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Kanamycin resistance of Pasteurella Multocida in rabbits

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Abbreviations

DNA= deoxyribonucleic acid

ERK= extracellular signal regulated kinases

LPS= Lipopolysaccharide

MRSA=Methicillin-resistant Staphylococcus aureus

MRL= Maximum residue level

MIC=Minimum inhibitory concentration

MS=Moderate sensitive

P. multocida = Pasteurella multocida

PM=Pasteurella multocida

PCR = Polymerase chain reaction

R=Resistant

REA= restriction endnuclease analysis

Rrna= Ribosomal ribonucleic acid

Sbsp. = subspecies

S=Sensitive

WHO= World Health Organization

1. Summary

Pasteurella belongs to the *Pasteurellaceae* family with a wide range group of gram-negative Gamma proteobacteria. The understanding of the molecular basis of *P. multocida* and the host predilection is still at a very early stage. *P. multocida* is a facultative pathogen. Infection with *P. multocida* can cause high mortality and production decrease in cattle, pig, chicken, and rabbit farming and major economic losses. The most common way for *P. multocida* to come in contact with humans is through animal bites, open wounds, skin lesions and mucous discharges from animals infected with Pasteurella strains. Kanamycin is classified as an aminoglycoside antibiotic that is extracted from *streptomyces kanamyceticus*. Some researchers have added kanamycin as a selective media for the culture and isolation of *Pasteurella multocida*. In this study we sampled 138 *P. multocida* strains from industrial rabbits' farms all over Hungary in 2021. A Kirby-Bauer disc diffusion method was used to determine the susceptibility of *P. multocida* to 19 different antibiotics based on the Clinical and Laboratory Standards protocols. The antibiotics tested were apramycin, doxycycline, enrofloxacin, florphenicol, gentamycin, colistin, marbofloxacin, neomycin, oxytetracycline, streptomycin, spiramycin, streptomycin, sulfamethoxazole, and trimethoprim, tilmicosin, kanamycin, tulathromycin, tiamulin and valnemulin. We found *P. multocida* showed a 100% resistance to spiramycin, 81.03% resistance to kanamycin, and 98.36% susceptibility to tulathromycin.

2. Introduction

Rabbit belong to the family *Leporidae* of the order *Lagomorpha* and are herbivores and terrestrial animals[66]. The domestication of rabbits' dates back about a thousand years, but the first recorded breeding took place in the 16th century. The first countries to begin breeding rabbits were France, Italy, and England[36]. Rabbits are kept for meat, fur, experimental laboratory studies, and as pets. However, even with the best genetics, feeding and hygienic environment, disease cannot be completely eliminated [71]. The rabbit meat's quality combined with its life cycle, short gestation and high feed conversion capacity makes it the ideal animal for agriculture. According to the Food and Agriculture Organisation, 1.4 million tonnes of rabbit meat are produced annually. China and Europe are the largest meat producing regions in the world [11]. Infectious and non-infectious pathogens such as bacteria, viruses, parasites, fungi, stress are the main problems causing economic losses in rabbit farming[43]. *P. multocida* is the most common pathogen in the rabbit industry that causes high morbidity and mortality in the herd [4].

The *Pasteurella* genus has more than 17 species. The most common *is P. multocida, canis, stomatitis, septica, dogmatis.* The above species are very common in humans [22]. *P. multocida* was first discovered in 1881 by the French scientist Luis Pasteur, who was investigating an outbreak of fowl cholera[48]. Smears stained with methylene blue and cultivation on rich medium under aerobic conditions can identify *Pasteurella* species. Molecular techniques such as multi-locus sequence typing and repeated extragenic palidromic PCR can distinguish subspecies of *P. multocida* [48].

The most common capsular serotype that we meet in rabbits is Type A and less common serotype D. Recently serotype F have been reported in rabbits too [24]. Serotype A of P. multocida can attach to the ciliated epithelial cells of the respiratory tract and serotype D to the nonciliated cells. Usually, *P. multocida* is not a primary pathogen, but infection is promoted by the presence of other pathogens [35].

Antibiotic therapy is the treatment of choice for illness caused by *P. multocida*. Ideally, the pathogen should be isolated, and sensitivity to antibiotics should be tested to obtain the most appropriate treatment plan. Since these diagnostic methods are time consuming, it is common practice to start antibiotic therapy before the results are available and depending on the clinical symptoms observed. Moreover, nowadays, with the increasing prevalence of unnecessary and

excessive antibiotic use, a proportional increase in antimicrobial resistance is observed due to selective pressure on the genes encoding resistance. In addition, the aforementioned pre diagnostic therapies are no longer as effective as they once were [56].

Kanamycin was advised as a suitable agent to selectively grow *Pasteurella* from rabbits [28]. In the following study we will analyse the kanamycin resistance of *P. multocida* in rabbits and if kanamycin is suitable agent for the *P. multocida* isolation.

3. Literature review

3.1 History of the pathogen

Pasteurella belongs to the *Pasteurellaceae* family with a wide range group of gram-negative gamma proteobacteria. Historical interaction between subspecies can be compared with their 16S Rrna genes. Genomic and phylogenetic properties of the *Pasteurella* species revealed that a lot of species have been poorly classified. For example *Actinobacillus , Manheimia, Bibersteina, Gallibacterium* and a lot of other species have been classified in the past under the name of Pasteurella species[69].

Pasteurella took its name by from the very popular French biologist Louis Pasteur. Louis Pasteur was the first person who isolated *Pasteurella* in the1880s as the cause of the fowl cholera which was named by the French as cholera des poules. *Pasteurella* species can be physiologically found as part of oropharyngeal microbes of almost all the animal species, furthermore it can be the cause of primary or secondary infection in the upper and lower respiratory tract.

While *P. multocida* has been studied for more than a century, many aspects of this pathogen are still being discovered. The understanding of the molecular basis of *P. multocida* and the host predilection are still at an early stage. Although full-genome sequences of Pasteurella strains have been identified, which give us a better understanding of the genetic mechanism relating to the virulence and host specificity of *P. multocid*a [48].

The term snuffles which is related to upper respiratory tract infection of rabbits was first used in 1920 by Webster who researched respiratory infections in rabbits [12].

3.2 General characteristics

P. multocida has 5 different capsular types (A, B, D, E, F) that can be typed serologically with indirect hemagglutination and gel diffusion precipitin test. Virulence of *P. multocida* involve adhesion factor, endo- and exotoxins, phagocyte resistance and iron regulation. The strains that have adhesion proteins or pili on the outside membrane tend to have propagate more. Type A strains can adhere on the respiratory mucosa more than type D. Iron deposits determine and regulates the growth of some strains. In most of the strains iron binding protein is produced by the outer membrane. The reason that *P. multocida* can invade and grow is due to the hyaluronic acid capsule. The capsule inhibits phagocytosis and the complement-activated bactericidal activity of the serum. The endotoxins produced *by P. multocida* increase the resistance of the bacteria and regulates the release of the inflammatory mediators like interleukins. The free endotoxin in the plasma is the reason behind the fever, depression and even shock due to the bacteraemia. Some D types of *P. multocida* produces an exotoxin. This exotoxin by the name dermonecrotic toxin is similar to the toxins of the D strain that cause atrophic rhinitis in pigs. The *P. multocida* toxin can cause pneumonia, lymphoid atrophy, pleuritic or in some cases even osteoclastic bone resorption in rabbits. Most Pasteurella stains invade the mucous membranes of the respiratory tract, when rabbits are predisposed due to stress and immunodeficiencies [24].

3.3 Pathogenicity

Causes and pathophysiology of respiratory diseases

Respiratory diseases can be caused by viral pathogens that multiply in the upper and lower respiratory tract, such as myxoma virus. Foreign bodies, trauma, irritants, and chemicals can be the cause of non-infectious diseases. Dental disease, such as deformities and infections of the upper incisor roots, can affect the nasolacrimal duct and nasal cavity and lead to respiratory infections. Metastatic neoplasms in the lungs can cause respiratory disease and secondary bacterial respiratory infections. Pathogens such as P. multocida , Mycoplasma and Chlamydophila species can also cause respiratory disease in rabbits [39].

Respiratory distress and dyspnoea are the most common clinical signs in case of respiratory diseases. Dyspnoea occurs due to the decrease in oxygen concentration in the lungs and increased concentration of carbon dioxide. As the animals' body tries to compensate this phenomenon hyperventilation can be observed.

Respiratory diseases can be either restrictive or obstructive. Usually, rabbits tend to acquire restrictive diseases, which lead to shortness of breath and tachypnoea. Those clinical signs can be linked with pneumonia, abscesses, lung oedema, tumours, and pleural effusions. Obstructive diseases occur due to narrowing of either the upper or lower respiratory tract. In case of obstruction of the upper respiratory tract increased respiratory effort can be observed, while in lower obstructive respiratory disease increased expiration can be mentioned. Wheezes are very characteristic during expiration and crackles during inspiration usually due to pneumonia and bronchial inflammation [24].

P. multocida can propagate on dextrose starch and blood agar but not on MacConkey's agar. Large and green colonies can be seen in horse blood media. *P. multocida* has characteristic odour and can grow under aerobic conditions. It is very sensitive to temperature and some strains are very sensitive to carbon dioxide. The incubation time on blood agar is between 1 and 2 days. The biochemical characteristics of *P. multocida* are as followed: oxidase +, catalase ±, indole ±, hydrogen sulphide –, urease –, ornithine decarboxylase +, hexose +, and carbohydrate fermentation of sugars [12]. The serotyping of *P. multocida* can identified according to the differences of the capsular polysaccharides. The organisms are subdivided in 16 serotypes based on the serological differences of LPS. Molecular typing such 16S rRna gene sequence, multi-locus- sequence typing and other PCR methods have been used for the identification of the *Pasteurella* species[51].

