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Original Research Article

Isolation and Molecular Detection of *E.coli* from Common Respiratory Infections of Poultry in A.P.

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Abstract: In the present work a total of 228 pooled nasal swabs, 228 pooled tracheal swabs samples from the ailing birds and 152 pooled tracheal tissues, 152 pooled lung tissues from the dead birds were collected from the 19 suspected poultry farms in A.P. and were labelled farm wise and specimen wise. The DNA was extracted from the samples by using Trizol method. The PCR and SYBR Green real time PCR tests was standardized by targeting *16 s rRNA* gene. The study found that 12 farms were found to be positive for *E.coli*, with percent positivity of 63.15% by PCR and 71.2% by SYBR Green Real Time PCR. Among the 12 positive farms, 92(40.35% nasal swabs),106(51.75% tracheal swabs),64 (42.10% tracheal tissues) and 72 (47.36% lung tissues) were positive for *E.coli* by PCR and 118(49.56% nasal swabs),128(56.14% tracheal swabs),68(44.73% tracheal tissues) and 77(50.65% lung tissues) were positive for *E.coli* by SYBR Green Real Time PCR, nasal swabs and tracheal swabs were positive with positivity of 40.35 and 51.75% respectively by PCR , 106 and 128, nasal swabs and tracheal swabs were positive with positivity 46.49 and 56.14% by SYBR Green Real Time PCR. This study found that the confirmatory diagnosis of respiratory infections in poultry is accurate when histopathology, isolation and molecular detection methods like PCR and SYBR Green Real time PCR are used.

Keywords: Poultry, *E.coli*, EMB agar, histopathology, PCR, SYBR Green Real time PCR.

INTRODUCTION

India is the world's third leading egg producer, trailing China and the USA, and the fourth largest broiler producer, following China, Brazil, and the USA(Kumar and Pandey, 2021). Andhra Pradesh poultry industry strands 1st in egg production. Total egg production is 23 billion and poultry meat production is 4.2 million tonnes per annum as per latest census, still the demand and need for production is not up to the point. So to meet the target egg production of 123 billion and poultry meat production of 6 million tonnes by 2023 country wide, being national top producer there need to be addressed on the commonly poultry problems. Avian diseases, which are considered as one of the most important factors affecting avian productivity, has hampered the development of the poultry business to its full potential. Growth in poultry sector is being challenged due to increased incidence and re-emergence of diseases caused due to evolution of several pathogens and use of live vaccines. Among the poultry diseases, the respiratory pathogens like *Mycoplasma*, *Infectious bronchitis virus*, *Infectious laryngotracheitis virus and E. coli* are the common pathogens causing economic losses to the industry.The etiology of respiratory disease is complex, often involving more than one pathogen at the same time (Roussan *et al*., 2008), which causes heavy economic losses both in terms of production and cost of treatment. The interaction between pathogens that have the same site of multiplication might be either synergistic or antagonistic determining the severity of the resulting clinical outcomes. Infected birds express respiratory and other lesions such as cough, respiratory distress, poor growth and production leading to high economic losses (Pang *et al*., 2002). And it can be achieved by controlling infectious diseases through confirmatory diagnosis and better control strategies. Avian colibacillosis is considered as one of the major bacterial diseases afflicting poultry industry worldwide (Singh *et al*., 2011). Inspite of regular control measures following, still the *E. coli* infections are major problem, resulting in decrease in productivity, increased mortality and increased economic losses (Otaki, 1995). *Escherichia coli*, a Gram-negative bacterium that belongs to family Enterobacteriaceae, it causes typical localized or systemic disease occurring mostly

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secondarily when host defense have been impaired. It is characterized by septicemia in acuteform, resulting in death while in its subacute form by pericarditis, airsacculitis and perihepatitis, reproductive tract infection like salpingitis and peritonitis resulting in huge mortality (Ozaki and Murase, 2009). In poultry, *E. coli* infections affects all the systems, resulting in a complex syndrome characterized by lesions in multiple organ including digestive, respiratory and reproductive tract, alone or in association with other pathogen (Singh *et al*., 2011). Because of similarities in exhibition of clinical signs and lesions in common respiratory infections, it is often difficult to diagnose the specific disease condition. At present there is a need for accurate diagnosis of respiratory pathogens from the common respiratory infections and is highly essential to reduce the significant economic losses to the poultry industry. Accurate diagnosis is required for implementation of control strategies, hence present work was under taken to isolate and identify the common respiratory *E.coli* pathogen from poultry.

