IJMAAS International Journal of Microbiology and Applied Sciences
Volume 2 May, 2023
Research Article

Analysis of Virulence Traits in *Escherichia coli* from Human and Poultry Faeces

Abeni Barira Azeez*., Frank-Peterside, Nnenna., and Otokunefor, Kome
Address: Department of Microbiology, Faculty of Science,
University of Port Harcourt, Nigeria.
*** Corresponding Author:** *prettybarrie@gmail.com*

ABSTRACT

Extraintestinal pathogenic *Escherichia coli* (ExPEC) strains are significant pathogens that cause a variety of illnesses in humans and livestock. It is widely assumed that *E. coli* isolates from poultry faeces include ExPEC-associated virulence genes and hence appear potentially dangerous. They might potentially be transferred to people by handling and/or consumption of contaminated meat. The current study was carried out to investigate the prevalence of *Escherichia coli* isolated from fifty (50) poultry faeces and 50 faeces samples of poultry workers from a poultry farm in Choba, Rivers State, Nigeria as well as to detect and compare the outcomes of five *E. coli* virulence features in both sources using phenotypic assays. Biochemical analysis found 57 *E. coli* isolated from 100 faecal samples of poultry and poultry workers. Confirmed isolates were subjected to virulence characterization using phenotypic assays and virulence results from both sources were compared. The study found the highest incidence of *E. coli* in human faeces (poultry workers/consumers) (62%), the lowest in poultry faeces (52%), and an overall *E. coli* prevalence of 57%. The poultry employees had a higher frequency of distribution of virulence features such as protease, colicin, and hemolysin than poultry faeces. None of the isolates tested positive for haemagglutination. It was discovered that human *E. coli* isolates have stronger virulence characteristics than poultry isolates. These data supported the concept that the more an organism's virulence characteristics, the greater its pathogenicity. Finally, the findings demonstrated the diversity of *E. coli* isolates but did not support the likelihood of zoonotic transmission.

Keyword: Faeces, poultry, poultry workers, phenotypic assays, *E. coli*, virulence features.

Introduction

The main and secondary habitats of *Escherichia coli* are the gastrointestinal tracts of warm-blooded organisms and the surrounding environment. In chicken, as in humans, *E. coli* colonises the lower digestive tract within the first 24 hours of laying or hatching (Ballou *et al*., 2016). *Escherichia coli* are mostly harmless and can be found in the regular flora of people and animals. It is, nevertheless, an opportunistic pathogen. This is because certain commensal bacteria develop virulence and thus become pathogenic. These virulence characteristics are traits that give pathogenic ability to produce illness states, and they are uncommon among commensal isolates. These virulence factors are required for infectious microbes to penetrate and avoid the body's tissues and immune systems reactions. They include hemolysin, which aids in the degeneration of red blood cells, colicin, which acts as an antibacterial to prevent other bacteria from colonising its host, adhesin, which aids in the binding and propagation of bacteria that are pathogenic to host cells, CNF1, which is accountable for apoptosis/programmed cell suicide (Soltani *et al*., 2018), and papC, which is an important element in the formation of the fimbriae (Samei *et al*., 2016).

Toxins, adhesins, and siderophores are the three types of virulence traits. Commensal *E. coli* that gains multiple virulence genes may result in significant illnesses of public health relevance when combined. Based on the foregoing, pathogenic infections caused by *E. coli* can be divided into intestinal (or diarrheagenic) and extra-intestinal *E. coli* infections (Ssekatawa *et al*., 2020).

Citation: Abeni *et al*. (2023). Analysis of virulence traits in *Escherichia coli* from human and poultry faeces *International Journal of Microbiology and Applied Sciences*. *2*: 121 - 127.

