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Original Research Article

Study of the Antimicrobial Resistance and Biofilm Formation of *Escherichia Coli* Isolated from Hens and Humans

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Abstract: *Escherichia coli* (*E. coli*) causes various infections in humans and animals. Animals and humans can get a variety of diseases from *E. coli*. Because of its tendency to build biofilms, Escherichia coli is more resistant to antibiotics and can cause chronic and recurrent infections. In connection to antibiotic resistance, this study assessed the biofilm-forming capacity of E. coli isolated from extra intestinal infections in humans and chickens. The numbers of total samples that isolated from humans 8(26.67%) and chickens 12(40%) were twenty and harvested to determine of the biofilm forming by the test tubes, congo red agar method, appear all E. coli isolated were positive to biofilm. The pattern of resistance of these strains mentioned that *E. coli* from chicken samples was resistant to Nalidixic acid (100%), Ciprofloxacin (58.4%), Amikacin (58.4%), Nitrofurantoin (58.4%) Cefixime (16.6%), Ceftazidime (25%), Tetracycline (25%) and Gentamicin (33.3%). E. coli from human clinical samples was resistant Amikacin100%, Nitrofurantoin100%, Nalidixic acid, Ciprofloxacin 62.5%, Tetracycline 62.5%, Cefixime 50%, Gentamicin 25% and Ceftazidime 12.5%. Additionally, both human and chiken samples frequenently contained multiresistanant *E.coli* isolates. According to the study's findings, hens may serve as reservoirs for the spread of microorganisms resistant to antibiotics to people. Moreover, any ability of *E. coli* to build biofilms can raise the isolates' profile of antibiotic resistance.

Keywords: Chickens; *E. coli*; biofilms; antibiotic resistance.

INTRODUCTION

E. coli was founds as a comment microbes in the humans and animals gastrointestinal tracts [1-3]. However, *E. coli* infectious pathologies can also function as a real or opportunistic pathogen, resulting in diseases like meningitis, peritonitis, and septicemia as well as intestinal, extraintestinal, urinary tract, and stomach infections [4]. The extra-intestinal pathogenic *E. coli* (ExPEC) subspecies Uropathogenic *Escherichia coli* (UPEC) is the main pathogen responsible for community-acquired (80–90%) and hospital-acquired (30–50%) urinary tract infections [5, 6]. One of these infectious agents is the gram-negative, facultative anaerobic bacteria *E. Coli*, which is a common intestine commensal bacterium found in both humans and animals [7, 8]. All systems in chickens are impacted by *E. Coli* infections, which lead to a complex condition with lesions in the digestive, respiratory, and reproductive tract, either on their own or in combination with other pathogens [9, 10]. According to earlier research, the extracellular matrix of biofilms helps *E. coli* cling to host cells, withstands the shear pressures brought on by urine flow, and adds to the bacteria's persistence and chronic infection [11, 12, 8]. The Antibiotic resistance genes in other dangerous *E. coli* strains were found to be activated by antibiotic-resistant variants of APEC strains, and it was discovered that these resistance genes may be easily transferred and disseminated between humans and animals [13, 14]. Investigating *E. coli* virulence genes, biofilm formation, antibiotic resistance, and phylogenetic groups is therefore essential for the therapeutic management of sheep. Previous research on bacterial isolates have demonstrated the connection between resistance genes and virulence factors in both human and veterinary medicine [15-17].

Finally, it should be noted that biofilm-forming E. Coli is more likely to exhibit antibiotic resistance, and

that poultry can potentially transmit these bacteria to humans.

METERIALS AND METHODS

Collection Samples

The numbers of samples were 60 spaceman from the humans and the avian that including 30 samples from human urine and 30 samples from liver of poultry, from December 2023 to March 2204 from Al-Qasim city, Babylon, Iraq. This poultry was suffer from signs of CRD, swabs were taken after postmortem and transferred to the microbiology laboratory for transplantation on the available transported culture media.