Bacterial species	**Hosts**	**Disease conditions**
Type A	Cattle	Associated with bovine pneumonic pasteurellosis (shipping fever). associated with enzootic pneumonia complex of calves; mastitis (rare)
	Sheep	Pneumonia, mastitis
	Pigs	Pneumonia, atrophic rhinitis
	Poultry	Fowl cholera
	Rabbits	Snuffles
	Other	Pneumonia
Type B	Cattle, Buffaloes	Haemorrhagic septicaemia
Type D	Pigs	Atrophic rhinitis, pneumonia
Type E	Cattle, Buffaloes	Haemorrhagic septicaemia
Type F	Poultry	Fowl cholera
	Calves	Rarely peritonitis

Table 1: The pathogenic *Pasteurella* species, their principal hosts and associated diseases [51].

3.3.1 Virulence factors

Capsule

As mentioned above, *P. multocida* has five capsule groups. The structure of the capsule of serotypes A, D and F is very close to the structure of mammalian glycosaminoglycans. This means that the capsule of *P. multocida* is mainly composed of hyaluronan, heparosan, and unsulfated chondroitin. In general, *P. multocida* strains with capsular processes tend to be more virulent than the A capsular strain. A-capsule mutants have been isolated from serogroups A and B in mice and serogroup A in chickens. These avirulent mutants were unable to grow on the muscles of chickens.

It is well known that the capsule is very important for resistance to phagocytosis. In addition, studies have shown that when mouse macrophages are used, the capsular type is more sensitive than the wild type of the parent. Resistance to complement-mediated lysis also plays an important role in virulence. Experiments revealed that serum resistance of *P. multocida* type A strains corresponded with capsular possession. The acapsular mutant could not resist normal serum compared to the type A parent strain. No decrease in resistance of the parent strain was observed when compared with the serotype B mutant[21].

Outer membrane

P. multocida consists of a uniform inner membrane and an uneven outer membrane divided by the peptidoglycan layer and the periplasmic space. The inner part of the outer membrane is composed of phospholipids and the outer part is composed of the LPS leaflet. The role of the outer membrane is to control the transport of nutrients and waste products inside and outside the cells. The integral membrane proteins and peripheral lipoproteins of the outer membrane ensure the adaptation of bacteria to different environments. The outer membrane proteins of *P. multocida* are actively involved in virulence.

The outer membrane proteins differ in their bioinformation. There are three groups of predictors for the outer membrane proteins: global predictors, predictors for the transmembrane B-barrel protein, and predictors for the lipoproteins[15].

Lipopolysaccharide

LPS is produced by gram-negative bacteria and has the ability to stimulate the immune response in animals [70]. LPS is formed from the outer membrane of bacteria and functions to interact

with the host and act as an endotoxin that causes sepsis in the host. The extracellular and intracellular pattern recognition receptors activate the proinflammatory interferons and cytokines of the immune system to fight the infection [61]. If the immune system cannot control the infection, there may be an overproduction of proinflammatory cytokines, leading to septic shock, tissue necrosis, and even death[49]. The LPS of *P. multocida* has no O antigen and contains 3 domains. Lipid A as the binding component of the membrane that stimulates the innate and adaptive immune system, and the inner and outer core that contain a variety of residues and oligosaccharide components[20].

Fimbriae and Adhesins

The bacteria usually colonize the surface of the host with adhesins or, even better, with fimbriae. The attachment of fimbriae to the host is closely related to the virulence of *P. multocida*. The preferred site of *P. multocida* colonization is the mucosal epithelium of the rabbit nasopharynx[53]. A characteristic subunit gene is ptfA, which has been isolated from some strains and shows remarkable differences between strains. It is worth noting that the relationship between fimbriae and virulence of *P. multocida* has not yet been elucidated. The region encoding proteins of the Pm70 genome of *P. multocida* shares similarities with the Flp pilin locus in *Actinobacillus actinomycetemcomitans*. 2 genes flp1 encodes a Flp pilin subunit and tadD, which presumably encodes a part of the secretion apparatus important for the construction of Flp pilins. The pfhaB1 and pfhaB2 genes of *P. multocida* share similarities with genes encoding filamentous hemagglutinins, which play a critical role in Bordetella pertussis colonization[21]. According to Fuller et al. the genetic mutation of this gene drastically reduced the virulence of *P. multocida* in mice [17]. Not long ago, in the poultry cholera strain P1059, the route of administration was shown to affect the attenuation of the pfhaB2 mutant, suggesting that pfhaB2 is important for colonization and invasion [21].

Iron regulated and iron acquisition proteins

Iron is an important factor in the growth of bacteria. One of the animal's defences against bacterial infection is that mucous membranes contain low concentrations of free iron to reduce bacterial proliferation as much as possible. Bacterial access to the iron source is essential for their survival in the host. Some pathogens, such as *E. coli* or *Salmonella typhimurium*, form siderophores that are responsible for removing iron molecules from the host. Other pathogens use the outer membrane for active transport of iron through the functional TonB system. Some

P. multocida strains produce siderophores and non-classical transferrin receptors for iron consumption [7]. A large portion of the *P. multocida* PM70 genome encodes identical proteins to those responsible for iron absorption. Except for the bovine strains of *P. multocida*, the *Pasteurellaceae* family contains 2 iron-binding receptors (TbpA and B) for transferrin receptor utilization. The TonB complex provides energy for iron sequestration[21].

Sialic acid metabolism and hyaluronidase

Many bacterial species produce enzymes called sialidases to cleave sialic acid from host lipids and glycosylated proteins and use it as a carbon source. The property of these enzymes may increase the virulence of the pathogen by disarming the effectiveness of host defences. *P. multocida* strains produce cell-bound and extracellular sialidase enzymes.

The role of hyaluronidase in the pathogenesis of *P. multocida* has not yet been elucidated. However, studies have shown that hyaluronidase is present in several strains of serotype B that caused haemorrhagic septicaemia in cattle[21].

Bacteriophages

Bacteriophages work to transmit virulence genes and influence the pathogenic potency of bacteria. Moderate bacteriophages fuse with bacterial chromosomes and become prophages. Through lysogenic transformation, bacteriophages alter the phenotype of the bacterium. Moderate bacteriophages with virulence gene change the characteristics of the bacterium to a pathogenic strain. Bacteriophages have the ability to transmit toxins, and phages encode adhesion, colonization, immune evasion, and serum-resistant genes. However, moderate bacteriophages of *P. multocida* are only used as a typing method in epidemiological studies because of the lack of information on the virulence and evolution of this bacterium. Different groups of phages belong to the families *Myoviridae, Siphoviridae* and *Podoviridae* [1].

Pasteurella multocida toxin

The toxin of *P. multocida* is a very potent mitogen for cells and cultures, especially Swiss 3T3 and fibroblast cells. The toxin is not produced by all strains, but infection with the toxigenic strain can lead to proliferation of the bladder epithelium. The toxin produces inositol triphosphates and diacylglycerol with the help of calcium mobilisation and protein kinase C activation. *P. multocida* toxin targets heterotrimeric G protein and leads to Ras-dependent

activation ERK. *P. multocida* toxin alters the cytoskeleton by disrupting focal adhesion and actin stress fibres. This leads to tyrosine phosphorylation and kinase adhesions[50]. The toxin of *P. multocida* was the first toxin recognised as an intracellular toxin leading to activation of phosphatidylinositol-specific phospholipase C. This toxin may be the starting point for studying the cell interaction of the pathway under free conditions[22].

Filamentous Hemagglutinin

The filamentous proteins FhaB1 and FhaB2 of *P. multocida* share similarities with the filamentous hemagglutinins LspA1 and LspA2 of *Haemophilus ducreyi*. These mutations probably influence the virulence of the different species. FhaB2 is actively involved in the virulence of *P. multocida* and in the nonmucoid *P. multocida* (al1114), FhaB2 was decreased fourfold. FhaB and its transporter are responsible for the formation of the two-partner system, which has similarities to the system of *Bordetella* species [69].

Pathogenicity test in mice

According to OIE, 2008, to determine the pathogenicity of *P. multocida*, 0.1 ml of the isolate was administered to 3 mice. The mice were kept for 3 days after administration and after 3 days the macro and microscopic pathological lesions were examined. Blood films and stained preparations were examined for the presence of bipolarity [16].

3.3.2 Clinical signs

P. multocida infection can lead to several clinical signs like rhinitis, sinusitis, conjunctivitis, otitis, pleuropneumonia, bacteraemia, and abscesses. White/yellowish mucopurulent nasal discharge can be seen sometimes during the infection. Sneezing is also very common due to the irritation of the upper airways. Alopecia, pyoderma and excessive tearing due to the occlusion of the lacrimal duct may occur too. In chronic cases clinical signs may not occur but may present as unspecific signs like depression, exsiccosis, anorexia and weight loss[4].