MATERIALS AND METHODS

Samples

From a total of 19 suspected farms, each poultry farm few live birds, few dead birds and pooled samples of 12 tracheal swabs, 12 nasal swabs, 8 tracheal tissues and 8 lung tissues were collected. The total pooled samples of all the poultry farms includes tracheal swabs (228), nasal swabs (228), trachea from dead birds (152) and lungs from dead birds (152) were labelled separately as per farm.

Isolation of *E.coli:*

The samples after collection were immediately inoculated into sterile test tubes containing sterile nutrient broth, incubated aerobically at 37^{o}C in Bacteriological incubator for 24 hrs. The cultures were tested for the presence of *E.coli* by PCR, SYBR Green Real time PCR and simultaneously plated on EMB agar medium and incubated aerobically at 37℃ in bacteriological incubator. The colonies were detected by macroscopic examination of characteristic metallic sheen Fig 1. The organisms were shown the IMViC pattern of ++--. The DNA extraction was carried out from the suspected samples by Trizol method. PCR tests were standardized by targeting 16s r RNA gene (Table 1). It was found that an annealing temperature of 62^{o}c for 30 seconds was optimum for amplification of *16SrRNA* gene yielding 585 bp PCR product (Fig 6). And further molecular detection was done by using SYBR Green Real time PCR by targeting the same gene and the optimized conditions were shown in the Table 3.

Molecular detection of *E. coli*

Table 1: Primers used for detection of *E.coli* (Wang *et al*., 1996)

Primers	Primer Name	Nucleotide Sequence	Amplicon Size
***16S rRNA* gene**	**16 s-F**	5'-GACCTCGGTTTAGTTCACAGA-3'	**585 bp**
	16s-R	5'-CACACGTGACGCTGACCA-3'	

Table 2: Cyclic conditions used for amplification of 16S*r*RNA gene of *E.coli*

S. No.	Step	Temperature (°C)	Time	No of cycles
1.	Initial Denaturation	95	5 min	1
2.	Denaturation	94	30 sec	35
3.	Annealing	62	30 sec	
4.	Extension	72	30 sec	
5.	Final extension	72	2 min	1

Table 3: SYBR Green Real Time PCR conditions optimized for the amplification of *16S rRNA* gene (Fig 7)

Sl. No	Step	Temperature (°C)	Time
1.	Initial Denaturation	94	3.59 min.
2.	Denaturation	94	20 sec
3.	Annealing	55	45 sec.
4.	Extension	72	1 min.
5.	Step 2-4 were 40 cycles		
6.	Final extension	72	10 min

Histopathology

A transverse section of tissue approximately 0.5cm in thickness was taken from trachea and lungs of birds showing respiratory and associated organ lesions. Tissues were fixed in 10per cent formalin and trimmed to a thickness of about 3mm. Tissues were dehydrated, cleared and embedded in paraffin in a routine manual processing. Tissues were cut at 3 to 5 mm thickness, mounted on glass slides, stained with haematoxylin and eosin and were places with DPX for histopathological examinations. The stained slides were read under microscope and histopathological changes were recorded.

RESULTS AND DISCUSSION

In the present study the clinical signs including nasal discharges, swelling of face and crest formation was observed in all poultry farms, similar type of observations were documented by De Carli *et al*., (2014) and Veeraselvam *et al*., (2019). The Postmortem examination of diseased birds revealed septicemia, blood vascular congestion, hemorrhagic enteritis and severe congestion of, Trachea and lungs were observed. Our finding coincided with Qamar *et al*., (2019) worked on molecular typing of E.coli in commercial ducks. In the present study some of the suspected poultry were also showed multiple organ lesions such as air sacculitis, pericarditis, peritonitis, salpingitis, synovitis, osteomyelitis and yolk sac infections, similar type of lesions were also observed by Redweik *et al*., (2020) and Halder *et al*., (2021).

In the present study, *E.coli* was detected after pre-enrichment on nutrient broth and and also observed the growth after 48hrs of incubation the typical characteristic metallic sheen colonies c on EMB (Fig 1) and all isolates were showed lactose fermentation (pink colonies) on MacConkey agar, Boro *et al*., (2019) was also observed similar type of growth pattern. All the isolates were showed the ++-- IMViC pattern (Fig 2) and similar type findings also observed by Amin *et al*., (2017) and. Veeraselvam *et al*., (2019).

