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INFLUENCE OF CARBON SOURCES ON THE PATHOGENICITY

OF MYCOPLASMA PNEUMONIAE

A Thesis

by

TRAVIS ANTHONY SALINAS

Submitted to Texas A&M International University
in partial fulfillment of the requirements
for the degree of

MASTER OF SCIENCE

Spring 2020

Major Subject: Biology

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Major Subject: Biology

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ABSTRACT

Influence of Carbon Sources on the Pathogenicity of *Mycoplasma pneumoniae* (Spring 2020)

Travis Anthony Salinas, Texas A&M International University;

Chair of Committee: Dr. Michael R. Kidd

Mycoplasma pneumoniae is a human respiratory tract pathogen and one of the best-characterized minimal bacteria. It is a serious problem because of health problems it can cause as it can be detrimental to humans and the occurrence and aggravation of systemic involvement in *M. pneumoniae* infection occur via multiple immunological pathways. With its major source of carbon and energy coming from phospholipids, *Mycoplasma pneumoniae* thrives on lung epithelia. In *Mycoplasma pneumoniae*, pathogenicity is closely related to carbon metabolism. By testing carbon sources in well plates and growth curves to analyze how growth is affected in *Mycoplasma pneumoniae* and measure optical density, carbon source effectiveness may be better understood. The hypothesis of this study is that other carbon sources similar to glycerol and glucose, such as mannose and sorbose, can be beneficial to the growth of *Mycoplasma pneumoniae* as well. With the use of well plates and culture flask, growth curves were developed to measure growth with optical density. Glucose and glycerol were shown to be carbon sources that were able to use by *Mycoplasma pneumoniae*, with carbon sources such as maltitol and xylitol showed positive results. The hypothesis in this study was shown to be not supported. While other carbon sources were able to be utilized by *Mycoplasma pneumoniae*, they were not as efficient as glucose and glycerol. Maltitol was a carbon source uncharacteristically grew with *Mycoplasma pneumoniae*. A two-way

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ANOVA that was done testing No Carbon Source against other carbon sources showed that glucose was the only carbon source that was significant. A two-way ANOVA of glucose against other carbon sources showed they were all significant, except for maltitol. It can be assumed with the data that hexose sugars, pentose sugars, and sugar alcohols that are similar to glucose and glycerol can be used by these lung bacteria. In turn, theoretically these carbon sources can be used to develop alternate treatments to treat afflictions that infect the lungs. In time, these carbon sources can be possibly be used to counter diseases and difficulties caused by *Mycoplasma pneumoniae*.

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INTRODUCTION

There are more than 200 known species in the genus *Mycoplasma*. Belonging to the class *Mollicutes*, a group of bacteria that have been evolved from a common gram-positive ancestor with low GC content (1). This bacterium has recently attracted considerable interest as a model organism in synthetic and system biology (2). Although the term *Mycoplasma* is widely used to refer to any organism in the class *Mollicutes*, only four species – *Mycoplasma pneumoniae*, *Mycoplasma hominis*, *Mycoplasma genitalium*, and *Ureaplasma urealyticum* – are known to be human pathogens (3, 4). *Mycoplasma pneumoniae* is a human respiratory tract pathogen and one of the best-characterized minimal bacteria (5). It is a rod-shaped bacterium and is one of the most common causes of atypical pneumonia in the United States, usually transmitted from person to person via respiratory droplets with an incubation period average of two to three weeks (6). As it has no bacterial cell wall, antibiotics that inhibit DNA synthesis, such as macrolides, fluoroquinolone, and tetracycline, are commonly used to treat infections (7-9). It is also one of the smallest self-replicating organisms that is able to grow independently from any hoist cell, with the entire machinery of the cell being increasingly more known in recent years due to increased research (10-12). It has shown to be a significant respiratory pathogen that is most common seen in children and the elderly, resulting in over 100,000 adult hospitalizations every year in the United States (3, 4). *Mycoplasma pneumonaie* is the causative agent of atypical pneumonia. It also causes a wide array of extrapulmonary infections – such as encephalitis, optic neuritis, acute psychosis, stroke, cranial nerve palsies, aseptic meningitis - and it has been implicated in immune mediated neurological diseases such as acute demyelinating encephalomyelitis, Guillain-Barre syndrome and transverse myelitis (13) - and autoimmune phenomena (14). While it is most common to see respiratory tract symptoms be a cause of *Mycoplasma pneumoniae*, extrapulmonary indications

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are not uncommon (15). It is also known that it causes cardiovascular, hematologic, dermatologic, hepatobiliary, and neurologic systems in children, which is occur as difficulties after pneumonia (16-18).

Among the genital mycoplasmas, Ureaplasma species are the most prevalent, potentially pathogenic bacteria isolate from the urogenital tract of both women and men (19). It penetrates the submucosa only during immunosuppression or instrumentation transmitted through sexual contact. It is also able to be transmitted from the mother to offspring vertically in the utero or through infected body fluids at the time of birth (19). Their involvement in the hyperactive bladders and interstitial cystitis and painful bladder syndrome is controversial. In most cases their role in any particular pathology cannot be proven, only theorized (20). Belonging to family *Mycoplasmataceae* and the class *Mollicutes*, it was first discovered in 1954 by Maurice Shepard (19). Since they produce small colonies, 7-15 micrometers in diameter, they were originally call T-strain Mycoplasmas. It was eventually considered to be unique among Mycoplasmas of human origin; thus, it was proposed that a new genus and species designation was to be made, now called order *Mycoplasmatales*. They were named *Ureaplasma urealyticum* by Maurice Shepard in 1974 (19). In general, the knowledge of how mycoplasma and Ureaplasma species in the urogenital tract is lacking, with not that much literature on the topic as of now, specifically dealing with its impact on women. Further studies are urgently needed (20).

Mycoplasma pneumoniae is particularly instructive, as it features an attachment organelle (AO) or terminal organelle, a polar extension of the cell involved in adherence, motility, and cell division (21). Its mechanisms, regulation, and construction have been studied, which show that various aspects of its basic biology and its pathogenicity are at the heart of major cell biological issues concerning *M. pneumoniae*. Pathogenic mycoplasmas, such as *Mycoplasma pneumoniae*,

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have evolved strategies to survive specific environments in their hosts. This process of surviving in the lungs involves mechanisms; for example, the loss of unnecessary genes via reductive evolution that allow essential genes in the mycoplasma to be able to survive longer (22). Another way this bacterium has adapted for survival in developing different pathways of transmission (23). Its pathogenicity is linked to carbon metabolism, using glucose and glycerol and primary carbon sources (24).

Mycoplasmas have a been known to be simple organisms when; however, studies have shown that this is not true. Having undergone reductive evolution from their ancestors, mycoplasma genomes are less complex than other bacteria and are capable of being grown in laboratory setting, which makes them a model organism (21). Their small genome size results from the loss of major anabolic pathways and reduction in the complexity of gene expression regulation. This can include assembly of macromolecular structures, adherence, cell polarity, cell division, and motility (21).

Mycoplasma pneumoniae is known to have a loss of the cell wall as a result of reductive evolution. It retains 54 of the 105 genes that are associated with cell wall construction, which may suggest that reductive evolution is ongoing (26). The loss of certain gene sets, such as lipid metabolism and amino acid biosynthesis, also shows a need of a transfer of dependence for nutrient acquisition from host organisms (26). *Mycoplasmas* seem to have evolved in this direction, from a large genome to a smaller one with reduced genes. This supports the theory that *Mycoplasma* genomes were altered in response to the environment (26).

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Virulence Factors

The formation of hydrogen peroxide, which is a product of glycerol metabolism, is crucial to host cell cytotoxicity (22). Hydrogen peroxide is a by-product of glycerol metabolism in mycoplasmas and has shown to cause cytotoxicity for cocultured eukaryotic cells (22). Because of this, there seems to be discerning pressure for mycoplasmas to preserve the genes needed for glycerol metabolism, leading to speculation as to their function during infection. Studies have shown that glycerol metabolism is active in many mycoplasma species, glycerol production and hydrogen peroxide production have been established to be linked with the virulence of any mycoplasma in vivo, meaning taking place in a living organism (22).

Asthma is a complex inflammatory disease of the lungs that has reached epidemic proportions in the developed world (27). As of 2018, it is estimated to affect 300 million worldwide and 10% of the United States and Europe. Until recently, no virulence factors of *Mycoplasma pneumoniae* have been directly linked to asthma pathogenesis (27). Atypical bacterial infections or colonization with *Mycoplasma pneumoniae* have been increasingly associated with the development of asthma and the worsening of asthma control. It has been identified that an ADP-ribosylating and vacuolating toxin producing by *Mycoplasma pneumoniae*, community-acquired respiratory distress (CARDS) toxin, which is strongly linked with *Mycoplasma pneumoniae*-associated disease in humans and directly linked to asthma-like inflammatory patterns in animal models (27). Colonization is seasonal, and CARDS toxin is readily detected in respiratory secretions in children in the winter months. Approximately 50% of hospitalized children with acute exacerbation of asthma had detectable CARDS toxin in their respiratory secretions (27).

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Epidemiology

Although *Mycoplasma pneumoniae* is known to cause community acquired pneumonia, it has seldom been documented as the causative organism of pneumonia because of the typical mild clinical course of mycoplasma infection (28). Apart from respiratory tract infections, it also causes various extrapulmonary manifestations in as many as 25% of patients that have a mycoplasma infection. Stevens-Johnson syndrome has been associated with *M. pneumoniae*, with inducing rash and mucositis symptoms being shown as indications patients exhibit (29-31). Histopathologically, *M. pneumoniae-caused* pneumonia is characterized by acute cellular bronchiolitis with edematous and ulcerative lesions of bronchial walls and by peribronchial and perivascular interstitial opacities containing lymphocytes, plasma cells, and macrophages (32). This can also manifest as renal, gastrointestinal, hematological, ocular, and osteoarticular infections, and appear at various intervals after the initial pulmonary infection. Pulmonary manifestations that occur are typically tracheobronchitis and pneumonia, with symptoms including coughing and wheezing (33-35). It can also occur in the absence of respiratory tract symptoms (28, 36). The immunopathogenesis of *M. pneumoniae* remains unknown, but it has been hypothesized that excessive immune reaction against the insults from infection is associated with lung cell injury (37).

