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Review of Detecting Virulence Associated Genes Isolated from Pasturella *multocida* Strains of Fowl Cholera

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Abstract
Avian Pasteurella multocida is the causative agent of fowl cholera, a highly transmissible illness that affects both domestic and wild bird species and causes enormous economic losses around the globe. Numerous genes encoding potential virulence factors that aid in Pasteurella multocida pathogenesis are found across the genomes of the bacteria. Pasteurella multocida contains a variety of virulence factors (VF), such as iron acquisition proteins, neuraminidases and superoxide dismutases, hyaluronidase, toxins (ToxA), lipopolysaccharides (LPS), capsular and outer membrane proteins, and proteins involved in adhesion and colonization. Pasteurella multocida can enter a host by way of the conjunctiva, cutaneous wounds, and mucous membranes of the upper respiratory tract. Stressors can alter how a disease progresses, including dietary changes, environmental changes, and injuries. To prevent and control both acute and chronic poultry cholera, practical measures include vaccination, disinfection, and management practices.
Key words: Fowl cholera, Virulence factors, Capsules, LPS
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1. INTRODUCTION

Gram-negative, facultative anaerobic bacteria belonging to the Pasteurellaceae family is called Pasteurella multocida. It is not motile. It is the underlying cause of chicken cholera, a disease that frequently strikes adult birds as acute, deadly septicemia, while it can also cause persistent, asymptomatic infections (Anon, 2015).
Poultry is one of the most popular food industries worldwide, with over 90 billion tons of chicken meat produced each year (FAO, 2017). The most widely farmed species is chicken. However, fowl cholera, which is caused by the extremely contagious avian P. multocida, affects both domestic and wild bird species and results in substantial economic losses worldwide (Sellyei et al., 2017). Poultry cholera disease can also be caused by P. multocida subspecies septica and gallicida, however P. multocida subspecies multocida is the most common cause.
(Peng *et al.,* 2018).
Pasteurella multocida is categorized into 16 Heddleston serotypes based on lipopolysaccharide (LPS) antigens and five serotypes, A, B, D, E, and F, based on the specificity of capsular antigens (Jilo *et al.,* 2020). Serotype A is known to have a broad host range among them (Wilson *et al.,* 2013). It is further separated into the serotypes 1: A, 5: A, and 9: A (Mohamed *et al*., 2012). However, poultry are typically not infected by strains isolated from nonavian hosts, and the underlying mechanisms are not well known. According to Pan et al. (2020), an avian P. multocida of serotype A, PmQ, that was isolated from the tissues of a deceased duck exhibits great virulence toward both poultry and mammals (*Pan et al., 2020*).
The primary lipopolysaccharide and capsule on the surface of P. multocida are critical for resistance to phagocytosis, complement inhibition, and antimicrobial peptide action (Wilkie *et al.,* 2012). Furthermore, several virulence factors contribute to the microorganism's survival within the infected host, and pathogenic bacteria typically possess multiple adhesins to unique cell types (Wilson & Ho 2013).
One of the many infectious diseases affecting Ethiopia's rapidly expanding chicken industry is fowl cholera. Nevertheless, neither the locally accessible vaccine nor the genotypes of P. multocida that cause poultry cholera are known. No actions were done to address the issue in spite of numerous complaints and reports from both public and private poultry farms connected to cholera outbreaks in poultry (Wubet *et al.,* 2019).
Because P. multocida is an opportunistic bacterium in vertebrates, studies on Pasteurella virulence genes and in-vitro transcription analysis have shown that the pathogenic strain is essential (Pilatti *et al.,* 2015). The several virulence factors of P. multocida are linked to its pathogenicity. P. multocida contains a variety of virulence

factors (VF), such as extracellular enzymes like neuraminidases (NanB and NanH) and superoxide dismutases (Soda, SodC, and TbpA), hyaluronidase (PmHAS), toxins (ToxA), lipopolysaccharides (LPS), capsular and outer membrane proteins (OmpA, Omph, Oma87, and PlpB), proteins related to adherence and colonization (PtfA, FimA, Hsf-1, Hsf-2, PfhA, and TadD); and iron acquisition proteins (ExbB, ExbD, TonB, HgbA, HgbB, and Fur), extracellular enzymes comprising capsular and outer membrane proteins (OmpA, Omph, Oma87, and PlpB), hyaluronidase (PmHAS), toxins (ToxA), lipopolysaccharides (LPS), neuraminidases (NanB and NanH), and superoxide dismutases (Soda, SodC, and TbpA) (Gharibi *et al*., 2017).

When there is an acute or peracute infection, the lung lesions that are primarily hemorrhagic may be indicative of poultry cholera. In the lungs of chicken challenged with avian P. multocida strain X73, which died from acute infection, there was a considerable rise in the expression levels of IL6, IL17, and IL22 (Petruzzi *et al*., 2018). The transcriptome of PmQ-infected chicken lungs further shows that cytokines, like IL6 and IL22, were considerably increased in PmQ-P compared to P-C, suggesting that PmQ infection causes a robust inflammatory response in the lungs. According to Pan *et al.* (2020), IL6 is a pleiotropic cytokine that, depending on the situation, can have both pro- and anti-inflammatory effects.

Because the disease has a major effect on chicken production, it must be controlled. From an economic and practical standpoint, vaccinations are the most effective control strategy when it comes to diseases that are endemic in a given area. This is because the sickness develops very quickly, and before the illness is identified and treated, a significant number of people die from it. The advent of multidrug-resistant bacteria has restricted the effectiveness of antibiotic treatment and prevention (Wubet *et al*., 2019).

OBJECTIVE:- To review the virulence associated genes of Pasturella *multocida* species

2. LITRUTURE REVIEW

2.1 Epidemiology

The FC is a highly contagious bacterial disease that affects both domestic and wild birds. It is well recognized as a significant issue for poultry globally. Because of the high death rate caused by the disease, the poultry businesses frequently suffer significant financial losses as a result (Akhtar et al., 2015). Many elements are thought to play a significant role in the emergence of an outbreak, such as pathogen virulence, environmental factors, host density, distribution, immunity, and nutrition. Poultry outbreaks usually happen in the late summer, early fall, and early winter (WHA, 2016).