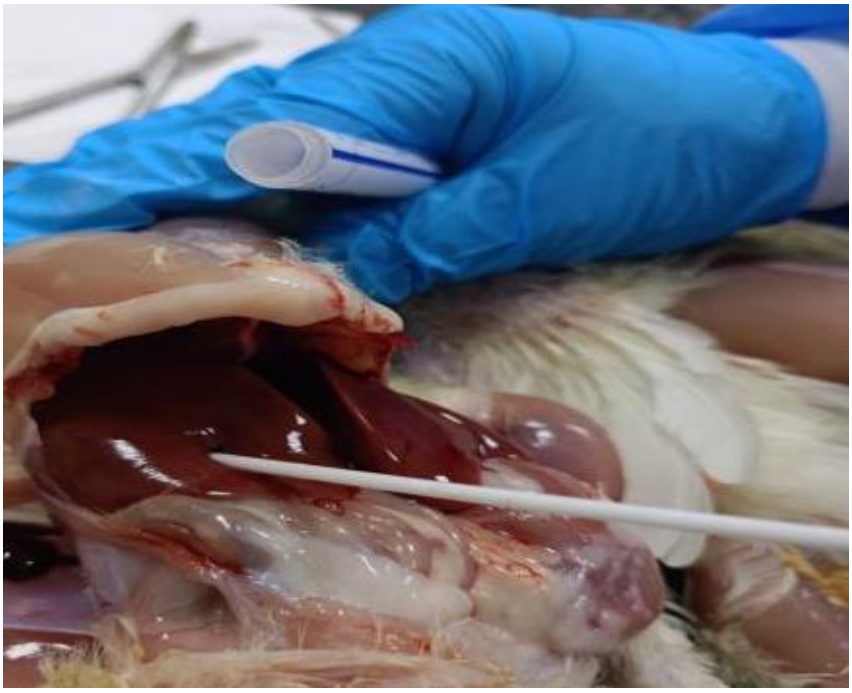

Fig 1: Samples collection

Bacteriology

All specimens were observed through macroscopic and microscopic analysis with gram staining [18]. They were later placed on MacConkey and Eosin methylene blue agar dishes and left to incubate at 37oC for 24 hours. Other bacteriological tests identified the colonies suspected to be E. coli [19-21].

DNA extraction:

E. Coli DNA was obtained in accordance with the PrestoTM Mini gDNA Bacteria Kit (Geneaid®, Taiwan) instructions. Using the Biodrop® (UK), the DNA concentration of E. coli was calculated and kept at -20°C.

PCR amplification:

Using the designated primer pairs, the target DNA was amplified by PCR. It involved three steps that were carried out over a predetermined number of cycles to produce an amplicon, or PCR result, that could be detected following agarose gel electrophoresis [22, 23].

RESULTS

Bacterial Identification

The results were shown in Table 1 indicated that growth was observed in 8(26.67%), 12(40%) *E.coli*, while 10(33.34%), 0(0%) *Staphylococcus aureus*, 7(23.33%), 13(43.34%) *Kellebsella spp*. and 5(16.66%), 5(16.65) *Pseudomonas aurogenosia* from60 total samples for human and hens respectively.

Table 1: Bacterial isolates of samples

Samples	Type of specimen	
	Human urine (No.30)	Chicken (liver No.30)
E.coli	8(26.67%)	12(40%)
Staphylococcus aureus	10(33.34%)	0(0%)
Kellebsella spp	7(23.33%)	13(43.34%)
Pseudomonas aurogenosia	5(16.66%)	5(16.66%)
Total	30(100%)	30(100%)

E.coli isolates are characterized by cultural, morphological and biochemical characteristics, as in (Fig 2).