Mycoplasma pneumoniae is a serious problem because of health problems it can cause as it can be detrimental to humans and the occurrence and aggravation of systemic involvement in *M. pneumoniae* infection occur via multiple immunological pathways (28). Radiological findings of this strain of pneumonia are diverse and nonspecific (38). The prevalence of infection with *M. pneumoniae* is generally miscalculated, as most patients infected as seldomly symptomatic and they infrequently seek medical attention (39). Due to advantages of PCR on its sensitivity,

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specificity, and early detection, a sizable range of studies have been published as able to detect *M. pneumoniae* (40). Laboratory diagnosis of this bacterial infection in a clinical practice study have been based on serology, polymerase chain reaction (PCR), and culture, although they have specific limitations (41). Serology using enzyme immunoassays is the most widely used test for the diagnosis for the infection, with IgM and IgA antibodies both appear in the early phase of infection and detection of any or both antibodies indicates an acute infection (42). The clinical aspect and tendencies observed suggest that higher *M. pneumoniae*-IgM titer leads to more severe presentation of diseases, as well as more serious laboratory and imaging changes including C-reactive protein, platelets, CT Scans, and chest x-rays (43). These infections seem to primarily involve pathogen adhesion to respiratory epithelium, followed by direct cell invasion (44). Natural immunity of *M. pneumoniae* infections are usually short-term, as evidenced by the frequency of reinfections in the same person over time, and organisms continue to be shed for variable periods after resolution of the clinical illness (45). More studies are still needed to understand mycoplasma infections and how to manage it in early stages to prevent serious ailments (38). *M. pneumoniae's* molecular characteristics are currently scarce, which don't benefit patients that have been hospitalized due to it. Researchers have performed molecular characterization of nasopharyngeal and oropharyngeal swabs from hospitalized children and adults. Results indicate that current methods are not able to notably distinguish *M. pneumoniae* because the majority of strains can be classified into only a few main types. (46). Differences in strain types do not appear to be associated with variances in clinical outcomes (46). Whole genome sequencing has been hypothesized to better identify ways to characterize these strains for better clinical treatment (46).

Mycoplasma pneumoniae infections can encompass both the lower and upper respiratory tract and occurred worldwide in an endemic fashion with an epidemic spurt at four to seven

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intervals (47). Seasonality, geography, and climate are not thought to be a major consequence. Most occurrences in the United States tend to occur in late summer and early fall (47). However, according to Japanese multi-center study, 403 typical pneumonia cases were studied, with 62 were caused by *M. pneumoniae* (48). Serological studies, such as studies by Lind *et al*, shows that *M. pneumoniae* has a pattern of endemic disease transmission over a period of 50 years. This is also punctuated with recurring epidemics every three to five years (47). Relatively large droplets may be required for transmission as evidenced by close personal contact. This is common in military barracks, institutions, and schools (47). Outbreaks of this strain of pneumonia is a significant public health concern since numerous strains exhibiting antibiotic resistance are emerging worldwide (49).

Mycoplasma pneumoniae has strong clinical associations with asthma exacerbations and morbidity in both children and adults (40). This strain of pneumonia causes up to 40% or more of community acquired pneumonia (CAP) cases and as many as 18% of cases requiring hospitalizations in children and young adults (47, 50-53). This strain of pneumonia is responsible for 15-20% of all cases of community-acquired pneumonia and cause of hospitalization and mortality in Chinese children (54). Infection with *M. pneumoniae* is mostly seen in outpatient settings and it is a significant cause of hospitalization due to pneumonia, especially in the elderly and immunocompromised patients (55). Although adults are not frequently affected, there are 5.6% of cases associated with respiratory failure that require ventilator management (56). It also accounts for 10 to 30% of all community-acquired pneumonia (57, 58). Fortunately, it has a lesser occurrence in children under 3 years old (59). *M. pneumoniae* is also detected as frequently as *Streptococcus pneumoniae* and *Haemophilus influenzae* (60). A common method of detection that has been used frequently is Real-time polymerase chain reaction (RT-PCR) (61). Studies have

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shown the *M. pneumoniae* strains are unusual in children less than five years. However, the risk of infection is shown to be in children age 5-15, decreasing after youth and into adulthood. Older individuals and young children under the age of 5 also may have *M. pneumoniae* occur endemically and sporadically epidemically. Serological studies in Denmark over a 50-year period showed that infections exhibit epidemic periodicity every 3-5 years (62). It often spreads through close contact in families, with the epidemic happening every 3 to 7 years, with the most recent one happening in Korea in 2016 (63, 64). Early and rapid diagnosis of interstitial pneumonia due to *M. pneumoniae* is of significance since certain antibiotics are unsuccessful against it (65). This strain of pneumonia is regularly a self-limiting disease, but some patients complain of getting a progressively severe pneumonia that is not responsive to antibiotic treatment (66). Factors normally associated with *M. pneumoniae* are vomiting diarrhea, and persistent cough have been identified as symptoms (67). Although *M. pneumoniae* is generally nonthreatening and self-limiting, numerous studies have specified that it can progress into severe deadly diseases such as acute respiratory distress syndrome, necrotizing pneumonitis, and fulminant pneumonia in children 5- 15 years (68). A condition known of central retinal artery occlusion (CRAO) is an arterial ischemic stroke, hardly happened in children inflicted with asymptomatic cerebral infraction and almost never involved in severe pneumonia related to *M. pneumoniae* infection (69).

Respiratory disease caused by *Mycoplasma pneumoniae* comes from the close association between the organism and the mucosal epithelium that occurs as a result of cytoadherence, which is considered as a major virulence factor (4). Subsequent to cytoadherence, it is also believed to partly cause disease through generation of peroxide and superoxide radicals. These act together with endogenous toxic oxygen molecules generated by the host to induce oxidative stress (4). The superoxide anions produced by *M. pneumoniae* act to inhibit catalase in host cells, resulting in

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reducing the enzymatic breakdown of peroxides at the point of origin. It is also reducing the enzymatic breakdown by the mycoplasma, rendering the hoist cell more vulnerable to oxidative harm (4). General physicians commonly encounter patients with *M. pneumoniae*-caused pneumonia who have a disproportionately low level of respiratory distress compared to the extent of lung involvement. This has caused concerns regarding the diagnostic process of *M. pneumoniae*-caused pneumonia (37).

Mycoplasma pneumoniae may also be implicated in immune mediated neurological diseases (13). Central nervous system infections and inflammatory or autoimmune disorders may cause secondary central nervous system vasculitis. Mycoplasma may cause secondary central nervous system vasculitis in children. In studies by Yimenicioğlu, S., et al. (13), one of their patients with encephalitis also had vasculitis of internal carotid artery and posterior circulation. Their conclusion in their tests came to that while neurological manifestations associated with *Mycoplasma pneumoniae* usually resolve completely, they also can result in chronic debilitating deficits in motor and mental function with various degrees of life-threatening complications (70). Neurological complications occurred in 11.5% of children hospitalized in our institution with PCR-confirmed *M. pneumoniae* infection (71). Recent studies demonstrated that the incidence of fatal or severe *M. pneumoniae* is gradually increasing which might be related to the excessive inflammation. However, the exact pathogenesis of this excessive inflammation is still unclear (72).

A mutual opinion in the treatment of *Mycoplasma pneumoniae* is that it really does not matter where any antibacterial solutions were given because the mortality rate is low and the infections it causes are often self-limiting (47, 73). However, studies from as early as the 1960's indicate that the treatment for mild *M. pneumoniae* infections do reduce the morbidity of pneumonia and shortens the duration of symptoms shown. In a Japanese study, telithromycin, a

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ketolide antibiotic was found to have a good activity against 41 clinical isolates of *Mycoplasma pneumoniae* (47). Macrolide-resistant *Mycoplasma pneumoniae* have also been reported worldwide as a focus as well (74). Since 200, the emerging of macrolide-resistant *M. pneumoniae* has been reported in China, Japan, Europe, and United States, affecting both children and adults (75, 76). Various antibiotics have been developed to react to the infections it may cause, with numerous developed depending on the age of the patients involved in the infection. Drug resistant *M. pneumoniae* is also a rising issue in the management of community-acquired pneumonia, as epidemiological monitoring is important for classifying resistant patterns of *M. pneumoniae* against several antibiotics in adult community-acquired pneumonia patients (77).

Carbon Metabolism

For *Mycoplasma pneumoniae* and other human pathogens, including *Mycobacterium tuberculosis* or *Listeria monocytogenes,* glycerol, glycerol-containing lipids, and glucose are the ideal carbon sources (2). In lung epithelium, the main habitat of *Mycoplasma pneumoniae*, the bacteria use available carbon sources in the surfactant, primarily phospholipids. Since phospholipids are the most plentiful potential carbon source for *Mycoplasma pneumoniae* living on lung epithelial surfaces, the possibility that these bacteria synthesize enzymes to cleave polar head groups from the glycerophosphodiesters to produce glycerol-3-phosphate that can be utilized by the enzymes of glycerol metabolism has been considered (77).

With its major source of carbon and energy coming from phospholipids, *Mycoplasma pneumoniae* thrives on lung epithelia (25). In *Mycoplasma pneumoniae*, pathogenicity is closely related to carbon metabolism (24). Cumulative information on the pathogenesis of human *Mycoplasma pneumoniae*-caused pneumonia to have been gathered from pathological

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examination of autopsy specimens (78). The chemical properties of other carbon sources affect the growth of *Mycoplasma pneumoniae* is not well known.

A study on carbon sources common to the lungs, where *M. pneumoniae* is most known to infect human hosts, would be beneficial to treating these afflictions. There appears to be selective pressure for mycoplasmas to retain their genes needed for glycerol metabolism. Experimental and clinical studies have implied that the pathogenesis of lung injuries in *Mycoplasma pneumoniae* infection is linked to cell-mediated immune reaction, and high receptiveness to corticosteroid therapy have been stated, specifically for severe diseases (79). In comparison to copious amount of published studies on the carriage of *Streptococcus pneumoniae* in children, a more common strain of pneumonia, studies that exclusively address asymptomatic carriage of *Mycoplasma pneumoniae* has not be been well researched (80). If studies can figure out carbon sources that can affect the growth of lung bacteria, then various medicines and treatments can be clinically tested to better help patients. Carbon sources - such as hexose sugars, pentose sugars, disaccharides, trisaccharides, alcohol sugars, sugar acids, amino acids, and fatty acids – can be tested to see the effectiveness in growth of the bacteria.

Glycerol Metabolism

The pathway of glycerol utilization is of great importance for *Mycoplasma pneumoniae* and related species not only due to relevance of glycerophospholipids as a major carbon source, but also since it generates hydrogen peroxide as a major virulence factor (81). *Mycoplasma pneumoniae* possesses the complete set of genes for glycerol utilization, and the bacteria do use this a carbon source (24). GlpF is the glycerol uptake facilitator, and GlpK and GlpO metabolize glycerol to the glycotic intermediate dihydroxyacetone phosphate. Hydrogen peroxide that forms

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by GlpQ is important for the effects of *M. pneumoniae* cytotoxicity (12). Glycerol metabolism is under dual control, as the genes involved in glycerol utilization are expressed only if glycerol or glycerol 3-phosphate is present in the medium, and they are not expressed in the presence of glucose, the preferred carbon source (24).