Histopatholocally, we examined the lung and tracheal sections from diseased birds were showed, inflammation withinfiltration of leukocytes and air capillaries were collapsed (Fig 3, 4, 5) similar type of findings also documented by Dwars *et al*., (2009). Heterophils are the first line of defense and die at the lesion and might be major cause for purulent inflammation. The inflammatory fluid from lungs tested by staining revealed higher levels of heterophils and macrophages indicating that the more recruitment of these inflammatory cells (Fig 3) for defense similarly observations also noticed by Wang *et al*., (2018).

Further confirmation was done by molecular based PCR (Fig 6) and SYBR Green Real Time PCR (Fig 7 & 8) by targeting 16S r RNA gene and produced 585bp product in positive samples and similarly Tonu *et al*., (2011) carried out detection of *E.coli* by PCR in chickens using ECO-f and ECO-r primers targeting 16S ribosomal DNA and found a specific amplicon of 585bp.

The study found that 12 farms were positive for *E.coli*, with percent positivity of 63.15% by PCR and 71.2% by SYBR Green Real Time PCR similarly Rahman *et al*., (2020) observed 65.67% of broiler and 61.33% of layer meat swabs tested positive for *E. coli and* Other research workers detected high frequency of *E. coli* in poultry Rasheed *et al*., (2013); Adeyanju and Ishola (2013) and Park *et al*., (2015). In Bangladesh, Jakaria *et al*., (2012) reported 78.67%, 82% and 70% the prevalence rates of *E. coli* in layer, broiler, and indigenous chicken were, respectively. In the present study, among the 12 positive farms, 92(40.35% nasal swabs),106(51.75% tracheal swabs),64 (42.10% tracheal tissues) and 72 (47.36% lung tissues) were positive for *E.coli* by PCR and 118(49.56% nasal swabs),128(56.14% tracheal swabs),68(44.73% tracheal tissues) and 77(50.65% lung tissues) were positive for *E.coli* by SYBR Green Real Time PCR, nasal swabs and tracheal swabs were positive with positivity of 40.35 and 51.75% respectively by PCR , 106 and 128, nasal swabs and tracheal swabs were found positive with positivity46.49 and 56.14% by SYBR Green Real Time PCR. Similarly 87.5% avian pathogenic ecoli in commercial poultry farms was observed by Abdelaziz *et al*., (2019). Our results indicating that respiratory *E.coli* might be localized in the respiratory organs causing the highest mortality in combination with other pathogens like mycoplasma (Thopi Reddy *et al*., 2021). Similarly Dashe *et al*., (2013) from Nigeria reported 15.8% from liver and 13% from the spleen suggesting that *E. coli* localizes most commonly in these organs. The APEC is a major cause of extensive economic loss in the poultry industry due to high morbidity and mortality. The high prevalence of *E. coli* infections chickens could be associated with the accumulation of *E. coli* aerosols in the atmosphere of chicken barns that are inhaled by chickens into the respiratory tract. Samples that gave negative bacterial culture may be collected from farms that used early antibiotic treatment policy. Rapid conformation and molecular identification were performed to reduce the false positive results.

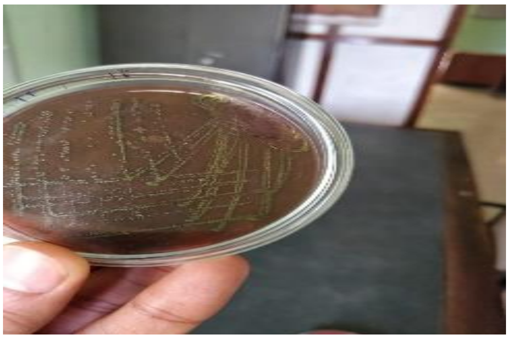

Fig 1: Showing Metallic sheen colonies on EMB agar

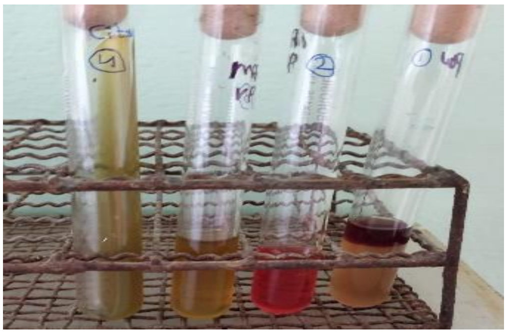

Fig 2: Showing IMVIC tests pattern for *E.coli* (1.citrate negative, 2.Vp test negative, 3.Methyl red test positive and 4.indole test positive)