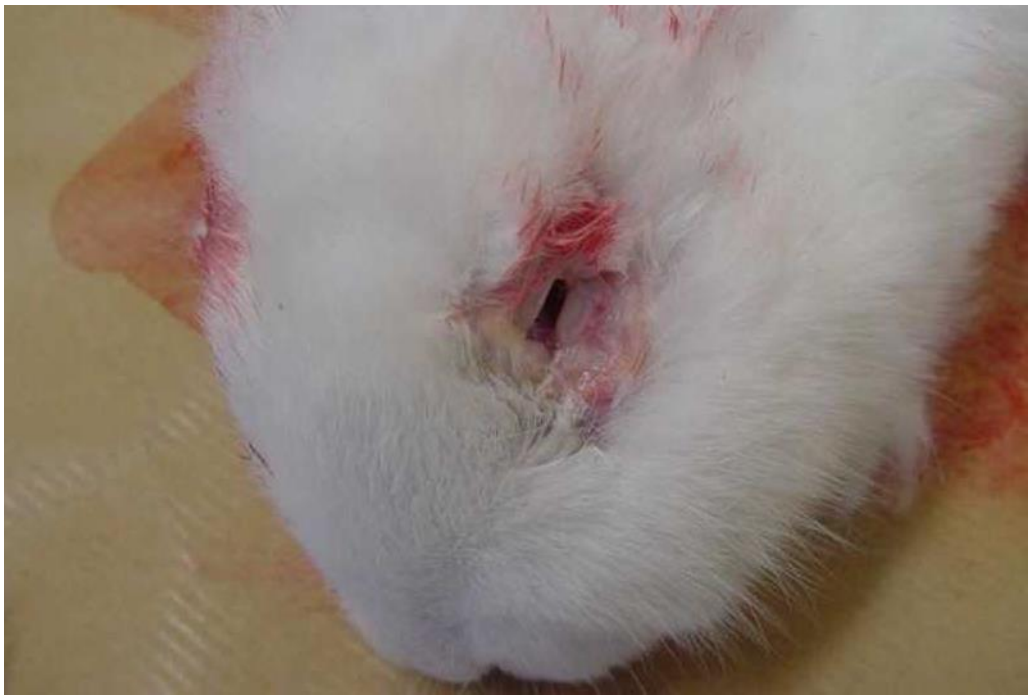


Figure 1. Purulent conjunctivitis in young Rabbit[38].

a) Respiratory form

The most common clinical signs are related to the respiratory system. Inflammation of the entire respiratory system, alopecia of the muzzle and forelegs, sneezing and splashing are the most common signs of this form. The acute inflammation may become chronic in certain cases. The final stage can be pneumonia or even pleuropneumonia, with other pathogens such as *staphylococcus, streptococcus* and *bordetella*, which can end up being fatal.

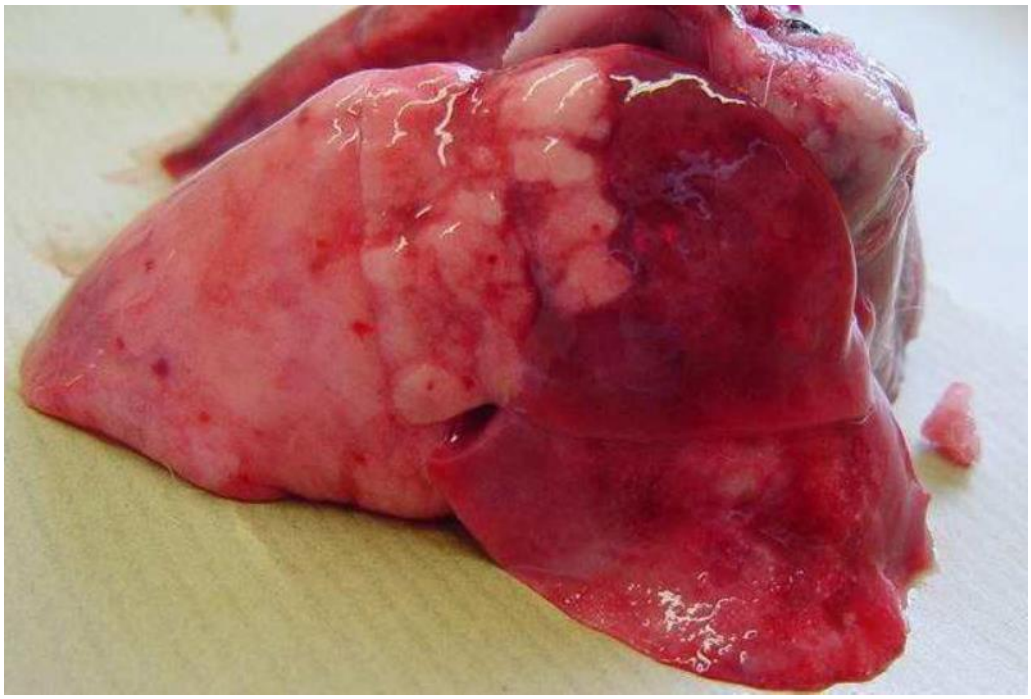


Figure 2. Pneumonia on the cranial lung lobes of a rabbit[38].

b) Septicaemic form

This form usually occurs when rabbits and poultry are kept together, and the rabbits catch it, especially from fowls. Sepsis is very rare in rabbits and the animal may die without showing any clinical signs. Usually, death occurs within 1-2 days

c)Abscess form

The size of the abscess varies from case to case. It may range from a small sized abscesses that only under the microspobe can be visible to an orange-sized one. The site of the abscesses is usually subcutaneous, subserosal, retrobulbar and in the genitalia. Some abscesses located in the middle ear may cause chronic otitis.

d) Otitis and encephalitis

This form usually occurs in young rabbits and is manifested by torticollis and vestibular syndrome. A high concentration of Pasteurella pathogens may be found in otitis media

Figure 3. Internal otitis and encephalitis [38].

e) Metritis and mastitis

Mastitis and metritis are usually uncommon in rabbits, but in recent years there has been a dramatic increase in Europe. One reason for this may be aseptic artificial insemination in some cases or embryonic lysis of some pregnant animals. This form usually leads to death due to poisoning from the toxins produces in the uterus [33].

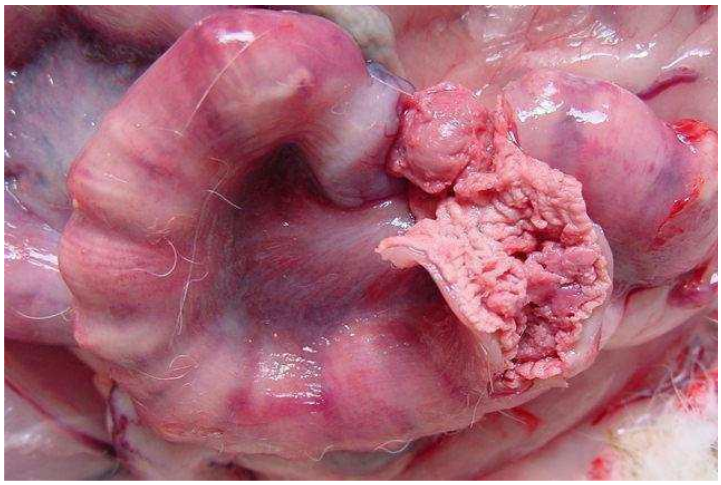


Figure 4. Caseous metritis in rabbits genital organs[38]

3.3.3 Pathology

On macroscopic examination purulent pleuropneumonia, purulent lesions may be observed. Microscopically multiple coagulative necrosis, necrotized alveolar walls, coagulative necrosis with neutrophils, macrophages and bacterial cells in the surrounding area and necrosis of bronchioles[64]. The affected organs are infiltrated with inflammatory mediators, granulocytes, lymphocytes and plasmocytes [38].

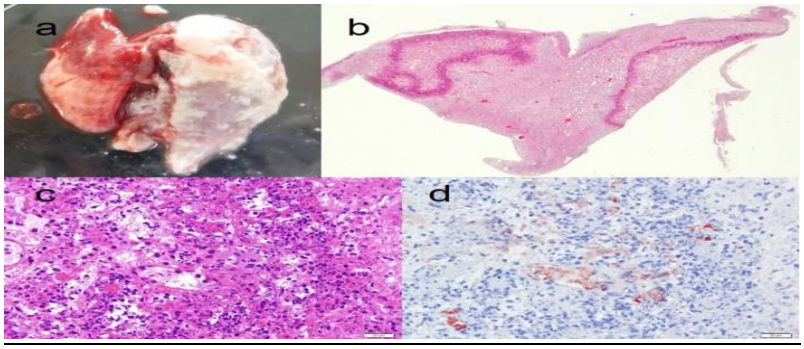


Figure 5. a) Gross necropsy examination of the rabbit revealed suppurative pleuropneumonia. The right lung was covered with sheets of fibrin .b) Low magnification of the right lung characterized by the multiple coagulative necrosis. c) Neutrophils and macrophages were observed in the surrounding area of the coagulative necrosis. d) Immunohistochemical analysis demonstrated that the rod-shaped bacteria reacted with the chicken antibody against *P. multocida* serotype 11 [64].

4. Epidemiology

4.1 *P. multocida* impact on the livestock industry

P. multocida is one of the principal causative agents of haemorrhagic septicaemia in cattle, fowl cholera in birds, atrophic rhinitis in pigs and snuffles in rabbits [20].Infection with P. multocida can cause high mortality and a significant decrease in productivity in cattle, pig, chicken and rabbit farms. *P. multocida* is responsible for 30% of mortality in cattle farms resulting billions of dollars losses in the industry. The farmers try to control *P. multocida* with antibiotic prophylaxis but this is not effective enough that's why the pharmaceutical industry tries to produce cross-protective vaccines to control the *P. multocida* infections [69]. Haemorrhagic septicaemia in ungulates is very fatal in countries of Africa and Asia and results in enormous economic losses to farms. When clinical signs such as fever, oedema, respiratory distress, and septic shock occur, the mortality rate of the infected flock can reach almost 100%. Avian cholera causes huge economic losses to farm and wild birds worldwide. *P. multocida* multiplies very rapidly in the liver spleen and often leads to fatal septicaemia [20]. The mortality rate due to *P. multocida* infection in rabbits can reach up to 50% of the infected population[72].