Diarrheagenic pathotypes (DEC) are a kind of *E. coli* with unique virulence characteristics. They are involved in the majority of *E. coli* infections of the gastrointestinal system, including diarrhoea. Furthermore, DEC strains account for the majority of diarrhoea cases, which are the primary causes of death and disease in children under the age of five in underdeveloped countries. obtaining communal immunity against DEC among youngsters is difficult due to the diversity of pathotypes and, by extension, variations. Extraintestinal virulent *Escherichia coli* (ExPEC) strains, on the other hand, constitute major pathogens that cause a variety of diseases in people and livestock. Some *E. coli* specimens from chicken faeces include ExPEC-associated virulence genes, making them potentially harmful; they could be transferred to humans by contaminated meat handling and/or eating. Extra-intestinal pathogens are the primary cause of all *E. coli* infections that develop outside the gastrointestinal system in humans (Daga *et al*., 2019). This includes, among other things, infections of the urinary system (UTI), pneumonia, meningitis, and septicemia (Ssekatawa *et a*l., 2020). Additionally, *Escherichia coli* is the major culprit in both community and hospital acquired UTI worldwide. As a result, the purpose of this study was to identify along with compare the distribution of *E. coli* and its detection of five virulence features in chicken faeces and stools of individuals who consume/handle poultry.

Materials and Methods

Study design, site and source of bacteria isolates

This was a cross-sectional laboratory study conducted at the Microbiology Laboratory of the University of Port Harcourt in Rivers State, Nigeria. The study was a comparative investigation to identify and distinguish between the presence of *E. coli* and five of its virulence features in chicken faeces and faeces of people who work on a poultry farm/ handle and also consume poultry products. All human and poultry faeces samples were collected and evaluated while still fresh. A total of 100 samples were obtained from poultry excrement and handlers/customers at a large poultry farm in Choba, Rivers State, Nigeria. Faecal swabs were collected from 50 broiler chickens to make one sample. Additionally, faecal sample of farm workers and some of its customers willing to be enrolled (n = 50) were collected from the farms. For sample collection, faecal swabs of both poultry and humans were collected using sterile swab sticks dipped in 1 ml Buffered Peptone Water (BPW).

Isolation and Phenotypic Assay of *E. coli* from Poultry and Human Faeces

Using conventional microbiological protocols, samples were pre-enriched with 1 ml sterile BPW before being transferred to nutrition broth (NB) and cultured for 24 hours at 37°C. After that, the culture was streaked onto Eosin methylene blue agar and incubated at 37°C for 24 hours. Three putative *E. coli* colonies were then subcultured to obtain pure culture, and Gram staining was used to identify them. Following that, biochemical assays such as catalase, oxidase, indole, methyl red, the Voges-Proskauer test, and sugar fermentation using triple sugar iron agar were performed. Following biochemical confirmation, isolates were submitted to 5 phenotypic assays to determine the presence of virulence characteristics. The phenotypic assays used in this study were colicin, curli fimbriae, hemolysin, haemagglutination, and protease. The chi-square test was used to compare associations between phenotypic virulence indicators and sample source, and a P-value of 0.05 was judged statistically significant.

Phenotypic Virulence Detection in *E. coli* Isolates derived from human and poultry faeces

The following virulence characteristics were determined phenotypically: Curli fimbriae expression was assessed by cultivating the tested isolates on agar plates comprising 0.1% tryptone, 0.05% yeast extract, 0.002% Coomassie brilliant blue, 0.004% Congo red, and 1.5% agar. Curli production was identified by the detection of red colonies, whereas white colonies were curli negative. Isolates were evaluated for hemolysis by inoculating them into 5% sheep blood agar plates. Hemolysin production is shown by clear zones around colonies. Protease testing was performed on the tested isolates by cultivating them on skim milk agar, and the appearance of clear zones surrounding colonies is regarded positive. The haemagglutination test was performed by combining one drop of blood group "O" with a drop of bacterial cultures on a slide, then rotating the slide for 5 minutes at 37 degrees Celsius. Haemagglutination was identified as the formation of clumps (Abd El-Baky *et al*., 2020). Total colicin production was determined using the double-agar diffusion method on Trypticase soy agar.

Citation: Abeni *et al*. (2023). Analysis of virulence traits in *Escherichia coli* from human and poultry faeces *International Journal of Microbiology and Applied Sciences*. *2*: 121 - 127.

After an overnight incubation at 37°C, the clearance zone diameters of the *E. coli* K-12 indicator strain 6092 (Mellata *et al*., 2009) were measured.