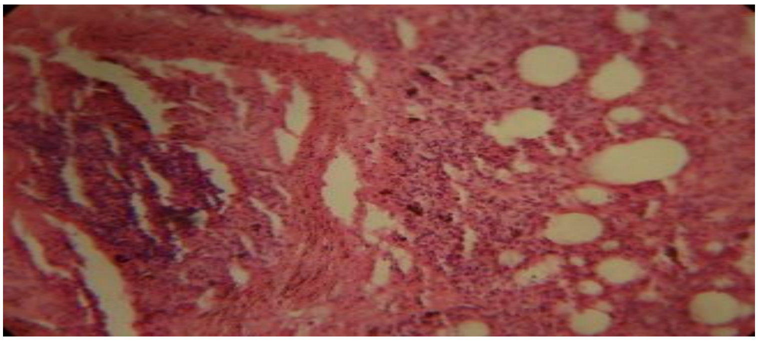

Fig 3: Showing thickened blood vessels filled with RBC in the lung

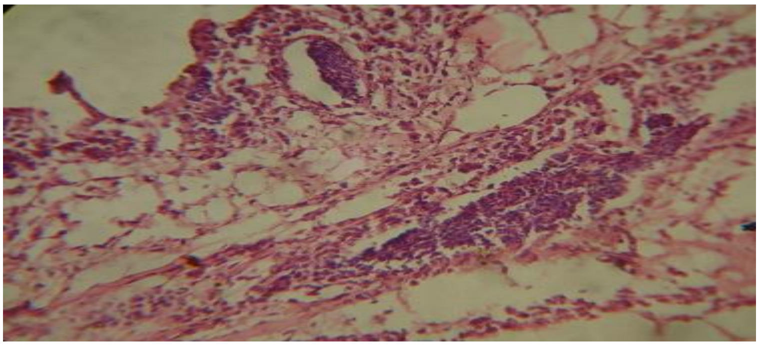

Fig 4: Showing degenerated tracheal epithelial cells and infiltration of inflammatory cells

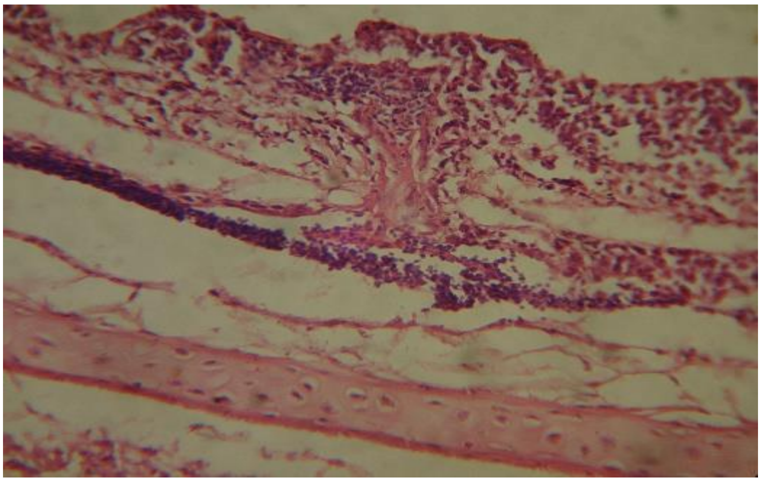

Fig 5: Showing infiltration of inflamatory cells and degeneration of tracheal epithelial cells