In rabbits *P. multocida* is highly infectious and can cause enormous economic losses to the meat and fur industries. According to Eady et al, half of the breeding rabbits infected with *P. multocida* died. The main reason for culling of young rabbits is also pasteurellosis [59].

4.2 Predisposing factors

It is a known fact *P. multocida* is a facultative pathogen. Multiple factors can predispose rabbits developing infection with the pathogen. Some of the internal factors the complexity of the rabbit sinuses and the fragility of their mucous membranes. Overcrowded houses increase the ammonia levels and decrease the ventilation, this leads rabbits to be more vulnerable to rhinitis. Feed with increased concentration of poorly agglomerated particles and bad hygiene have the same effect in rabbits [37].

Extrinsic factors

Extrinsic factors play an important role in the onset of the disease. The respiratory tract of rabbits has an efficient defence mechanism against external factors such as pathogens. In combination with inadequate hygiene such as dirty cages, drinkers and hoppers it can lead to a higher prevalence of mastitis in does. In farms where new rabbits are introduced without epidemiological measures such as quarantine, new *Pasteurella* strains could appear[33].

Intrinsic factors

It is difficult to differentiate between environmental and genetic causes of *P. multocida* infections. According to Coudert and Brun, they have observed that the California breed is more susceptible to pasteurellosis than crossbreeds. Moreover, age is considered a predisposing factor. Usually during the first month of life rabbits are free from *Pasteurella*. The highest incidence is seen after the first parturition. As it is believed that in the last term of pregnancy the does immune system is compromised which makes them more susceptible to infections [33].

4.3 Transmission and Zoonosis

P. multocida is usually transmitted through direct contact, air, and some fomites. Less frequently transmission can be haematogenous or venereal [26]. In rabbits the acute form is seen more often than the chronic form. New-born rabbits could get infected if the mother suffers from metritis caused by *Pasteurella*. The point of entry of *Pasteurella* is through open wounds and through the nostrils of the host [12].

The most common route by which *P. multocida* comes into contact with humans is through animal bites, open wounds, skin lesions, and mucus excretions from animals infected with *Pasteurella* strains. In the last 30 years, about 30 human deaths have been reported due to Pasteurellosis, but this number is increasing every year. *Pasteurella* species are widely distributed throughout the world. In human cases reported, the mortality rate due to bit wounds is around 30%. *P. multocida* strains are the most important pathogens of all *Pasteurella* species in humans. *P. canis* is commonly found when a human is bitten by a dog [69].

Oedema, inflammation, redness, pain, and purulent discharge on the side of the wound are the most common symptoms people face with a dog bite. Leukocytosis and neutrophilia develop

rapidly, and local inflammation progresses to systemic inflammation. Without appropriate treatment, the inflammation can affect other organs and cause bacterial sepsis, osteomyelitis, endocarditis, and eve meningitis. Compared to rabbits and other species susceptible to *Pasteurella*, humans acutely infected with *Pasteurella* do not show respiratory signs. However, in chronic cases, lymphadenopathy, pneumonia, and lung disease may occur[69].

4.4 Pathogenomics of *P. multocida*

The identification of genetic information through in vivo expression, mutagenesis and whole genome expression are essential information to understanding the virulence of *P. multocida*. All the *P. multocida* strains have been express with outer membrane proteins, thiamine metabolism genes, iron additional genes and adhesion pilus gene cluster. Important role on the biofilm production of the *Pasteurella* species are the homologs of the tad gene locus. Some virulence genes of *P. multocida* genes are correlated phylogenetically with the virulence gene of *Haemophilus influenza* [69].

5. Control and Treatment

Prophylactic measures

There are several predisposing factors that may influence *P. multocida* infection. Some of them are the environmental and management factors. Optimization of ventilation, feeding hygiene and climatic conditions is necessary for prophylaxis of *P. multocida* infection. Vaccines are sometimes ineffective due to the diversity of serotypes of the different species.

Control of *P. multocida* infections is very difficult mainly for 2 reasons. First, despite control of isolates, the causes of the disease cannot be eliminated and second, *Pasteurella* species have a high rate of resistance to treatment with antibiotics. Unfortunately, it takes several days to send a sample to the lab, isolate the bacteria, and test for sensitivity to the correct antibiotic, resulting in huge economic losses. Some veterinarians choose pre- and post-operative antibiotics based on recommendations from textbooks or other sources. This can easily lead to antimicrobial resistance of certain pathogens[29]. Vaccines against serotypes A and D are also available. They vaccinate rabbits at 4 weeks of age and then at 7 and 10 weeks of age. After the first rounds then every 6 months. The older vaccinations consist of an initial vaccination and 1

booster at 3-week intervals. Some large farms can also produce autologous vaccines against some pathogens as a prophylactic or treatment measure [24].

Control

The first rabbit herds free of *Pasteurella* were achieved by Webster and Burn in the 1920s. Some of these methods are still in use. Selection of uninfected rabbits according to their bacteriological and serological status and isolation from other untested rabbits. Preventing the movement of personnel and materials from infected to uninfected rabbits is important for maintaining Pasteurella-free rabbits. Caesarean section and rearing of young in Pasteurella-free animals is recommended to prevent exposure of the young to the pathogen. Early weaning with or without antibiotics is recommended for infected animals. Unfortunately, all domestic rabbits must be tested for *P. multocida* infection, even if they do not show clinical signs. Sometimes it is easier to stop the infection in younger animals before the infection becomes chronic. In cases where animals show clinical signs such as nasal discharge, the affected rabbits must be isolated. Rabbits with chronic infections appear to transmit the pathogen more slowly than in acute cases. *P. multocida* is very sensitive to germicides, so controlling the spread of infection with disinfectants is critical. Predisposing factors such stress, sudden temperature changes, bad husbandry practices and inappropriate ventilation have to avoided since all those factors can facilitate the onset of the infection[12].

Treatment

Broad-spectrum antibiotics are usually used to treat *P. multocida* infections. *P. multocida* shows moderate sensitivity to erythromycin, lincosamides, and B-lactams. A combination of amoxicillin with clavulanic acid, doxycycline, and fluoroquinolones are the most common antibiotics used against *P. multocida* infections[69].

6. Genetic identification

6.1 Molecular identification and differentiation of *P. multocida*

For many years, the detection and identification of P. multocida was based on cultivation and purification methods. However, with the development of technology over the years, cultivation and purification were no longer preferred for the identification of P. multocida because they are slow and expensive.

Methods such as nucleic acid amplification and DNA hybridization offered us the advantage of reducing the time required for pathogen identification and significant efficacy in detecting and identifying pathogens such as *P. multocida* [23].

6.2 *P. multocida* specific PCR assays

PCR is an important method for the rapid production of large numbers of target sequences. It is one of the most important tools in the laboratory arsenal. The advantage of PCR is that analysis of clinical samples can be performed, and bacterial pathogens can be identified in a short time, while multiplex PCRs can provide comprehensive information on pathogenesis, diagnosis and resistance

Nowadays, rational, and random are the 2 approaches that have been used for the procedure of *P. multocida*-specific PCR. Oligonucleotide primers are used to amplify the psl gene that encodes the P6-like proteins of *P. multocida*. This gene can be used as a starting point for specific detection of *P. multocida*. With this technique, sensitivity can reach up to 10 organisms, after which additional hybridization with psl is required [23].

6.3 Restriction endonuclease analysis

The REA analysis could help with outbreaks of pasteurellosis. This method is so reliable because it is not affected by phenotypic characteristics that limit the sensitivity and specificity of simplified typing methods. REA can distinguish isolates of the same serotype. The most informative restriction enzymes that have been used for DNA fingerprinting of *P. multocida* strains are HhaI and HhaII, which can distinguish a variety of serotypes B [23].

6.4 Species-specific PCR and Capsular PCR

For accurate identification of pasteurellosis in livestock, rapid identification of *P. multocida* is important for the farmer from an economic point of view and for the veterinarian to treat the affected individual as soon as possible. The advantage of the *P. multocida*-specific PCR test is that the test takes less time and does not depend on phenotypic differentiation. To identify the *P. multocida* isolates we used direct colony preparations as described by Townsend et al. (1998).

The primer used for identification were KMTT1T7 (ATCCGCTATTTACCCAGTGG) and KMT1SP6 (GCTGTAAACGAACTCGCCAC). Capsular PCR was used for the capsule, serogroup according to Townsend et al.

Type A, CAPA-FWDTGCCAAAATCGCAGTCAG and CAPA-REV TTGCCATCATTGTCAGTG.
Type B, CAPB-FWD CATTTATCCAAGCTCCACC and CAPB-REVGCCCGAGAGTTTCAATCC.
Type D, CAPD-FWD TTACAAAAGAAAGACTAGGAGCCC and CAPD- REV CATCTACCCACTCAACCATATCAG.
Type E, CAPE-FWD TCCGCAGAAAATTATTGACTC and CAPE-REV GCTTGCTGCTTGATTTTGTC.
Type F, CAPF-FWD AATCGGAGAACGCAGAAATCAG and CAPF-REV TTCCGCCGTCAATTACTCTG.
Cycling conditions were used for all reagents [63] [62]. For one cycle, an initial inactivation heat of 95C for 5 minutes was applied, 35 cycles of 94C for 1 minute, 55C for 1 minute, and 72C for 1 minute. Finally, one cycle at 72C for 10 minutes [16]. Agarose gel electrophoresis with 1.5% agarose (Sigma, USA) was used to amplify the gene results [59].

