Research Article

Prevalence and Sequence Analysis of *E. coli* and *Shigella* Strains Isolated from Ostrich Feces

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Abstract | Important economic losses for both humans and animals have been attributed to *E. coli* and *Shigella*. Prevalence of pathogenic bacteria is a major threat to ostrich industry. Present study was carried out to evaluate the prevalence *E. coli* and *Shigella* in ostrich feces samples by morphological and molecular sequence analysis. *E. coli* and *Shigella* were isolated after the samples were inoculated on EMB and SS agar respectively. Molecular sequence analysis was performed to assess the similarities among the two species. Results determined that the prevalence of *E. coli* and *Shigella* was 50% in ostrich feces which means that half of the ostrich feces samples were found to be positive for *E. coli* and *Shigella*. It is therefore concluded that *E. coli* and *Shigella* were the most prevalent bacteria in ostrich feces, thus effective treatment strategies are recommended to minimize the risk of infection caused by these pathogens.

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1. Introduction

Ostrich is the largest living bird in the world. They have captured the attention of people since antiquity. The ostrich hen is slightly smaller than the adult male, which stands 2.4 meter tall and can weigh well over 100 kg. Ostriches are flightless birds due their large bodies and small wings, which prevent them from flying. They have two toes, a long neck and long, bare legs. When necessary, their powerful legs enable them to run at speeds of up to 70 km per hour with strides as long as 8 meters. The muscles in the neck and thighs are strong and unfeathered (Brassó *et al*., 2020; Cooper *et al*., 2010).

Diseases attributed to *E. coli* cause important economic losses for both humans and animals. The primary cause of shigellosis is *Shigella*, a bacterial agent from the Enterobacteriaceae family, which primarily affects children under the age of five (Hosangadi *et al*., 2017). Through the parallel acquisition of important virulence factors, such as the invasion plasmid pINV, *Shigella* has evolved from *Escherichia coli* to become pathogenic (Yang *et al*., 2005). Currently, there are 4 species of *Shigella* as a genus. More than

50% of all cases of shigellosis diagnosed in low- and lower middle-income countries are caused by *Shigella flexneri*, while *S. sonnei* is more common in high-income countries (Liang *et al*., 2022). In developing nations with poor sanitation, *Shigella* infection is a serious public health issue. Although other primates may also be infected, humans are the disease's natural reservoir. Although no naturally occurring food products contain endogenous *Shigella* species, many different foods could become contaminated. Shigellosis is transmitted through faeces and the mouth. Other means of transmission include contact with a contaminated object, fomites, ingestion of contaminated food or water (untreated wading pools, interactive water fountains), and specific forms of sexual contact. By moving infected faeces, vectors like houseflies can physically spread the illness.

Different bacterial agents are associated with high morbidity and mortality rates in ostriches and ostrich eggs (Hemmatinezhad *et al*., 2015). Young ostrich chicks may develop diarrhea due to enteritis caused by pathogenic strains of *E. coli*, *Pseudomonase*, *Salmonllae*, *Pasteurella* and *Klebsiella* (Farghaly and Erfan, 2012). A few particular strains, like avian pathogenic *E. coli* can result in colibacillosis in poultry which is treated and controlled with the help of antimicrobial agents (Salari *et al*., 2021; Hasani *et al*., 2017). The umbilicus can become infected with *E. coli* when it is not cleaned (Foggin, 1992). If an infection develops in the egg, very frail chicks hatch. An inflamed, reddened yolk sac with occasional strands of pus is one of the pathological symptoms. Ostrich faeces also contain other pathogens. Bacillosis (*E. coli* infection) spreads through contaminated faeces in the hatchery bedding or neonatal-chick house. Cloacal swabs are used to identify the pathogen (Tully *et al*., 1996). *Pasteurella multocida* bacterium causes pausterllosis that affects the air sac in ostriches (Huchzermeyer *et al*., 1997). Virulent *Shigella* can withstand the acidic gastric pH and is a very resilient pathogen (Nato *et al*., 2007; Niyogi, 2005; Jin *et al*., 2002).