Statistical analysis

The chi-square test or Fisher's exact test was used to compare the frequencies of dispersion of phenotypic traits between *E. coli* isolated from human faeces and poultry faeces. If the P-value is less than 0.05, it is considered.

Results

The results of the frequency of *E. coli* in human faeces, chicken litter, and combined, the total prevalence of *E. coli* in human and poultry faeces are shown in Figure 1, Figure 2, and Figure 3 respectively. Following *E. coli* verification using traditional biochemical assays, 57 isolates (31 from human faeces and 26 from poultry litters) were confirmed as *E. coli*. According to the findings, the rate of *E. coli* in human faeces was 62% (Fig. 1), while it was 52% in chicken litter (Fig. 2). When all sample sources were combined, the total prevalence of *E. coli* in the research was 57% (Fig. 3).

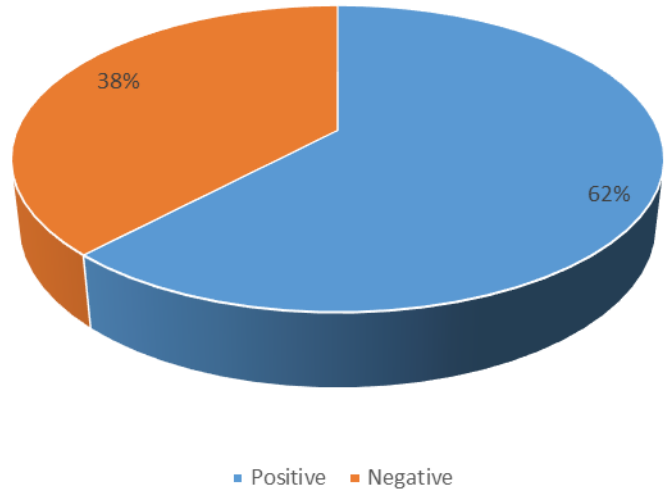

Fig. 1: Total prevalence of *E. coli* in human faeces

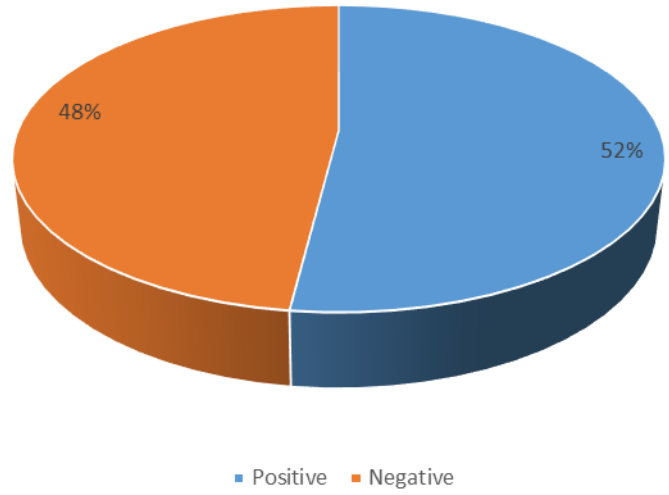

Fig. 2: *E. coli* prevalence in poultry faeces

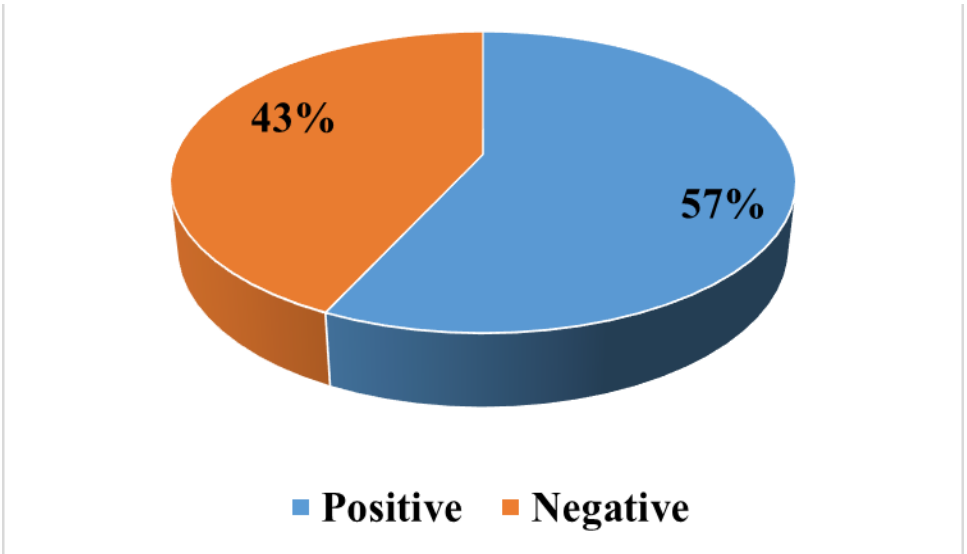

Figure 3: Overall prevalence of *E. coli* in poultry and human faeces