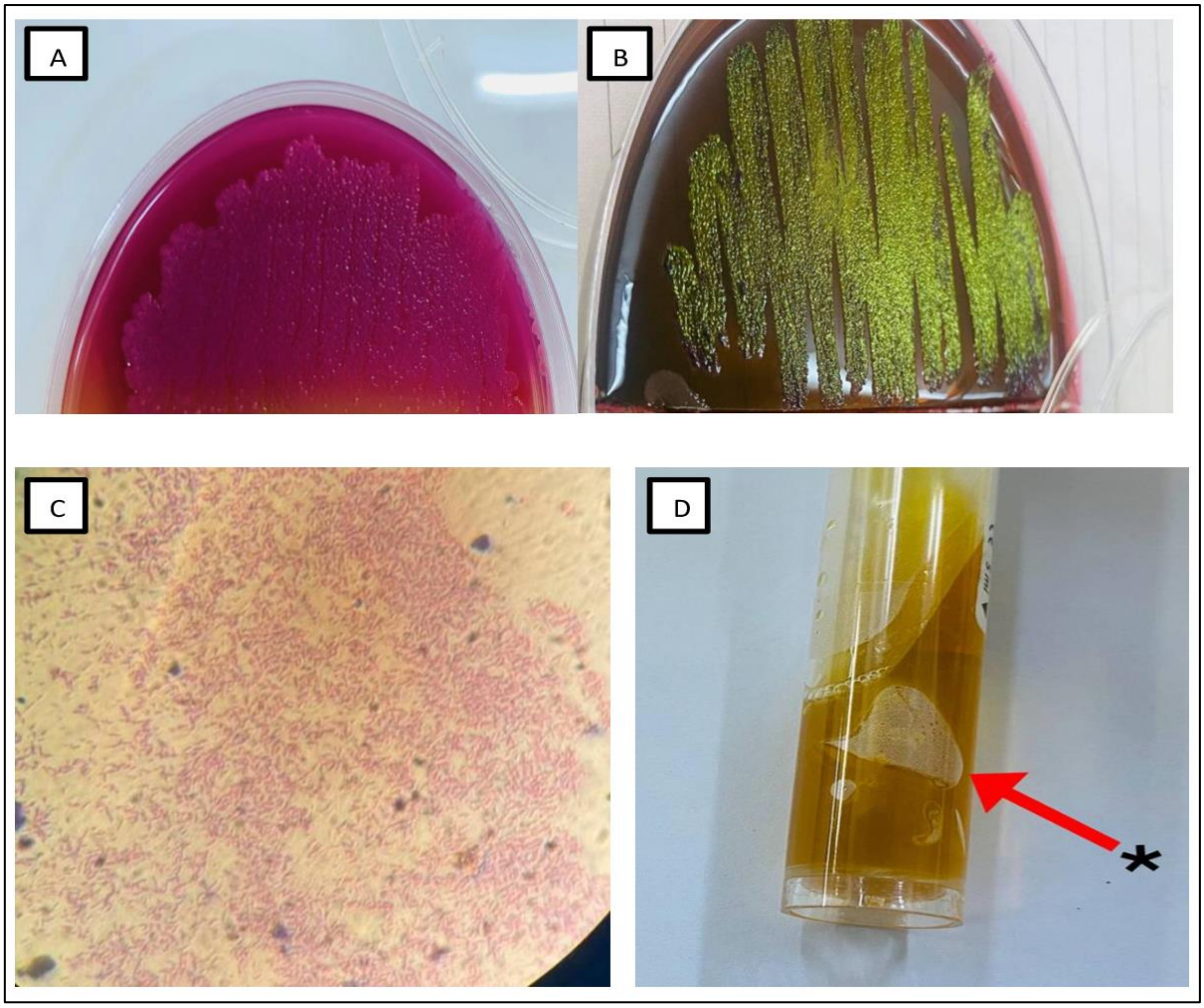

Fig 2: *E. coli* culture depiction. A) E. coli colonies display a distinctive metallic green sheen on Eosin methylene blue agar. B) Pink colonies of E. coli were observed on MacConkey Agar. C) Microscopic images of *E.coli,* Gram-negative rod-shaped bacteria. Gram-staining. Magnification: 10 × 100 immersion. D) *E. coli* on triple sugar iron (TSI) appear fermentation glucose, lactose and sucrose, produce gas.*

The current study's findings demonstrate that all positive *E. coli* isolates had bands with a molecular weight of 480 bp (Fig 3).

