ISOLATION, IDENTIFICATION, ANTIMICROBIAL SUSCEPTIBILITY AND GENOTYPING OF *PASTEURELLA MULTOCIDA* FROM DIFFERENT REGIONS OF INDIA

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Abstract

Pasteurella multocida (P. multocida) demonstrates exceptional versatility as a pathogen, being capable of causing infections in a wide range of hosts. These include domestic and wild animals, humans, non-human primates, and numerous avian species, including poultry. The study was performed to determine the prevalence of *P. multocida* in poultry flocks using a prospective method following commercial poultry farms over a specific time period to examine genotyping, antibiotic sensitivity, and pathogenicity of *P. multocida* establishing causal relationships between risk factors and events that provide valuable insights into disease progression in different regions of India. A total of 191 bone samples were collected from poultry suspected for fowl cholera (FC) originating from nine Indian states. Initially, all the samples were inoculated onto blood agar and MacConkey agar for isolation and biochemical characterization. Further, the confirmation of *P. multocida* was done by PCR using universal primer and genotyping was performed using eight (Genotype-1 to 8) sets of primers. Out of 191 samples, 48 were identified as *P. multocida* and tested for antibiotic sensitivity using the Kirby/Bauer disc diffusion method with 13 antibiotics. The strains displayed the highest susceptibility to Enrofloxacin (100%), Co-trimaxazole (100%), and chloramphenicol (100%). Ampicillin (87.50%), Amoxycillin (87.50%), and Gentamicin (87.50%) showed slightly lower susceptibility rates. Pathogenicity of the strains was checked by inoculating the pure isolates of *P. multocida* in specific pathogen free (SPF) chickens. Following experimental infection in SPF chickens, the affected birds exhibited symptoms characteristic of fowl cholera, and *P. multocida* was successfully re-isolated from the heart blood, liver, and femur bone marrow of the deceased birds. After inoculation, mortality and other clinical symptoms were observed. Out of the 48 confirmed *P. multocida* samples, 34 were identified as Lipopolysaccharide (LPS) genotype-1, 6 as LPS genotype-3, 1 as LPS genotype-4, and 7 as LPS genotype-5. This study provides valuable insights into the prevalence of various genotypes and highlights the emergence of multidrug-resistant strains of *P. multocida* within commercial chicken flocks in India.

Keywords: Antimicrobial susceptibility, Fowl cholera, India, LPS genotyping, *P. multocida*.

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Introduction

The acute and fatal septicemic disease known as fowl cholera causes enormous financial losses to the global chicken industries, which can infect all avian species (Pruimboom*et al*., 1999), caused by the bacteria *P. multocida*. *P. multocida* is a small, non-motile, Gram negative, and coccobacillary facultative anaerobic organism. It can also infect the reptiles and humans in addition to avian species, causing acute diseases such as hemorrhagic septicemia in buffalo and cattle, enzootic bronchiolitis in cattle, sheep, and goats, necrotic rhinitis in swine, snuffles in rabbits, and fowl cholera in chickens. *P. multocida* has also been reported as an emerging zoonotic pathogen which could endanger health of the public. *P. multocida* is recognized as a commensal bacterium found in the respiratory tract of numerous wild and domestic birds. However, it can also become pathogenic and cause diseases, particularly when the birds are subjected to stressful conditions (Harper *et al.,* 2006; Dziva*et al.,* 2008; Register and Brockmeier, 2019).

P. multocida has been consistently found in the upper respiratory tract, spleen, lungs, blood, and liver of the infected birds (Ozbey and Muz, 2006; Peng*et al.,* 2019). Contact to mucous discharges from animals and pets with pulmonary infestation or disease can cause severe pulmonary infection in human. *P. multocida*'s incubation time typically ranges from 12 to 48 hours, with 100% death most frequently occurring between 24 and 72 hours post infection (Sarkozy, 2002).

