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Molecular identification of bacterial pathogen in suspected cases of respiratory tract infection from chicken

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Abstract
The respiratory illness is characterized by weakness, gasping, pump-handled respiration, dyspnea, mucous discharge, mortality, sinus swelling, facial edema, tracheitis, exudative pneumonia, pleuritis, pericarditis, sinusitis, decrease in egg production and poor egg quality (Zorman *et al*., 2000; Canal *et al*. 2005). Air sacculitis, higher mortality rates, higher medical expenses, and decreased egg production are the most common outcomes of respiratory illnesses in poultry. Total of 52 tissue samples of lungs and tracheae were collected from 52 birds suffering from respiratory tract infections during the period from December 2022 to March 2023. Out of 52 tissue samples, 98.08% tissue samples were found positive for bacterial pathogen isolation, while 1.92% wound swab samples were negative. Molecular detection of bacteria from tissue samples 98.07%, 59.61%, 9.61%, 7.69%, 3.84% and 1.92% isolated *E. coli*, *Staphylococcus spp.*, *M. gallisepticum*, *O. rhinotracheale*, *A. paragallinarum*, and *M. synoviae* respectively. In this study, none of the tissue samples detected *B. avium*, *P. aeruginosa*, and *P. multocida*.

Keywords: Respiratory infections, chicken, bacteria, polymerase chain reaction

Introduction
Poultry are domesticated birds that are primarily raised for their meat, egg, and feathers. According to the most recent census, there are 23 billion eggs produced worldwide and 4.2 million tonnes of poultry meat are produced annually (Thopireddy, 2023) [13]. Compared to illnesses that affect other organs, respiratory tract illnesses constitute a major part of diseases that affect poultry and lead to significant economic losses for the industry globally (Glisson, 1998; Blackall and Soriano-Vargas, 2020) [5, 2]. The respiratory organs of chicken include the lungs, syrinx, trachea, larynx, air sacs, nasal cavity, and nostrils. The respiratory illness is characterized by weakness, mucous discharge, gasping, dyspnea, mortality, sinus swelling, facial edema, tracheitis, exudative pneumonia, pump handled respiration, pleuritis, pericarditis, sinusitis, poor egg quality, and decrease in egg production (Zorman *et al*., 2000; Canal *et al*., 2005) [16, 3]. Various bacterial pathogens affecting the respiratory system include *Pasteurella multocida* (*P. multocida*), *Avibacterium paragallinarum (A.paragallinarum)*, *Escherichia coli* (E. *coli*), *Bordetella avium* (*B. avium*), *Ornithobacterium rhinotracheale (O.rhinotracheale)*, *Klebsiella pneumoniae (K. pneumoniae)*, *Mycoplasma gallisepticum (M.gallisepticum)*, and *Staphylococcus* spp. (Popy *et al*., 2011) [9]. Molecular PCR methods that are more sensitive, specific, and effective could be more useful in the diagnosis of respiratory infections (Prabhu *et al*., 2021) [10].

Materials and Methods
Sample Collection
Samples (lungs and trachea) were collected from 52 birds from commercial farms delivered to the department of Veterinary Pathology for the post-mortem examination. Samples were collected from 40 chicken flocks. The birds were of different ages and came from different regions of the Anand, Gujarat.

DNA extraction

The DNeasy® blood & tissue kit (50), Cat. No. 69504, Lot No. 172049243 was used to isolate bacterial genomic DNA directly from lung and trachea tissue samples of chickens, according to Kit's instructions. The negative control in the DNA extraction was the Tris buffer used for sample preparation. The quantity and quality of the DNA was determined using the Nanodrop 1000 system (Thermo Fisher Scientific, Waltham, MA, USA).

Molecular identification of bacteria by PCR

PCR was carried out in 25 µl volumes. The reaction mixture consisted of 2X PCR Master Mix (PCR Master Mix) 12.50 µl, Forward Primer (10pmol/µl) 1.0 µl, Reverse Primer (10pmol/µl) 1.0 µl, Template DNA 3.0 µl, and Nuclease Free Water 7.50 µl.