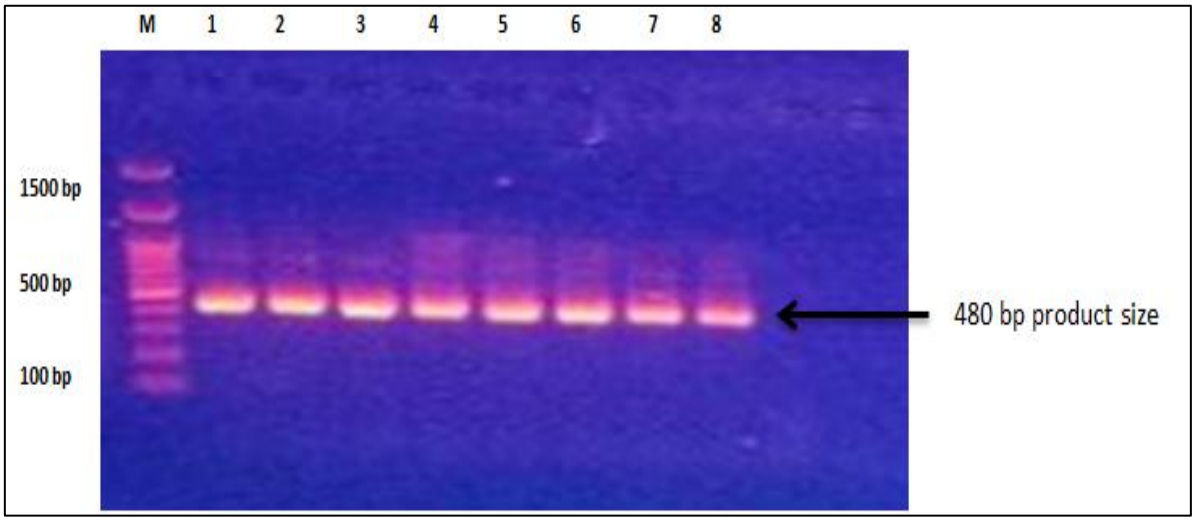

Fig 3: Molecular evidence of *E. coli* isolates according to the sequence of 16S rRNA (M: Ladder, 1-8 positive for *E. coli* at 480 bp)

Biofilm Formation:

1. Congo Red agar

Among (12) *E.coli* bacterial isolates from hens and (8) human isolates were cultured on CRA, the isolates (20) (100%) were showed biofilm formation through formation of the black colonies with a dry crystalline consistency, as shown in Figure (4).

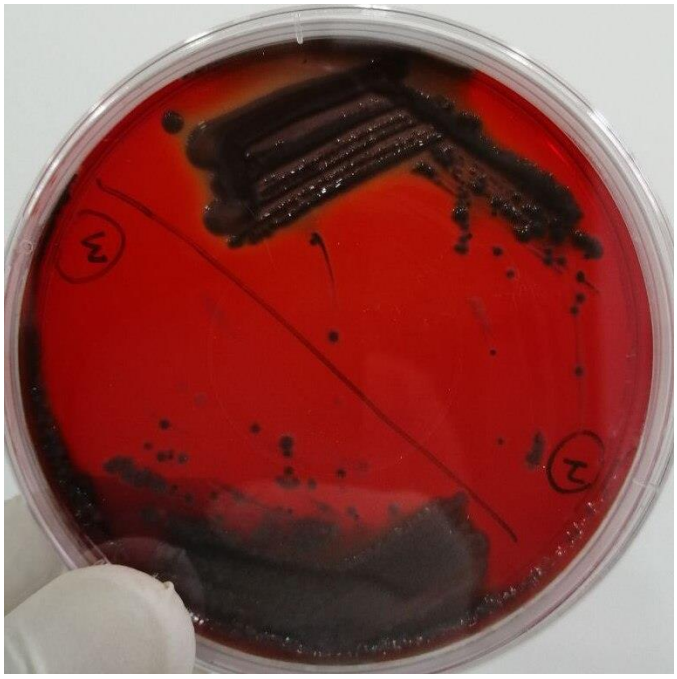

Fig 4: *E.coli*. biofilm formation on CRA

2. Tube method

In this study, differentiation between bacteria forming biofilms and those that do not was achieved through the tube method. Bacterial biofilms lead to challenging chronic diseases. In total, (20) isolates from E.coli bacteria were examined for their capacity to form biofilm. Strong biofilm was observed in 9 isolates, while moderate biofilm formation was seen in 5 isolates, and weak/none biofilm formation was noted in 6 isolates, as depicted in Figure 6.