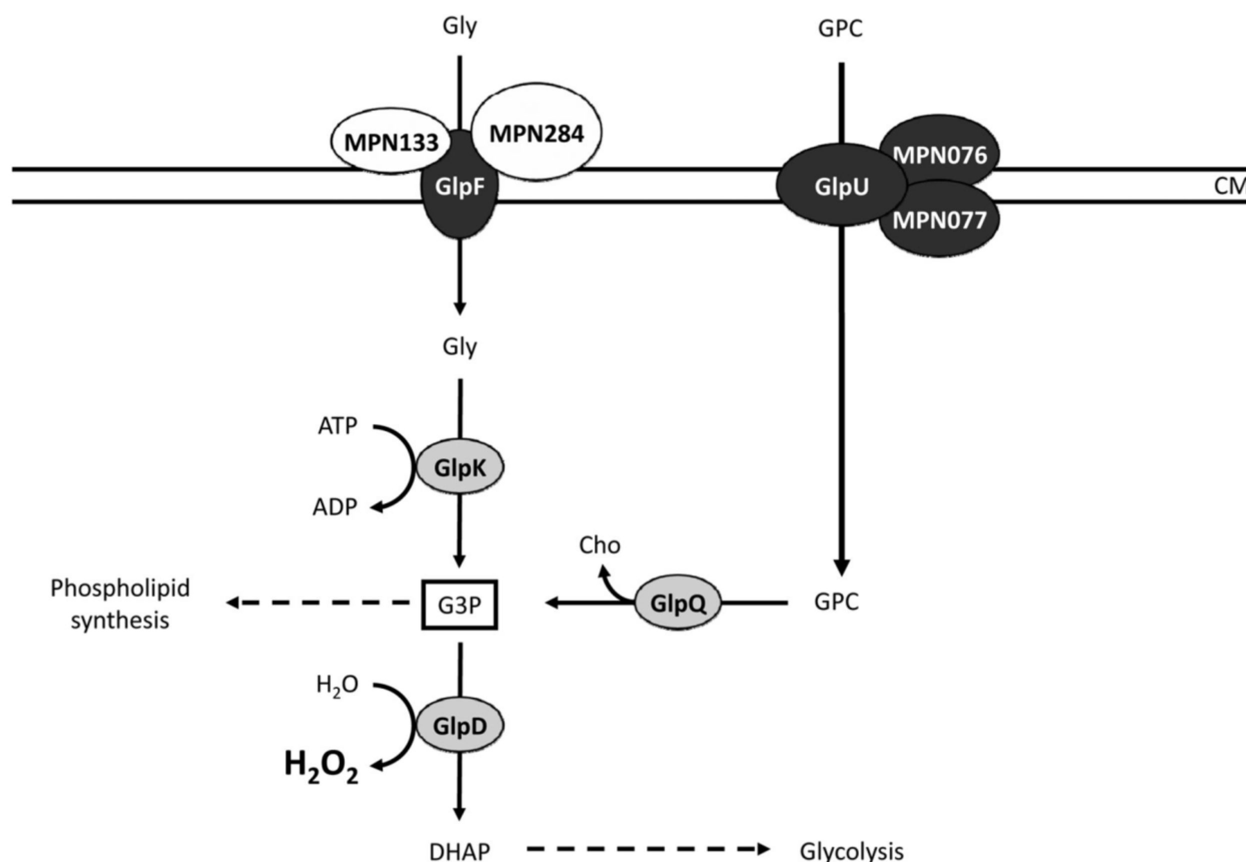

FIG 1: shows an illustration of metabolic pathway for the uptake and conversion of carbohydrates leading to the formation of glycerol-3-phosphate in *Mycoplasma pneumoniae* (25).

Mycoplasmas must acquire macromolecular precursors and high-energy compounds, such as sugars from their environment. This is done to maintain their life cycle and to produce active metabolic intermediates (90). Glycerol is one of the few carbon sources that can be used efficiently by *Mycoplasma pneumoniae* (24). Researchers have analyzed genes involved in glycerol

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metabolism and detected constitutive expression in the presence of glycerol or selected carbon sources. The enzymes of glycerol metabolism are critical for the pathogenicity, but also for other currently undisclosed functions of the cell (24).

The newly identified GlpU transport protein is responsible for the uptake of the glycerophosphodiester glycerophosphocholine (25). Data suggests that the proteins MPN133 and MPN 284, may act as binding proteins for glycerol and deliver glycerol molecules to the glycerol facilitator GlpF (25). Other studies show that there appears to be no link between glycerol metabolism, hydrogen peroxide, cytotoxicity, and virulence in Mycoplasma species in a natural host (12).

In *Mycoplasma pneumoniae*, as well as other *Mollicutes*, pathogenicity is closely linked to carbon metabolism (24). Glycerol being a major carbon source involves the oxidation of glycerol 3-phosphate oxidase, which produces hydrogen peroxide rather than $NADH_2$, which is generated by glycerol 3-phosphate dehydrogenase in most other bacteria. In addition to the induction of autoimmune responses, the formation of hydrogen peroxide is the only established mechanism by which mycoplasmas cause damage to their hosts (24).

For the colonization of epithelial lung cells, *Mycoplasma pneumoniae* has developed different mechanisms for attachment, virulence, and utilization of host-derived metabolites (2). This is where its utilization of glucose, fructose, and glycerol as a carbon source come into the picture. These carbohydrates are catabolized via glycolysis as the major pathway for ATP generation, and this is very important as high efficiency is crucial. The importance of glycolysis for *Mycoplasma pneumoniae* and other mollicutes un underlined by the lack of the oxidative part of the pentose phosphate pathway and of the citric acid cycle (2).

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Scientists have explored the interactions between glycolytic enzymes using the bacterial adenylate cyclase-based two-hybrid system and determined that most of the glycolytic enzymes perform self-interactions (82). Furthermore, enolase as the central glycolytic enzyme of *M. pneumoniae* was able to be identified due to its ability to directly interact with all other glycolytic enzymes. Results support the idea of the development of a glycolytic complex in *M. pneumoniae* (82). The formation of this complex might ensure higher fluctuations through the glycolytic pathway than would be possible with isolated non-interacting enzymes (82).

Mycoplasma pneumoniae contains MPN420 (GlpQ), which is the only active glycerophosphodiesterase. GlpQ metabolizes in this organism glycerophosphocholine from the lung to generate free choline. Chlorine also serves as a substrate for renewed synthesis of phosphatidylcholine. Glycerol-3-Phosphate is also a product of this reaction. While all of this data that has been discovered is not directly contributed to *Mycoplasma*, infection with GlpQ2 mutant decreased the severity of pneumonia compared with the parent strain (83). GlpQ2 also develops surface phosphorylcholine expression, which contributes to the severity of pneumonia by stimulating adherence and host cell cytotoxicity (84). GlpQ gene expression levels were noticeably higher than in under other growth conditions (83).

In *Mycoplasma pneumoniae*, GlpQ is not only important for virulence but also growth in the commonly used medium in the laboratory, such as Hayflick medium with glucose as the added carbon source (12). Studies by Schmidl et al (12), no difference between the wild type strain and the GlpQ mutant was observed during the growth in the presence of glycerol. Carbon metabolism is linked to virulence in pathogenic bacteria, including *Mycoplasma pneumoniae* and other mollicutes, and the utilization of glycerol and phospholipids play a significant role in the virulence of *Mycoplasma pneumoniae* and other mycoplasma species. Hydrogen peroxide, which was

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mentioned before as the major cytotoxic substance produced by these bacteria, is generated as a product of glycerol metabolism, and both GlpD and GlpQ mutants are severely affected in pathogenicity (12).

Even with this known pathway, *Mycoplasma pneumoniae* still lacks many other metabolic pathways, forcing it to acquire the necessary building blocks, amino acids, nucleobases, and fatty acids, from its environment (85). Accurate representation of cellular networks through mathematical models is central goal of a discipline such as integrative systems biology, the study of disease and how it affects at systems level (85). For example, ATP generation is completed by simple organic acid fermentation because of the absence of the TCA cycle and a functional respiratory chain. The absence of most pathways and anabolic processes make *M. pneumoniae* an ideal organism to study basic metabolic functions and study its energy charges (86).

The lack of most anabolic processes and rescue pathways known for more complex organisms and the predictable high linearity of its metabolic network make *Mycoplasma pneumoniae* an model organism to study essential metabolic functions and analyze energy expenses (86). Studies have shown that the main subsystems of the metabolic network are energy-producing pathways, amino acid, nucleotide, lipid, and cofactor metabolism, as well as transport reactions. Wodke et al. (86), found in their studies that flux activity analysis at diverse time points of the exponential growth phase and in silico knock-outs, or in a computer simulations, coincide, signifying that the same metabolic pathways are responsible for adaptation in *Mycoplasma pneumoniae*, namely the nucleotide metabolism and pentose phosphate pathway. Metabolic gene essentiality analysis also identified the genes connected with main catabolic pathways – the glycolysis + pentose phosphate pathway – among the non-essential genes as most vital for metabolic performance. This further shows the simplicity of the metabolic network of *Mycoplasma*

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pneumoniae (86).

Carbon sources refer to any carbon contain molecule - carbohydrate, amino acid, fatty acid, carbon dioxide – used by an organism for the synthesis of its organic molecules. Carbon is a basic element for sustain life and is mainly used for cell growth and product formation. *Mycoplasma pneumoniae* shows the fastest growth with glucose (23). For the colonization of epithelial lung cells, *M. pneumoniae* has developed different mechanisms for attachment, virulence and utilization of host-derived metabolites, utilizing glucose, fructose, and glycerol as a carbon source (87) These carbohydrates are catabolized via glycolysis as the major pathway for ATP generation. Glycolysis for *M. pneumoniae* and other mollicutes are significant, so high efficiency is essential (87). Phospholipids and derived metabolites, such as glycerol, are the major sources of carbon and energy for the bacteria on lung epithelia. The oxidation of the common intermediate glycerol 3-phosphate gives rise to the production of hydrogen peroxide, which is a major factor in the virulence in *Mycoplasma pneumoniae*.

With the use of these sugars, the possibility of other hexose sugars being successful is worth studying. Other hexose sugars, such as galactose, mannose, rhamnose, and sorbose, may also yield results that show they can also be utilized. Additionally, other carbon sources like pentose sugars and alcohol sugars need to be tested to see their effect on *Mycoplasma pneumoniae* growth and pathogenicity. In laboratory experiments, *Mycoplasma pneumoniae* can be grown in rich and defined medium. In both conditions, it metabolizes glucose as a major carbon and energy source (85). A variety of other reduced carbon compounds, such as fructose, mannose, ribose, ascorbic acid, glycerol, glycerol 3-phosphate, and glycerolphosphocholine can be metabolized (85).

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Two things this study will be examining in *Mycoplasma pneumoniae* are carbon source metabolism by testing bacteria using bacterial well plates and by cell growth curves. By testing carbon sources in well plates and growth curves to analyze how growth is affected in *Mycoplasma pneumoniae* and measure optical density, carbon source effectiveness may be better understood. The hypothesis of this study is that other carbon sources similar to glycerol and glucose, such as mannose and sorbose, can be beneficial to the growth of *Mycoplasma pneumoniae* as well. If more carbon sources are revealed to be advantageous to lung bacteria such as *Mycoplasma pneumoniae*, then professionals in the medical field will have more data to be able to create improved treatments to combat these carbon sources that aid infectious bacteria.