2.2 Subspecies identification for clinical isolates of P. multocida

Pasteurella multocida is easily recovered, frequently in pure culture, via the caseous exudate typical of chronic fowl cholera lesions, or from internal organs such as the lung, liver, and spleen, bone marrow, gonads, or cardiac blood of chickens that die from the acute bacteraemic type of the disease. The ideal growth temperature for this facultative anaerobic bacterium is 37°C. Media such as blood agar, trypticase-soy agar, and dextrose starch agar are typically used for primary isolation. The addition of 5% heat-inactivated serum may enhance isolation. After 18 to 24 hours of incubation, colonies are distinct, round, convex, transparent, and butyraceous, with a diameter ranging from 1 to 3 mm. The cells are 0.2–0.4 × 0.6–2.5 µm in size, coccobacillary or short rod-shaped, staining Gram negative, and usually happen alone or in pairs. Wright or Giemsa stains clearly show bipolar staining (OIE, 2018)

The following four subspecies are identified as: gallicida, septica, multocida, and tigris. These subspecies are distinguished by sugar fermentation tests. The subtype gallicida has been isolated from cattle and is known to be the cause of chicken cholera. Dogs, cats, birds, and people have all been found to be isolated from the subspecies Septica. Numerous serious illnesses affecting several domestic animal species as well as humans are caused by the subspecies multocida. Only human wounds from tiger bites have been reported to belong to the subspecies tigris (Huberman and Terzolo 2016). Differential properties and identity of 45 clinical isolates of *Pasteurella is* mentioned in table 1 in the following.

	P. *multocida ssp multocida*	P. *multocida ssp.septica*	P. *multocida ssp. gallicida*
Dulcitol	-	-	+
Sorbitol	+	-	+
Trehalose	+	+	-
Arabinose	-	-	+
Xylose	+	+	-
Indole	+	+	+

Table 1: Differential properties and identity of 45 clinical isolates of *Pasteurella* (Zhangcheng *et al*., 2018).

According to (Wubet *et al.,* 2019), Phenotypic characteristics the *P. multocida* type A is isolated in following table 2.

	Isolate		
Test	**1**	**2**	**3**
Hemolysis on blood agar	No hemolytic	No hemolytic	No hemolytic
Growth on MacConkey agar	No growth	No growth	No growth
Gram's reaction	-	-	-
Cellular morphology	Coccobacilli	Coccobacilli	Coccobacilli
Indole production	+	+	+
Catalase test	+	+	+
Oxidase test	+	+	+
MP test	-	-	-
VP test	-	-	-
TSI citrate testtest	Y/Y	Y/Y	Y/Y
Urease test	+	+	+
Interpretation	*P. multocida*	*P. multocida*	*P. multocida*

+= Positive, -=negative, Y/Y= yellow slant and yellow butt

Table 2. Phenotypic characteristics the *P. multocida* type A (Wubet *et al.,* 2019)

2.3 Routes of infection and persistence of the pathogen on farms

Pasteurella multocida can enter the host through the mucous membranes of the upper respiratory tract, conjunctiva, and cutaneous wounds. Stressors, including dietary changes, injury, and changes in the environment, can alter the course of the disease. Carriers and convalescent birds that are chronically infected are a major source of infection because carrier birds shed the bacteria for life. Chronic conditions can arise where the majority of infections are localized. P. multocida can survive for up to two weeks in the environment and up to eight days in dried blood. If separated from organic material, *P. multocida* is easily destroyed by common disinfectants, sunlight, drying, and at temperatures above 56°C (Julia *et al.,* 2019, WHA 2016).

The disease can spread swiftly and infect a large percentage of the herd in a short amount of time, especially if animals are kept in close quarters on trains with insufficient ventilation or for extended periods in holding pens and feed lots (Legesse *et al*., 2018). According to Tadesse *et al.* (2017), it is contracted by breathing contaminated nasal secretions, droplets, coughed up, or exhaled from infected animals or recovered carriers, in which case the infection remains in the upper respiratory tract. Animal bites, scratches, or licks from cats or dogs are common ways for people to contract P. multocida infection (Elane P and Elane M, 2019).

2.4. Virulence factors

Different virulence factors have independently entered the *P. multocida* strains at different times in the evolutionary history of the species and at multiple positions within the phylogenetic tree. Since pathogenic behavior is predicted by both virulence factor repertoire and sero-group, the clonal associations of virulence factors must be evaluated (Haghnazari *et al.,* 2017). The virulence of a particular strain of P. multocida varies greatly depending upon several factors, including the presence of a capsule, endotoxin production, outer membrane proteins, heat shock proteins, and antibody cleaving enzymes (Julia *et al.,* 2019)

As a result, virulence genotyping based on the identification of various virulence gene profiles has also been developed and used as a practical genotyping method (Devi *et al.,* 2018). Many genes in Pasturella multocida genomes encode potential virulence factors that aid in P. multocida pathogenesis (Peng *et al.,* 2016). However,

some virulence genes are mainly linked to particular disease types and/or host species because they are found in a small number of isolates. For example, only isolates from bovine and ovine sources have tbpA, and only bacteria linked to progressive atrophic rhinitis contain toxA (Peng *et al.,* 2019).

Numerous crucial functions have been reported about the correlation between adhesion proteins and bacterial virulence, provoking adhesion and colonization. Sialic acid can be found in a conjugated form to glycolipids and glycoproteins of eukaryotic cells. The presence of sialidases enzymes is crucial in removing these compounds. Additionally, OmpH and PlpE are protective antigens which have been detected in A:1, A:3, and A:4 serotypes from poultry suffered from fowl cholera and cattle with shipping fever (Gharibi *et al.,* 2017).

There is distribution of virulence associated genes of *Pasteurella multocida* in strains isolated from breeders with fowl cholera by multiplex-PCR (*Almeida et al., 2018*)., The distribution of virulence associated genes of *P. multocida detected by PCR according to host species (*Quedi *et al., 2016)*

2.4.1. Detection Of Fimbrae and adhesion Typing Virulence Associated Genes

The pathogenicity of *P. multocida* is associated with fimbriae and adhesions biosynthesis Adhesins have been targeted in the recent virulence genotyping studies, which demonstrated that *fimA* (fimbriae), *hsf*-1, 2 (auto transporter adhesins), *pfhA* (filamentous hemagglutinin), *tad* (nonspecific tight adherence protein), *ptfA* (subunit of type 4 fimbriae), are believed to play an important role in the pathogenesis of *P. multocida* (Zhangcheng *et al*., 2018). Genes and their adhesive factors are characterized according to the table 3 below.