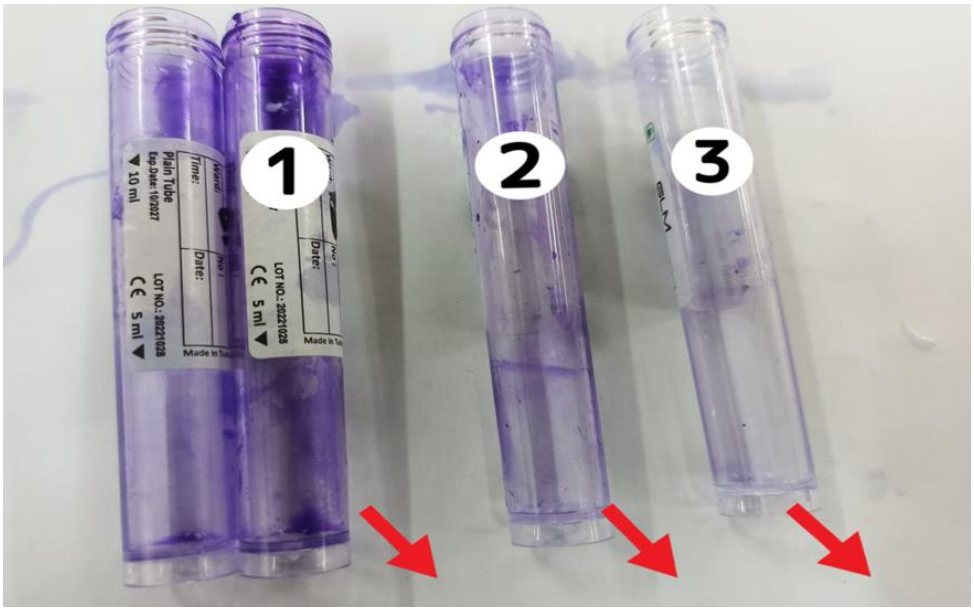

Fig 5: Formation of biofilm in *E.coli* by tube method (appear 1 strong and 2 moderate and 3 weak)

Antibiotic Resistance

Twelve samples of E. coli isolates from chickens were compared to eight samples as shown in Table 2 and Figure 6. Table 2 displays the proportion of vulnerable and resistant isolates for every antimicrobial agent.

Table 2: The proportions of vulnerable (S) and immune (R) E. coli samples from human and bird samples using the disk diffusion technique

Antimicrobial agent	Avian isolates (n=12)		Human isolates (n=8)	
	S	R	S	R
Nalidixic acid	0(0%)	12(100%)	3(37.5%)	5(62.5%)
Gentamicin	6(50%)	4(33.3%)	6(75%)	2(25%)
Ciprofloxacin	5(41.6%)	7(58.4%)	3(37.5%)	5(62.5%)
Amikacin	5(41.4%)	7(58.4%)	0(0%)	8(100%)
Nitrofurantoin	5(41.4%)	7(58.4%)	0(0%)	8(100%)
Tetracycline	9(75%)	3(25%)	3(37.5%)	5(62.5%)
Cefixime	10(83.4%)	2(16.6%)	4(50%)	4(50%)
Ceftazidime	9(75%)	3(25%)	7(87.5%)	1(12.5)

