organisms isolated throughout this study were most in danger of gentamicin, streptomycin and chloramphenicol. This will be often altogether chance because of less usage of these antibiotics in feeds leading to less exposure of the organism to the antibiotics. Additionally, the drugs are disinfectant in their modes of action. Variety of those bacterial contaminants will grow or survive throughout food process and storage. Food contamination with these pathogens will occur at multiple steps on the food chain, alongside production, processing, distribution, retail marketing, and handling or preparation. This study shows the importance of maintaining hygiene and sanitation to regulate the expansion of Salmonella and E. coli for public safety.

From the study we found bacteria which is harmful to the human. Results shows that there is lack of sanitization and proper hygiene management. The *Salmonella* and *E. coli* bacteria found in the water fed to the chickens makes them a carrier and then the bacteria are transferred to humans through the consumption of poultry meat. Various gastrointestinal diseases are caused in humans by the consumption of contaminated poultry meat. Poor management of farming, overcrowding, dirty sanitation environment, bad ventilation, poor feed quality and stress will cause chicken to infect with diseases. Antibiotics should be used carefully so that a bacterium doesn't become resistant to antibiotics. Therefore, the way to take effective measures to stop and control infectious diseases from chicken is the task. Hence, it is required to implement a stricter hygiene and sanitation standard in poultry farms. For further studies bacteriophage may be thought of for the reduction of contamination in poultry feeds. Bacteriophages are viruses that are specific obligate bacterial parasites and typically possess high specificity for one bacterial species. Generally, once the bacteriophage inserts its genome into the bacteria, it'll use the bacteria's replicating machinery to produce more phages that are released upon bacterial cell lysis.

Table.1 Biochemical characterization of *Salmonella* and *E.coli* from Water sample

Biochemical tests	Bacteria			
	Sample 1		Sample 2	
	Salmonella	*E. coli*	*Salmonella*	*E. coli*
Triple sugar agar test	K/A+ , H_2S +	K/A+,H_2S+	K/A+ , H_2S+	K/A+,H_2S+
Indole test	+	+	+	+
Citrate test	+	-	+	-
Urease test	-	-	-	-
Catalase test	+	+	+	+
Methyl red test	+	+	+	+

Notes: K, Alkali (red); A, Acid (yellow);
+, positive; - , negative;±, positive/negative

Antibiotic susceptibility test (Sample.1)

Table.1.1 *Salmonella*

Antibiotics (µg)	Zone measurement (mm)
Gentamicin (50)	7
Streptomycin (10)	6
Ampicillin (10)	0
Penicillin (2)	0
Chloramphenicol (50)	7

Table.1.2 *E. coli*

Antibiotics (µg)	Zone measurement (mm)
Gentamicin (50)	7
Streptomycin (10)	5
Ampicillin (10)	0
Penicillin (2)	0
Chloramphenicol (50)	2

Antibiotic susceptibility test (Sample.2)

Table.2.1 *Salmonella*

Antibiotics (µg)	Zone measurement (mm)
Gentamicin (50)	8
Streptomycin (10)	7
Ampicillin (10)	0
Penicillin (2)	0
Chloramphenicol (50)	5

Table.2.2 *E. coli*

Antibiotics (µg)	Zone measurement (mm)
Gentamicin (50)	7
Streptomycin (10)	8
Ampicillin (10)	0
Penicillin (2)	0
Chloramphenicol (50)	3