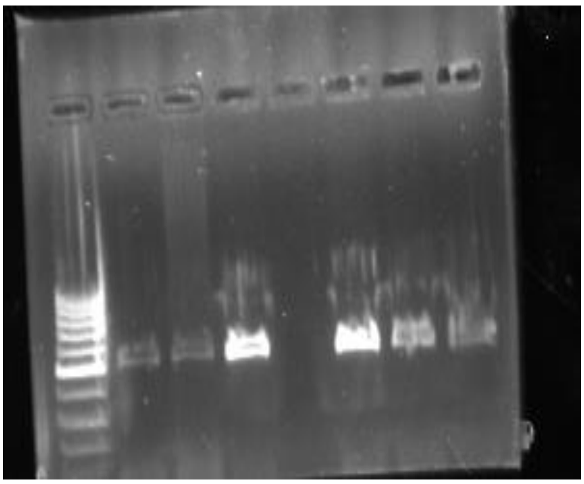

Fig 6: Gel image showing the predicted size of 585 bp of 16s r RNA gene

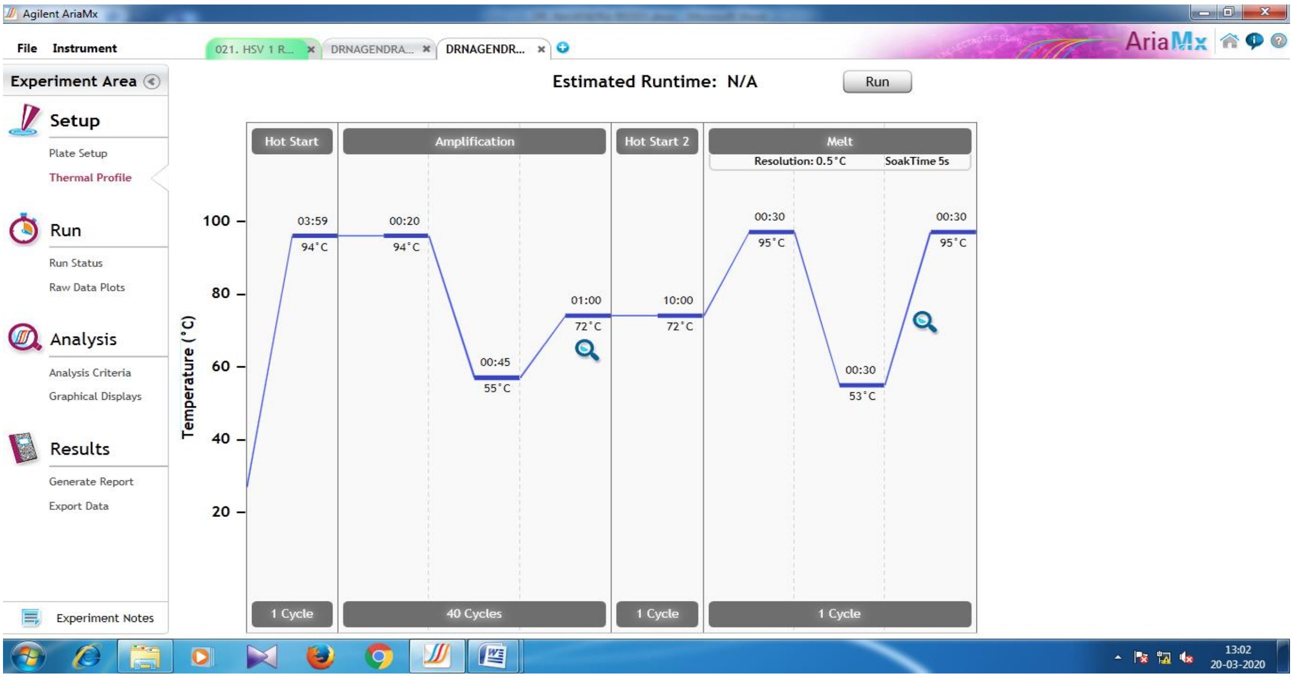

Fig 7: Showing optimized conditions for amplification of 16S r RNA gene in SYBR Green Real Time PCR