Genes	Adhesin factors
cap A	Capsule A
ompH	Outer membrane protein H
Hsf1	Auto transporter adhesion
prfA	Type IV fimbriae
pfhA	Filamentous hemagglutinin
radD	Putative nonspecific tight adherence
fimA	Fimbriae A
toxA	Dermonecrotic toxin

Table 3: Adhesin-coding genes among avian *P .multocida* (Haghnazari *et al.,* 2017).

One of the most crucial stages in the infection and colonization of a vulnerable host is the adhesion of pathogenic bacteria to epithelial surfaces. Bacteria frequently employ sugar-binding proteins, like lectins and the adhensins pili or fimbriae, as part of their infection strategy to identify and bind to host glycoconjugates (Magda *et al*., 2015). Consequently, pathogenicity and their presence on bacterial surfaces are typically associated (Haghnazari *et al*., 2017).

The primary subunit of type 4 fimbriae, or pili, is encoded by the ptfA gene and is a distinctive feature of P. multocida isolates from various hosts and serotypes/genotypes. For example, the P. multocida strains P1059 (type A; avian isolate), X73 (type A; avian isolate), and 3480 all contain the gene ptfA (PM0084) from Pm70. Additionally, the genomes of these strains contain the putative adhesin-encoding comE1 (Pm70-PM1665) (Johnson *et al*., 2013).

The filamentous hemagglutinin (FhaB) protein encoded by the pfhB gene is comparable to the Bordetella FhaB protein, which is known to be essential for the bacterium's adherence to the respiratory epithelium (Scheller and Cotter, 2015). The Pm70 genome contains two copies of the pfhB gene. Strains P1059, X73, and 3480 include pfhB1 (PM0057), while strains P1059, X73, and 36950 contain pfhB2 (PM0059), but neither HN06 nor 3480 do. A few isolates of P. multocida have extra FhaB-encoding genes. While pfhB4 is present in strains HN06 and 3480 but absent from Pm70, X73, or 36950 (16), pfhB3 is present in strains X73 and 36950 but absent from Pm70, HN6, or 3480.

The tad locus is essential for biofilm development, colonization, and illness in a number of Pasteurellaceae species. Low-molecular-weight fimbrial adhesion protein assembly also requires it. According to Magda et al. (2015), the 14 genes that comprise the tad locus are typically flp1-flp2-tadV-rcpCAB-tadZABCDEFG. Pm70, X73, or 36950.

2.4.2 Detection of Capsule Typing and Virulence-Associated Genes (Vags)

The externally placed, highly hydrated polysaccharides known as capsules stick to the bacterial cell wall. According to Quedi *et al.* (2016), the genes hyaD-hyaC, dcbF, and bcbD are linked to P. multocida capsular

biosynthesis that is particular to serogroups A, D, and B, respectively. Pathogenic bacteria can access a range of heme iron sources from the infected host with the aid of genes related with virulence (capA, capB, capD, capE, and capF) and capsule biosynthesis (Zhangcheng *et al.,* 2018).
Hyaluronic acid makes up the majority of capsule A, heparin makes up capsule D, and chondroitin makes up capsule F. The precise molecular structure of the polysaccharide in capsules types B and E is still unknown. According to Huberman and Terzolo (2016), strains with type A capsule are linked to most poultry cholera outbreaks, type B and E to bovine hemorrhagic septicemia, and type D to porcine atrophic rhinitis. The capsule is associated with the pathogenesis and host predilection. Capsular genotypes A, D, and F are commonly associated with conjunctivitis, respiratory diseases such as rhinitis, pneumonia, and shipping fever, as well as poultry cholera (Peng *et al.,* 2019).

One of the most significant virulence factors for P. multocida is the presence of its capsule, a polysaccharide structure that facilitates invasion and multiplication within the host (Wilkie *et al*., 2012). The capsule is said to have antiphagocytic activity, complement system interaction, and resistance to desiccation (Furian *et al*., 2014). Furthermore, contradictory observations about the potential contribution of the capsule to the adherence to host cells and tissues may be found in the literature (Harper *et al*., 2012). The strain and type of host cell may have an impact on the significance of the capsule in P. multocida adhesion (Furian *et al.,* 2014).

While complete lipopolysaccharide is essential for bacterial survival in the host, the capsule is obviously implicated in the bacteria's resistance to complement and avoidance of phagocytosis. P. multocida strains produce protean toxins in capsular types A and D. Since the hyaluronic acid structure of the type A capsule is identical to that of the tissue structure of the bird, it is thought that the capsule could act as a mask against the immune system. (Terzolo and Huberman 2016).

Polysaccharides make up each P. multocida capsule. Chondroitin, heparin, and hyaluronic acid make up the A-, D-, and F-type capsules, respectively. Despite their close association, the capsular polysaccharide components of types D and F are chemically distinct from the hyaluronic acid component of type A. Mannose, arabinose, and galactose make up the type B capsule. The contents of the type E capsule are still unknown (Peng *et al*., 2019)

A single locus on the genome known as the "cap locus" contains the genes necessary for the production of the many P. multocida capsule types. Three distinct functional gene clusters were identified by analyzing the genetic structure of the cap loci for capsular types A, B, D, and F. These clusters are in charge of capsule production, phospholipid substitution, and capsule export.
. There are at least two different kinds of genomic organization known for P. multocida's cap loci. The ten genes that make up the type I loci, which are specific to the P. multocida type A, D, and F strains, are phyBA-hyaEDCB-hexDCBA. PhyBA is involved in phospholipid substitution, hyaEDCB is involved in capsule manufacturing, and hexDCBA is involved in capsule export. The 15 genes that make up the type II loci, which are specific to P. multocida type B strains, are lipB-bcbABCDEFGHI-lipA-cexDCBA. LipB and lipA are involved in phospholipid substitution, bcbABCDEFGHI in capsule biosynthesis, and cexDCBA in capsule export.
The capsular type E cap region was found to have homologs of bcbA, bcbB, bcbC, bcbE, bcbF, bcbG, and bcbI, as those identified in a type B cap locus, despite the lack of a P. multocida type E genome. The DNA sequences of the capsule export genes of type A were shown to be highly homologous with those of types B, D, and F based on sequence alignments. Although there was reduced homology between the phospholipid substitution genes of types A, D, and F, they were all substantially conserved. The genes encoding glycosyltransferase, such as hyaD in type A, dcbF in type D, and fcbD in type F, were found to differ across the strains of types A, B, D, and F. There were no hyaD, dcbF, or fcbD homologs found at the type B cap locus. Rather, capsular type-specific target sequences were represented by the ecbJ gene in the type E cap locus and the bcbD gene in the type B cap locus. Despite their close relationship, P. multocida capsular types A, D, and F's capsular polysaccharides differ greatly from type B's. Capsular type B strains might be distinguished from other strains using this particular area. (Peng and associates, 2019)

Capsular genotyping of P. multocida isolates:
Due to their comparable cultural, morphological, and biochemical traits, all P. multocida isolates shared a same phenotype that made it impossible to distinguish them using accepted techniques. For the purpose of capsular serogrouping of isolates, multiplex PCR was thus carried out. Elalamy *et al.*, (2020) reported that all P. multocida isolates exhibited an amplicon of 1044 bp for the hyaD-hyaC gene, which is linked to capsular biosynthesis of serogroups A. However, no amplicon was detected for the genes of the other capsular types.