2. Materials and Methods

2.1. Sampling

Ostrich faecal samples were obtained from the W. A. Apparel factory, which is a short distance from the ostriches' captivity in Youhanabad Lahore, Pakistan. The samples were taken in the early hours of the day. At the time of collection, the weather conditions such as temperature, rain, humidity, and wind were observed. The pickup time was 9:34 AM. The conditions were as follows; 17°C temperature, 0% precipitation, 61% humidity, and a 14 km/h wind. Faecal samples were collected from the surface layer (0–15 cm), which were then placed in sterile polythene bags and appropriately labelled. To isolate the bacteria, the faecal samples were brought to the lab.

2.2. Media preparation and sterilization

EMB media was prepared in a conical flask by dissolving 3.6g EMB agar in 100 ml distilled water. The prepared media was then placed in an autoclave at 121°C and 15 psi pressure. For the preparation of the SS media, 6.302 grams of SS agar and 100 ml of distilled water were allowed to stir continuously on a magnetic porcelain-top hot plate for 30 to 40 minutes.

Sample handling, microbial isolation and propagation were carried out in a laminar hood with extreme care taken to avoid any kind of contamination or cross-contamination by the test organisms. One hour before working in the Laminar Hood, the UV light was turned on. Glassware, including petri dishes was autoclaved for 20 minutes at 121°C and 15 psi pressure for sterilization.

2.3. Microbial isolation and identification

Using distilled water, 10g of faecal sample was serially diluted to a concentration of 10-6 while suspended in 90 ml of sterile, distilled water. 50µl of samples from test tubes labelled 10-2 and 10-4 were pipetted out using a micro-pipette following dilutions. Using a micro-pipette, 50µl of the samples were inoculated onto freshly made petri plates of EMB Agar and SS Agar. Plates were incubated at 37°C for 48 to 72 hours. Numerous bacterial colonies appeared on cultured plates.

Test organisms were transferred from pure cultures and kept in an aseptic environment under a laminar air cabinet. The bacteria are transferred via a loop to create new, pure cultures. For the best possible growth, the inoculated strains were then incubated for 24 hours at 37°C. The selected bacterial colony was picked and streaked using the standard streaking technique, and then incubated for 48–72 hours at 37 °C. *Shigella* and *E. coli* were identified from the isolated faecal strains by morphology. Pinkish colonies of *Shigella* on the SS media and green *E. coli* colonies with metallic sheen on EMB media were observed.

2.4. Molecular identification and sequencing

Purified strains cultured on petri plates were sent to Islamabad for molecular identification and sequencing. *Escherichia coli* and *Shigella spp*. both had their sequences analysed. Five out of the ten samples tested positive for *E. coli* and *Shigella*, the prevalence rate for both *Shigella* and *E. coli* was therefore 50%.

3. Results

Following study was conducted to find out the prevalence of bacterial *E. coli* and *Shigella* strains in ostrich feces. Ostrich feces samples were gathered. After the collection, the bacteria of interest were isolated. The bacteria were isolated on EMB and SS Agar. The specific colonies were streaked on petri plates and the streaking results are shown in figure 1 and figure 2.

Figure 1: Petri plate of EMB Agar showing bacterial growth after streaking at 37ºC after 24 hours

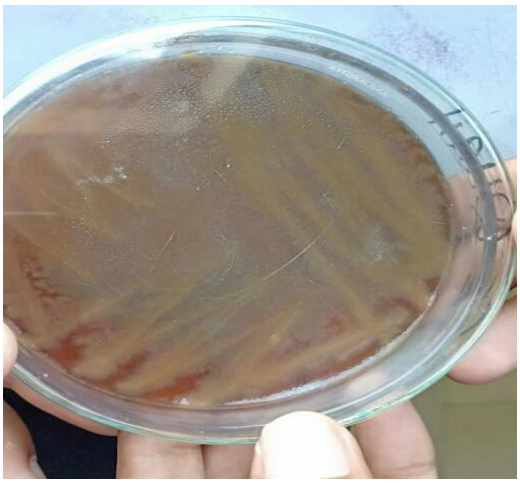

Figure 2: Petri plate of SS Agar showing bacterial growth after streaking at 37ºC after 24 hours.