LPS is an immunogenic and extremely variable carbohydrate antigen that forms the outside kernel of Gram-negative microbes outer membrane and has a definite role in strains' propensity to produce high virulence (Harper *et al.,* 2012, 2004). According to Harper *et al.,* study, only eight different genetic loci designated L1 through L8 are present in the 16 Heddleston type strains. These genomic loci are responsible for the biosynthesis of LPS outer core (Harper *et al.,* 2015). Traditional phenotypic techniques have some drawbacks, which genotypic approaches of bacterial identification have helped to overcome in recent years (Townsend *et al.,* 1998). The Heddleston system is used to classify the 16 lipopolysaccharide (LPS) serovars (1–16) of *P. multocida* strains into serovars A, B, D, E and F based on capsule antigens (Heddleston*et al.,* 1972). Using such serological typing approaches, which have been extensively used in epidemiological research, the serotypes of *P. multocida* isolates that are proliferating in different host species and that are associated to diverse types of illnesses have been found. Many poultry farmers in India continue to employ autogenous bacterins, it is widely assumed that killed whole cell vaccine induce powerful protective immunization against strains with similar LPS structures. In order to figure out if the already utilized bacterin provide the defense against newly found outbreak strains, Heddleston serotyping has been employed to estimate the LPS type of both outbreaks and vaccination strains. Capsular genotyping, LPS genotyping, MLST, and virulence genotyping based on the identification of several virulence gene profiles are the most used molecular typing techniques for *P. multocida*(García-Alvarez *et al.,* 2017; Peng*et al.,* 2018).

Several serovars are genetically related; for example, serovars 1 and 14 have the same LPS outer core biosynthetic loci. The type L2 outer core biogenesis locus is shared by serovar 2 and strain 5, the type L3 locus is shared by serovars 6 and 7, the type L4 locus is shared by serovar 9, the type L5 locus is shared by serovars 10, 11, 12 and 15, the type L6 locus is shared by serovar 8 and strain 13, and the type L7 and the type L8 locus is shared by serovar 16 strains (Harper *et al.,* 2012). The genetic organisation of the LPS outer core biosynthetic loci was used to develop a multiplex PCR test that allowed the classification of the 16 LPS serotype strains of *P. multocida* into L1, L2, L3, L4, L5, L6, L7 and L8 eight genotypes (Harper *et al.,* 2015). This LPS-build genotyping approach is a quick and efficient means to identify *P. multocida* for epidemiological

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investigations. Because of the importance of the capsule and LPS in *P. multocida*'s virulence and host specificity, the use of genotyping techniques that incorporate the capsular and LPS genotyping processes would be beneficial for the analysis of clinical isolates and the investigation of the pathogen's molecular evolution in epidemiological aspects. This study offers significant insights into the prevalence of diverse genotypes of *P. multocida* and sheds light on the emergence of multidrug-resistant strains within commercial chicken flocks in India. These findings provide crucial information for understanding the genetic diversity of the pathogen and its resistance patterns. The identification of multidrug-resistant strains emphasizes the need for effective control measures and antimicrobial stewardship in the poultry industry. This research contributes to the ongoing efforts to combat fowl cholera and underscores the importance of continuous surveillance and monitoring of *P. multocida* genotypes for effective disease management strategies.

Materials and Methods
Sample collection
Selection Criteria
The affected birds exhibited ruffled feathers, a faster breathing rate, and later on, diarrhoea before they died. They also seemed clinically anorexic, dull, and melancholic. Acutely deceased birds' post-mortem examination revealed petechial hemorrhages, overall hyperaemia, and an enlarged liver with necrotic foci. There were noticeable hemorrhages on the subepicardial and subserosal regions. Lung congestion as well as modest renal enlargement with inflated tubules was seen (Figure 1 A, B, C, & D).
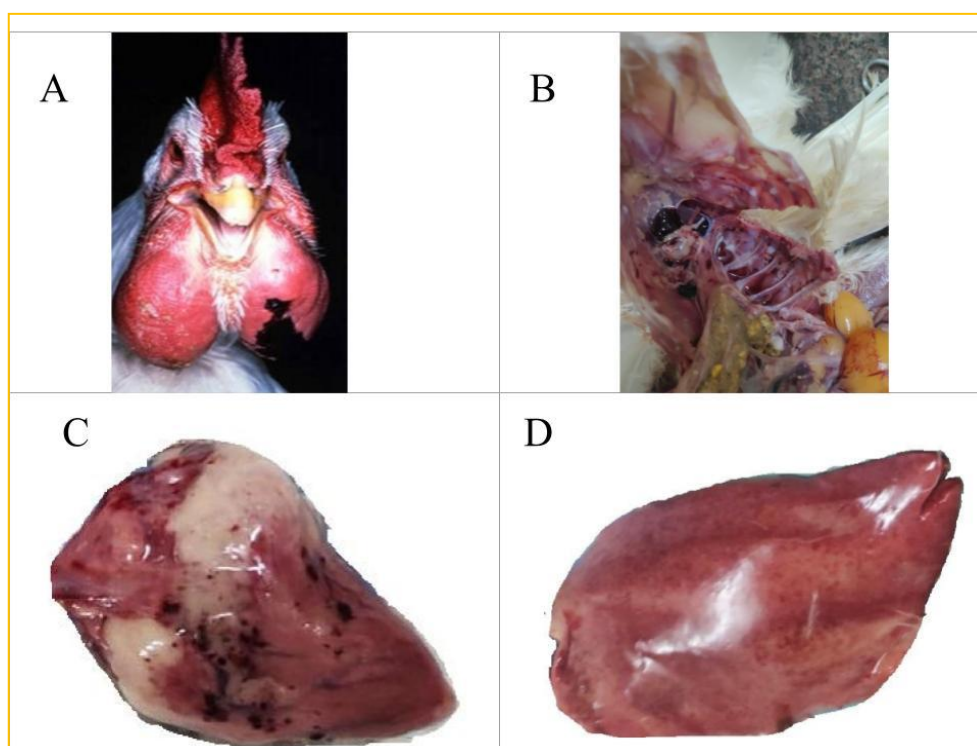