Citation: Abeni *et al*. (2023). Analysis of virulence traits in *Escherichia coli* from human and poultry faeces *International Journal of Microbiology and Applied Sciences*. *2*: 121 - 127.

The results of the virulence attributes in samples of human faeces, and in samples of poultry litter are shown in Figure 4 and Figure 5 respectively. While the results of the comparison of the virulence frequencies from the numerous phenotypic in both faeces human and poultry is shown in Figure 6.

According to the results of the numerous phenotypic tests used to determine virulence attributes in both samples, human faeces had a greater virulence trait for colicin and a lower trait for hemolysin (Fig 4). Poultry litter, on the other hand, has more curli fimbriae virulence and less proteas *E. coli*cin, hemolysin, and haemagglutination traits were not seen (Fig. 5). When the virulence frequencies from the numerous phenotypic tests done in both samples were compared, it was discovered that human faeces exhibited higher virulence features than poultry litters (Fig. 6).

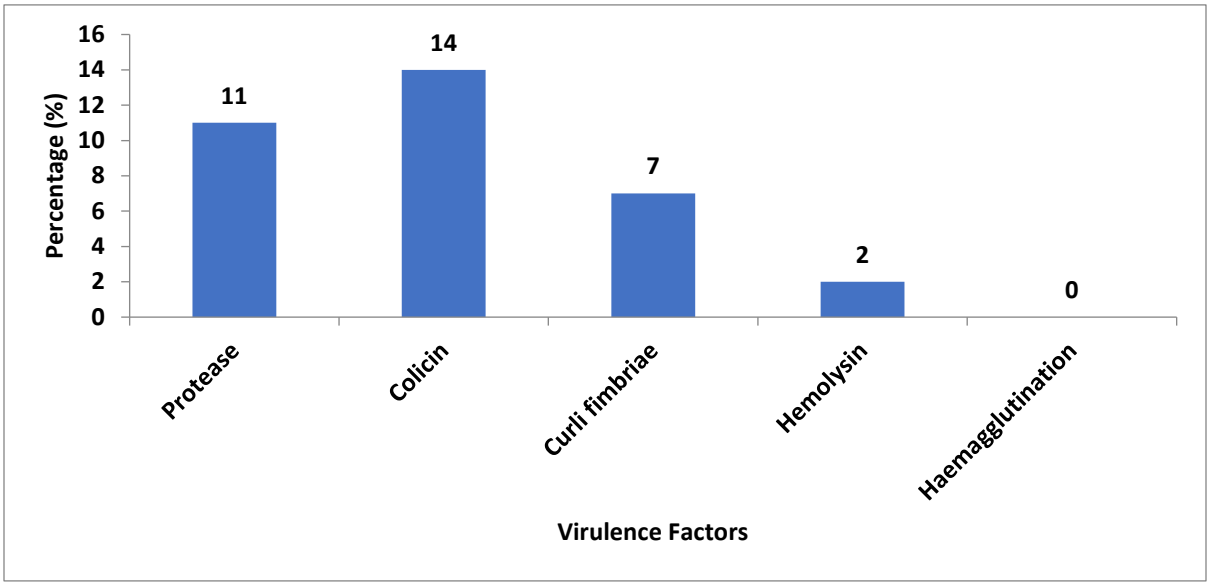

Figure 4: Distribution of phenotypic virulence traits of *E. coli* from human faeces in the study