Table 1: Genus-specific, Species-specific primer sequences and size of amplified products

Sr. No.	Name of targeted organism	Primer		Primer sequences (5'-3')	size (bp)	References
1.	*Staphylococcus spp.*	*TStaG422* *TStaG765*	F R	GGCCGTGTTGAACGTGGTCAAATCA TTACCATTTCAGTACCTTCTGGTAA	370	Martineau *et al.* (2001) [8]
2.	*Pasteurella multocida*	*KMT1T7* *KMT1SP6*	F R	ATCCGCTATTTACCCAGTGG GCTGTAAACGAACTCGCCAC	460	Townsend *et al.* (1998) [14]
3.	*Escherichia coli*	*Eco 223* *Eco 455*	F R	ATCAACCGAGATTCCCCCAGT TCACTATCGGTCAGTCAGGAG	232	Riffon *et al.* (2001) [11]
4.	*Avibacterium paragallinarum*	*HPG-2*	F R	TGAGGGTAGTCTTGCACGCGAAT CAAGGTATCGATCGTCTCTCTACT	500	Anne *et al.* (2022) [1]
5.	*Bordetella avium*	*N-avium* *C-avium*	F R	GGCGCCGTCAACACATACTCTTGAT AGGGAGGTCAGATAGCTCTAGAAT	520	Savelkoul *et al.* (1993) [12]
6.	*Ornithobacterium rhinotracheale*	*OR16S*	F R	GAGAATTAATTTACGGATTAAG TTCGCTTGGTCTCCGAAGAT	784	Van Emple and Hafez (1999) [15]
7.	*Pseudomonas spp.*	*algD*	F R	ATGCGAATCAGCATCTTTGGT CTACCAGCAGATGCCCTCGGC	1310	Lanotte *et al.* (2004) [6]
8.	*Mycoplasma gallisepticum*	*mgc2*	F R	CGCAATTTGGTCCTAATCCCCAACA TAAACCCACCTCCAGCTTTATTTCC	300	Garcia *et al.* (2005) [4]
9.	*Mycoplasma synoviae*	*MS-16SrRNA*	F R	GAGAAGCAAAATAGTGATATCA CAGTCGTCTCCGAAGTTAAAA	205	Lauerman (1998) [7]

Table 2: Steps and conditions of thermal cycling for different genus-specific, species-specific, and antimicrobial-resistant genes primer pair used in PCR

Name of targeted organism	Primers (Forward and Reverse)	Cycling conditions				
		Initial Denaturation	Denaturation	Annealing	Extension	Final Extension
Staphylococcus spp.	TStaG422 TStaG765	94 °C 5 minute	94 °C 30 second	52 °C 1 minute	72 °C 1 minute	72 °C 10 minute
			Repeated for 40 cycles			
Pasteurella multocida	KMT1(F) KMT1(R)	95 °C 5 minute	95 °C 1 minute	58 °C 1 minute	72 °C 1 minute	72 °C 6 minute
			Repeated for 30 cycles			
E. coli	Eco 223 Eco 455	95 °C 5 minute	94 °C 45 second	64 °C 45 second	72 °C 90 second	72 °C 10 minute
			Repeated for 35 cycles			
Avibacterium paragallinarum	HPG-2 (F) HPG-2 (R)	95 °C 5 minute	94 °C 1 minute	65 °C 1 minute	72 °C 2 minute	72 °C 10 minute
			Repeated for 25 cycles			
B. avium	N-avium (F) C-avium (R)	95 °C 5 minute	95 °C 30 second	50 °C 30 second	72 °C 30 second	72 °C 5 minute
			Repeated for 35 cycles			
O. rhinotracheale	OR16S (F) OR16S (R)	94 °C 7 minute	94 °C 30 second	53 °C 1 minute	72 °C 2 minute	72 °C 7 minute
			Repeated for 30 cycles			
Pseudomonas spp.	*alg*D (F) *alg*D (R)	94 °C 5 minute	94 °C 45 second	62 °C 1 minute	72 °C 1 minute	72 °C 7 minute
			Repeated for 30 cycles			
M. synoviae	16SrRNA (F) 16SrRNA (R)	94 °C 5 minute	94 °C 30 second	55 °C 30 second	72 °C 1 minute	72 °C 5 minute
			Repeated for 30 cycles			
M. gallisepticum	*mec* 2 (F) *mec* 2 (R)	93 °C 3 minute	94 °C 30 second	58 °C 30 second	72 °C 1 minute	72 °C 5 minute
			Repeated for 30 cycles			
Pseudomonas spp.	*opr*D (F) *opr*D (R)	93 °C 3 minute	93 °C 1 minute	55 °C 1 minute	72 °C 1 minute	72 °C 7 minute
			Repeated for 40 cycles			
Staphylococcus spp.	*mec*A (F) *mec*A (R)	94 °C 5 minute	94 °C 30 second	55 °C 30 second	72 °C 1 minute	72 °C 5 minute
			Repeated for 40 cycles			
Staphylococcus spp.	*Coa (F)* *Coa (R)*	94 °C 45 second	94 °C 20 second	57 °C 15 second	70 °C 15 second	72 °C 2 minute
			Repeated for 30 cycles			