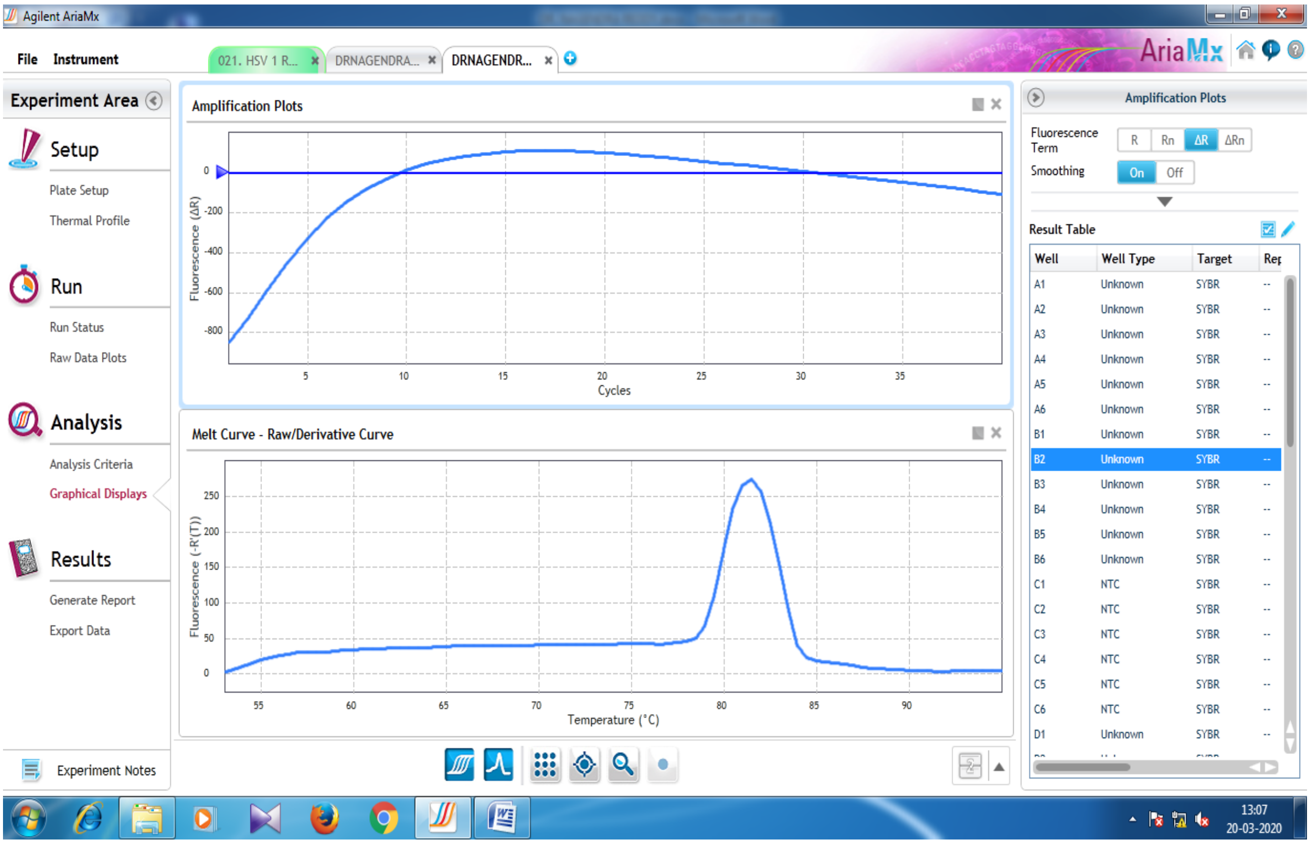

Fig 8: Showing melt curve and amplification plot for 16S r RNA gene in SYBR Green Real Time PCR

CONCLUSION

In conclusion, respiratory infection due to *E.coli* causes significant economic losses accompanied by high mortality rates. In this study, *E.coli* molecular detection by targeting 16s rRNA gene is confirmatory rapid diagnostic technique and more useful in common respiratory infections whereas the isolation procedures and histopathology are the time taking. Regular investigation of the currently circulating respiratory infections in both backyard and commercial flocks, as well as the evaluation of vaccination programs, is necessary for the improvement of disease prevention and control.