Figure 1: A) Swollen wattles, B) Hemorrhages in lung cavity, C) Pin point hemorrhages on heart, & D) Focal Necrosis on liver

In the present investigation, 191 femur bone samples (Table 1) from commercially raised poultries from nine states of India (Andhra Pradesh, Haryana, Gujarat, Telangana, Tamil Nadu, Kerala,

Karnataka, Maharashtra and Punjab) were collected and transported in charcoal to M/s Globion India Pvt. Ltd., Hyderabad, Telangana during January 2019 to February 2021, from the deceased birds that showed signs of depression, diarrhea, increased respiratory rate, and hemorrhagic septicemia.

Table 1: State wise sample collection

S/N	States	Total samples collected
1	Andhra Pradesh	29
2	Gujarat	13
3	Haryana	26
4	Karnataka	52
5	Kerala	8
6	Maharashtra	12
7	Punjab	9
8	Tamil Nadu	24
9	Telangana	18
	Total	191

Isolation and identification of bacteria

All the 191 femur bone samples were opened using a sterilized bone cutter and cultured onto blood agar and MacConkey's agar plates using sterile swabs and wire loops (Fig. 2 A& B). Swabs with the remaining samples were inoculated in brain heart infusion broth for bird inoculation to verify Pathogenicity and to produce a pure culture in case of mixed and/or contaminated cultures were observed on plates. At 37°C, all of the above inoculated plates and broth were incubated for 24 hours and 18 hours respectively (Cowan, 1974).
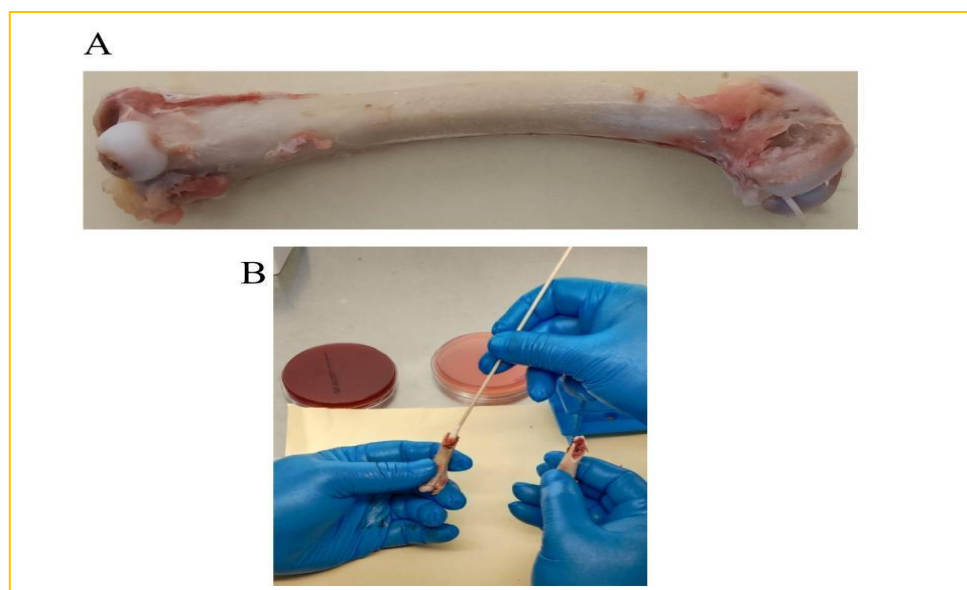

Figure 2: A) Femur Bone sample & B) Bone marrow swab sample collection

To confirm *P. multocida* strains, standard procedures described by Cowan and Steel (Cowan, 1974) and Cruickshank *et al*., (Cruickshank *et al.,* 1975)were followed, including cultural, morphological, biochemical, and sugar fermentation tests.