PCR products were detected by running a 5 µl sample in 2% agarose gel containing ethidium bromide for 45 min at 80 V and visualization under UV light.

Results and Discussion

The present study was undertaken to study the presence of bacteria in respiratory tract infections from chickens. To find out the bacterial organisms from the respiratory tract of chickens using direct culture and molecular methods. In the present investigation samples were collected from the dead birds presented for post-mortem disease diagnosis at the Department of Veterinary Pathology, Veterinary College, Anand from December 2022 to March 2023. In the present study, 52 bird tissue samples (lung and trachea) were collected from chickens suffering from respiratory tract infections. Out of 52 tissue samples, 51 (98.07%) tissue samples were found positive for bacterial pathogen by PCR, while 01 (1.92%) tissue sample was negative for the presence of any pathogen. PCR-based detection of tissue samples revealed positivity for *Escherichia coli* 98.08% (51/52) followed by *Staphylococcus* spp. 59.61% (31/52), *Mycoplasma gallisepticum* 9.61% (05/52), *Ornithobacterium rhinotracheale* 7.69% (04/52), *Avibacterium paragallinarum* 3.85% (02/52), and *Mycoplasma synoviae* 1.92% (01/52). None of the samples found to be positive for *Bordetella avium*, *Pasteurella multocida*, and *Pseudomonas aeruginosa* through PCR.

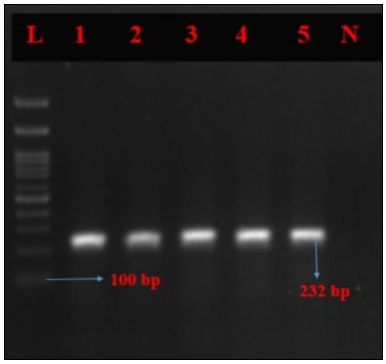

Fig 1: Agarose gel showing amplified product for *Eco* gene PCR product of *E. coli* of 232bp. L- 100-1000bp N- Negative, 1-5 bacterial isolates

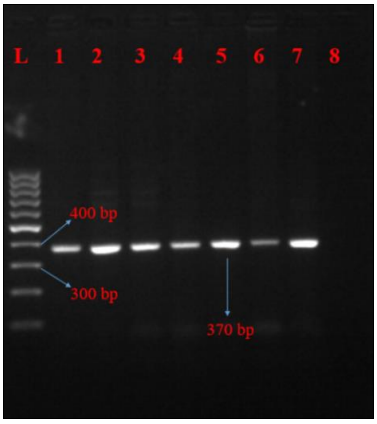

Fig 2: Agarose gel showing amplified product for *TStaG* gene PCR product of *Staphylococcus* spp. of 370bp. L- 100-1000bp 8-Negative, 1-7 bacterial isolates

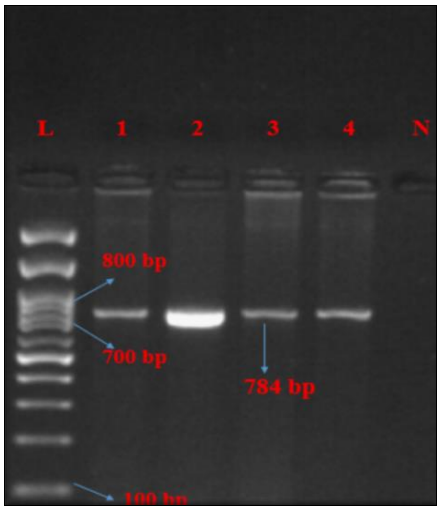

Fig 3: Agarose gel showing amplified product for *OR16S* gene PCR product of *O. rhinotracheale* of 784bp. L- 100-1000bp N- Negative, 1-4 bacterial isolates