2.4.3 Detection of LPS Typing and Virulence Associated Genes

Lipopolysaccharides (LPS) are important in the disease's etiology. A non-repeating inner and outer core oligosaccharide and the hydrophobic lipid A domain combine to generate the structure of LPS for P. multocida. All strains had twenty genes that encoded the enzymes needed for the production of LPS (Raquel *et al.,* 2018).

The outer membrane of the bacteria anchors LPS. However, LPS needs to be extracted from the membrane or other membranous structures in order for the host immune system to recognize it. Lipid A-binding protein (LBP), a soluble host protein that functions as a lipid transferase and may extract LPS molecules from the bacterial membrane, can cause this directly or indirectly. LPS is released when bacterial cells die. To detect the presence of bacteria in the blood, LBP is present in the serum (Steimle *et al.,* 2016).

When LPS and carrier protein are mixed, antibodies are produced that shield birds from fowl cholera. In contrast to other bacteria, P. multocida contains two LPS, A and B, which are quite identical to one another and only slightly different in terms of internal structure. The pathogenic characteristics of endotoxins are caused by LPS A. Additionally, certain P. multocida strains generate a third LPS known as C. It is thought that the simultaneous expression of several LPS increases the bird's ability to survive P. multocida (Huberman and Terzolo 2016).

Based on their LPS outer core biosynthesis locus, the eight LPS genotypes (L1 to L8) are currently being assigned to the sixteen Heddleston serovars using an LPS multiplex PCR (LPS-mPCR) [Harper et al., 2015]. Turni et al. (2018) have identified LPS types L1 (common to Heddleston serovars 1 and 4) and L6 (comprising Heddleston serovars 10, 11, 12, and 15) as the next most common LPS types linked to poultry cholera in Australia. LPS type 3 is comprised of Heddleston serovars 3 and 4.
The three genetically, biologically, and chemically distinct domains that make up lipopolysaccharides (LPS) are (I) the phosphorylated and more or less acylated lipid A, which is anchored in the bacterial outer membrane; (II) the core oligosaccharide connected to lipid A by 3-deoxy-D-manno-oct-ulosonic acid (Kdo); and (III) the so-called O-antigen or O-specific polysaccharide, which is linked to lipid A. The latter two indicate the aqueous environment. Smooth (S)-form lipopolysaccharides are those that have all three regions, whereas rough (R)-form lipopolysaccharides, also known as lipoligosaccharides (LOS), are those that do not contain the O-antigen. Steimle *et al*. (2016) state that the lipid A structure is generally highly conserved, which is crucial for host receptor recognition.

A heptosyltransferase gene that codes for the addition of heptose, the first outer core sugar, is the starting point for both LPS outer core biosynthesis loci L1 and L3. This is followed by a gene that codes for a bifunctional (Lida *et al.,* 2020) glycosyltransferase (Harper and Boyce, 2017). Six glycosyltransferase-coding genes—htpE, gctC, gatF, natB, gatG, and natC—make up the LPS type L3. (Harper *et al.,* 2013).

Six genes make up the LPS outer core biosynthesis locus L1, including two glycosyltransferases, gatA and htpE (for the addition of the outer core heptose and a bifunctional galactosyltransferase that adds Gal I and Gal II to the heptose, respectively), and four genes involved in the biosynthesis of phosphocoline (PCho), pcgD, pcgA, pcgB, and pcgC. The Heddleston serovars 1 and 14 serovar reference strains share this kind of LPS. The Heddleston serovar 1 reference strain X-73's structural analysis shows that the strain's LPS outer core is made up of a single heptose with two galactoses joined to its positions 4 and 6.
Additionally, two galactose molecules have one PCho and one PEtn molecule connected to positions 3 and 6, respectively (Harper and Boyce, 2017). Previous research has indicated that the majority of the poultry cholera isolates expressing LPS type L1 resemble VP161 (Lida *et al*., 2020) (2PCho, 2Hex, Hep), which is devoid of the two PEtn identified in X_73 (Harper *et al*., 2015). P. multocida has been found to include a number of PEtn transferases, some of which are in charge of adding PEtn to the outer core biosynthetic loci galactose and the inner core, lipid A (Harper *et al.,* 2017). Recently, the gene petG was identified as the one responsible for adding the PEtn molecule (Lida *et al*., 2020).

P. multocida strains only share eight unique loci for the generation of the outer core of LPS, despite the fact that their outer cores differ. Serovar 1 and 14 strains of LPS share the type L1 outer core biosynthesis locus, while strains classified as type L2 and 5 strains, type L3 and 4 strains, type L4 and 7 strains, type L5 and 10, 11, 12, and 15 strains, strains classified as type L6 and 8 and 13 strains, and strains classified as type L7 and 16 strains share the type L8 locus (Peng *et al.,* 2019).

It is also acknowledged that one of the key elements connected to P. multocida pathogenesis is LPS. Though it lacks the typical extended O-antigen structure of LPS observed in other Gram-negative bacteria, P. multocida's LPS is constituted of an inner and outer core oligosaccharide backbone and a highly hydrophobic lipid A moiety.

The two LPS glycoforms (A and B) that P. multocida strains typically produce simultaneously have different inner cores but the identical outer core structure.

The genes necessary for the production of P. multocida lipid A (lpxA, lpxB, lpxC, lpxD, lpxH, lpxK, lpxL, lpxM, kdsA, kdsB, and kdsC) and the inner core oligosaccharide (kdkA, kdtA, gmhA, gmhB, gmhC, hptA, and hptB) are conserved among various P. multocida strains, according to a comparison of representative genomes. These genes are spread throughout the genome at various points. The synthesis of the inner core of the LPS is controlled by two of these genes, hptA and hptB, which encode the heptosyltransferases HptA and HptB, respectively. Accordingly, the kinds of inner core LPS structures that a specific strain of P. multocida produces can be determined by the existence of these two genes and whether or not they are intact in the genome (Moustafa *et al.,* 2015).