6.5 Multiplex PCR

A multiplex PCR to determine the 5 serogroups of *P. multocida* as described by Townsend et al. 2001 [62]. The multiplex PCR contained primers specific for all five serogroups. The mixture contained six primer sets at 3.2 Mm cc, 1 U Taq DNA polymerase, 2 Mm MgCl2, each deoxy-nucleoside triphosphate at 200 Mm cc, and a PCR buffer. A cycling program was used to separate the products and electrophoresis was performed in 2% agarose, which was then visualized by ethidium bromide staining [62].

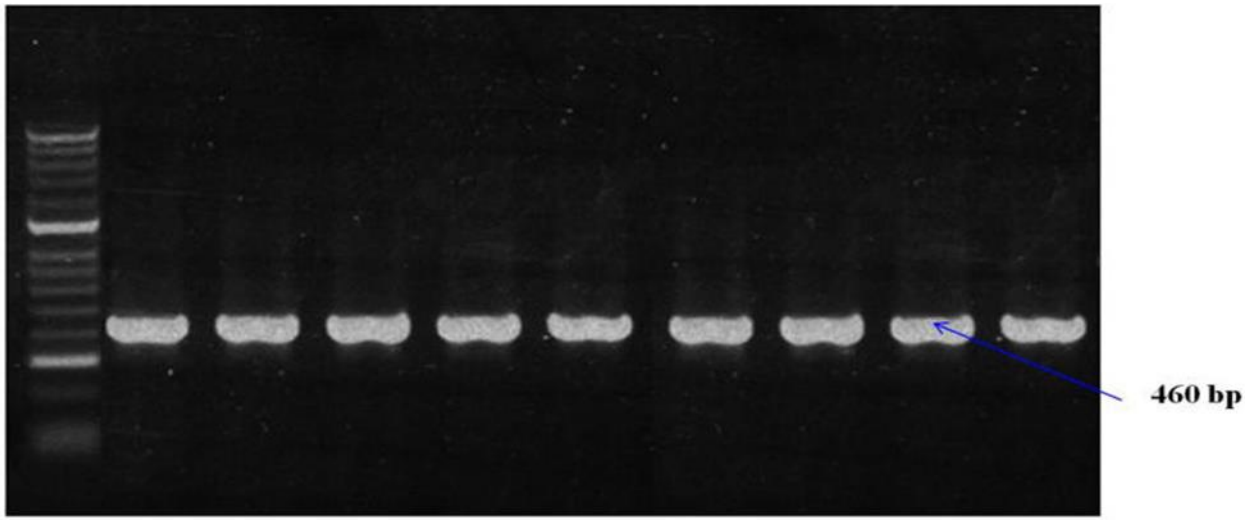


Figure 8: interpreted the amplification of 460bp fragments specific to *P. multocida* field isolates by agarose gel electrophoresis[5]

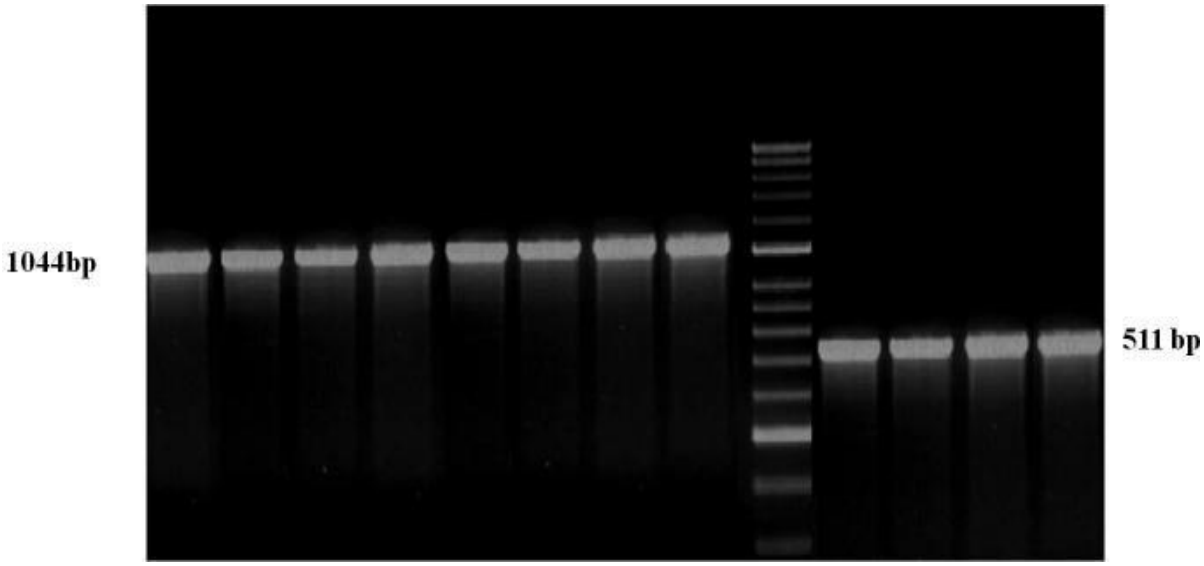


Figure 9: Agarose gel electrophoresis with amplification of 1044bp fragments specific to capsular antigen type A and 511 Bp fragments specific to type E [5].

7. Aminoglycosides

The aminoglycosides are one of the oldest groups of antibiotics. The first antibiotic in this class was streptomycin, which was discovered in 1940[13]. Aminoglycosides are broad-spectrum antibiotics whose mechanism of action is bactericidal [31]. They have the ability to penetrate the three domains of the LPS and increase the permeability of the membrane [34]. Aminoglycosides are highly ototoxic and nephrotoxic when ingested by humans through meat, so a maximum residue level has been established in animal products almost everywhere in the

world [31]. Aminoglycosides are produced naturally by *Streptomycetes* and *Microspora* or chemically. Aminoglycosides are water soluble and heat stable. They are active over a wide PH range and at different temperatures. Their molecular range varies from 400 to 500 g/mol [8]. The use of aminoglycosides in combination with beta-lactams has shown very good results against a wide range of bacterial pathogens[13].

Aminoglycosides require one or more aminated sugars glycosidically linked to a dibasic cyclitol to exert their potency and antibiotic spectrum. Initially, aminoglycosides inhibit bacterial protein synthesis by binding to prokaryotic ribosomes. The transport of aminoglycosides along the cytoplasmic membrane is subordinate to electron transport and is referred to as energy-dependent phase I. The disadvantage of aminoglycosides is that they are easily inhibited by low PH, hyperosmolarity and divalent cations. Aminoglycosides bind to the aminoacyl site of 16s rRNA in the 30s ribosomal subunit in energy-dependent phase II. The deviant proteins penetrate the cell membrane, altering permeability and stimulating aminoglycoside transport. The 2-deoxystreptamine and the prepared amino sugar cause mistranslation. The nucleotide responsible for aminoglycoside binding formed an irregular inner loop. This irregular loop is caused by the asymmetric base pairs.

While all aminoglycosides affect prokaryotic protein synthesis at optimal concentrations, they can also affect protein synthesis of mammalian cells at high concentrations. Possibly through unknown binding to eukaryotic ribosomes. Agents such as kanamycin C, the antibiotic G418 and gentamicin A with a hydroxyl function on the C-6' amino side are more potent inhibitors of eukaryotic protein synthesis [42].

7.1 Kanamycin

Kanamycin extracted from *Streptomyces kanamyceticus* [60] [45]. The structure of kanamycin consists of 3 components, deoxystreptamine, 3-amino-3-deoxydglucose and 1 amino sugar[32]. Kanamycin is effective against gramme-positive and -negative bacteria and mycobacteria [9]. Kanamycin successfully induces mistranslation and inhibits translocation of the prokaryotic ribosome by affecting the 30 subunit [58]. It has been successfully tested for tuberculosis in mice and guinea pigs [65]. The advantage of kanamycin over other drugs in the same class is that kanamycin has fewer side effects and less toxicity than the others [10].

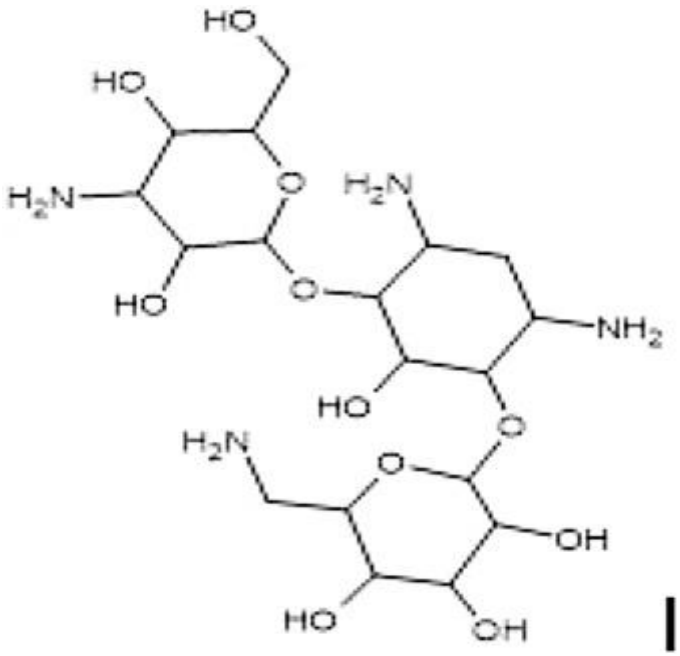


Figure 6: Kanamycin chemical structure [30].