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RESULTS

Carbon Source Utilization

Carbon sources in this study were chosen based on their similarities to glucose and glycerol. Hexose sugars and sugar alcohols, such as fructose and mannitol, were tested in well plates and culture flasks and incubated at 37°C to allow for *Mycoplasma pneumoniae* to growth with the inoculated carbon source. Day cycles are needed to see how over six to eight days the trend these carbon sources go through. The well plates were photographed on certain days to see how the well and the various concentrations affect *Mycoplasma pneumoniae.* Each well plate has the medium and the correct carbon source in it. The contents of each well starts out the color of the Hayflick medium, which is red. If the color turns lighter, such as an orange or yellow, this signifies that the carbon source is reacting favorably with *Mycoplasma pneumoniae*. If the color turns darker, such as purple or black, this means that it is overkilling the bacteria, making it unable to thrive. The cell culture flasks were photographed in two-day periods to track their growth and then tested at the end of day eight. A spectrophotometer was used to measure the optical density of these carbon sources, with the starting OD being 0.01.

Two phases of methodology were done to test carbon sources: testing well plates to see change in color and cultivating culture flasks to test optical density. With glucose being a known carbon source that *Mycoplasma pneumoniae* is able to utilize, many other carbon sources have been shown to also be useful in the processes it needs. A variety of additional reduced carbon compounds, such as fructose, mannose and ribose can be metabolized (85). Mannitol has a reduced amount of growth and cannot be utilized by *Mycoplasma pneumoniae*, even though a utilization pathway has been predicted (86).

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Well plates with several sugar alcohols were tested to see how *Mycoplasma pneumoniae* would react in different concentrations in the incubator. MPN in ribose showed the most growth over the six-day period, with xylose showing a little growth as well. MPN in arabinose, sorbitol, mannitol, and glycerol demonstrated little to no growth, even in the diverse concentrations they were tested in. Assumptions that can be from these results is that *Mycoplasma pneumoniae* can utilize ribose and xylose in some way for its various processes.

Hexose Sugars

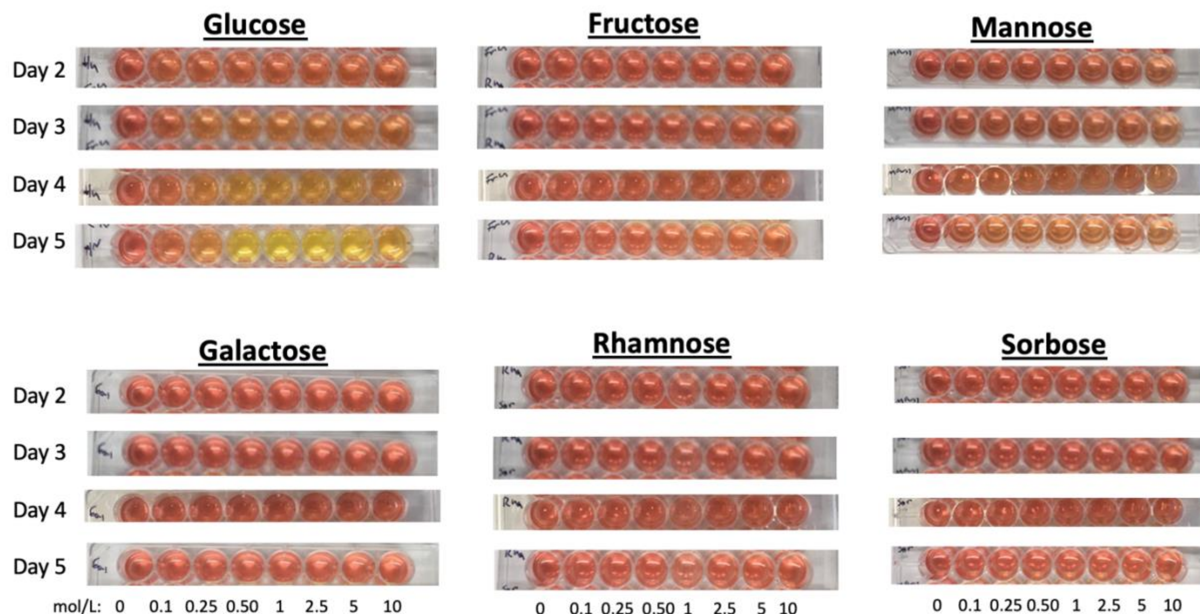

FIG 2*:* Hexose sugars well plate. Sugar concentrations are as indicated: 0, 0.1, 0.25, 0.50, 1, 2.5, 5, and 10. From pictures top to bottom: Day 2, Day 3, Day 4, Day 5.

By Day 2, glucose, fructose, and mannose have grown in concentrations 0.25 and higher (Figure 2). Galactose has seen some growth, but rhamnose and sorbose show no growth at all. Day 3 growth shows that Galactose starts to be utilized by *Mycoplasma pneumoniae* in a more efficient

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way, while glucose, fructose, and mannose are stable. Day 4 and day 5 have the top four carbon sources stable out in their growth, showing that an eight-day cycle is effective. Rhamnose and sorbose show little to no growth over the cycle.

Hexose sugars were tested at different concentrations to see how they would affect over several days in an incubator. MPN in glucose grew the fastest, as by Day 4 the color of the well was already yellow in the wells with 0.5% or higher concentrations. MPN in mannose showed the second most growth of the hexoses tested, turning orange by Day 4 when exposed to *Mycoplasma pneumoniae*. MPN in fructose is the 3rd hexose that showed some growth through the days in the conditions in the incubator, specifically the higher concentrations tested. MPN in galactose, rhamnose, and sorbose showed little to no growth, even by Day 10. From these results it can be assumed that *Mycoplasma pneumoniae* will react similarly with mannose and fructose as it does with glucose, albeit not as well as glucose.

Pentose Sugars

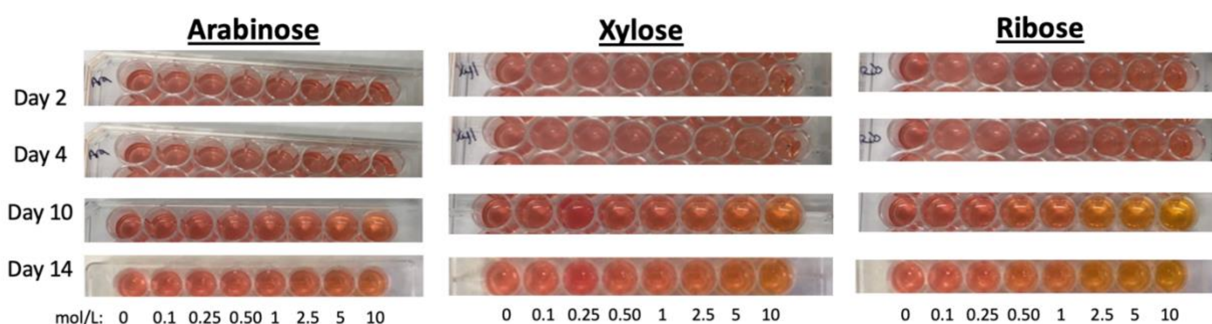

FIG 3*:* Pentose sugars well plate. Sugar concentrations are as indicated: 0, 0.1, 0.25, 0.50, 1, 2.5, 5, and 10. From pictures top to bottom: Day 2, Day 4, Day 10, Day 14.

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Figures 3 shows that growth for sugar alcohols are not as proficient hexose sugars. Ribose, and to a lesser extent xylose, start to show growth by Day 10 at concentrations of 1 or higher. Arabinose, sorbitol, mannitol, and glycerol show little to no growth over a two-week timeframe. Glycerol does show growth by day 14, but the time it takes to get any growth with the current concentrations is not an effective use of a carbon source.

Sugar Alcohols

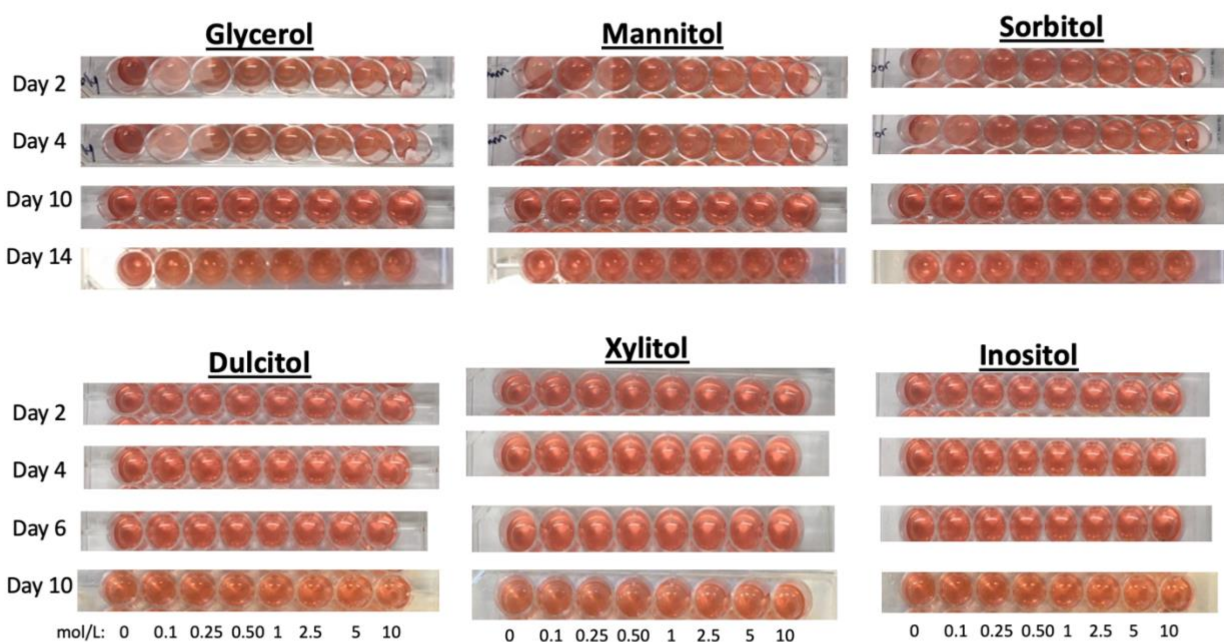

FIG 4: Sugar Alcohols well plate. Sugar concentrations are as indicated: 0, 0.1, 0.25, 0.50, 1, 2.5, 5, and 10. From pictures top to bottom: Day 2, Day 4, Day 6, Day 10.

Figure 4 shows xylitol, dulcitol, and inositol show little to no growth over a ten-day cycle. On day 10, there seems to have a little growth for the sugar alcohols. However, the growth is not as efficient as other carbon sources, such as glucose and fructose.