Testing for Pathogenicity

P. multocida isolates were cultured in brain heart infusion (BHI) broth for 18 hours at 37° C in an incubator. To determine pathogenicity, 0.2 ml of each culture, holding approximately 2.4×10^8 CFU/ml, was inoculated intramuscularly into each of three specific pathogen free (SPF) Chickens aged 6-8 weeks and monitored for 72 hours   (Abood*et al.,* 2021). The organism was again isolated among the heart blood, liver, and femur bones of deceased birds.

Antibiotic sensitivity test

Thirteen different antibiotic discs (Table 3) were used in total to test each strain's susceptibility to antibiotics using the Kirby/Bauer disc diffusion method (Bauer *et al.,* 1966. All the 48 *P. multocida* strains after identification were inoculated in to BHI broth and incubated at 37°C for 18 hrs. The turbidity was adjusted to 0.5 on the scale of McFarland standards.

The disc diffusion test was conducted on Mueller-Hinton agar plates. To perform the antimicrobial susceptibility test, 200 µl of an 18 hour culture for each strain was evenly spread on plates. After allowing the culture to absorb onto the plate for 10 minutes, antimicrobial discs were placed on the plates at suitable distances from each other. The plates were then incubated at 37°C for 24 hours. The diameter of the inhibition zone was measured and compared to the corresponding standard zone diameters provided by the antibiotic disc manufacturer (Himedia, Mumbai, India). Based on this comparison, the test culture was interpreted as resistant, intermediate, or sensitive, following the manufacturer's procedures.

PCR assay for isolate identification

All the *P. multocida* isolates used in the study underwent rapid detection by extracting genomic DNA from a 1.5 ml overnight culture of *P. multocida* using the Himedia genomic DNA purification kit (HiPurA® Bacterial Genomic DNA Purification Kit).The specific detection of *Pasteurella multocida* was conducted using a PCR method based on the standard procedure described by Miflin and Blackall in 2001. The primer sets used for PCR amplification were designed from the sequence of the 23S ribosomal RNA, obtained through automated DNA sequencing. To prepare the sequencing template, PCR amplification was performed using the primers identified by Van Camp et al. in 1993 (Van Camp *et al.,* 1993). These primers targeted universally conserved regions flanking highly variable regions, enabling the specific detection of *P. multocida* strains (Miflin and Blackall, 2001). The PCR cycling conditions are as follows: initial denaturation at 98°C for 2.5 min, followed by 30 cycles of denaturation at 94°C for 1 min, annealing at 69°C for 1 min, extension at 72°C for 1 min with a final extension at 72°C for 10 min using Veriti[TM] 96-well fast thermal cycler (Applied Biosystems, Foster City, USA).

Genotyping

After obtaining positive samples using universal primers, further analysis was performed using eight distinct genetic loci primers, corresponding to Genotype - L1, L2, L3, L4, L5, L6, L7, and L8. This analysis aimed to confirm the genotype of the samples individually. The method developed by Harper et al. in 2015 was employed for this purpose (Harper *et al.,* 2015). The PCR cycling conditions used in this study were as follows: an initial denaturation step at 95°C for 10 minutes, followed by 30 cycles of denaturation at 95°C for 30 seconds, annealing at 54°C for 30 seconds, and extension at 72°C for 2.5 minutes. A final extension step was performed at 72°C for 2 minutes. The Veriti TM 96-well fast thermal cycler was utilized for carrying out these PCR cycling conditions (Applied Biosystems, Foster City, USA). The PCR products obtained from the LPS-mPCR were subjected to analysis using gel electrophoresis. A 2% agarose gel prepared in 1X

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Tris-acetate-EDTA (TAE) buffer was used for this purpose. The gel was run at a constant voltage of 70 V for 90 minutes to separate and visualize the PCR products. Each primer pair generates a distinct amplicon size for optimal electrophoretic separation.

Results

It was discovered that *P. multocida* was present in all of the morbid samples that were analyzed shortly after the collection. 48 bacterial cultures collected from outbreaks of fowl cholera were identified as *P. multocida.* It was found in 48 of the 191 samples (Table 2) collected between January 2019 and February 2021, ranging in age from 14 to 100 weeks.