Genes necessary for the manufacture of the LPS outer core are found within a single locus on the chromosome, situated between the two conserved genes fpg and priA, in contrast to the genes required for the assembly of the inner core. The eight distinct loci responsible for LPS outer core biosynthesis (L1 through L8) are determined by this gene cluster, which exhibits varying degrees of DNA similarity among strain types. There is only a little amount of sequence similarity between these loci; the L6 locus has a considerable degree of sequence homology with the L3 locus, and the L4 locus has minor sequence homology with the L8 locus. (Peng and colleagues, 2019) (Moustafa & Associates, 2015)

Lipopolysaccharides have two main purposes. First, the LPS anchored in the outer bacterial membrane serves as a defensive mechanism for Gram negative bacteria against adverse environmental conditions by performing a protective role for them. Because it acts as a buffer against external stressors, LPS is essential for bacterial survival in a variety of unique environments (Silipo *et al*., 2012).
By changing the lipid by combining sugar molecules with phosphate groups, the bacteria are able to produce an outer membrane that is negatively charged. This allows it to interact with divalent cations that are present in the surrounding environment. This contributes significantly to the outer membrane's rigidity and tightness, which in turn helps bacteria resist external stressors. Second, among all Gram-negative bacterial species, LPS is one of the most conserved structures.
Because of this, LPS is a crucial pathogen-associated molecular pattern (PAMP) that the mammalian innate immune system can identify and use to start the process of eliminating a bacterial infection. The appearance of comparatively conserved molecular structures in animals for the identification of this PAMP is facilitated by this significant immune response. A prompt identification and detection of LPS of encroaching Gram negative (Steimle and others, 2016). Figure 1 is identify Phylogenetic tree of 16 *P. multocida* strains with different LPS serotypes, constructed based on the DNA sequences of the LPS outer core biosynthetic genes

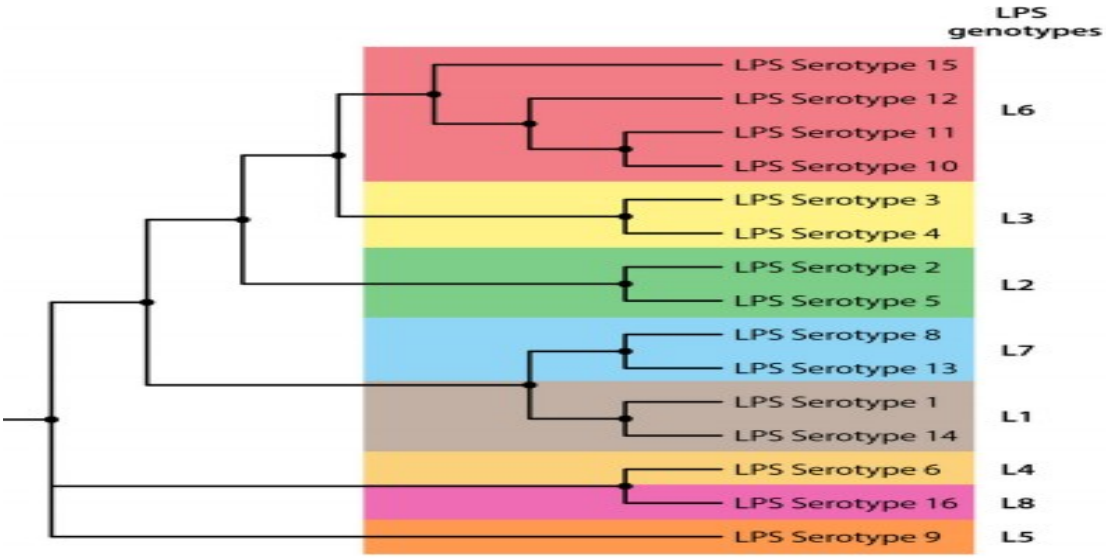

Figure 1: Based on the DNA sequences of the genes involved in the biosynthesis of the outer core of LPS, a phylogenetic tree of 16 P. multocida strains with distinct LPS serotypes was created (Peng *et al.,* 2019).

2.4.4 Detection of iron-regulated and iron acquisition proteins

Because of its vital role in metabolic transport networks, iron is an essential component for the majority of organisms. Bacteria utilize a variety of mechanisms, including siderophores and outer membrane proteins, to get iron. While some outer membrane proteins can also obtain iron from the host Fe-binding molecules, including as lactoferrin, heme, ferritin, hemoglobin, and transferring, siderophores are iron ligands that compete with protein carriers to bind to ferric iron (Motahare *et al*., 2019).

Shirzad and Tabatabaei (2016) reported a correlation between certain VAGs and the pathogenic processes of P. multocida. The iron acquisition genes (tonB, hgbA, hgbB, tbpA, and fur) are among these crucial virulence genes that can aid pathogenic bacteria in obtaining a range of heme iron sources from the infected host. Zhangcheng & colleagues, 2018

Genomic islands (S1) provide descriptions of a set of CDSs involved in fructose, galactitol, citrate transport, and metabolism, as well as proteins belonging to the avian group. Furthermore, virulence-associated proteins such as UDP-glucose 6-dehydrogenase, an enzyme involved in the synthesis of various surface structures, and succinyl ornithine transaminase, a protein that encodes a hemoglobin receptor of the TonB-dependent family (Prasannavadhana *et al*., 2014)

According to Wilson BA and Ho M. (2013), iron uptake has been identified as a crucial biological step for P. multocida survival and host pathogenicity. The tonB-exbB-exbD operon, which encodes proteins necessary for energizing the iron transport process, the fecABCDE operon, which encodes proteins involved in the synthesis of the TBDT system, and the tbpA gene, which encodes transferrin-binding protein A, which is crucial for iron uptake in Pasteurellaceae, are among the predicted genes or operons shown to have roles in iron metabolism. Most of these iron acquisition methods are well conserved among P. multocida strains that produce similar kinds of capsular polysaccharide (types A, D, and F) (Peng *et al.,* 2019). Interestingly, iron uptake is often considered a major host specificity limiting factor (Wilson BA and Ho M. 2013). One noteworthy protein is TbpA, which is only present in specific isolates from bovine and ovine species and is encoded by tbpA. P. multocida contains only one transferrin receptor, TbpA (Peng *et al.,* 2018).
The genes linked to sialidases (nanB), dismutases (sodA, sodC), and iron mebatolism (exbD-tonB, fur, hgbA) (Quedi *et al.,* 2015). Pathogenic bacteria can obtain several heme iron sources from infected hosts with the aid of iron acquisition genes, including tonB, hgbA, hgbB, tbpA, and fur (Zhangcheng *et al.,* 2018).