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Hexose Sugars Growth Curves

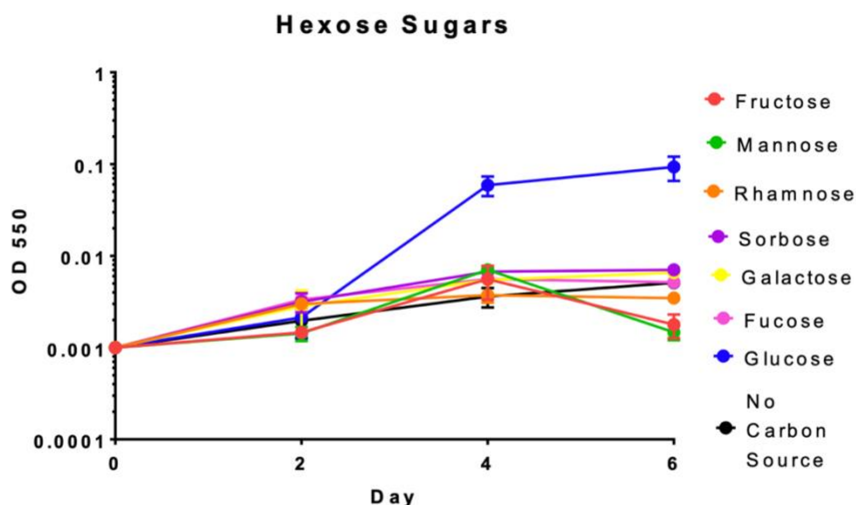

FIG 5: A graph of hexose sugars being tested against No Carbon Source. For all carbon sources in the figure, 3 of the trials were put together to show a standard deviation for each hexose sugars. All the carbon sources – Glucose, Fructose, Mannose, Rhamnose, Sorbose, Galactose, and Fucose – were then graphed out over the 6-day cycle at OD_{500}. Concentrations of carbon sources are as indicated: 40% Fructose; 25% Mannose, Rhamnose, Sorbose, Galactose, Fucose; & 50% Glucose.

Glucose showed the most growth over a six-day growth curve cycle than the other hexose sugars. Glucose seems to more efficient that the others, although the other carbon sources are not any less significant. Fructose, mannose, rhamnose, sorbose, galactose, and fucose all had growth over the six days, but they were all about the dame range by Day 6. Glucose, as other studies have stated, seems to be one of the most efficient carbon sources for *Mycoplasma pneumoniae*.

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Pentose Sugars Growth Curves

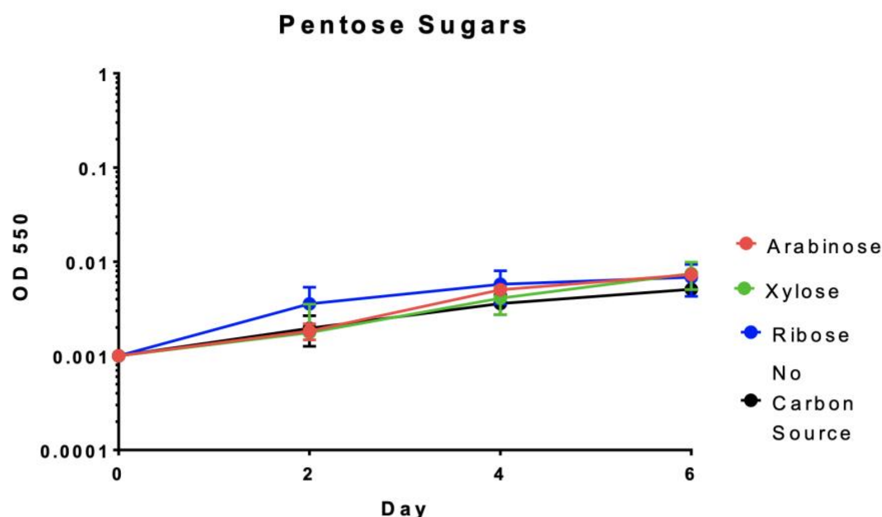

FIG 6: A graph of pentose sugars being tested against No Carbon Source. For all carbon sources in the figure, 3 of the trials were put together to show a standard deviation for each pentose sugars. All the carbon sources – Arabinose, Xylose, Ribose – were then graphed out over the 6-day cycle at OD_{500}. Concentrations of carbon sources are as indicated: 25% Arabinose. Xylose, & Ribose.

Arabinose, xylose, and ribose all has similar trajectories in the six-day growth curve cycle. Ribose had the most growth on day 2, but over the next four days arabinose and xylose evened out around the same range as ribose. Compared to glucose, the growth is significant as glucose grew considerably more than the pentose sugars. *Mycoplasma pneumoniae* was able to utilize the carbon sources in the culture flask environment, but not as well as some of the hexose sugars.

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Sugar Alcohols Growth Curves

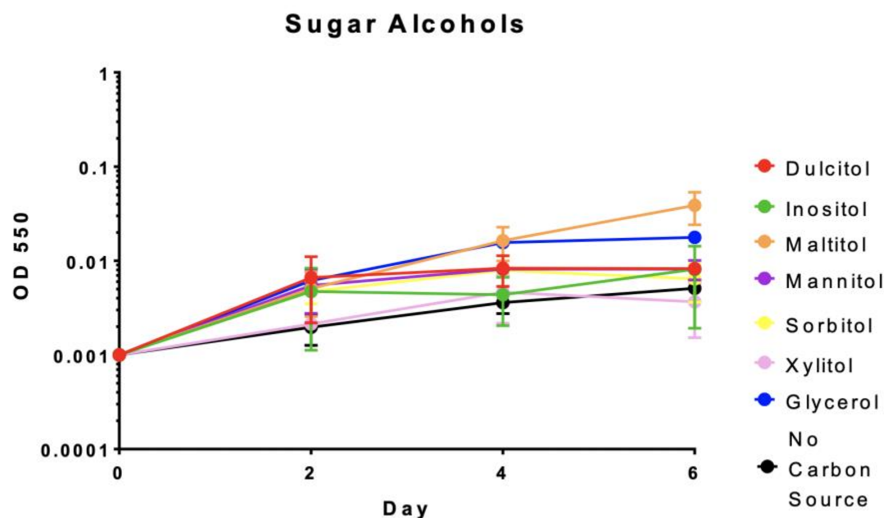

FIG 7: A graph of sugar alcohols being tested against No Carbon Source. For all carbon sources in the figure, 3 of the trials were put together to show a standard deviation for each sugar alcohols. All the carbon sources – Glycerol, Dulcitol, Inositol, Maltitol, Mannitol, Sorbitol, & Xylitol – were then graphed out over the 6-day cycle at OD_{500}. Concentrations of carbon sources are as indicated: 10% Dulcitol, Inositol, Maltitol, Mannitol, Sorbitol, & Xylitol; & 50% Glycerol.

Over the six-day growth curve cycle for sugar alcohols, maltitol had the most amount of growth of them. However, the growth of the other carbon sources was not any less proficient as maltitol. Glycerol and dulcitol has almost the same amount of growth by day 6, with xylitol having the least amount of growth. Sugar alcohols such as xylitol and inositol seem to have a decreased at certain points in the cycle, showing that possibly *Mycoplasma pneumoniae* was done utilizing the carbon source, or no more was able to be used at the point in the cycle. While the sugar alcohols

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grew in mostly positive way, they were still not as efficient as glucose or some of the hexose sugars.

All Carbon Sources Growth Curves

Figure 8 shows all the carbon sources in the same graph to show the comparison between all the carbon sources in the groups together. Figures 9-11 show the Day 2, Day 4, and Day 6 growth respectively.

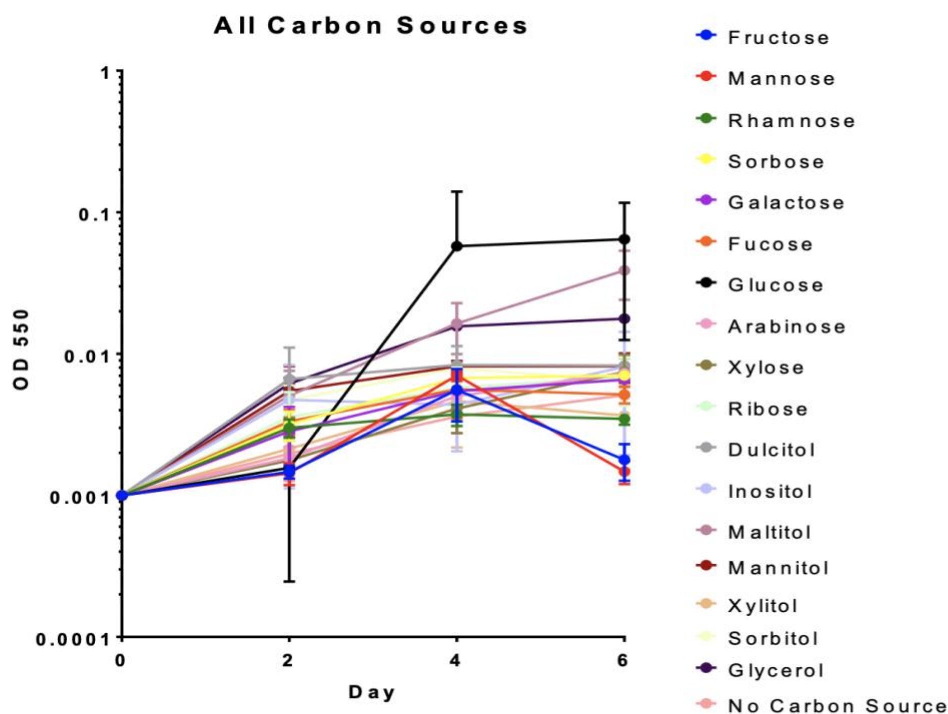

FIG 8: A graph of all carbon sources compared to each other. For all carbon sources in the figure, 3 of the trials were put together to show a standard deviation for each. All the carbon sources were then graphed out over the 6-day cycle at OD_{500}.

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Six-Day Cycle Comparisons

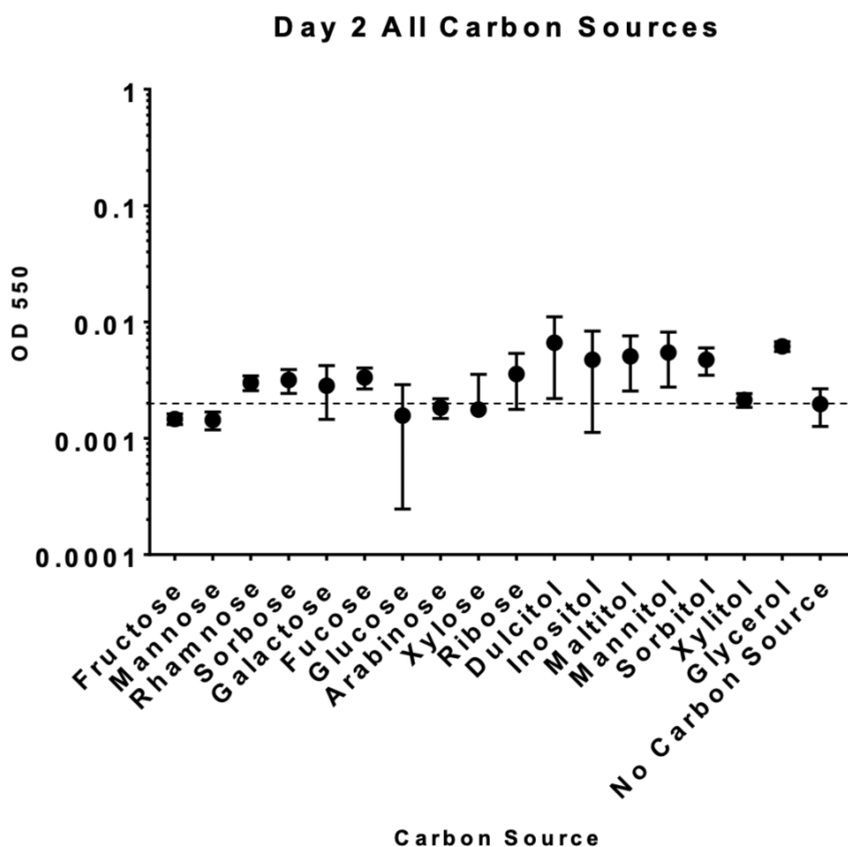

FIG 9: A graph showing all the data points for Day 2 of the cycle for all carbon sources.