Table 2: Sate wise details of PCR positive samples

S/N	State	Total samples collected	PCR positive
1.	Andhra Pradesh	29	4 (13.79 %)
2.	Gujarat	13	3 (23.08 %)
3.	Haryana	26	8 (30.77 %)
4.	Karnataka	52	23 (44.23 %)
5.	Kerala	8	1 (12.50%)
6.	Maharashtra	12	1 (8.33 %)
7.	Punjab	9	1 (11.11 %)
8.	Tamil Nadu	24	1 (4.17 %)
9.	Telangana	18	6 (33.33%)
	Total	191	48 (25.13%)

Isolation

After 18 hours of incubation at 37°C, small, glistening, dew drop-like colonies were discovered on blood agar plates. Gram-negative cocco-bacilli and bipolar organisms were also detected after gram's staining, Giemsa staining respectively and capsules were demonstrated with indirect India-ink method staining. Oxidase, catalase and the nitrate reduction and Indole tests were all positive, which was generated by all strains. Tests for gelatin liquefaction, methyl red, Voges-Proskauer, and citrate showed no response. The organisms were identified as non-hemolytic on blood agar but failed to develop on McConkey's agar. None of the strains interacted with salicin, raffinose, inositol, or rhamnose when fructose, glucose, galactose, mannitol, and sucrose were fermented.

Colony morphology and staining

The homogenous colonies were observed on blood agar plates after the incubation period is over. Typical colonies on blood agar plates are circular in shape, smooth, greyish in colour, butyrous, and have a distinct odour.All isolates produced indole and tested positive for ornithine decarboxylase, catalase, oxidase, and nitrate reduction. Citrate, the methyl red (MR) and VogesProskauer (VP) tests, and the gelatin liquefaction test revealed no reaction.

On blood agar, the organism was discovered to be non-motile and non-haemolytic and did not grow on McConkey's agar. Salicin, raffinose, inositol, and rhamnose were not fermented and the isolates were reacted with fructose, glucose, galactose, mannitol, and sucrose. Gram negative bacilli were observed in gram's staining. Bipolar staining was observed in bacteria that are usually rods, found to be single or in pairs (Fig. 3). Capsules were demonstrated using an indirect India-ink method.
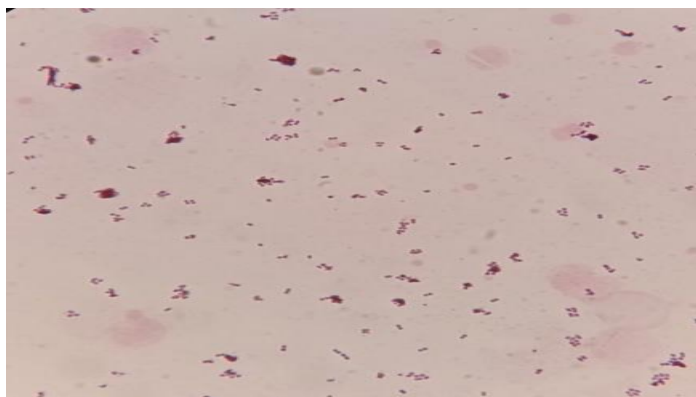
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Figure 3: Gram's stained *P. multocida*

Pathogenicity

The SPF chickens presented with ongoing anorexia and despondency, as well as an extreme exhaustion and death after the inoculation of *P. multocida* culture broth. Within 24 to 72 hours of the infection, chickens also showed enlarged wattles, localized necrosis, and pin-point hemorrhages on the heart. Re-isolated bacteria from the heart blood, liver, and femur bone marrow of the deceased birds exhibited a *P. multocida* culture in the expected manner.

Antibiotic sensitivity test

Table 3 presents the sensitivity profiles of *P. multocida* strains to 13 antimicrobial discs. The strains showed the highest susceptibility to Enrofloxacin (100%), Co-trimaxazole (100%), and Chloramphenicol (100%). Ampicillin (87.50%), Amoxycillin (87.50%), and Gentamicin (87.50%) exhibited slightly lower susceptibility rates.