Fig.1 TSI test result

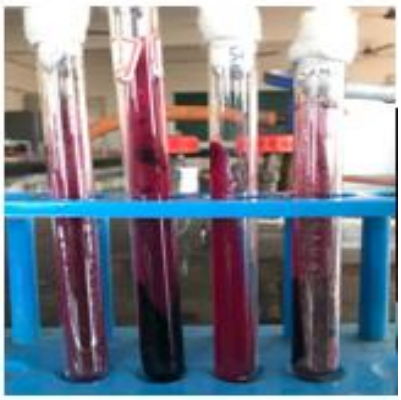


Fig.2 Indole test result

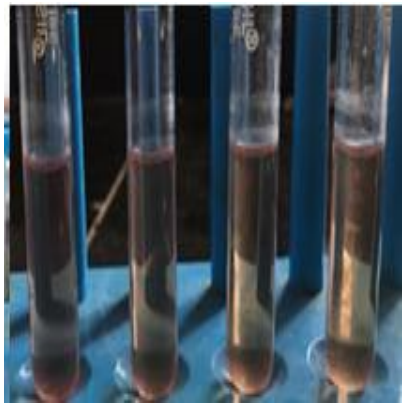


Fig.3 Citrate test result

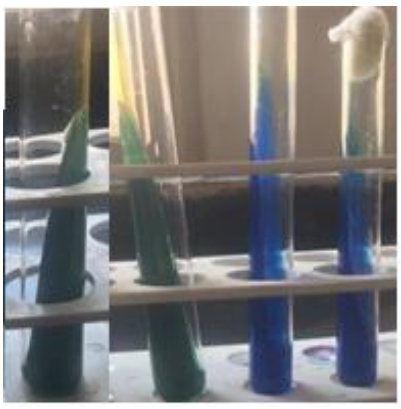


Fig.4 Urease test result

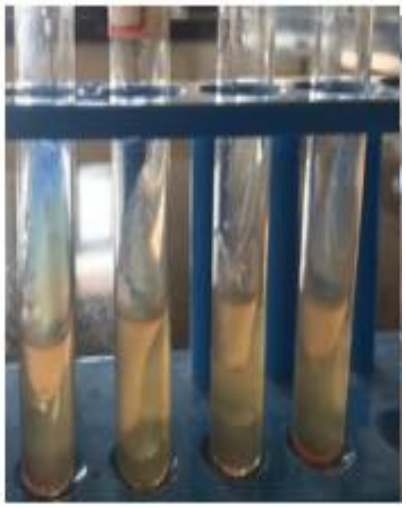
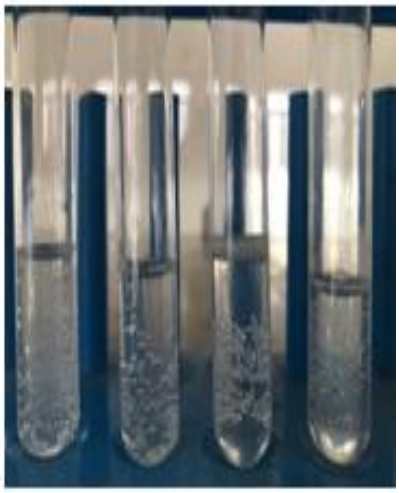