7.2 Optimal antimicrobial treatment

Optimal antimicrobial treatment is the choice of the right drug for the appropriate pathogen at the right time with the optimal route of administration and dosage. The duration of treatment must also be considered. Toxicity, pharmacodynamics and pharmacokinetics, animal species, size, physiological status, and drug interactions are various factors for appropriate selection of antimicrobial agents. For food-producing animals, holding time and herd productivity are the major concerns of farmers. Basic logical process requires model can help to reach the optimal antimicrobial treatment.

a) Veterinary advice on the establishment and management of specific cases

b) Alternative treatments for the prevention, control, and treatment of bacterial diseases

c) If there is no alternative, only the first-choice agent should be used

d) Safe and proper use of the selected antimicrobial agent

In addition, in the case of antibiotic treatment, constant monitoring is necessary to avoid side effects and to maintain the health of the herd [18].

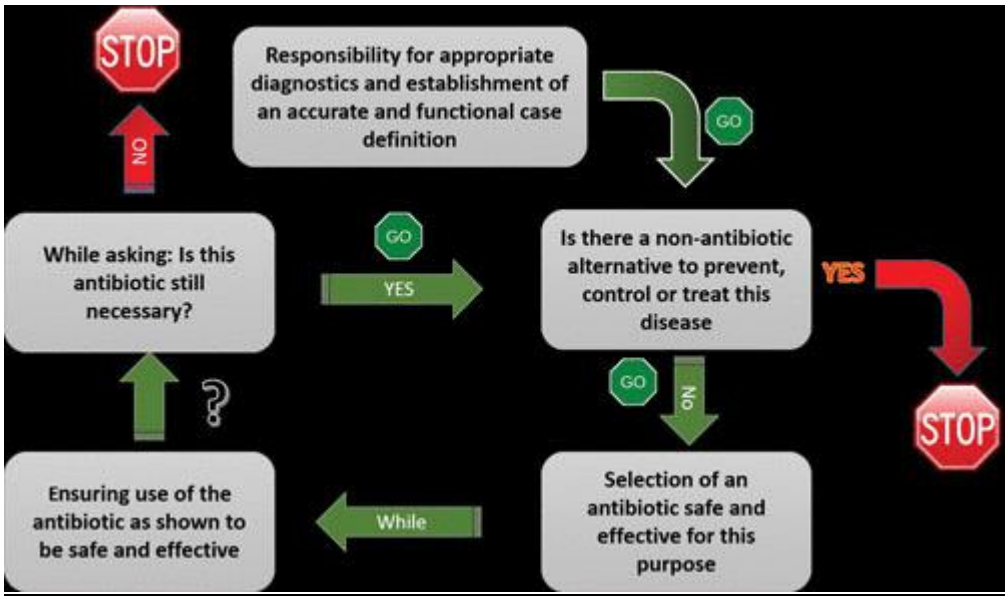


Figure 7: shows the logical thinking for the optimal antimicrobial treatment[18]

4 important objectives for defining optimal antimicrobial treatment in animal husbandry:

1. decrease of antimicrobial consumption
2. improved diagnostic testing and identification of the pathogen
3. careful use of second-line antimicrobials
4. optimization of dosage regimens

8. Antimicrobial Resistance

Antimicrobial resistance has been targeted WHO due to increasing incidences in the last decade and requires drastic action. Each year, approximately 700,000 people die from AMR, and it is estimated that this number will increase 15-fold by 2050 [54]. The food industry plays a central role in the epidemiological background of antimicrobial resistance, as agricultural workers are the first to come into contact with animal pathogens. In 2005, the European Union banned the use of some specific antibiotics as growth promoters rather than treatments. On rabbit farms, rabbits and farmers share the same multidrug-resistant genotypes because the rabbit industry, among other industries, has the highest drug levels [2]. The overuse of antibiotics and their residues has increased antibiotic resistance genes. Many methods have been tried to study the antibiotic resistance genes, but these methods are very limited. Detection of the occurrence of antimicrobial resistance genes in the microbes by meta-sequencing or PCR only qualitatively links the antimicrobial resistance gene[25].

Different plasmids of *P. multocida* express resistance to different antibiotics. The most common are B-lactams, chloramphenicol, tetracycline, and sulphonamides. Some antibiotic resistance genes may carry plasmids capable of mobilization, cleavage, or replication. Other plasmids of *Pasteurellaceae* species can transfer to other Gram-negative species.

Multidrug-resistant genes can invade the chromosome of some strains. Such genes were identified in the chromosome of strain *P. multocida* 36950, which was resistant to all commonly used antibiotics for respiratory disease in cattle.12 of the 88 genes of 85-kb ICEPmul were resistance gene and they were dispensed in 15.7-kb and 9.8-kb end of the element regions [69].

8.1 Biology of the Antimicrobial resistance

Genetic AMR results from mutation of the bacterial genome. The ability of bacterial isolates to replicate in the presence of an antibiotic agent defines AMR. If genetic AMR to a particular antibiotic occurs, a replacement antimicrobial should be used. Phenotypic AMR is the change in individual cells of the bacterium without subordination on genes. Phenotypic AMR against a particular antibiotic stops the growth of the bacteria above the MIC. The increase in survival in the presence of an antibiotic agent without an increase in the MIC is what we define by the term 'tolerance'. Phenotypic AMR is the ability of a subunit of the bacterial cell to be killed more slowly or not at all. This is what we refer to as 'persistence'. Another way to confirm antibiotic efficacy is MBC. The MBC defines the minimum antibiotic concentration that can kill 99% of the bacterial population in a given time. However, sometimes it is difficult to detect the presence of genetic or phenotypic AMR[55].

8.2 Antibiotic Resistance Transmission through the food chain

Through contact with or consumption of animal products, there is a great possibility that humans will be exposed to resistant bacteria. It is well known that almost all foods of animal origin in all species contain a certain number of resistant bacteria and their genes. For example, Alexander et al. discovered drug-resistant E. coli on bovine carcasses after removal of organs, which were observed even after one day. MRSA detected in 12% of animal meat production in the Netherlands and Italy [40].

8.3 Resistance of Pasteurella species to aminoglycosides focusing on kanamycin

Resistance of Pasteurella to aminoglycosides is usually achieved by inactivation of the antibiotic by enzymes through adenylation, acetylation, and phosphorylation. Resistance is also possible due to some chromosomal mutations of this antibiotic group[41].

The first aminoglycoside resistance in Pasteurella was observed in 1978 by Berman and Hirsch in streptomycin in turkeys [6].

Kanamycin resistance in *P. multocida* is closely related to the alphA1 gene, which is responsible for encoding the aminoglycoside-3-phosphotransferase that causes resistance. The alphA1 gene was recognized in most cases from chromosomal DNA. In one *P. multocida* strain, alphA1 is located on the 5,955-bp plasmid pCCK3152 along with str A and B and sul2. Multidrug-resistant plasmids carrying these genes were first identified in Actinobacillus isolates in China. AphA3 is another resistance gene expressed on the 5.1-kb plasmid Pcck411 from *P. multocida* [41].

4. Aims to study

This study aimed the following:

1. To determine the prevalence of multi resistant *P. multocida* strains in rabbit specifically resistance to kanamycin.
2. The way a higher prevalence would affect the economy in the rabbit meat and fur industry.
3. The impact of prophylactic use of antibiotic on human consumers.

5. Materials and methods

5.1 Bacterial strains

138 *P. multocida* strains were collected from industrial rabbit farms throughout Hungary in 2009-2016. The samples were collected during pathological examination of respiratory tissues of rabbit carcasses. The strains were isolated from the infected carcasses using standard methods. *P. multocida* was determined by the following characteristics: no haemolysis on sheep blood agar, no growth on MacConkey agar, distinct odour of colonies, positive indole production, positive catalase activity, negative urease activity, positive ornithine decarboxylase activity, negative lactulose, and maltose but positive sucrose. For the D-trehalose, D-xylose, L-

arabinose very different results. Lung samples were homogenised and 50% suspended in saline. We used brain heart infusion agar plates with 5% sheep blood. We then allowed them to culture at 37C for 1-2 days. [68].

5.2 Antimicrobial Susceptibility test

A Kirby-Bauer disc diffusion method was used, based on the Clinical and Laboratory Standards protocols. We used a total of 19 antibiotics for the test (apramycin, doxycycline, enrofloxacin, florphenicol, gentamycin, colistin, marbofloxacin, neomycin, oxytetracycline, streptomycin, spiramycin, streptomycin, sulfamethoxazole and trimethoprim, tilmicosin, kanamycin, tulathromycin, tiamulin and valnemulin). The Kirby-Bauer disc is typically used to test rapidly multiplied aerobic bacteria. Small, circled papers contain a specific amount of an antibiotic and are placed evenly on the agar plate, including the test bacterium. We measure the diameter of the zone of inhibition based on the Clinical and Laboratory Standards Institute [51].

Table 1&2. Percentage of the resistance of the *P. multocida* to the different antibiotic's agents.