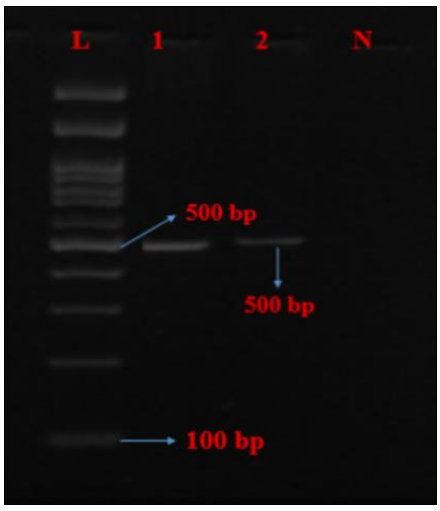

Fig 4: Agarose gel showing amplified product for *HPG-2* gene PCR product of *A. paragallinarum* of 500bp. L- 100-1000bp N- Negative, 1-2 bacterial isolates

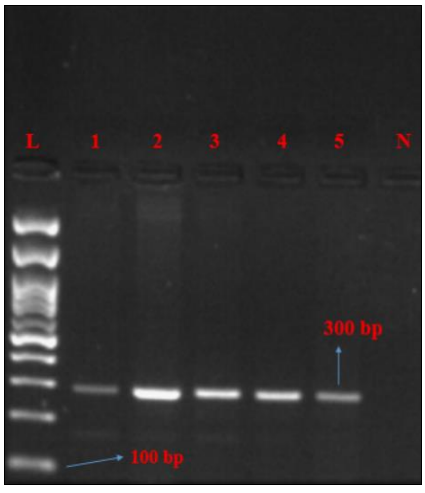

Fig 5: Agarose gel showing amplified product for *mec* gene PCR product of *M. gallisepticum* 300bp. L- 100-1000bp N- Negative, 1-5 bacterial isolates

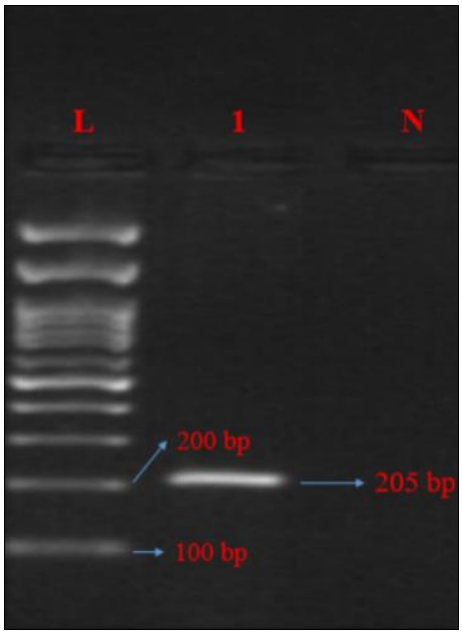

Fig 6: Agarose gel showing amplified product for 16S-rRNA gene PCR product of *M. synoviae* of 205bp. L- 100-1000bp N- Negative, 1 bacterial isolates

Conclusion

Our study highlights the prevalence of bacterial pathogens in chickens suffering from respiratory tract infections. Utilizing molecular methods, notably PCR, we detected a high incidence of Escherichia coli and Staphylococcus spp. in lung and trachea tissue samples. This underscores the significance of these bacteria in poultry respiratory diseases. Importantly, the absence of *Bordetella avium*, Pasteurella multocida, and Pseudomonas aeruginosa suggests their limited involvement in the sampled cases. Our findings emphasize the necessity of thorough surveillance and targeted control measures against prevalent bacterial pathogens to mitigate the impact of respiratory infections in poultry populations. Future research could explore the molecular characteristics and virulence factors of the identified pathogens to enhance understanding and develop more effective control strategies.