REFERENCES

- Abdelaziz, A. M., Mohamed, M. H., Fayez, M. M., Al-Marri, T., Qasim, I., & Al-Amer, A. A. (2019). Molecular survey and interaction of common respiratory pathogens in chicken flocks (field perspective). *Veterinary world*, *12*(12), 1975.
- Adeyanju, G. T., & Ishola, O. (2014). Salmonella and Escherichia coli contamination of poultry meat from a processing plant and retail markets in Ibadan, Oyo State, Nigeria. *Springerplus*, *3*(1), 1-9.
- Das, A., Sen, A., Dhar, P. K., Nath, S. K., Ghosh, P., & Saifuddin, A. K. M. (2017). Isolation of Escherichia coli from the liver and yolk sac of day-old chicks with their antibiogram. *British Journal of Biomedical and Multidisciplinary Research*, *1*(1), 19-25.
- Dashe, Y. G., Raji, M. A., Abdu, P. A., & Oladele, B. S. (2013). Aeromonas hydrophila infections in chickens affected by fowl cholera in Jos Metropolis, Nigeria. *Int J Microbiol Immunol Res*, *1*, 32-36.
- De Carli, S., Ikuta, N., Lehmann, F. K. M., da Silveira, V. P., de Melo Predebon, G., Fonseca, A. S. K., & Lunge, V. R. (2015). Virulence gene content in Escherichia coli isolates from poultry flocks with clinical signs of colibacillosis in Brazil. *Poultry Science*, *94*(11), 2635-2640.
- Halder, S., Das, S., Nath, S. K., Kundu, S. K., Islam, M. S., Chowdhury, S., & Masuduzzaman, M. (2021). Prevalence of some common bacterial diseases in commercial poultry farm. *Ukrainian Journal of Veterinary and Agricultural Sciences*, *4*(2), 44-51.
- Jakaria, A. T. M., Islam, M. A., & Khatun, M. M. (2012). Prevalence, characteristics and antibiogram profiles of Escherichia coli isolated from apparently healthy chickens in Mymensingh, Bangladesh. *Microbes and Health*, *1*(1), 27-29.
- Pang, Y., Wang, H., Girshick, T., Xie, Z., & Khan, M. I. (2002). Development and application of a multiplex polymerase chain reaction for avian respiratory agents. *Avian diseases*, *46*(3), 691-699.
- Park, S. Y., & Ha, S. D. (2015). Ultraviolet-C radiation on the fresh chicken breast: Inactivation of major foodborne viruses and changes in physicochemical and sensory qualities of product. *Food and Bioprocess Technology*, *8*(4), 895-906.
- Qamar, A., Mohyuddin, S. G., Hamza, A., Lartey, K. A., Shi, C. Q., Yang, F., & Chen, J. J. (2019). Physical and chemical factors affecting chicken meat color. *Pakistan Journal of Science*, *71*(2), 82.
- Rahman, M., Husna, A., Elshabrawy, H. A., Alam, J., Runa, N. Y., Badruzzaman, A. T. M., & Ashour, H. M. (2020). Isolation and molecular characterization of multidrug-resistant Escherichia coli from chicken meat. *Scientific Reports*, *10*(1), 1-11.
- Redweik, G. A., Stromberg, Z. R., Van Goor, A., & Mellata, M. (2020). Protection against avian pathogenic Escherichia coli and Salmonella Kentucky exhibited in chickens given both probiotics and live Salmonella vaccine. *Poultry science*, *99*(2), 752-762.
- Roussan, D. A., Khwaldeh, G. Y., Haddad, R. R., Shaheen, I. A., Salameh, G., & Al Rifai, R. (2008). Effect of ascorbic acid, acetylsalicylic acid, sodium bicarbonate, and potassium chloride supplementation in water on the performance of broiler chickens exposed to heat stress. *Journal of Applied Poultry Research*, *17*(1), 141-144.
- Saadaoui, I., Rasheed, R., Aguilar, A., Cherif, M., Al Jabri, H., Sayadi, S., & Manning, S. R. (2021). Microalgal-based feed: promising alternative feedstocks for livestock and poultry production. *Journal of Animal Science and Biotechnology*, *12*(1), 1-15.
- Singh, M., Reynolds, D. L., & Das, K. C. (2011). Microalgal system for treatment of effluent from poultry litter anaerobic digestion. *Bioresource technology*, *102*(23), 10841-10848.
- Nagendra Reddy, T., Sreedevi, B., & Vinod Kumar, N. (2021). Simultaneous Detection of Mycoplasma gallisepticum, Mycoplasma synoviae, Infectious bronchitis virus and Infectious laryngotracheitis virus in Poultry by Multiplex SYBR Green Real Time PCR, 2021. *EAS J Vet Med Sci*, 3(6), 72-76.
- 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.
- Veeraselvam, M., Senthilkumar, N. R., Vairamuthu, S., & Ramakrishnan, V. (2019). Isolation and identification of bacterial agents causing respiratory infection in native chicken. *Journal of Entomology and Zoology Studies*, *7*(4), 162-167.
- Wang, W., Chen, M., Jin, X., Li, X., Yang, Z., Lin, H., & Xu, S. (2018). H2S induces Th1/Th2 imbalance with triggered NF-κB pathway to exacerbate LPS-induce chicken pneumonia response. *Chemosphere*, *208*, 241-246.
- Umar, S., Guerin, J. L., & Ducatez, M. F. (2017). Low pathogenic avian influenza and coinfecting pathogens: a review of experimental infections in avian models. *Avian Diseases*, *61*(1), 3-15.

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