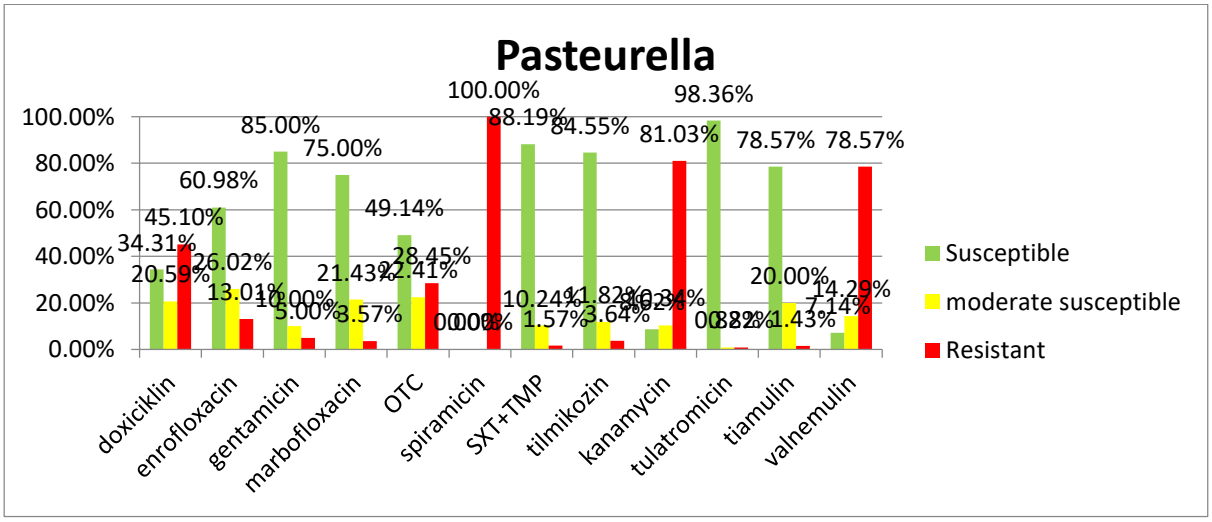
	Doxycycline	Enrofloxacin	Florfenicol	Flumequine	Gentamycin	Marbofloxacin	Oxytetracycline
Sensitive	35	75	2	0	102	21	57
	34.31%	60.98%	66.67%	0.00%	85.00%	75.00%	49.14%
Moderate	21	32	0	0	12	6	26
	20.59%	26.02%	0.00%	0.00%	10.00%	21.43%	22.41%
Resistant	46	16	1	2	6	1	33
	45.10%	13.01%	33.33%	100.00%	5.00%	3.57%	28.45%
Total	102	123	3	2	120	28	116

	Spiramycin	SXT & TMP	Tilmicosin	Kanamycin	Tulathromycin	Tiamulin	Valnemulin
Sensitive	0	112	93	5	120	55	2
	0.00%	88.19%	84.55%	8.62%	98.36%	78.57%	7.14%
Moderate	0	13	13	6	1	14	4
	0.00%	10.24%	11.82%	10.34%	0.82%	20.00%	14.29%
Resistant	25	2	4	47	1	1	22
	100.00%	1.57%	3.64%	81.03%	0.82%	1.43%	78.57%
Total	25	127	110	58	122	70	28

6. Results

45.1% of PM samples were resistant to doxycycline, while 34.31% were sensitive and 20.59% were moderately sensitive. On the other hand, only 13.01% were resistant to enrofloxacin while 60.98% and 26% were sensitive and moderately sensitive respectively. 85% of the samples were sensitive to gentamycin, 10% moderately sensitive and 5% resistant. Of the samples containing marbofloxacin, 75% were sensitive, 21.43 moderately sensitive and 3.57% resistant. 49.1% samples were sensitive to oxytetracycline, 22.4% moderately sensitive and 28.45% resistant.

Table 3. Comparison graphic of the sensitivity of the different Antibiotics



All samples were resistant to spiramycin, 88.19% of PM samples were sensitive to sulfamethoxazole and trimethoprim in combination, with 10.24% moderately sensitive and 1.57% resistant. When tilmicosin was used, 84.55% of PM samples were sensitive, 11.82% moderately sensitive and 3.64% resistant. When kanamycin was used, 81.03% samples were insensitive, 10.34% moderately sensitive and 8.62% sensitive. At the highest rate, 98.36% of PM samples were sensitive to tulathromycin, with 0.82% moderately sensitive or sensitive. Only 1.43% of the 138 samples were resistant to tiamulin, 20% of which were moderately sensitive and 78.57% were sensitive. Valnemulin also showed a high rate of resistance with 78.57% of the samples resistant to it, 7.14% sensitive and 14.29% moderately sensitive.

7. Discussion and Conclusion

It is well known that comparison of disk diffusion antibiotic susceptibility test results from different laboratories and countries is difficult due to lack of similarities. However, the Kirby-Bauer method is one of the most reliable methods for diagnosing animal diseases. Based on studies conducted in Ibadan, Nigeria, 100% of rabbits infected with *P. multocida* were sensitive to kanamycin. The P. *multocida* isolates tested for enrofloxacin in Nigeria were almost identical to our results[46]. Kanamycin is a relatively new and expensive drug. As we know, Nigeria is a third world country, and it is quite possible that kanamycin is used less frequently in Nigeria than in Hungary. So also, the resistance rate. Another study in Japan found that 40 *P. multocida* isolates were all sensitive to gentamycin and kanamycin[28].

Of samples collected in the UK and France in 2004, 100% of isolates were sensitive to marbofloxacin and enrofloxacin, and 99.1% and 93.4% were sensitive to gentamycin and sulfamethoxazole and trimethoprim, respectively. The *P. multocida* isolates we collected from rabbits in Hungary are generally far more resistant to oxytetracycline than in the study in the UK and France. Doxycycline resistance of *P. multocida* isolates was significantly lower (2.8%) than in our study, where resistance was almost half[52]. In Rougier's study, all strains of *P. multocida* were sensitive to fluoroquinolones and over 90% to tetracycline and trimethoprim-sulfamethoxazole [52].

Studies also show that *P. multocida* has almost the same sensitivity to tulathromycin in different animals. Based on study comparisons also in Hungary, tulathromycin has almost 100% efficacy in rabbits, pigs and poultry [56].

Aminoglycosides are generally the best weapon against Gramm-negative bacteria. But in recent years, increasing resistance to aminoglycosides has been observed in *P. multocida*. Our study revealed that 81.03% of isolates were resistant to kanamycin and 5% to gentamycin. Also the study on pigs and poultry showed that about 30% of *P. multocida* isolates were also resistant [56].

From a study in Bulgari, the kanamycin resistance of *P. multocida* in samples from birds and other mammals including rabbits was 52.7% and 47.3% were only moderately sensitive [27].

A study in Fujian, China, also investigated the antimicrobial susceptibility of *P. multocida* strains from 205 isolates. Our interest is focused on the susceptibility of *P. multocida* to kanamycin. Of the 205 isolates, 19 were resistant, 131 were intermediate resistant, and only 55 were sensitive to kanamycin. This shows that only 26.89% of the isolates were sensitive to kanamycin. A high rate of sensitivity to gentamycin was also found in China, with 63% and 61% being sensitive to enrofloxacin. [67].

If we compare the data and results, we can say that over the years there has only been a rapid increase in the overuse of antibiotics. Another aspect is that the overuse of antibiotics that do not belong to the first class of use can lead to the resistance of many strains. When farmers or veterinarians use broad-spectrum antibiotics for mild infections and without antimicrobial susceptibility testing or isolation of bacterial strains, it can lead to uncontrollable resistance of many bacteria and especially *P. multocida*.

Unfortunately, the use of antibiotics to treat *P. multocida* is sometimes unavoidable due to disillusioned or ineffective prophylactic measures. Moreover, resistance genes are linked to mobile genetic elements and can be exchanged between bacteria. Resistance genes such as catIII and sulII have also been found in other Gram-negative bacteria [29]. This could lead to new genetic mutations that cause antibiotics previously used against certain bacteria to become ineffective.

However, the public health threat from pasteurellosis is low. But it would be inappropriate if we did not monitor AMR daily and always choose the right antibiotic treatment[29].

7.1 Selective media

Kanamycin has been used for the cultivation and isolation of *P. multocida*[28]. However, our study shows that *P. multocida* strains in Hungary are not completely resistant to kanamycin, which may lead to false negative results in various studies where kanamycin was used for pathogen isolation. On the other hand, spiramycin could be ideal agent for the *P. multocida* isolation strains in Hungary since in our study the resistance of *P. multocida* was on 100%.

7.2 Measures for reduction of antibiotic resistance

In many countries, farmers or any other person can use antibiotics without a veterinary prescription. This situation is not the best type of antibacterial treatment as it increases the antimicrobial resistance rate. In Europe, any systemic antibiotic can only be purchased on veterinary prescription. This measure can be beneficial and very important in the fight against multi-resistant bacteria. Another measure to reduce antibiotic use is to restrict certain drugs and benchmarking approaches to ensure uncontrolled use and discipline farmers who use large amounts of antibiotics. These two measures require adequate control programming to be achieved.

The most efficient way to reduce antibiotic use is to ban antibiotics used for growth promotion. A good example of this measure is Denmark. In Denmark, growth promotion was banned and antibiotic consumption decreased by 80 tonnes between 1994 and 2015. In Europe, growth promotion was banned in 2006.

Another measure to consider is controlling the unnecessary use of prophylactic antibiotics in certain cases. Metaphylaxis is also a risk factor for antibiotic resistance. The use of antimicrobials in a herd containing healthy and sick animals can quickly lead to multidrug resistance. Sick animals should be treated individually.

Unnecessary antimicrobial therapies are the number one factor of concern. Treatment of some viral infections and self-limiting bacterial or parasitic infections also increases the risk of resistance [18].