Pasteurella multocida uses two proteins, HgbA and HgbB, to directly get iron from the hemoglobin component. While the incidence of the hgbB gene fluctuates among strains based on the origin of the host and the state of animal disease, the hgbA gene is believed to be distributed more frequently among isolates. Another protein, transferrin binding protein TbpA, is crucial for the extraction of iron from transferrin and has been identified as an epidemiological marker in cattle (Sarangi *et al.,* 2014). All avian and ovine isolates had the fur, hgbA, exbB, exbD, and tonB genes, with the exception of tbpA (Motahare *et al.,* 2019).

The conserved area known as the tonB box, which is present in transferrin, siderophore, and hemoglobin, is what allows the iron carrier components to connect with the TonB protein. Iron is passed through the TonB pore and the TonB protein shape changes as a result of the TonB box binding to the outer membrane receptor. According to reports, the expression levels of tonB, exbD, and exbB alternation are 2.5, 2.3, and 4.7 times higher in response to low iron conditions (Motahare *et al*., 2019). Iron-dependent regulation and acquisition are mediated by virulence factors, such as the ferric iron transport-related genes fecCD. Involved virulence factors include heme iron consumption and exporter proteins such ccmABCEF (Raquel *et al.,* 2018).

2.4.5 Enzaymes

Apart from the immunological response, P. multocida infection also had an impact on metabolism. For instance, PmQ-infected chicken had significantly higher levels of GAD1, a crucial enzyme that converts L-glutamate to GABA, which is then used for succinic semialdehyde synthesis and ultimately succinate, which is the primary route of M1 macrophage and essential for stabilizing HIFα (Meiser *et al*., 2016). On the other hand, PmCQ2-infected chicken had higher levels of GLUL, also known as glutamine synthetase, or GS, which is highly expressed in M2 macrophages and crucial for the M2 phenotype (Palmieri *et al.,* 2017). All NADH dehydrogenase subunits (ND1–ND6), however, were similarly reduced as a result of PmCQ2 infection, suggesting that chicken oxidative phosphorylation was compromised, which was detrimental to M2 macrophage activation (Viola *et al.,* 2019).

According to a recent study, arachidonic acid can bind to myeloid differentiation factor-2 (MD2) and limit the activation of MD2/TLR4 signaling, hence suppressing the inflammatory response. PGD2 (Prostaglandin D2), which can connect to its receptor DP2 and exacerbate the inflammatory response in asthma, is one of the several metabolites that are overproduced in PmQ-infected hens due to the upregulation of ten genes linked to arachidonic acid metabolism (Brightling *et al*., 2020). Suggesting that chicken's oxidative phosphorylation was compromised, which was detrimental to the activation of M2 macrophages (Viola *et al.,* 2019).

A group of genes called kdtA, kdkA, opsX, rfaF, and waaC code for enzymes that catalyze the inner core's production (Johnson et al., 2013). The addition of the Kdo molecules to lipid A requires the 3 deoxy D manno octulosonic-acid transferase, which is encoded by the kdtA gene. KdkA is a kinase that phosphorylates the first Kdo residue. The acylation of the sugar nucleotide UDP-GlcNAc by LpxA is the initial reaction in the conserved biosynthesis of lipid A that was found. The Kdo donors are the kdsA and kdsB. The production and transfer of L, D heptose require the transferases and L, D heptose precursors; they include phosphatase, gmhA, rfaD, and rfaE isomerases.

The Six genes encoding proteins (P1059_00080 to P1059_00085) are found in a unique region that is present in the genome of strain P1059 (107.9 to 115.7 kb), but is absent from the genomes of strains C48-1 and HB02; these genes are predicted to be involved in the transport and modification of citrate, as well as the subsequent conversion of citrate to oxaloacetate via citrate lyase (P1059 *et al.,* 2013). A putative prophage with similarity to a bacteriophage resembling Mannheimia lambda, vB_MhS_1152AP2 (GenBank accession no. NC_028956), is found in a second region (773.1 to 804.3 kb) (Niu *et al*., 2015). Ten genes encoding proteins involved in the transport and utilization of L-fucose (P1059_01496 to P1059_01503), a crucial component of host mucin, are found in a third region (1,608.2 to 1,618.9 kb), which may provide P. multocida strains with a fitness advantage in low-nutrient environments (Johnson *et al*., 2013). Genes encoding proteins involved in xylose metabolism and transport are found in the last unique region, which spans 1,647.4 to 1,656.9 kb (P1059_01535 to P1059_01541) (Peng *et al.,* 2019).

Sialic Acid Metabolism

The nan operon (nanRATEK yhcH), nanB, nanH, neuS2, neuA, and the siaPT-nanM cluster are potential genes linked to the sialometabolism of P. multocida. NeuS2 encodes a protein homologous to a sialyltransferase found in Haemophilus influenzae; neuA encodes a CMP-sialic acid synthetase that functions similarly to that which initiates the precursor scavenging pathway involved in the uptake of environmental sialic acid in E. coli; and nanB and nanH of these genes encode the two significant sialidases NanB and NanH that facilitate sialic acid uptake. Members of the Nan operon (nanRATEK-yhcH) encode structural (NanATEK) and regulatory (NanR) proteins involved in sialic acid catabolism, just like those in H. influenzae. The siaPT-nanM cluster, which encodes proteins that make up the TRAP system required for sialic acid uptake, is located downstream of the Nan operon on the opposite strand (Peng *et al.,* 2019).

In avian samples, two genes, nanB and nanH, that encode sialidases were found. The eukaryotic cells' glycoproteins and glycolipids that have sialic acid attached to them are broken down by enzymes called sialidases. Subsequently, sialic acid is either integrated into the outer membrane of the bacterium or used as a carbon source, facilitating infection and suppressing the host immune system (Thales *et al.,* 2013).