The Day 2 growth curves (Figure 9) for the carbon sources in the groups show a wide range of values. While glucose and glycerol are known to be major carbon sources for *Mycoplasma pneumoniae* to utilize, dulcitol has the most growth on day 2 than the other carbon sources. Mannitol, maltitol and inositol are the next carbon sources with the highest growth after dulcitol. Curiously, glucose, mannose, and fructose have the lowest growth on day 2 out of the carbon sources.

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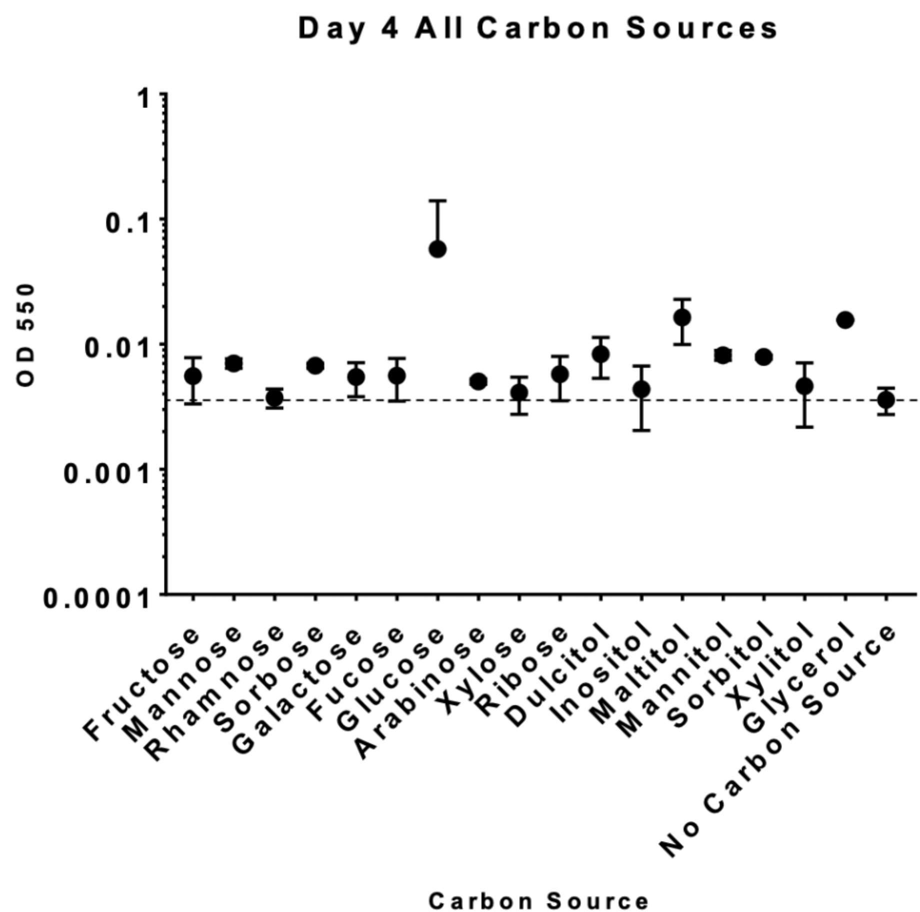

FIG 10: A graph showing all the data points for Day 4 of the cycle for all carbon sources.

On Day 4 (Figure 10), the growth curves for the carbon sources change drastically. Glucose becomes the carbon source with the highest growth by far out of the groups, with mannose and fructose growing more significantly over two days. Dulcitol stays about the same, while mannitol and maltitol grow slightly more.

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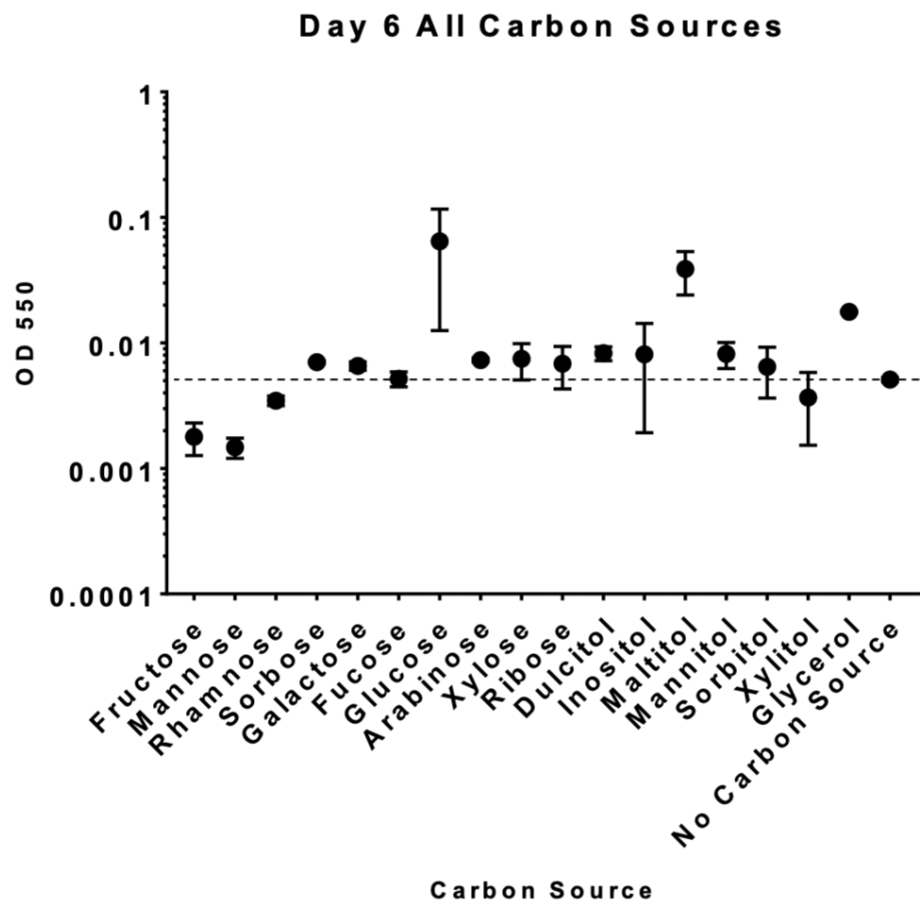

FIG 11: A graph showing all the data points for Day 6 of the cycle for all carbon sources.

By the final day of the growth curve cycle (Figure 11), glucose is still the highest growing carbon source on the growth curve, followed by maltitol and xylitol. Fructose and mannose decrease a little, while the other carbon sources stay around the same range as day 4. While all the carbon sources had some level of growth in the six-day growth curve cycle, glucose was the clear number one option that *Mycoplasma pneumoniae* used to growth at a more exponential rate.

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Statistical Analysis

Statistical analysis was done to find if the carbon sources were significant compared to no carbon source and glucose. Using the program GraphPad Prism, these tests were able to be done once data was added for comparisons. A Dunnett's test was done to compare a number of treatments with a single control to see that difference in the groups. When a Two-Way ANOVA test has significant findings, it doesn't show which pairs are significant compared to other pairs. A Dunnett's test can be used after an ANOVA test run the pairs to find the significance, if any can be found.

A Two-Way ANOVA test was done for no carbon source vs. carbon sources and glucose vs. carbon sources. A Dunnett's test was done afterwards to see if there is any significance in the carbon sources tested and compare these multiple comparisons. Comparing tests with no carbon sources to all the other carbon sources (Table 1) gave us data that the only carbon source that was statistically significant was glucose. While other carbon source grew in the condition the culture flasks, they were calculated to not be as significant as glucose. Comparing tests glucose to all the other carbon sources (Table 2) gave us data that the only carbon source that was not statistically significant was maltitol. While maltitol grew in the condition the culture flasks, it was calculated to not be as significant as glucose or the other carbon sources. Comparing tests glucose to all the other carbon sources (Table 2) gave us data that the only carbon source that was not statistically significant was maltitol. While maltitol grew in the condition the culture flasks, it was calculated to not be as significant as glucose or the other carbon sources. Comparing tests with glucose to all the other carbon sources (Table 3) only on day 6 gave us data that the only carbon source that was not statistically significant was maltitol.

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TABLE 1: Two-Way Anova of carbon sources comparing No Carbon Source against all the carbon sources