N=number

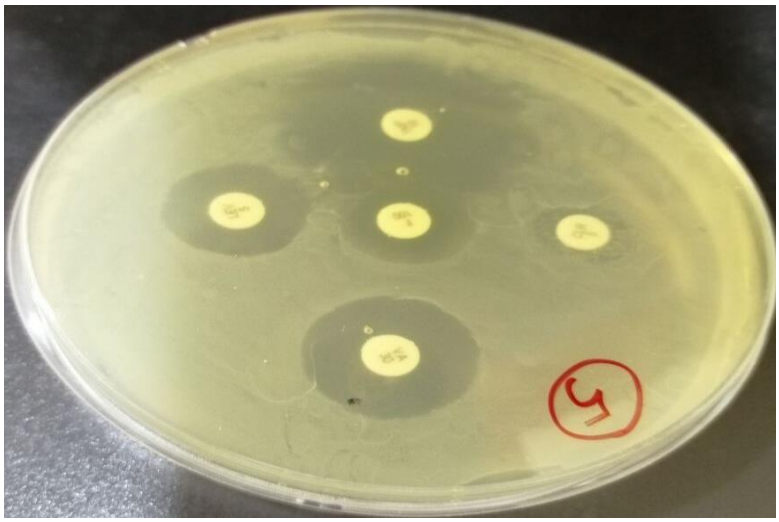

Fig 6: Effect of antibiotics on *E coli* using the Kirby pure method

DISCUSION

E. coli was identified in the study by culturing on MacConkey agar and EMB agar for morphological and biochemical identification of the characteristic colonies. All E. coli strains displayed lactose fermentation (pink colonies) on MacConkey agar and green metallic sheen colonies on EMB in this study. Out of, *E. coli* was isolated from 40% chicken. The frequency of isolation in this study was agree to that reported in Malaysia (48%) [24] and Egypt (34%) [25]. But disagree that reported in Ethiopia (32.5%) [26]. While *E.coli* isolated from 26.67% human , these agree Thangavelu *et al.*, [27] it found *E.coli* 28.09% and disagree that reported Abduljabar and Hasan [28] in Dehok, Iraq (10.66%). The breeding strategy, sample type, and sampling size may all have an impact on the variation in isolation frequency. Additional validation was conducted using molecular PCR targeting the 16S rRNA gene, resulting in a 480 bp product in positive samples. Tonu *et al.*, [29] also detected E. coli in chickens by PCR using ECO-f and ECO-r primers targeting 16S ribosomal DNA, yielding a specific 585bp amplicon. *E. coli* ability to build biofilms facilitates the onset of many illnesses, making them challenging to treat. There are precise controls over colonization and the development of mature biological envelopes [30, 31]. In this study, we report the production of biofilm by all strains of 100% *E. Coli* recovered from hens and humans. One crucial component of *E. coli* pathogenicity is its capacity to produce biofilms. The creation of biofilm-harboring bacteria, which are typically more resistant to routine cleaning and sanitizing methods, may result from its persistence on equipment or in clinical settings. It proved that the process of bacterial biofilm formation adds to the pathogenicity of microorganisms [32]. Previous research has also shown that because biofilm-forming bacteria have a thick polymeric matrix that prevents antibiotic penetration, they typically show better resistance than planktonic cells [9, 33]. Antimicrobial resistance is an important problem in human and veterinary medicine, and the administration of antibiotics is the main factor in the emergence of antibiotic-resistant bacteria. In chickens, resistance rates for Gentamicin and Tetracycline were 33.3% and 25%, respectively. This is in line with earlier studies reporting similar resistance rates in Brazil, Jordan, and Thailand, as well as a study by Al-Marri et.al showing resistance rates of 39.5% and 29.1% for Gentamicin and Tetracycline. According to the study conducted by Raum *et al.*, [40], it was concluded that there was a significant increase in the prevalence of E. coli isolates during antibiotic treatment. Multi-drug resistance was discovered in both human and animal *E. coli*, but E. coli from chicken samples showed a higher frequency and proportion of this resistance. This

is consistent with previous studies carried out in various places [41].

CONCLUSION

The study's findings indicated that hens may serve as reservoirs for the spread of germs resistant to antibiotics to people. Additionally, every producer of *E. coli* biofilm with avian and human origins exhibited characteristics of the multidrug resistance in addition to the biofilm production and capacity to raise the *E. coli* isolates' profile of antibiotic resistance.