Table 3: Antimicrobial susceptibility pattern of 48 *P. multocida* strains isolated from poultry and tested with the disc diffusion method

S/N	Antimicrobial agent	Concentration	*n*	% of strains		
				Sensitive	Intermediate	Resistant
1	Ampicillin	30 µg	48	87.50	12.50	0.00
2	Amoxycillin	10 µg	48	87.50	12.50	2.08
3	Cephalexin	10 µg	48	31.25	68.75	0.00
4	Chloramphenicol	10 µg	48	100.00	0.00	0.00
5	Chlortetracycline	30 µg	48	85.42	14.58	0.00
6	Ciprofloxacin	5 µg	48	66.67	33.33	0.00
7	CoTrimoxazole	25 µg	48	100.00	0.00	0.00
8	Enrofloxacin	10 µg	48	100.00	0.00	0.00
9	Gentamicin	10 µg	48	87.50	12.50	0.00
10	Neomycin	10 µg	48	85.42	0.00	14.58
11	Oxytetracycline	30 µg	48	83.33	6.25	10.42
12	Sulphametho-xazole	30 µg	48	81.25	0.00	18.75
13	Tetracycline	30 µg	48	81.25	4.17	14.58

Universal primer PCR and Genotyping results

Out of the 48 samples analyzed, 34 samples exhibited bands at 1307 bp, indicating the presence of LPS genotype-1. Additionally, 6 samples displayed bands at 474 bp, corresponding to LPS

genotype-3. Furthermore, 1 sample showed bands at 550 bp, indicative of LPS genotype-4, while 7 samples revealed bands at 1157 bp, representing LPS genotype-5. These findings suggest that the predominant LPS genotype causing poultry infections is LPS-1 (refer to Figure 4 A& B). The LPS genotypes in avian *P. multocida* exhibit a greater diversity. This LPS-based genotyping technique offers a rapid and precise method for determining *P. multocida* LPS genotypes, facilitating epidemiological research.
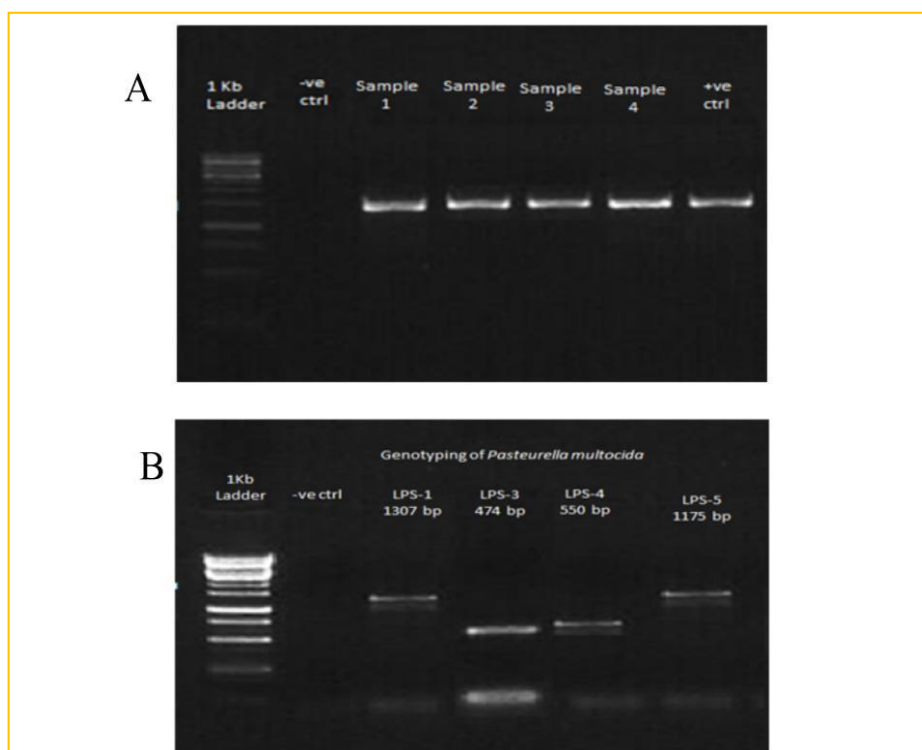

Figure 4: A) *P. multocida* **PCR with universal primer & B) Genotyping of** *P. multocida*

Table 4. State wise data of the LPS genotypes of the current study during 2019 to 2021

S/N	States	n	Year			Percentage (%) of Genotypes			
			2019	2020	2021	LPS GEN-1	LPS GEN-3	LPS GEN-4	LPS GEN-5
1	Andhra Pradesh	4	4	-	-	100	0	0	0
2	Gujarat	3	1	-	2	100	0	0	0
3	Haryana	8	4	2	2	87.5	0	0	12.5
4	Karnataka	23	2	11	10	56.52	26.09	4.35	13.04
5	Kerala	1	-	1	-	100	0	0	0
6	Maharashtra	1	1	-	-	100	0	0	0
7	Punjab	1	1	-	-	100	0	0	0
8	Tamil Nadu	1	1	-	-	100	0	0	0
9	Telangana	6	2	4	-	50	0	0	50
	Total	48	16	18	14	70.83	12.50	2.08	14.58

*GEN refers to genotype

Discussion

In the current investigation, testing of Pathogenicity, genotyping, and antibiotic resistance trends of *P. multocida* isolates found in commercial chicken farms over a period of years from various locations of India were examined.