1	Compare column means (main column effect)								
2									
3	Number of families	1							
4	Number of comparisons per family	17							
5	Alpha	0.05							
6									
7	Dunnett's multiple comparisons test	Mean Diff.	95.00% CI of diff.	Significant?	Summary	Adjusted P Value			
8									
9	0.001 OD No Carbon Source vs. 0.001 OD Fructose	0.0004625	-0.0194 to 0.02032	No	ns	0.9999			
10	0.001 OD No Carbon Source vs. 0.001 OD Mannose	0.0001817	-0.01968 to 0.02004	No	ns	0.9999			
11	0.001 OD No Carbon Source vs. 0.001 OD Rhamnose	0.0001167	-0.01974 to 0.01998	No	ns	0.9999			
12	0.001 OD No Carbon Source vs. 0.001 OD Sorbose	-0.001567	-0.02143 to 0.01829	No	ns	0.9997			
13	0.001 OD No Carbon Source vs. 0.001 OD Galactose	-0.00105	-0.02091 to 0.01881	No	ns	0.9998			
14	0.001 OD No Carbon Source vs. 0.001 OD Fucose	-0.0008583	-0.02072 to 0.019	No	ns	0.9999			
15	0.001 OD No Carbon Source vs. 0.001 OD Glucose	-0.02825	-0.04811 to -0.008391	Yes	**	0.0017			
16	0.001 OD No Carbon Source vs. 0.001 OD Arabinose	-0.000875	-0.02073 to 0.01898	No	ns	0.9998			
17	0.001 OD No Carbon Source vs. 0.001 OD Xylose	-0.0006667	-0.02053 to 0.01919	No	ns	0.9999			
18	0.001 OD No Carbon Source vs. 0.001 OD Ribose	-0.001375	-0.02123 to 0.01848	No	ns	0.9997			
19	0.001 OD No Carbon Source vs. 0.001 OD Dulcitol	-0.003142	-0.023 to 0.01672	No	ns	0.9993			
20	0.001 OD No Carbon Source vs. 0.001 OD Inositol	-0.001637	-0.0215 to 0.01822	No	ns	0.9997			
21	0.001 OD No Carbon Source vs. 0.001 OD Maltitol	-0.01238	-0.03224 to 0.007475	No	ns	0.4587			
22	0.001 OD No Carbon Source vs. 0.001 OD Mannitol	-0.002783	-0.02264 to 0.01708	No	ns	0.9994			
23	0.001 OD No Carbon Source vs. 0.001 OD Sorbitol	-0.0021	-0.02196 to 0.01776	No	ns	0.9996			
24	0.001 OD No Carbon Source vs. 0.001 OD Xylitol	5.833e-005	-0.0198 to 0.01992	No	ns	0.9999			
25	0.001 OD No Carbon Source vs. 0.001 OD Glycerol	-0.007208	-0.02707 to 0.01265	No	ns	0.9522			
26									
27									
28	Test details	Mean 1	Mean 2	Mean Diff.	SE of diff.	N1	N2	q	DF
29									
30	0.001 OD No Carbon Source vs. 0.001 OD Fructose	0.002917	0.002454	0.0004625	0.006567	12	12	0.07042	36
31	0.001 OD No Carbon Source vs. 0.001 OD Mannose	0.002917	0.002735	0.0001817	0.006567	12	12	0.02766	36
32	0.001 OD No Carbon Source vs. 0.001 OD Rhamnose	0.002917	0.0028	0.0001167	0.006567	12	12	0.01776	36
33	0.001 OD No Carbon Source vs. 0.001 OD Sorbose	0.002917	0.004483	-0.001567	0.006567	12	12	0.2386	36
34	0.001 OD No Carbon Source vs. 0.001 OD Galactose	0.002917	0.003967	-0.00105	0.006567	12	12	0.1599	36
35	0.001 OD No Carbon Source vs. 0.001 OD Fucose	0.002917	0.003775	-0.0008583	0.006567	12	12	0.1307	36
36	0.001 OD No Carbon Source vs. 0.001 OD Glucose	0.002917	0.03117	-0.02825	0.006567	12	12	4.302	36
37	0.001 OD No Carbon Source vs. 0.001 OD Arabinose	0.002917	0.003792	-0.000875	0.006567	12	12	0.1332	36
38	0.001 OD No Carbon Source vs. 0.001 OD Xylose	0.002917	0.003583	-0.0006667	0.006567	12	12	0.1015	36
39	0.001 OD No Carbon Source vs. 0.001 OD Ribose	0.002917	0.004292	-0.001375	0.006567	12	12	0.2094	36
40	0.001 OD No Carbon Source vs. 0.001 OD Dulcitol	0.002917	0.006058	-0.003142	0.006567	12	12	0.4784	36
41	0.001 OD No Carbon Source vs. 0.001 OD Inositol	0.002917	0.004553	-0.001637	0.006567	12	12	0.2492	36
42	0.001 OD No Carbon Source vs. 0.001 OD Maltitol	0.002917	0.0153	-0.01238	0.006567	12	12	1.886	36
43	0.001 OD No Carbon Source vs. 0.001 OD Mannitol	0.002917	0.0057	-0.002783	0.006567	12	12	0.4238	36
44	0.001 OD No Carbon Source vs. 0.001 OD Sorbitol	0.002917	0.005017	-0.0021	0.006567	12	12	0.3198	36
45	0.001 OD No Carbon Source vs. 0.001 OD Xylitol	0.002917	0.002858	5.833e-005	0.006567	12	12	0.008882	36
46	0.001 OD No Carbon Source vs. 0.001 OD Glycerol	0.002917	0.01013	-0.007208	0.006567	12	12	1.098	36

Hexose sugars, pentose sugars, and sugar alcohols. A Dunnett's test was also done to see multiple comparisons between the carbon sources. The mean difference, adjusted p-value, and if the carbon source is significant are shown in the table.

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TABLE 2: Two-Way Anova of carbon sources comparing Glucose against all the carbon sources

1	Compare column means (main column effect)								
2									
3	Number of families	1							
4	Number of comparisons per family	17							
5	Alpha	0.05							
6									
7	Dunnett's multiple comparisons test	Mean Diff.	95.00% CI of diff.	Significant?	Summary	Adjusted P Value			
8									
9	0.001 OD Glucose vs. 0.001 OD Fructose	0.02871	0.008854 to 0.04857	Yes	**	0.0014			
10	0.001 OD Glucose vs. 0.001 OD Mannose	0.02843	0.008573 to 0.04829	Yes	**	0.0016			
11	0.001 OD Glucose vs. 0.001 OD Rhamnose	0.02837	0.008508 to 0.04823	Yes	**	0.0016			
12	0.001 OD Glucose vs. 0.001 OD Sorbose	0.02668	0.006825 to 0.04654	Yes	**	0.0034			
13	0.001 OD Glucose vs. 0.001 OD Galactose	0.0272	0.007341 to 0.04706	Yes	**	0.0027			
14	0.001 OD Glucose vs. 0.001 OD Fucose	0.02739	0.007533 to 0.04725	Yes	**	0.0025			
15	0.001 OD Glucose vs. 0.001 OD Arabinose	0.02738	0.007516 to 0.04723	Yes	**	0.0025			
16	0.001 OD Glucose vs. 0.001 OD Xylose	0.02758	0.007725 to 0.04744	Yes	**	0.0023			
17	0.001 OD Glucose vs. 0.001 OD Ribose	0.02688	0.007016 to 0.04673	Yes	**	0.0031			
18	0.001 OD Glucose vs. 0.001 OD Dulcitol	0.02511	0.00525 to 0.04497	Yes	**	0.0065			
19	0.001 OD Glucose vs. 0.001 OD Inositol	0.02661	0.006755 to 0.04647	Yes	**	0.0035			
20	0.001 OD Glucose vs. 0.001 OD Maltitol	0.01587	-0.003992 to 0.03573	No	ns	0.1851			
21	0.001 OD Glucose vs. 0.001 OD Mannitol	0.02547	0.005608 to 0.04533	Yes	**	0.0056			
22	0.001 OD Glucose vs. 0.001 OD Sorbitol	0.02615	0.006291 to 0.04601	Yes	**	0.0042			
23	0.001 OD Glucose vs. 0.001 OD Xylitol	0.02831	0.00845 to 0.04817	Yes	**	0.0017			
24	0.001 OD Glucose vs. 0.001 OD Glycerol	0.02104	0.001183 to 0.0409	Yes	*	0.0324			
25	0.001 OD Glucose vs. 0.001 OD No Carbon Source	0.02825	0.008391 to 0.04811	Yes	**	0.0017			
26									
27									
28	Test details	Mean 1	Mean 2	Mean Diff.	SE of diff.	N1	N2	q	DF
29									
30	0.001 OD Glucose vs. 0.001 OD Fructose	0.03117	0.002454	0.02871	0.006567	12	12	4.372	36
31	0.001 OD Glucose vs. 0.001 OD Mannose	0.03117	0.002735	0.02843	0.006567	12	12	4.329	36
32	0.001 OD Glucose vs. 0.001 OD Rhamnose	0.03117	0.0028	0.02837	0.006567	12	12	4.319	36
33	0.001 OD Glucose vs. 0.001 OD Sorbose	0.03117	0.004483	0.02668	0.006567	12	12	4.063	36
34	0.001 OD Glucose vs. 0.001 OD Galactose	0.03117	0.003967	0.0272	0.006567	12	12	4.142	36
35	0.001 OD Glucose vs. 0.001 OD Fucose	0.03117	0.003775	0.02739	0.006567	12	12	4.171	36
36	0.001 OD Glucose vs. 0.001 OD Arabinose	0.03117	0.003792	0.02738	0.006567	12	12	4.168	36
37	0.001 OD Glucose vs. 0.001 OD Xylose	0.03117	0.003583	0.02758	0.006567	12	12	4.2	36
38	0.001 OD Glucose vs. 0.001 OD Ribose	0.03117	0.004292	0.02688	0.006567	12	12	4.092	36
39	0.001 OD Glucose vs. 0.001 OD Dulcitol	0.03117	0.006058	0.02511	0.006567	12	12	3.823	36
40	0.001 OD Glucose vs. 0.001 OD Inositol	0.03117	0.004553	0.02661	0.006567	12	12	4.052	36
41	0.001 OD Glucose vs. 0.001 OD Maltitol	0.03117	0.0153	0.01587	0.006567	12	12	2.416	36
42	0.001 OD Glucose vs. 0.001 OD Mannitol	0.03117	0.0057	0.02547	0.006567	12	12	3.878	36
43	0.001 OD Glucose vs. 0.001 OD Sorbitol	0.03117	0.005017	0.02615	0.006567	12	12	3.982	36
44	0.001 OD Glucose vs. 0.001 OD Xylitol	0.03117	0.002858	0.02831	0.006567	12	12	4.31	36
45	0.001 OD Glucose vs. 0.001 OD Glycerol	0.03117	0.01013	0.02104	0.006567	12	12	3.204	36
46	0.001 OD Glucose vs. 0.001 OD No Carbon Source	0.03117	0.002917	0.02825	0.006567	12	12	4.302	36

Hexose sugars, pentose sugars, and sugar alcohols. A Dunnett's test was also done to see multiple comparisons between the carbon sources. The mean difference, adjusted p-value, and if the carbon source is significant are shown in the table.

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TABLE 3: One-Way Anova of carbon sources comparing Glucose against all the carbon sources –