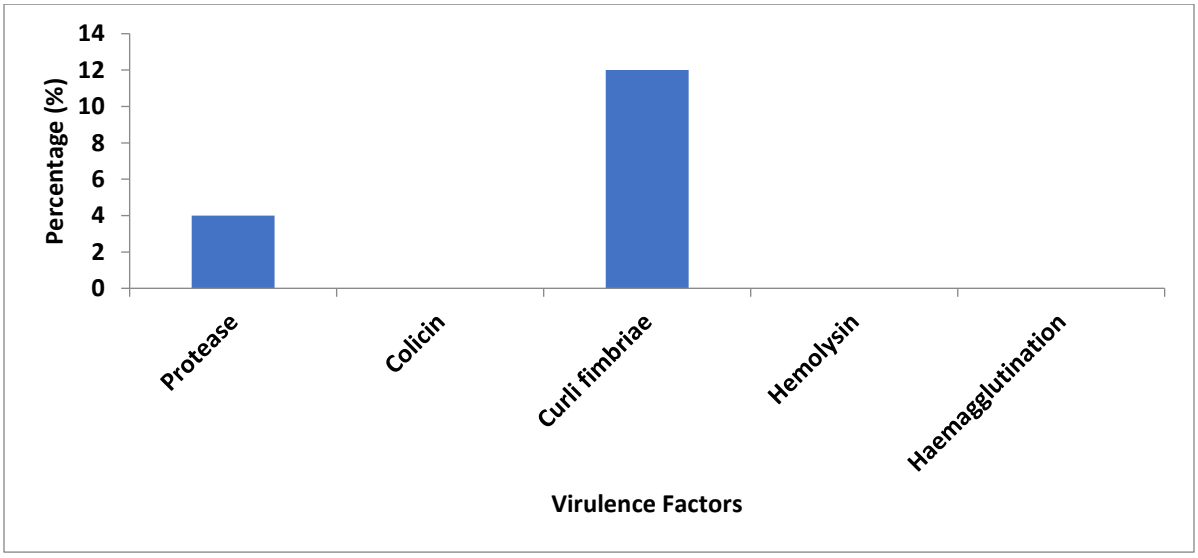

Figure 5: Distribution of phenotypic virulence traits of *E. coli* from poultry faeces in the study

Citation: Abeni *et al*. (2023). Analysis of virulence traits in *Escherichia coli* from human and poultry faeces *International Journal of Microbiology and Applied Sciences*. *2*: 121 - 127.

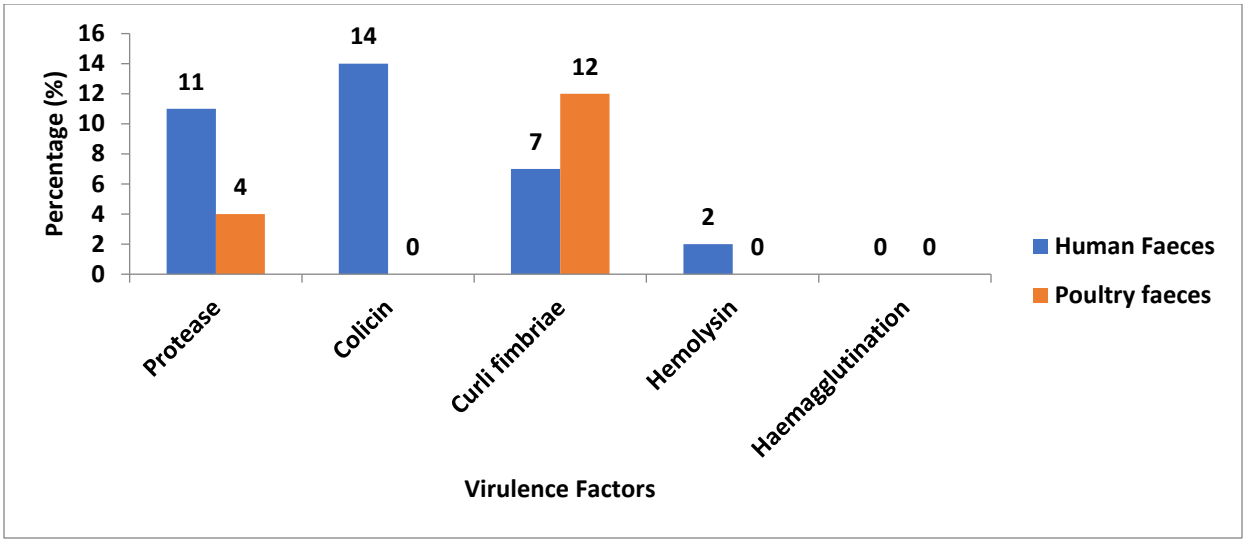

Figure 6: Comparison of phenotypic virulence traits in *E. coli* between human faeces and poultry faeces