REFERENCES

1. Leimbach, A., Hacker, J., & Dobrindt, U. (2013). E. coli as an all-rounder: the thin line between commensalism and pathogenicity. *Between pathogenicity and commensalism*, 3-32.
2. Croxen, M. A., & Finlay, B. B. (2010). Molecular mechanisms of Escherichia coli pathogenicity. *Nature Reviews Microbiology*, *8*(1), 26-38.
3. Sadat, A., Ramadan, H., Elkady, M. A., Hammad, A. M., Soliman, M. M., Aboelenin, S. M., ... & Awad, A. (2022). Phylotypic Profiling, Distribution of Pathogenicity Island Markers, and Antimicrobial Susceptibility of Escherichia coli Isolated from Retail Chicken Meat and Humans. *Antibiotics*, *11*(9), 1197. https://doi.org/10.3390/antibiotics11091197.
4. Denamur, E., Clermont, O., Bonacorsi, S., & Gordon, D. (2021). The population genetics of pathogenic Escherichia coli. *Nature Reviews Microbiology*, *19*(1), 37-54.
5. Foxman, B. (2010). The epidemiology of urinary tract infection. *Nature Reviews Urology*, *7*(12), 653-660.
6. Ramírez Castillo, F. Y., Guerrero Barrera, A. L., Harel, J., Avelar González, F. J., Vogeleer, P., Arreola Guerra, J. M., & González Gámez, M. (2023). Biofilm formation by Escherichia coli isolated from urinary tract infections from Aguascalientes, Mexico. *Microorganisms*, *11*(12), 2858.
7. Jang, J., Hur, H. G., Sadowsky, M. J., Byappanahalli, M. N., Yan, T., & Ishii, S. (2017). Environmental Escherichia coli: ecology and public health implications—a review. *Journal of applied microbiology*, *123*(3), 570-581.
8. Qian, W., Li, X., Yang, M., Liu, C., Kong, Y., Li, Y., ... & Zhang, Q. (2022). Relationship between antibiotic resistance, biofilm formation, and biofilm-specific resistance in Escherichia coli isolates from Ningbo, China. *Infection and Drug Resistance*, 2865-2878.
9. Singh, S., Singh, S. K., Chowdhury, I., & Singh, R. (2017). Understanding the mechanism of bacterial biofilms resistance to antimicrobial agents. *The open microbiology journal*, *11*, 53.
10. Thopireddy, N. R. (2023). Isolation and Molecular Detection of E. coli from Common Respiratory Infections of Poultry in AP. *EAS J. Vet. Med. Sci*, *5*, 1-6.
11. Delcaru, C., Alexandru, I., Podgoreanu, P., Grosu, M., Stavropoulos, E., Chifiriuc, M. C., & Lazar, V. (2016). Microbial biofilms in urinary tract infections and prostatitis: Etiology, pathogenicity, and combating strategies. *Pathogens*, *5*(4), 65.
12. Behzadi, P., Urbán, E., & Gajdács, M. (2020). Association between biofilm-production and antibiotic resistance in uropathogenic Escherichia coli (UPEC): an in vitro study. *Diseases*, *8*(2), 17.
13. Kabiswa, W., Nanteza, A., Tumwine, G., & Majalija, S. (2018). Phylogenetic groups and antimicrobial susceptibility patterns of Escherichia coli from healthy chicken in Eastern and Central Uganda. *Journal of Veterinary Medicine*, *2018*(1), 9126467.
14. Afayibo, D. J. A., Zhu, H., Zhang, B., Yao, L., Abdelgawad, H. A., Tian, M., ... & Wang, S. (2022). Isolation, molecular characterization, and antibiotic resistance of avian pathogenic Escherichia coli in Eastern China. *Veterinary Sciences*, *9*(7), 319.
15. Pan, Y., Zeng, J., Li, L., Yang, J., Tang, Z., Xiong, W., ... & Zeng, Z. (2020). Coexistence of antibiotic resistance genes and virulence factors deciphered by large-scale complete genome analysis. *Msystems*, *5*(3), 10-1128.
16. Pérez, V. K. C., da Costa, G. M., Guimarães, A. S., Heinemann, M. B., Lage, A. P., & Dorneles, E. M. S. (2020). Relationship between virulence factors and antimicrobial resistance in Staphylococcus aureus from bovine mastitis. *Journal of global antimicrobial resistance*, *22*, 792-802.
17. Zhao, X., Lv, Y., Adam, F. E. A., Xie, Q., Wang, B., Bai, X., ... & Yang, Z. (2021). Comparison of antimicrobial resistance, virulence genes, phylogroups, and biofilm formation of Escherichia coli isolated from intensive farming and free-range sheep. *Frontiers in Microbiology*, *12*, 699927.
18. Abdul Rahaman Shariff AR., Shenoy S., Taruna Y. and Radhakrishna M. (2013)"The antibiotic susceptibility patterns of uropathogenic Escherichia coli, with special reference to the fluoroquinolones." *Journal of clinical and diagnostic research: JCDR* 7.6: 1027.
19. Hammerum, A. M., & Heuer, O. E. (2009). Human health hazards from antimicrobial-resistant Escherichia coli of animal origin. *Clinical infectious diseases*, *48*(7), 916-921.
20. Miller, M. B., & Tang, Y. W. (2009). Basic concepts of microarrays and potential applications in clinical microbiology. *Clinical microbiology reviews*, *22*(4), 611-633.
21. Tadesse D., Zhao SH., Tong E., Ayers SH., Singh A., Bartholomew, M. J., & McDermott PF. (2012). Antimicrobial drug resistance in Escherichia coli from Humans and food animals, united states, 1950-2002. Emerging Infectious Diseases Journal, 18(5), 741-749.