7.3 Development of rabbit resistant to pasteurellosis

A 2016 study in Toulouse showed that it is possible to breed genetically resistant rabbits to pasteurellosis, or rabbits that show no clinical signs during infection. This will reduce the morbidity and mortality of rabbits infected with pasteurellosis and subsequently reduce the use of antimicrobials in rabbit herds[57].

Eady et al. Also studied the contribution of maternal genetic effects for disease traits in meat rabbits. They observed that disease incidence decreased with increasing maternal litter parity by attempting to breed genetically resistant rabbits[14].

Increasing the genetic resistance of rabbits improves survival rates. However, to achieve this, the indicator trait should have a genetic variation component and the trait must be economically efficient for farmers[14].

Another study by Gunia M. et al. has successfully shown that additive genetic variation for resistance to bacterial diseases and certain disease syndromes is possible in rabbit breeding [19].

7.4 Alternative solutions rather than antibiotics

b-glucans

Probiotics, prebiotics, and phytonutrients are unfortunately not as effective. However, with our alternatives, we must conclude B-glucans. B-glucans occur naturally in the cell wall of bacteria, plants, and fungi. The b-glucans of fungi 1-3 and 1-6 behave as biological reaction modifiers. Mammalian cells recognise the b-glucans as pathogen-associated molecular patterns that help the host enhance its immune defences. Studies have shown that 1-3 and 1-6 b-glucans have beneficial effects on humans and mammals against bacteria, viruses, and fungi.

Extensive research has shown that the properties of b-glucans can help individual organisms against bacterial infections and even neoplasms. B-glucans act on immune priming and bind to the CR -3 receptor on innate immune cells and increase chemotaxis, phagocytosis and killing of microorganisms. Another study showed that after rabbits were exposed to *P. multocida* virulence genes, the only effective means of fighting infection other than antibiotics was b-glucans. More specifically, administration of b-glucans delayed the death of rabbits but was very effective as a prophylactic agent against *P. multocida* exposure[47].

Egyptian propolis

Propolis is obtained from the excreta and buds of honeybees and mixed with wax and bee enzymes. The composition of Egyptian raw propolis consists of aromatic acids such as benzoic acid, cinnamic acid, trans-p-cinnamic acid, 3,4-dimethoxycinnamic acid, ferulic acid and caffeine. It contains among others 11 caffeic acid esters, flavonoids, sugars and aliphatic acids.

Propolis acts in the body as an antimicrobial, anti-inflammatory, antioxidant, antiparasitic and immunostimulant. A study shows that the combination of propolis and inactivated formalized *P. multocida* vaccine reduces the mortality rate of rabbits. To be more specific, 4 groups of rabbits were tested. The first group with saline. The second with propolis only, the third with *P. multocida* vaccine and the fourth with a combination of vaccine and propolis. Surprisingly, group 4 showed no clinical signs and no histopathological lesions.

Propolis proved to be very beneficial in combination with vaccines against *P. multocida* infections and furthermore showed that alone it can improve the general health status even of flocks infected with *P. multocida* [44].

Immunization

PASSIVE

Studies in the 1990s showed that Clemson University vaccine against P. multocida, potassium thiocyanate extract of P. multocida, and heat-killed bacterium were effective against P. multocida in rabbits, mice, and baby turkeys. Monoclonal antibodies developed against the membrane proteins of P. multocida and helped inhibit the proliferation of the bacteria in the lungs. Further studies revealed that ammonium sulphate-perceptible protein fractions and outer membrane protein H were highly effective against homologous infection of P. multocida in rabbits, cattle, and rabbits[3].

ACTIVE

Vaccines are the most efficient way, from an economic point of view, to prevent P. multocida infections or to reduce the development of clinical symptoms of pasteurellosis.

Multiple emulsion vaccine was first used successfully against haemorrhagic septicaemia in buffaloes and subsequently in rabbits and calves.

Cross-protection against rabbit pasteurellosis has also been achieved by using bacterin from iron-deficient Pasteurella[3].

Future perspectives

While the previous sections have described how we can avoid the use of antimicrobials and minimise their consumption, education and research are needed to achieve this goal. The key to achieving this goal, according to the European Food Safety Authority, is education of veterinarians and users. Veterinary and agricultural universities must provide students with all relevant information on the use of antimicrobials and teach them about the impact of the safe use of antimicrobials on human and animal health. You must educate them on the general principles of antimicrobial stewardship, infection control, and prevention. They need to focus on prevention of bacterial load as this is the main reason for reducing antimicrobial resistance. International and national organisations need to support and monitor the schools so that the guidelines can be successfully implemented.

Finally, new studies need to be funded to discover new innovative pharmaceuticals such as vaccines, new antibiotics, and alternative treatments. The new antibiotics must have appropriate pharmacokinetics and pharmacodynamics and target narrow spectrum pathogens or even better antibiotics for specific pathogens.

In food-producing animals, understanding metaphylaxis is very important. Early diagnosis and treatment are very important for controlling disease and avoiding the use of antimicrobials. Constant monitoring of feeding and watering systems and other precautions in animal husbandry can help control AMR[18].

8. Acknowledgements

Many thanks to my family for their support all over these years and to my friend how supported me in every step of my life.

9. Appendix

Table 1. Interpreted which isolates of the *P. multocida* isolates were resistant, sensitive, and moderate sensitive to the 20 antibiotics have been tested.

		Doxycycline	Florphrnicol	Gentamycin	Marbofloxacin	OTC	Spiramycin	SXT+TMP	Tilmicosin	Kanamycin	Tulathromycin	Tiamulin	Valnemulin
1	PM	R	M	M		M		S	S	S	S	nv	S
2	PM	R	M	S		M		S	S	M	S	nv	S
3	PM	S	S	S		S		S	S	M	S	nv	S
4	PM	S	S	S		S		S	S	R	S	nv	nv
5	PM	S	nv	S		M		S	S	R	S	nv	nv
6	PM	R	nv			M		S	S	S	S	nv	nv
7	PM	MS	nv	R		R		S	S	R	S	nv	nv
8	PM		nv	nv		nv		nv	nv	nv	Nv	nv	nv
9	PM	MS	nv	S		R		S	S	R	S	nv	nv
10	PM	S	nv	S		S		S	S	M	M	nv	nv
11	PM	S	nv	S		S		S	S	M	S	nv	nv
12	PM		nv	nv		nv		Nv	nv	nv	nv	nv	nv
13	PM	S	nv	S		S		S	S	S	S	nv	nv
14	PM	R	nv	M		nv		R	nv	nv	nv	nv	nv
15	PM	S	nv	S		S		S	nv	nv	nv	nv	nv
16	PM		nv	nv		nv		nv	nv	nv	nv	nv	nv
17	PM	S	nv	S		S		S	S	M	S	nv	nv
18	PM	S	nv	S		S		S	M	R	S	nv	nv
19	PM	MS	nv	S		S		S	S	M	S	nv	nv

20	PM		nv	S		R		S	S	M	R	nv	nv
21	PM	R	nv	M		S		S	S	M	S	nv	nv
22	PM	S	nv	S		S		S	S	R	S	nv	nv
23	PM		nv	nv		nv		nv	nv	nv	nv	nv	nv
24	PM	S	nv	S		S		S	S	R	S	nv	nv
25	PM	S	nv	S		S		S	M	R	S	nv	nv
26	PM	S	nv	S		S		S	S	M	S	nv	nv
27	PM	S	nv	S		S		S	S	R	S	nv	nv
28	PM		nv	S		S		S	S	R	S	nv	nv
29	PM	S	nv	R		S		S	S	R	S	nv	nv
30	PM	S	nv	S		S		S	S	R	S	nv	nv
31	PM	S	nv	S		S		S	S	R	S	nv	nv
32	PM	S	nv	M		S		S	S	M	S	nv	nv
33	PM	S	nv	M		S		S	S	R	S	nv	nv
34	PM	S	nv	S		S		S	S	R	S	nv	nv
35	PM	MS	nv	S		S		S	S	R	S	nv	nv
36	PM	S	nv	S		S		S	S	M	S	nv	nv
37	PM		nv	S		S		S	S	M	S	nv	nv
38	PM		nv	nv		nv		nv	nv	nv	nv	nv	nv
39	PM		nv	nv		nv		nv	nv	nv	nv	nv	nv
40	PM		nv	M		R		S	S	R	S	nv	nv
41	PM	S	nv	S		S		S	S	R	S	nv	nv
42	PM	S	nv	S		M		M	S	R	S	nv	nv
43	PM		nv	S		S		S	S	R	S	nv	nv
44	PM	S	nv	S		M		S	S	R	S	nv	nv
45	PM	MS	nv	S		S		S	M	R	S	nv	nv
46	PM		nv	S		S		S	M	R	S	nv	nv
47	PM		nv	S		S		S	nv	R	S	nv	nv
48	PM		nv	S		R		S	nv	R	S	nv	nv

49	PM		nv	S		S		S	nv	R	S	nv	nv		
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108	PM	R		S		R	R	S	S	-	S	S	-	
109	PM	R		S	-	MS	-	S	S	-	S	S	R	
110	PM	R		MS	-	S	-	S	S	-	S	S	R	
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116	PM	S		S	-	S	R	S	S	-	S	S	-	
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136	PM	S		S	-	S	R	S	R	-	S	MS	-	

137	PM	S		S	-	S	R	S	MS	-	S	MS	-	
138	PM	MS		MS	-	MS	R	S	MS	-	S	S	-	

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