Discussion

Virulence factors are required to get past the immune system's defences and increase bacteria's ability to live under harsh environments (Beceiro *et al*., 2013). The existence of virulent genes in the environment is a new concern in public health around the world, and the growing incidence of these genes poses an immediate threat to public health because they are transferrable in the environment. In this investigation, which included 100 samples taken equally from human and poultry poo, human faeces had a 62% prevalence. This is slightly lower than the 78% *E. coli* prevalence reported by Perewari *et al*. (2022). Singh et al. (2016) reported a similar prevalence to that seen in this study, but Mapanguy *et al*. (2019) reported a lower rate of 51%.

While human faeces had an *E. coli* prevalence of 62% in our study, poultry faeces had a lesser *E. coli* occurrence of 52%. Perewari et al., 2022 and Al-Bahry *et al*., (2016) showed far lower percentages, with 44% and 23.7% prevalence, respectively. Given the fact that Abd El Tawab *et al*. (2015) found a greater incidence of *E. coli* in poultry samples (72%), this suggests that poultry could function as reservoirs that encourage the transfer of virulence determinants to humans through ingestion.

In this investigation, an overall *E. coli* prevalence of 57% was found. This *E. coli* prevalence is comparable to other research, which found rates of prevalence that varied between 48% to 100% (Sarker *et al*., 2019). However, Jakaria *et al*. (2012) and Bashar *et al*. (2011) found 82% and 100% prevalence of *E. coli*, respectively. Other research, however, revealed a substantially lower occurrence rate of 26.8% (Enany et al., 2019). Amit *et al*. (2021) and Kwoji *et al*. (2019) found an overall prevalence percentage of 76% and 67.8% in *E. coli* cultured from humans and animals, respectively.

Virulence factors needed to conquer the human immune response and improve the bacteria's ability to flourish and persist in hostile environments with low resources (Beceiro *et al*. 2013). The presence of numerous virulence factors in our isolates may worsen this problem. Surprisingly, a great deal of the factors associated with virulence were found in human excrement rather than poultry poo. Our findings revealed a high level of variety among and within human isolates. Our findings contradict those of Natallie *et al*. (2015), who discovered a greater virulence predominance of *E. coli* in poultry faeces and speculated on zoonotic transmission of this virulent bacteria. Brink *et al*. (2015) discovered greater levels of colicin-producing *Escherichia coli* strains among poultry employees than in poultry or poultry processing industries. Bray *et al*. (2016) discovered that Salmonella enterica isolated from humans has more curli fimbriae than Salmonella enterica isolated from chickens. The researchers provided many explanations for this observation. One theory is that human hosts provide a more pleasant atmosphere for the development of bacteria with curli fimbriae than poultry hosts. Another idea is that curli fimbriae aids *Salmonella enterica* infecting people.

Citation: Abeni *et al*. (2023). Analysis of virulence traits in *Escherichia coli* from human and poultry faeces *International Journal of Microbiology and Applied Sciences*. *2*: 121 - 127.

More research is needed to confirm the findings and clarify the significance of curli fimbriae in Salmonella enterica infections, according to the researchers.

In generally, the increased incidence of *E. coli* virulence features in human faeces among poultry farm employees and consumers can be attributed to the fact that people are more vulnerable to specific pathogens than chickens. Humans, for example, have a lengthier gastrointestinal system than chicken, which may make certain bacteria colonise the human gut more easily. Furthermore, humans' immunological responses to bacteria may differ from those of poultry, which may influence the predominance of particular virulence features. Furthermore, humans and poultry may have different cleanliness practises, which may alter the incidence of certain virulence features. Another explanation is that some virulence features are more advantageous to bacteria in a human host than in a chicken animal. A virulence characteristic that allows bacteria to elude the human immune system, for example, may not be as relevant in a fowl host. Furthermore, certain virulence features may aid bacteria in causing disease in humans but have no effect on poultry. When bacteria exist in a human host, they are more inclined to acquire and preserve these characteristics.