For morphological identification of EMB isolates, EMB agar media was used and ostrich fecal samples were loaded after dilution. After the incubation period (24 hours), green colonies with metallic sheen were observed which indicated the presence of *E. coli*. For morphological identification of SS isolates, SS agar media was used and ostrich fecal samples were loaded after dilution. After incubation

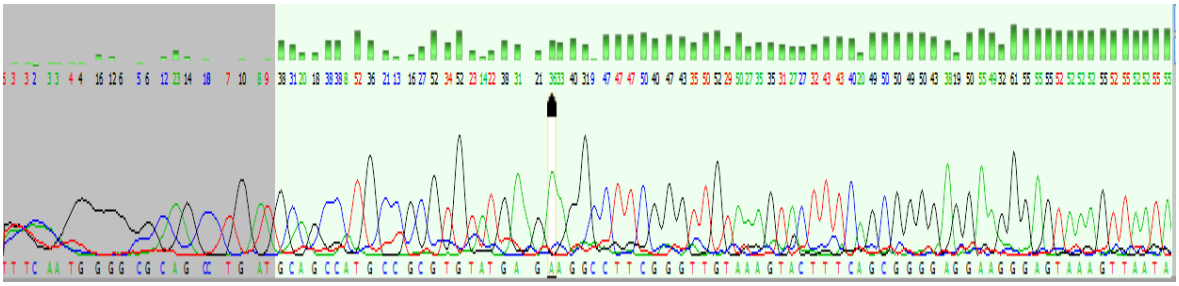

Figure 3: Sequencing results of *E.coli*

TTTCAATGGGGCGCAGCCTGATGCAGCCATGCCGCGTGTATGAGAAGGCCTTCGGGTTGT
AAAGTACTTTCAGCGGGGAGGAAGGGAGTAAAGTTAATACCTTTGCTCATTGACGTTACC
CGCAGAAGAAGCACCGGCTAACTCCGTGCCAGCAGCCGCGGTAATACGGAGGGTGCAAGC
GTTAATCGGAATTACTGGGCGTAAAGCGCACGCAGGCGGTTTGTTAAGTCAGATGTGAAA
TCCCCGGGCTCAACCTGGGAACTGCATCTGATACTGGCAAGCTTGAGTCTCGTAGAGGGG
GGTAGAATTCCAGGTGTAGCGGTGAAATGCGTAGAGATCTGGAGGAATACCGGTGGCGAA
GGCGGCCCCCTGGACGAAGACTGACGCTCAGGTGCGAAAGCGTGGGGAGCAAACAGGATT
AGATACCCGGGTAGTCCA