2.4.6 Detection of toxin typing and virulence associated genes

Serogroup D is positively correlated with the genes toxA and hsf-1, while serogroup A is favorably correlated with the genes pmHAS and pfhA. However, it was found that hsf-1 and serogroup A were negatively correlated with pmHAS, pfhA, and tadD, as well as with serogroup D (Quedi *et al*., 2016).

Despite the fact that both varieties are capable of causing infection, they are further separated into toxigenic and nontoxigenic strains based on whether or not they produce the toxin (PMT) and possess the toxin-encoding gene toxA. While the genome sequence for P. multocida serogroup E is currently unknown, the genome sequences of a number of isolates of P. multocida serogroups A, B, D, and F that have unique traits are now publicly available (Peng *et al.,* 2019).

2.1.7 Detection of Outer Membrane Protein typing and virulence associated genes

P. multocida strains commonly share the bulk of virulence-associated genes. Ompha87, plpB, psl, and ompH are the genes linked to outer membrane proteins (Quedi *et al.,* 2016). According to Raquel *et al.* (2018), the outer membrane proteins, which include lipoprotein, lolD, IcsA, metQ, an immunogenic lipoprotein A, two secretory proteins called secretory immunoglobulin A-binding protein EsiB, and a protein E of the Type II secretion system, improve P. multocida's capacity to colonize the host. Their coding genes have been identified in the core genome.

Bacterial OMPs play key roles in nutrient acquisition, molecule and ion transport, and bacterium-host interactions. According to their functional characteristics, *P. multocida* OMPs are mainly categorized into structural proteins, transport proteins, binding proteins, adhesins, protein assembly machinery, and membrane-associated enzymes. Among those OMPs categorized into , structural proteins (PM0786 and PM0966), transporters (PM0527 and PM1980), binding lipoproteins (PM1730 and PM1578), and membrane-associated enzymes (PM1426, PM1000, and PM1992) are relatively conserved in *P. multocida* isolates associated with a range of clinical manifestations in different hosts, while those OMPs with predicted functions, such as hemoglobin receptors (PM0040, PM0236, PM0300, PM0337, PM0576, PM0592, PM1081, PM1282, PM1622, and PM1188) or adhesins (PM0056, PM0057, PM0058, PM0059, PM0084, PM1665, PM0844, PM0846, PM0852, PM0853, and PM0855), are relatively heterogeneous (Peng *et al.,*2019)

2.4.8 Biofilm formations

When it comes to less virulent strains of the bacteria or P. multocida that is engaged in secondary infections, biofilms can play a significant role in their virulence and ability to survive in the host under unfavorable conditions (Rajagopal *et al*., 2013). The microbe's persistence is aided by its capacity to evade the human immune system in these circumstances and to withstand high dosages of antibiotics (Silva *et al.,* 2014). Since P. multocida is a significant respiratory pathogen and the formation of these structures may boost the organism's pathogenicity, microorganisms' ability to adhere is crucial (Rajagopal *et al.,* 2013).
Additionally, a number of genetic factors contribute to the production of biofilms, which are controlled by environmental parameters like pH, temperature, and the amount of nutrients present in the medium (Flemming *et al.,* 2016). Therefore, depending on the media and growth conditions, these genetic factors—especially those that play a role in the early phases of biofilm formation can be functionally substituted or overridden by others (Brunna *et al.,* 2017).

Hyaluronidase was added to the growing medium of all the strains examined, and this resulted in the generation of a more robust biofilm and less capsular polysaccharide when a highly encapsulated, poor-biofilm-forming strain was passed under conditions that encouraged biofilm formation. Enzymatic digestion, nuclear magnetic resonance, and gas chromatography-mass spectrometry all revealed that the biofilm's matrix material was primarily made up of a glycogen exopolysaccharide (EPS). However, quantitative reverse transcriptase PCR showed that there was no differential regulation of a putative glycogen production locus when the bacteria were cultured as a biofilm or planktonically. As a result, the negatively charged capsule may obstruct surface adhesion or the EPS matrix's ability to encase a significant number of bacterial cells, hence impeding the formation of biofilms. This is the first detailed description of biofilm formation and a glycogen EPS by P. multocida (Petruzzi *et al*., 2017).

Three isogenic CPS-deficient mutants with hyaE or hyaD mutations provided additional proof that CPS hindered the production of biofilms. Thus, by inhibiting surface proteins necessary for adhesion, the initial stage of biofilm development, CPS may prevent biofilm formation (Nagar and Schwarz, 2015).

2.4.9 Antibiotic resistance

Antibiotic use is a successful strategy for managing P. multocida infections, but it is seriously hampered by the rise of drug-resistant strains (Kangpeng *et al*., 2016). Testing the antimicrobial efficacy is necessary to identify the most effective antimicrobial agents for treatment and to modify the use of antimicrobials for prophylaxis and at subtherapeutic levels in chicken feed as growth promoters in order to prevent the emergence of extensively drug-resistant P. multocida strains (Elalamy *et al.,* 2020).
Research conducted in Europe on isolates from pigs, cattle, and poultry has demonstrated that tiny (4–7 kb size) plasmids are typically the mediating factor for resistance in P. multocida. The spread of resistance is thought to be caused by horizontal plasmid transfer rather than clonal dissemination, as evidenced by the discovery that a larger plasmid (pVM111) contains multiple genes conferring resistance against tetracycline, sulfonamides, and streptomycin resistance (tetR-tet (H), sul2, and strA) (Bushura and Meksud, 2019). According to earlier research by Wang *et al*. (2017), isolates containing aphA-1 typically displayed resistance to kanamycin, amikacin, and neomycin.
The absence of genes that mediate the resistance to macrolide suggests that resistance to the drug may be conferred by genes not found in this screening or by another mechanism, such as plasmid-mediated resistance or mutations in the macrolide's specific target region in the bacterial ribosome (Zhu *et al*., 2019).
The mobilization genes that are similar to ColE1 are absent from certain plasmids. The pJR1 (6.79 kb) and pJR2 (5.25 kb) plasmids were isolated from isolates connected to Taiwanese poultry cholera outbreaks. While plasmid pJR2 contains genes that confer resistance to ampicillin and carbenicillin (blaP1), streptomycin and spectinomycin (aadA1), and tetracycline (tetG), plasmid pJR1 contains three genes that confer resistance to

sulfonamides (sul2), tetracycline (tetG), and chloramphenicol (catB2). Two further plasmids, pCCK1900 (10.2 kb) and pCCK381 (10.9 kb), were recovered from florfenicol-resistant P. multocida strains. The phenotype that is resistant to florfenicol is caused by the floR gene, which is found in both plasmids and also imparts resistance to chloramphenicol. Additionally, pCCK1900 has genes that confer resistance to streptomycin (strA and strB) and sulfonamides (sul2). Interestingly, the mobilization region of pCCK1900 is similar to that of the broad-host-range plasmid RSF1010, while that of pCCK381 is similar to that of pDN1 from Dichelobacter nodosus (Peng *et al.,* 2019).