Finally, it's plausible that the bacteria are selecting for specific virulence features. Some bacteria, for example, can engage in horizontal gene transfer, which is the process by which bacteria acquire new genes from other bacteria. Certain virulence features may grow more frequent over time if they have a greater likelihood to be passed across bacteria. This could boost the incidence of certain characteristics in bacteria that infect humans. More research is needed, however, to completely understand the mechanisms underlying the differences in virulence features between humans and poultry. As a result, the results of our study did not support the likelihood of zoonotic transmission among our isolates. This outcome could be attributed to a lack of host adaptation, as well as the possibility of contamination between both hosts and the diverse character of the *E. coli* genome (Bendary *et al*., 2016).

In conclusion, the variability of human and poultry faecal isolates reported in this present study indicated the need for stringent regulated guidelines to avoid the pathogen's evolution of commensal *E. coli*.

Furthermore, due to the widespread expansion of virulence phenotypes, which raises the risk of more severe infections, novel alternative medicines are desperately needed. We discovered that human isolates had higher levels of virulence, whereas poultry isolates had lower levels of virulence. These data supported the concept that the higher an organism's virulence characteristics, the greater its pathogenicity. Finally, our findings indicated the diversity of *E. coli* isolates but did not support the likelihood of zoonotic transmission. As a result, additional research is required to obtain more information on the epidemiological characteristics of DEC isolates, identify potential sources of their infections, and apply control measures to prevent their spread.

Acknowledgement

We thank the University of Port Harcourt's Microbiology Laboratory in Rivers State, Nigeria for their technical assistance.

References

Abd El Tawab, A. A., Ammar, A. M., Nasef, S. A., and Reda, R. M. (2015). Prevalence of *E. coli* in diseased chickens with its antibiogram pattern. *Benha Veterinary Medical Journal*. *28(2):* 224-230.

Abd El-Baky, R. M., Ibrahim, R. A., Mohamed, D. S., Ahmed, E. F. and Hashem, Z. S. (2020). Prevalence of virulence genes and their association with antimicrobial resistance among pathogenic *E. coli* isolated from Egyptian patients with different clinical infections. *Infection and Drug Resistance*. *13:* 1221–1236.

Al-Bahry, S., Al-Sharji, N., Yaish, M., Al-Musharafi, S., and Mahmound, I. (2016). Diversity of Tetracycline Resistant Genes in *Escherichia coli* from Human and Environmental sources. *The open Biotechnology Journal*. *10:* 289-300.

Ballou, A. L., Ali, R. A., Mendoza, M. A., Ellis, J. C., Hassan, H. M., & Croom, W. J. (2016). Development of the chick microbiome: how early exposure influences future microbial diversity. *Front Vet Sci*. *3:* 2.

Beceiro, A., Tomás, M., and Bou, G. (2013). Antimicrobial resistance and virulence: A successful or deleterious association in the bacterial world? *Clin. Microbiol. Rev.*, 26: 185-230.

Citation: Abeni *et al*. (2023). Analysis of virulence traits in *Escherichia coli* from human and poultry faeces *International Journal of Microbiology and Applied Sciences*. *2*: 121 - 127.

Bendary, M. M., Solyman, S. M., Azab, M. M., Mahmoud, N. F., and Hanora, A. M. (2016). Characterization of Methicillin Resistant *S. aureus* isolated from human and animal samples in Egypt. *Cell. Mol. Biol. 20:* 112-117.

Bettelheim, K. A., and Lennox-King, S. M. (1976). The acquisition of *Escherichia coli* by new born babies. *Infection*. *4(3):* 174–179.