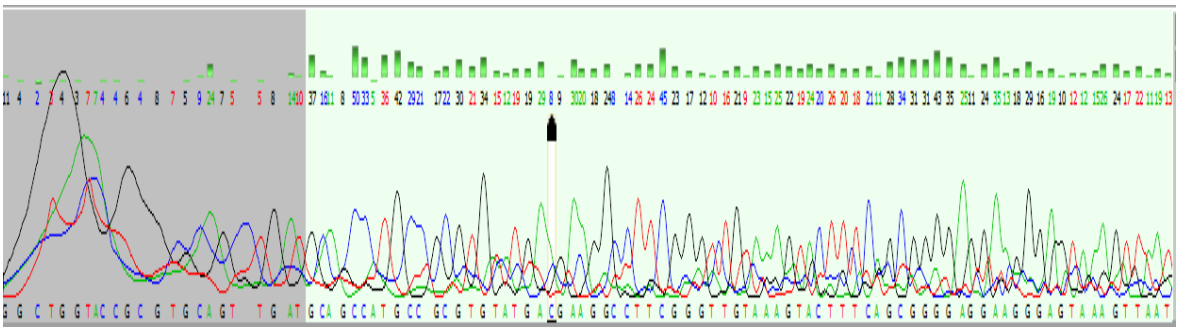

Figure 4: Sequencing results of *Shigella*

GGCTGGTACCGCGTGCAGTTGATGCAGCCATGCCGCGTGTATGACGAAGGCCTTCGGGTT
GTAAAGTACTTTCAGCGGGGAGGAAGGGAGTAAAGTTAATACCTTTGCTCATTGACGTTA
CCCGCACAAAAAGCACCGGCTAACTCCGTGCCAGCAGCCGCGGTAATACGGAGGGTGCAA
GCGTTAATCGGAATTACTGGGCGTAAAGCGCACGCAGGCGGTTTGTTAAGTCAGATGTGA
AATCCCCGGGCTCAACCTGGGAACTGCATCTGATACTGGCAAGCTTGAGTCTCGTAGAGG
GGGGTAGAATTCCAGGTGTAGCGGTGAAATGCGTAGAGATCTGGAGGAATACCGGTGGCG
AAGGCGGCCCCCTGGACGAAGACTGACGCTCAGGTGCGAAAGCGTGGGGAGCAAACAGGA
TTAGATACCCGCGGTAGTCCA

4. Discussion

period (24 hours), pinkish colonies were observed which indicated the presence of *Shigella*. For molecular identification, purified culture was sent to Alpha Genomics lab (Islamabad) for sequencing. The sequence obtained were blast using NCBI. Molecular identification showed 98% similarity to the *E. coli* while 95% for *Shigella* as shown in figure 3 and figure 4.

Consuming raw or undercooked poultry meats such as ostrich, turkey and quail poses a serious public health risk due to the high prevalence of Shiga toxin-producing *E. coli* (STEC) serogroups, virulence factors and their antibiotic resistance properties. The aim of earlier studies in the United States by Gallagher

et al., 2003 and Switzerland by Al-Saigh *et al*., 2004 was to provide a guideline for healthy foods with an animal origin. Similar studies have identified STEC strains as a pathogenic agent for human poisoning (Ranjbar *et al*., 2017; Beutin and Martin, 2012; Barkocy-Gallagher *et al*., 2003).

Current study aimed to assess the prevalence of bacteria such as *E. coli* and *Shigella* isolated from ostrich faeces. Results from present study showed that 50% of ostrich faecal samples contained *E. coli* and *Shigella* species. In a previously reported study, *E. coli* strains were found in meat samples from quail, turkey and ostrich 27.77%, 23.52%, and 9.33%, respectively (Hemmatinezhad *et al*., 2015). Significant variations in the prevalence of *E. coli* may be linked to variations in the types of meat samples tested (chicken, turkey, quail, ostrich, etc), the final acidic pH of the meat samples, the nutritional dietary and maintenance conditions of various birds, the quantity of samples, the sampling technique, the experimental methodology, the geographic location and climate variations in the areas where the samples were collected, which would have varied between each study (Momtaz and Jamshidi, 2013).

In present study molecular identification showed 98% similarity to *E. coli* while 95% for *Shigella*. Based on the highly conserved 16S rRNA gene, Kabali *et al*., 2021 stated that the clustering on phylogenetic analysis implied that the examined sections of the 16S rRNA gene in both *E. coli* and *Shigella* bacteria were very similar to each other and demonstrated how closely related some *Shigella* species are to the *E. coli* (Yang *et al*., 2007).

5. Conclusion

It was concluded that *E. coli* and *Shigella* were the most prevalent bacteria in ostrich feces. Hence, effective treatment strategies are needed to minimize the risk of infection caused by these pathogens. Molecular identification of isolated samples showed 98% similarity to the *E. coli* while 95% for *Shigella*.

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7. Author's Contribution

All authors contributed equally.

8. Conflict of Interest

The authors declare no conflict of interest.

9. Novelty Statement

Isolation and identification of pathogenic bacterial agents isolated from ostrich feces can be used for veterinary intervention and consequently efficient poultry production.

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