22. Freeman, D. J., Falkiner, F. R., & Keane, C. T. (1989). New method for detecting slime production by coagulase negative staphylococci. *Journal of clinical pathology*, *42*(8), 872-874.
23. Hassan, A., Usman, J., Kaleem, F., Omair, M., Khalid, A., & Iqbal, M. (2011). Evaluation of different detection methods of biofilm formation in the clinical isolates. *Brazilian journal of infectious diseases*, *15*, 305-311.
24. Ibrahim, S., Wei Hoong, L., Lai Siong, Y., Mustapha, Z., CW Zalati, C. S., Aklilu, E., ... & Kamaruzzaman, N. F. (2021). Prevalence of antimicrobial resistance (AMR) Salmonella spp. and Escherichia coli isolated from broilers in the East Coast of Peninsular Malaysia. *Antibiotics*, *10*(5), 579.
25. Ibrahim, W. A., Marouf, S. A., Erfan, A. M., Nasef, S. A., & El Jakee, J. K. (2019). The occurrence of disinfectant and antibiotic-resistant genes in Escherichia coli isolated from chickens in Egypt. *Veterinary world*, *12*(1), 141.
26. Sarba, E. J., Kelbesa, K. A., Bayu, M. D., Gebremedhin, E. Z., Borena, B. M., & Teshale, A. (2019). Identification and antimicrobial susceptibility profile of Escherichia coli isolated from backyard chicken in and around ambo, Central Ethiopia. *BMC Vet Res*, *15*(1), 85.
27. Thangavelu, S., Dhandapani, R., Arulprakasam, A., Paramasivam, R., Chinnathambi, A., Ali Alharbi, S., ... & Shrestha, A. (2022). Isolation, identification, characterization, and plasmid profile of urinary tract infectious Escherichia coli from clinical samples. *Evidence-Based Complementary and Alternative Medicine*, *2022*(1), 7234586.
28. Abduljabar, Y. A., & Hasan, H. K. (2023). Isolation and antibiotic susceptibility of E. coli and S. aureus from urinary tract infections in Dohuk city, Iraq. *J Popul Ther Clin Pharmacol*, *30*(14), e262–e267.
29. Tonu, N. S., Sufian, M. A., Sarker, S., Kamal, M. M., Rahman, M. H., & Hossain, M. M. (2011). Pathologicalstudy on colibacillosis in chickens and detection of Escherichia coli by PCR. *Bangladesh Journal of Veterinary Medicine*, *9*(1), 17-25.
30. Beloin, C., Roux, A., & Ghigo, J. M. (2008). Escherichia coli biofilms. *Bacterial biofilms*, 249-289.
31. Ma, K., Wang, H., Lv, Z., Hu, Y., Wang, H., Shu, F., ... & Xue, T. (2023). The Two-Component System CpxRA Affects Antibiotic Susceptibility and Biofilm Formation in Avian Pathogenic Escherichia coli. *Animals*, *13*(3), 383.
32. Rinser, A., Krogfelt, K. A., Klein, B. M., Zechner, E. L., & Molin, S. W. (2006). In vitro biofilm formation of Commensal and pathogenic Escherichia coli strains: Impact of Environmental and genetic factors, *Journal of Bacteriology*, *188*(10), 3572-3581.
33. Katongole, P., Nalubega, F., Florence, N. C., Asiimwe, B., & Andia, I. (2020). Biofilm formation, antimicrobial susceptibility and virulence genes of Uropathogenic Escherichia coli isolated from clinical isolates in Uganda. *BMC Infectious Diseases*, *20*, 1-6.
34. Sayah, R. S., Kaneene, J. B., Johnson, Y., & Miller, R. (2005). Patterns of antimicrobial resistance observed in Escherichia coli isolates obtained from domestic-and wild-animal fecal samples, human septage, and surface water. *Applied and environmental microbiology*, *71*(3), 1394-1404.
35. Nouri Gharajalar, S. (2017). Evaluation of antimicrobial resistance patterns of biofilm forming Escherichia coli isolated from humans and chickens. *Journal of Zoonotic Diseases*, *2*(2), 17-26.
36. Braga, J. F. V., Chanteloup, N. K., Trotereau, A., Baucheron, S., Guabiraba, R., Ecco, R., & Schouler, C. (2016). Diversity of Escherichia coli strains involved in vertebral osteomyelitis and arthritis in broilers in Brazil. *BMC Vet Res*, *12*(1), 1-12.
37. Abu-Basha, E., Gharaibeh, S., & Thabet, A. J. (2012). In vitro susceptibility of resistant Escherichia coli field isolates to antimicrobial combinations. *J Appl Poult Res*, *21*(3), 595-602.
38. Chansiripornchai, N., Mooljuntee, S., & Boonkhum, P. J. (2011) Antimicrobial sensitivity of avian pathogenic Escherichia coli (APEC) isolated from chickens during 2007-2010. *Thai J Vet Med*, *41*(4), 519.
39. Al-Marri, T., Al-Marri, A., Al-Zanbaqi, R., Al Ajmi, A., & Fayez, M. (2021). Multidrug resistance, biofilm formation, and virulence genes of Escherichia coli from backyard poultry farms. *Veterinary World*, *14*(11), 2869.
40. Raum, E., Lietzau, S., Von Baum, H., Marre R., & Brenner, H. (2008). Changes in Escherichia coli resistance patterns during and after antibiotic therapy: a longitudinal study among outpatients in Germany. *Clinical Microbiology and Infection*, *14*(1), 41-48.
41. Kibert, M., & Abera, B. (2011). Antimicrobial susceptibility of E. coli from clinical sources in northeast Ethiopia. *African Health Science*, *11*(1), 40-44.