A previous study from India to reported the use of bone marrow sample for *P. multocida* isolation (Shivachandra *et al.,* 2005). Considering the delay in transportation of the samples from various states of India, we preferred femur bone samples in charcoal to avoid the chances of putrefaction with the organ samples. One of the most helpful aspects in studying *P. multocida* is its colonial morphology as seen with obliquely transmitted light. Colonies may even be iridescent, sectored with varying degrees of iridescence, or blue with little to no iridescence upon first isolation from birds with fowl cholera (Glisson*et al.,* 2008). This study's *P. multocida* colony morphology also exhibits obliquely transmitted light and iridescence. According to Shiva Chandra *et al.,* biochemical characterization study of *P. multocida*, there is no reaction with the subsequent tests of citrate, MR and VP, as well as the liquefaction of gelatin. On blood agar, the organism was non-motile and non-hemolytic, and no growth was seen on a plate of McConkey's agar. Salicin, raffinose, inositol, and rhamnose were not fermented, and instead, fructose, glucose, galactose, mannitol, and sucrose were used to react with the isolates.

The isolates used in this investigation produced the same outcomes in biochemical and sugar fermentation testing (Shivachandra *et al.,* 2006). According to a 2017 study conducted in Bangladesh, the affected birds were diagnosed with FC by inspecting their clinical symptoms, which included enlarged wattles and combs, lameness, respiratory rales, diarrhoea, and abrupt fatality. In contrast, during our investigation, the FC suspected birds displayed the same symptoms, and after re-isolating the bacteria, we discovered the same clinical signs in SPF chickens as well (Saha *et al.,* 2021).The results of our investigation demonstrated that *P. multocida* was effectively identified in every sample using the 23S rRNA-based PCR assay developed by Miflin and Blackall (2001). Other researchers had documented the use of similar primers (Singh *et al.,* 2014; Harper *et al.,* 2015; Nobrega *et al.,* 2021).

Antibacterial treatment is still commonly used to control fowl cholera but has been compromised by the emergence of resistant strains. Disinfection management is essential to controlling fowl cholera, and antibacterial therapy has been widely used to treat infected chickens (Jeong *et al.,* 2021). The emergence of multi-antibiotic resistance has been attributed to the sub therapeutic use of antibiotics in animal feed and metaphylactic antibiotic therapy. The ability of *P. multocida* to mimic host molecules through its LPS is believed to play a crucial role in its survival within specific host niches. Moreover, *P. multocida* LPS demonstrates notable resistance against chicken cathelicidins, which are antimicrobial peptides. Additionally, *P. multocida* LPS acts as a potent stimulator of host immune responses (Harper and Boyce, 2017). The conjugative R-plasmids play a role in spreading multidrug resistance across different species and genetic backgrounds (Stone, 1975), potentially causing epidemic infections. According to Rajini *et al.,* (1995), in vitro drug sensitivity testing showed that chloramphenicol demonstrated high effectiveness (96.6%), followed by doxycycline-HCl, chlortetracycline, nitrofurantoin, penicillin, and co-trimoxazole with sensitivities of 80.0%, 73.3%, 73.3%, 66.66%, and 60%, respectively. Over the past two decades, there has been an observed shift in the antibiotic sensitivity pattern of avian-origin Pasteurella strains. The emergence of resistance to conventional antibiotics has become a significant challenge in the treatment of birds affected by Pasteurellosis (Shivachandra*et al.,* 2004). However, based on the current results, it is found that chloramphenicol, enrofloxacin, and

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Co-trimoxazole can still be considered as effective treatment options for avian pasteurellosis. This finding is consistent with the study conducted by Sarangi*et al.,* where 94% of *P. multocida* isolates showed sensitivity to enrofloxacin and chloramphenicol. In the previous study conducted by Hirsh *et al.,* in 1989, the susceptibility of avian strains of *P. multocida* to various antibiotics was tested.