Many Gram-negative bacteria use lipid A changes to evade the vertebrate host's antimicrobial peptide response. Catalytic antimicrobial peptides' affinity for binding to the bacterial outer membrane is decreased when the surface charge of the bacteria is changed, for example, by adding positively charged moieties. Calprotectin is a significant antimicrobial factor. It is an essential part of the nutritional immunity process that the host uses to withhold nutrients (Gaddy *et al*., 2015). The host-induced limitation of bacteria's access to vital metals is one mechanism of nutritional immunity (Steimle *et al.,* 2016). High affinity binding of Zn2+ and Mn2+ by calprotectin deprives bacteria of these vital metals, resulting in an environment where Zn2+ and Mn2+ are scarce. Furthermore, calprotectin has antibacterial activity against a variety of bacteria, both Gram-positive and Gram-negative (Damo *et al*., 2013).

2.5 Symptoms and lesions

According to Ali and Sultana (2015), fowl cholera is characterized by wattles discharges from the nose, mouth, and eyes, labored breathing, lack of coordination, and grayish diarrhea. According to Mohammed *et al.* (2012), the gross lesions included petechial bleeding in cardiac muscle, necrotic parenchymatous hepatitis, massive and necrotic foci on the spleen and liver, widespread congestion, and hemorrhages in the intestinal mucosa. Hemorrhage, congestion, and lymphoid cell infiltration in the liver, heart, and spleen were the histological indicators of FC (Panna *et al*., 2015).

It is easy to confuse the acute illness with poultry typhoid. Affected birds often exhibit depression and decreased appetite in chronic types of P. multocida infection. There will be a rise in deaths, however chronic chicken cholera does not have a significant death rate. A frequent sign of chronic poultry cholera is a bloated face that includes the comb and wattle (Habte *et al.,* 2017)

2.6 Diagnosis

Identification of the bacterium that causes the illness, P. multocida, after it has been isolated from birds exhibiting symptoms and lesions typical of the condition, is necessary for diagnosis. A presumed diagnosis can be made based on the presence of characteristic symptoms and lesions, as well as microscopic evidence of bacteria exhibiting bipolar coloring in tissue smears from the liver, spleen, or blood. There could be mild variations of the illness (OIE, 2018). Apart from the standard techniques including staining, culture procedures, and biochemical tests, the molecular approach of PCR utilizing primers specific for P. multocida was employed to validate the identification of the isolated organisms as P. multocida (Akhtar *et al*., 2015).

The diagnosis is suspected in sensitive species, during known environmental "hot spots," and following a typical mortality event. Confirmation is achieved by examining carcasses grossly and histologically and isolating the agent, usually from bacterial cultures of liver tissues (WHA 2016). Agar gel immunodiffusion (AGID) is used to detect serotype, while restriction enzyme analysis (REA) is commonly used to assess genotype. (Julia and others, 2019). Seldom are serological testing utilized to diagnose avian cholera. The requirement for sero-diagnosis is typically eliminated by the simplicity of getting a conclusive diagnosis by the isolation and identification of the causal bacterium. (OIE, 2018)

2.7 Prophylaxis and vaccination

When the right medications are used, sickness frequently returns after treatment ends. However, chronic carrier birds may continue to live in flocks of hens following treatment, which can effectively decrease mortality and restore egg production. Antimicrobial treatments frequently don't work. Similar to Salmonella, it's critical to remove carrier birds from flocks and stop the spread of the illness from bird to bird. In order to stop the virus from spreading again, rodent control is also crucial (Habte *et al*., 2017).

Vaccinating poultry would shield them from infection with P. multocida. Bacteriochins and live attenuated vaccines are the two currently available treatments for poultry cholera; the former offers only restricted protection against homologous serotypes, while the latter were created empirically and have been shown to revert to the virulent strain (Kangpeng *et al.,* 2016).

Preventive poultry vaccination is the best course of action, and antibiotic use should be kept to a minimum. It is advised to carry out susceptibility testing, or sensitivity testing, wherever possible prior to therapy. On occasion, though, it is vital to take quick action and start giving the birds antibiotics while you wait for the results of the lab tests. In these situations, tetracycline, trimethoprim-sulfamethoxazole, or florfenicol would be the best options for a quick therapy. Second, colistin, enrofloxacin, ampicillin, and kanamycin may be utilized, contingent on availability. In general, giving out gentamicin, neomycin, or streptomycin is not advised (Huberman and Terzolo, 2016).

3. CONCLUSION AND RECOMMENDATION

The bacterial disease known as FC is highly contagious and affects both domestic and wild birds. It is well recognized as a significant issue affecting poultry globally. A number of characteristics, such as the presence of a capsule, the ability to produce endotoxins, outer membrane proteins, heat shock proteins, and antibody cleaving enzymes, can significantly affect a strain of P. multocida's virulence. One of the many infectious diseases affecting Ethiopia's rapidly expanding chicken industry is fowl cholera. Nevertheless, neither the locally accessible vaccine nor the genotypes of P. multocida that cause poultry cholera are known. Treatment with the right antibiotics can successfully stop mortality and increase egg production, however following treatment, chronic carrier birds may continue to live in hen flocks.

Depends on the above conclusion the following recommendations are forwarded:

- To reduce the economic losses due to high mortality by providing good protection and vaccinated flocks
- Study genotype of P. *multocida* to develop locally vaccine
- Limit to a minimum the use of antibiotics.

6. DECLARATIONS

Ethical Approval and consent to participate

This review was performed following the recommendations of Ambo University Research and Ethical review guideline.

Consent of publication: Not applicable in the manuscripts

Data Availability

Not applicable (this manuscript does not report data generation or analysis)

Competing of Interests

The all contents of this review belongs to the authors (Wesenu Berhanu Enoro, Bacha Abata Chala) report no conflicts of interest for this work.

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Bacha Abata Chala did review collection and writing up the review. Wesenu Berhnau Enoro did the edition of the review for publication and will be responsible for email exchange with the journal to which the review was sent (corresponding author)

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