Bray, A. R., Calderwood, S. J., Magrini, R. L., Davis, L. S., *et al*. (2016). Comparative Genome Sequencing Identifies Lineage-Specific Features Linked to Host Adaptation and Virulence among Clinically Relevant *Salmonella enterica* Serovars. *Antimicrobial Agents and Chemotherapy*. *125(3):* 536-548.

Brink, D., Lawrence, E., Murray, M. M., Fonarow, P., Kay, J., Lore, C., Cannon, C., Angulo, M., and Mulvey, M. G. (2015). Higher Levels of Colicin-Producing *Escherichia coli* Strains among Poultry Workers Than in Poultry or Poultry Processing Plants. *Foodborne Pathogens and Disease*. *12(2)*: 103-111.

Daga, A. P., Koga, V. L., Soncini, J. G. M., De Matos, C. M., Perugini, M. R. E *et al*. (2019). *Escherichia coli* Bloodstream Infections in Patients at a University Hospital: Virulence Factors and Clinical Characteristics. *Frontiers in Cellular and Infectious Microbiology*. *9:* 191.

Enany, M. E., Algammal, A. M., Nasef, S. A., Abo-Eillil, S. A., Bin-Jumah, M., Taha, A. E., and Allam, A. A. (2019). The occurrence of the multidrug resistance (MDR) and the prevalence of virulence genes and

QACs resistance genes in *E. coli* isolated from environmental and avian sources. *AMB Express*. *9(1):* 1-9.

Kwoji, I. D., Musa, J. A., Daniel, N., Mohzo, D. L., Bitrus, A. A., Ojo, A. A., and Ezema, K. U. (2019). Extended-spectrum beta-lactamase-producing *Escherichia coli* in chickens from small-scale (backyard) poultry farms in Maiduguri, Nigeria. *Int. J. One Health*. *5*: 26-30.

Mellata, M., Touchman, J. W., and Curtiss, R. (2009). Full sequence and comparative analysis of the plasmid pAPEC-1 of avian pathogenic *E. coli* chi7122 (O78:K80:H9). *PLoS One*. *4: e4232.*

Natalie, M. M., James, R. J., Brian, J., Roy, C., and Melha, M. (2015). Zoonotic Potential of *Escherichia coli* Isolates from Retail Chicken Meat Products and Eggs. *Appl Environ Microbiology*, 81(3): 1177–1187.

Perewari, D. O., Otokunefor, K., and Agbagwa, O. E. (2022). Tetracycline-Resistant Genes in *Escherichia coli* from Clinical and Nonclinical Sources in Rivers State, Nigeria. *Int .J. Microbiol. 9192424*.

Samei, A., Haghi, F., and Zeighami, H. (2016). Distribution of pathogenicity island markers in commensal and uropathogenic *Escherichia coli* isolates. *Folia Microbiologica (Praha)*. *61(3)*: 261–8.

Sarker, M. S., Ahad, A., Ghosh, S. K., Mannan, M. S., Sen, A., Islam, S., Bayzid, M and Bupasha, Z. B. (2019). Antibiotic-resistant *Escherichia coli* in deer and nearby water sources at safari parks in Bangladesh. *Veterinary World*. *12*(*10)*: 1578.

Singh, N., Pattnaik, D., Neogi, D. K., Jena, J., and Mallick, B. (2016). Prevalence of ESBL in *Escherichia coli* isolates among ICU patients in a tertiary care hospital. *Journal of clinical and diagnostic research*. *10*(*9)*: DC19.

Soltani, S., Emamie, A. D., Dastranj, M., Farahani, A., Davoodabadi, A., and Mohajeri, P. (2018). Role of toxins of uropathogenic *Escherichia coli* in development of urinary tract infection. *British Journal of Pharmaceutical Research*. *21(1)*: e.JPRI.39188.

Ssekatawa, K., Denis, K., Byarugaba, Jesca, L. N., Charles, D. K., Francis, E., Robert, T., and Eddie, M. (2021). Carbapenem Resistance Profiles of Pathogenic *Escherichia coli* in Uganda. *European Journal of Biology and Biotechnology*. *2 (2)*: 171.

Citation: Abeni *et al*. (2023). Analysis of virulence traits in *Escherichia coli* from human and poultry faeces *International Journal of Microbiology and Applied Sciences*. *2*: 121 - 127.