Fig.5 Catalase test result

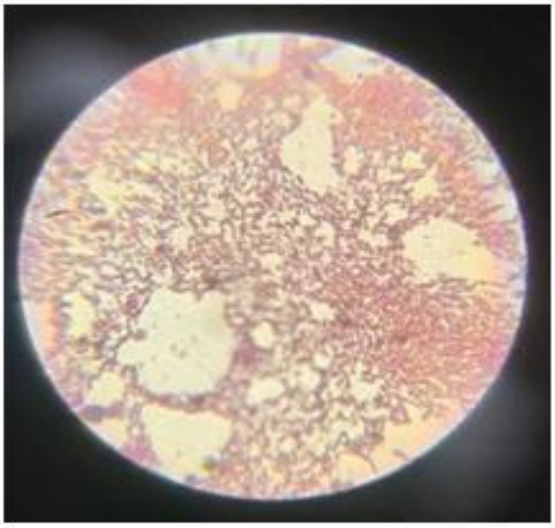
Fig.6 MR test result



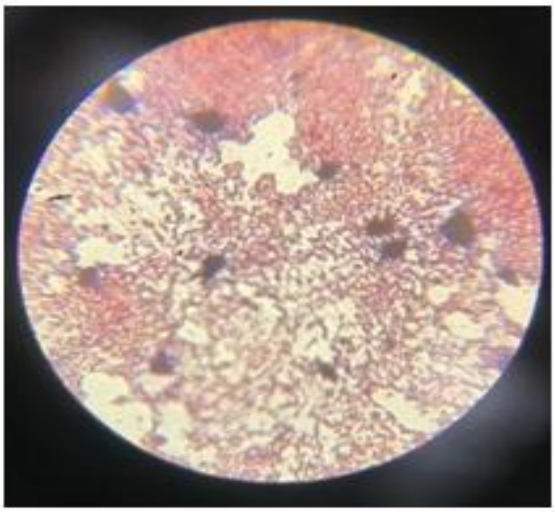
Gram staining

Sample.1

Salmonella

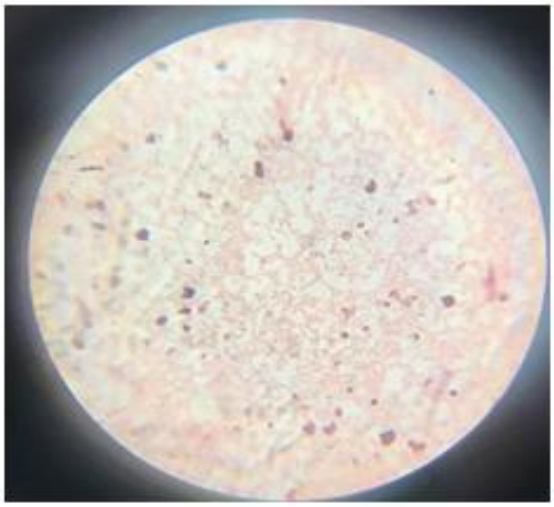
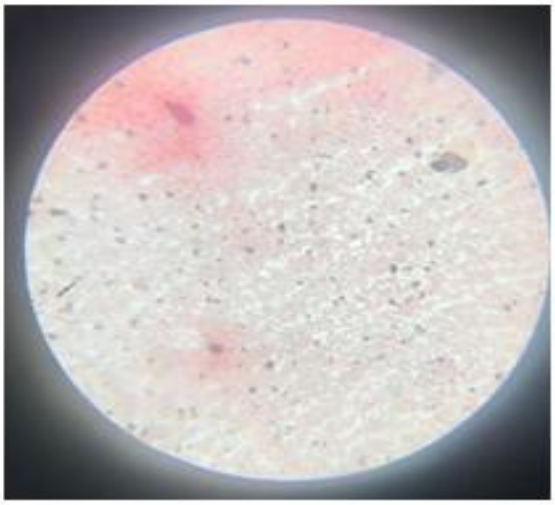


E. coli



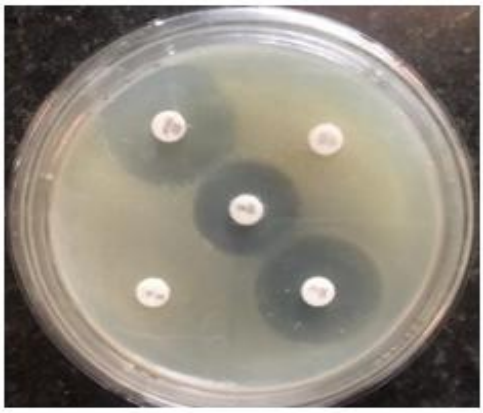
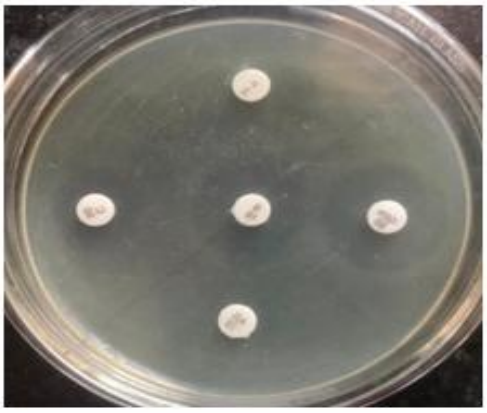
Sample.2

Salmonella ***E. coli***

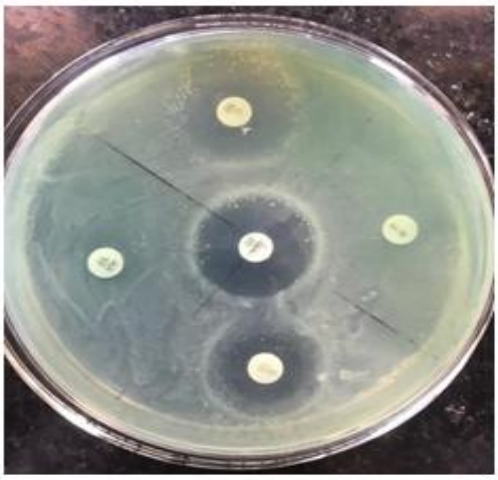
Antibiotic susceptibility test (Sample.1)

Salmonella ***E. coli***

Antibiotic susceptibility test (Sample.2)

Salmonella ***E. coli***



Lytic bacteriophages infect bacteria leading to rapid host death with minimal probability of phage transduction (Monk *et al.*, 2010). The virucidal impact of phage and also the pH of water needed for bacteriophage to survive may be further study area.

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