The antibiotics included chloramphenicol, gentamicin, tetracycline, kanamycin, penicillin G, streptomycin, sulphonamides, and trimethoprim. Additionally, the susceptibility of transconjugants and transformants was determined using the disc diffusion assay (Hirsh *et al.,* 1989). In the current study 14% of the strains found to be multidrug resistant out of the total 48 *P. multocida* isolates. The majority of isolates (68.75%) indicating intermediate sensitivity to the antibiotic cephalexin. Sulphamethoxazole had the highest rate of resistance among the isolates (18.75%), followed by Tetracycline (14.58%), Neomycin (14.58%), and Oxytetracycline (10.42%). *P. multocida* isolated from various animals was found to be susceptible to enrofloxacin, chloramphenicol, and co-trimoxazole, according to almost all earlier research (Shivachandra *et al.,* 2004; Kumar *et al.,* 2009; Katsuda*et al.,* 2013; Sarangi*et al.,* 2015).

In this study, 48 avian isolates were genotyped for *P. multocida* from 9 states of India, where as in a similar study by Sarangi *et al.,* (2016) from India reported 3 avian isolates that were genotyped for *P. multocida* from Tamil Nadu (2002, 2006) and Gujarat (2010). Although completion of genotyping for *P. multocida* isolates has lagged behind those of many states in India, the recent availability of genotyping data from multiple states has significantly not sufficient in understanding the pathogenesis of *P. multocida*. The strains are distributed in India's nine states in a diverse range of ways. The majority of the strains were isolated from Karnataka with 23 isolates out of a total of 48, followed by Haryana with 8 isolates, Telangana with 6 isolates, and Andhra Pradesh with 4. LPS genotypes 3 and 4 were exclusively found in the state of Karnataka, but LPS genotype 5 was found in the states of Karnataka, Telangana, and Haryana. The only one isolate of LPS genotype 4 was observed in Karnataka (Table 4).

Even when the conventional serotyping approach fails, the LPS genotyping method offers the benefit of being able to detect strains more rapidly (Harper *et al.,* 2015). LPS is acknowledged as one of the most essential components involved in the pathogenesis of *P. multocida* (Harper *et al*., 2006; Harper and Boyce, 2017). The significant proportion of the strains were identified as capsular LPS genotypes L1 (71%), L5 (50%), L3 (38%), and L4 (6%) from a total of 48 isolates. Whereas the majority of the genotyped avian *P. multocida* were A:L1 and A:L3 types, according to a research by Peng *et al.* that included the capsular genotypes with the LPS genotypes (Peng *et al.,* 2021). In this experiment, *P. multocida* LPS genotype L1 represents the most isolated genotype with a 71% isolation rate. One such study suggests that L3 are the most prevalent LPS genotypes associated with porker respiratory problems in China. LPS genotype L3 accounts for the third-highest genotype proportion in this investigation, at 38%.The L1, L3, L4, and L6 strains of fowl cholera were found among Australia's isolates, whereas only L1 and L3 were discovered in the fowl cholera strains from southwest China. Turni *et al.,* 2018. In this investigation, L1, L3, L4, and L5 LPS genotypes were discovered in various Indian locations.

The current study was especially focused on Indian commercial poultry farms to rescue the layer, broiler and breeder chickens from the different genotypes of *P. multocida* causing acute disease fowl cholera. A state wise data covering the country with multiple locations is unique for understanding the fowl cholera status in India. This study has the limitations as follows, due to the

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lack of sample resources availability and less number of poultry farms in the other states the sample collection was only conducted in nine states of India. The precise genotypic data is only possible to get when the samples were collected from all the states.

Conclusion

In order to develop an effective fowl cholera vaccine, it is crucial to have a thorough understanding of the circulating genotypes. Creating a vaccine from a strain obtained from the same location will assist in preventing fowl cholera in India. The current study not only helps veterinarians comprehend the prevalence of *P. multocida* in India, but it also provides accurate data for formulating a vaccine targeted towards the prevailing fowl cholera genotypes. Additionally, antimicrobial susceptibility testing has identified specific antibiotics that show promise in combating fowl cholera infections.

Acknowledgement

Ethical Approval

The research project complied with professional and institutional animal welfare policies and was approved by Committee for the Purpose of Control and Supervision of Experiments on Animal (CPCSEA) by the government of India with registration number 1326/c/10/CPCSEA.

Conflict of Interest: The authors declare no conflict of interest.

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