References

1. Anne NS, Malmarugan S, Prabhu M, Rajeswar JJ. Isolation and molecular serotyping of *A. paragallinarum* from desi birds. Indian Journal of Animal Health. 2022;61(1):78-83. DOI: https://doi.org/10.36062/ijah.2022.12021
2. Blackall PJ, Soriano-Vargas E. Infectious coryza and related bacterial infections. Diseases of Poultry. 2020:890-906. DOI: https://doi.org/10.1002/9781119371199.ch20
3. Canal CW, Leao JA, Rocha SLS, Macagnan M, Lima-Rosa CAV, Oliveira SD, Back A. Isolation and characterization of *O. rhinotracheale* from chickens in Brazil. Research in veterinary science. 2005;78(3):225-230. DOI: https://doi.org/10.1016/j.rvsc.2004.10.003
4. García M, Ikuta N, Levisohn S, Kleven SH. Evaluation and comparison of various PCR methods for detection of *M. gallisepticum* infection in chickens. Avian diseases. 2005;49(1):125-132. DOI: https://doi.org/10.1637/7261-0812204R1
5. Glisson JR. Bacterial respiratory disease of poultry. Poultry Science. 1998;77(8):1139-1142. DOI: https://doi.org/10.1093/ps/77.8.1139
6. Lanotte P, Watt S, Mereghetti L, Dartiguelongue N, Rastegar-Lari A, Goudeau A, *et al*. Genetic features of Pseudomonas aeruginosa isolates from cystic fibrosis patients compared with those of isolates from other origins. Journal of medical microbiology. 2004;53(1):73-81. DOI: https://doi.org/10.1099/jmm.0.05324-0
7. Lauerman LH, Hoerr FJ, Sharpton AR, Shah SM, Van Santen VL. Development and application of a polymerase chain reaction assay for *M. synoviae*. Avian Diseases. 1993:829-834. Doi: https://doi.org/10.2307/1592037
8. Martineau F, Picard FJ, Ke D, Paradis S, Roy PH, Ouellette M, Bergeron MG. Development of a PCR assay for identification of staphylococci at genus and species levels. Journal of clinical microbiology. 2001;39(7):2541-2547. Doi: https://doi.org/10.1128/jcm.39.7.2541-2547.2001
9. Popy N, Asaduzzaman M, Miah MS, Siddika A, Sufian MA. Pathological study on the upper respiratory tract infection of chickens and isolation, identification of causal bacteria. The Bangladesh Veterinarian. 2011;28:60-69. DOI: https://doi.org/10.3329/bvet.v28i2.10677
10. Prabhu M, Malmarugan S, Sweetline Anne N, Parthiban S, Balakrishnan G, Johnson Rajeswar J. Detection of *M. gallisepticum* infection in turkey and chicken farms of Tamil Nadu, India. International Journal of Current Microbiology and Applied Sciences. 2021;10(01):3151-3158. DOI: https://doi.org/10.20546/ijcmas.2021.1001.367
11. Riffon R, Sayasith K, Khalil H, Dubreuil P, Drolet M, Lagacé J. Development of a rapid and sensitive test for identification of major pathogens in bovine mastitis by PCR. Journal of clinical microbiology. 2001;39(7):2584-2589. DOI: https://doi.org/10.1128/jcm.39.7.2584-2589.2001
12. Savelkoul PH, de Groot LE, Boersma C, Livey I, Duggleby CJ, Van der Zeijist BA, *et al*. Identification of *Bordetella avium* using the polymerase chain reaction. Microbial Pathogenesis. 1993;15(3):207-215. DOI: https://doi.org/10.1006/mpat.1993.1071
13. Thopireddy NR. Isolation and Molecular Detection of E. coli from Common Respiratory Infections of Poultry in AP. 2023. DOI: 10.36349/easjvms.2023.v05i01.001
14. Townsend KM, Frost AJ, Lee CW, Papadimitriou JM, Dawkins HJ. Development of PCR assays for species- and type-specific identification of *Pasteurella multocida* isolates. Journal of Clinical Microbiology. 1998;36(4):1096-1100. Doi: https://doi.org/10.1128/jcm.36.4.1096-1100.1998
15. Van Empel PCM, Hafez HM. Ornithobacterium rhinotracheale: a review. Avian pathology. 1999;28(3):217-227. DOI: https://doi.org/10.1080/03079459994704.
16. Zorman-Rojs O, Zdovc I, Bencina D, Mrzel I. Infection of turkeys with *O. rhinotracheale* and *M. synoviae*. Avian Disease. 2000;44:1017-1022. DOI: https://doi.org/10.2307/1593082.