	1way ANOVA Multiple comparisons								
1	Number of families	1							
2	Number of comparisons per family	17							
3	Alpha	0.05							
4									
5	Dunnett's multiple comparisons test	Mean Diff.	95.00% CI of diff.	Significant?	Summary	Adjusted P Value	G-?		
6									
7	0.001 OD Glucose vs. 0.001 OD Fructose	0.06275	0.03093 to 0.09457	Yes	****	0.0001	A	0.001 OD Fructose	
8	0.001 OD Glucose vs. 0.001 OD Mannose	0.06306	0.03124 to 0.09488	Yes	****	0.0001	B	0.001 OD Mannose	
9	0.001 OD Glucose vs. 0.001 OD Rhamnose	0.06107	0.02925 to 0.09289	Yes	****	0.0001	C	0.001 OD Rhamnose	
10	0.001 OD Glucose vs. 0.001 OD Sorbose	0.0575	0.02568 to 0.08932	Yes	****	0.0001	D	0.001 OD Sorbose	
11	0.001 OD Glucose vs. 0.001 OD Galactose	0.05797	0.02615 to 0.08979	Yes	****	0.0001	E	0.001 OD Galactose	
12	0.001 OD Glucose vs. 0.001 OD Fucose	0.05937	0.02755 to 0.09119	Yes	****	0.0001	F	0.001 OD Fucose	
13	0.001 OD Glucose vs. 0.001 OD Arabinose	0.05723	0.02541 to 0.08905	Yes	****	0.0001	H	0.001 OD Arabinose	
14	0.001 OD Glucose vs. 0.001 OD Xylose	0.05707	0.02525 to 0.08889	Yes	****	0.0001	I	0.001 OD Xylose	
15	0.001 OD Glucose vs. 0.001 OD Ribose	0.0577	0.02588 to 0.08952	Yes	****	0.0001	J	0.001 OD Ribose	
16	0.001 OD Glucose vs. 0.001 OD Dulcitol	0.05627	0.02445 to 0.08809	Yes	****	0.0001	K	0.001 OD Dulcitol	
17	0.001 OD Glucose vs. 0.001 OD Inositol	0.05642	0.0246 to 0.08824	Yes	****	0.0001	L	0.001 OD Inositol	
18	0.001 OD Glucose vs. 0.001 OD Maltitol	0.02577	-0.006053 to 0.05759	No	ns	0.1736	M	0.001 OD Maltitol	
19	0.001 OD Glucose vs. 0.001 OD Mannitol	0.05637	0.02455 to 0.08819	Yes	****	0.0001	N	0.001 OD Mannitol	
20	0.001 OD Glucose vs. 0.001 OD Sorbitol	0.0581	0.02628 to 0.08992	Yes	****	0.0001	O	0.001 OD Sorbitol	
21	0.001 OD Glucose vs. 0.001 OD Xylitol	0.06087	0.02905 to 0.09269	Yes	****	0.0001	P	0.001 OD Xylitol	
22	0.001 OD Glucose vs. 0.001 OD Glycerol	0.04683	0.01501 to 0.07865	Yes	**	0.0011	Q	0.001 OD Glycerol	
23	0.001 OD Glucose vs. 0.001 OD No Carbon Source	0.05943	0.02761 to 0.09125	Yes	****	0.0001	R	0.001 OD No Carbon Source	
24									
25									
26	Test details	Mean 1	Mean 2	Mean Diff.	SE of diff.	n1	n2	q	DF
27									
28	0.001 OD Glucose vs. 0.001 OD Fructose	0.06453	0.001783	0.06275	0.01052	3	3	5.963	36
29	0.001 OD Glucose vs. 0.001 OD Mannose	0.06453	0.001473	0.06306	0.01052	3	3	5.993	36
30	0.001 OD Glucose vs. 0.001 OD Rhamnose	0.06453	0.003467	0.06107	0.01052	3	3	5.803	36
31	0.001 OD Glucose vs. 0.001 OD Sorbose	0.06453	0.007033	0.0575	0.01052	3	3	5.464	36
32	0.001 OD Glucose vs. 0.001 OD Galactose	0.06453	0.006567	0.05797	0.01052	3	3	5.509	36
33	0.001 OD Glucose vs. 0.001 OD Fucose	0.06453	0.005167	0.05937	0.01052	3	3	5.642	36
34	0.001 OD Glucose vs. 0.001 OD Arabinose	0.06453	0.0073	0.05723	0.01052	3	3	5.439	36
35	0.001 OD Glucose vs. 0.001 OD Xylose	0.06453	0.007467	0.05707	0.01052	3	3	5.423	36
36	0.001 OD Glucose vs. 0.001 OD Ribose	0.06453	0.006833	0.0577	0.01052	3	3	5.483	36
37	0.001 OD Glucose vs. 0.001 OD Dulcitol	0.06453	0.008267	0.05627	0.01052	3	3	5.347	36
38	0.001 OD Glucose vs. 0.001 OD Inositol	0.06453	0.008113	0.05642	0.01052	3	3	5.362	36
39	0.001 OD Glucose vs. 0.001 OD Maltitol	0.06453	0.03877	0.02577	0.01052	3	3	2.449	36
40	0.001 OD Glucose vs. 0.001 OD Mannitol	0.06453	0.008167	0.05637	0.01052	3	3	5.357	36
41	0.001 OD Glucose vs. 0.001 OD Sorbitol	0.06453	0.006433	0.0581	0.01052	3	3	5.521	36
42	0.001 OD Glucose vs. 0.001 OD Xylitol	0.06453	0.003667	0.06087	0.01052	3	3	5.784	36
43	0.001 OD Glucose vs. 0.001 OD Glycerol	0.06453	0.0177	0.04683	0.01052	3	3	4.451	36
44	0.001 OD Glucose vs. 0.001 OD No Carbon Source	0.06453	0.0051	0.05943	0.01052	3	3	5.648	36

Hexose sugars, pentose sugars, and sugar alcohols – on Day 6 only. A Dunnett's test was also done to see multiple comparisons between the carbon sources. The mean difference, adjusted p-value, and if the carbon source is significant are shown in the table.

While maltitol grew in the condition the culture flasks, it was calculated to not be as significant as glucose. A simplified version of the statistical analysis (Table 4) corroborated with the previous tests that comparing tests with no carbon sources to all the other carbon sources gave us data that the only carbon source that was statistically significant was glucose.

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TABLE 4: Dunnett's Multiple Comparison Tests

Dunnett's Multiple Comparison Tests	Mean Difference	Significant?	Adjusted P-Value
0.001 OD No Carbon Source vs. 0.001 OD Fructose	0.0004625	No	0.9999
0.001 OD No Carbon Source vs. 0.001 OD Mannose	0.0001817	No	0.9999
0.001 OD No Carbon Source vs. 0.001 OD Rhamnose	0.0001167	No	0.9999
0.001 OD No Carbon Source vs. 0.001 OD Sorbose	-0.001567	No	0.9997
0.001 OD No Carbon Source vs. 0.001 OD Galactose	-0.00105	No	0.9998
0.001 OD No Carbon Source vs. 0.001 OD Fucose	-0.0008583	No	0.9999
0.001 OD No Carbon Source vs. 0.001 OD Glucose	-0.02825	Yes	0.0017
0.001 OD No Carbon Source vs. 0.001 OD Arabinose	-0.000875	No	0.9998
0.001 OD No Carbon Source vs. 0.001 OD Xylose	-0.0006667	No	0.9999
0.001 OD No Carbon Source vs. 0.001 OD Ribose	-0.001375	No	0.9997
0.001 OD No Carbon Source vs. 0.001 OD Dulcitol	-0.003142	No	0.9993
0.001 OD No Carbon Source vs. 0.001 OD Inositol	-0.001637	No	0.9997
0.001 OD No Carbon Source vs. 0.001 OD Maltitol	-0.01238	No	0.4587
0.001 OD No Carbon Source vs. 0.001 OD Mannitol	-0.002783	No	0.9994
0.001 OD No Carbon Source vs. 0.001 OD Sorbitol	-0.0021	No	0.9996
0.001 OD No Carbon Source vs. 0.001 OD Xylitol	5.833e-005	No	0.9999
0.001 OD No Carbon Source vs. 0.001 OD Glycerol	-0.007208	No	0.9522

A simplified version of Dunnett's Multiple Comparison Tests showing the mean difference, adjusted p-value, and if the carbon source is significant or not.

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DISCUSSION

Carbon Sources Trials

For the trials with hexose sugars (Figure 5), fructose, mannose, rhamnose, sorbose, galactose, and fucose all showed growth over the 6-day cycle. Sorbose, galactose, fucose, and rhamnose were observed on day 4 reaching their highest peak before staying about even for day 6. Galactose has an extremely low ability to incorporate *in vivo*, due to either the lack of a proper membrane uptake system, or the UDP-glucose is the source for UDP-galactose *in vivo* by epimerization (88, 89). Fructose and mannose showed that after day 4 a slight dip in their growth as they were going on a downward trend by day 6. Glucose showed the same growth on day 2 as the other hexose sugars tested, but by day 4 saw a massive jump in growth that far surpassed the other carbon sources. This data concurs with Wodke's (86) findings, as while *in silico*, mathematical models, there is no difference in energy yield for *Mycoplasma pneumoniae* growth on different carbon sources. However, *in vivo*, observed in a living organism, the doubling times differed considerably, and only glucose and mannose allowed for healthy growth. *Mycoplasma pneumoniae* grew more proficiently with the help of glucose then any of the other hexose sugars. While the other hexose sugars saw considerable growth, they were not at the range that glucose saw in the growth curve cycle.

Glucose being the hexose sugar that grew the most is consistent with previous studies that were done into *Mycoplasma pneumoniae* carbon source utilization. While mannose and the other hexose sugars have moderate to little growth, it is not as efficient and stable as what glucose provides for the bacteria's processes and metabolism. The pathway for glucose is defined, and *Mycoplasma pneumoniae* seems to rely on this more than most other carbon sources to go into glycolysis and other systems.

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The pentose sugar trials (Figure 6) showed a consistent growth for arabinose, xylose, and ribose. Ribose showed the most growth on day 2, before becoming even with arabinose and xylose. By day 6 the pentose sugars had evened out at positive rate, showing that the growth was steady throughout the 6-day cycle. While not as massive a growth as glucose previously, these carbon sources were shown to be able to be utilized by *Mycoplasma pneumoniae*. While the glucose-specific uptake protein (MPN207) has high copy numbers (~385/cell), the known transport proteins for other sugars (fructose, ribose, mannitol, mannose, glycerol, G3P, and ascorbate) are 8-20 times less abundant. To allow for pertinent growth of *Mycoplasma pneumoniae* on fructose *in vivo*, for example, the cells have to be adapted over several successive passages to show any substantial overexpression of proteins involved in fructose import and metabolism (85).

Pentose sugars grew as well as hexose sugars, but glucose is still the sugar that is most efficiently used by *Mycoplasma pneumoniae*. Out the tested pentose sugars, only ribose had a carbon source pathway that was able to be found to be dependable to the study. Ribose is theorized to be a carbon source that this bacterium is able to use competently. However, it seems from this study and others of a similar to it, that glucose is still the preferred carbon source to be used by this lung bacteria.

The sugar alcohols that were tested (Figure 7) showed a wide range of growth between the carbon sources that were tested. Maltitol showed the most growth by day 6, while xylitol showed the least amount of growth by day 6. Glycerol and dulcitol reached their highest peak of growth by day 4, steadying by day 6, while xylitol, sorbitol, and mannitol were going in a downward trend by day 6. Maltitol and inositol stayed in positive growth throughout, never losing growth. Glycerol and maltitol saw the most growth out of all the sugar alcohols over the six-day cycle, with maltitol growing slightly more than glycerol.

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While glycerol is a known carbon source that *Mycoplasma pneumoniae* can utilize, the fact the maltitol grew is interesting. Maltitol is not a carbon source that other studies and findings noted as a potential carbon source for *Mycoplasma pneumoniae* to use, but with this data the possibility of future testing with maltitol is definitely a prospect of future testing. For comparison, Grembecka (90) found that sugar alcohols, such as maltitol, were found to increase mineral bioavailability in humans and rats. Maltitol is partially digested in the intestines, and a non-absorbed part is metabolized by colonic bacteria in the lower gut. Its absorption ranges from 5 to 80%, but it must be proceeded by hydrolysis which leads to glucose and sorbitol. It is slowly digested in the small intestine and the non-absorbed part passes to the colon where it undergoes fermentation by bacteria (90).

Maltitol Utilization

Maltitol is a common sugar alcohol used as a sugar replacement that is found in many sweeteners for foods and drinks we consume in our society (91). After ingestion, maltitol is slowly hydrolyzes by the enzymes of the small intestine into its constituent monomers, glucose and sorbitol (92). It has a very slow digestion rate because it is fermented in the colon (91). This is similar to *Mycoplasma pneumoniae*, as glucose and sorbitol are carbon sources that can be utilized in some way. *Mycoplasma pneumoniae* is known to use glucose and glycerol, and its pathways for utilization are well known and covered in many studies. Maltitol, however, has not seen the same research. The enzymes and pathways needed to be utilized by *Mycoplasma pneumoniae*'s processes are not well documented at this present time. Further studies into maltitol may be clear up how it is able to be utilized so efficiently by *Mycoplasma pneumoniae*.

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Glycerol being a carbon source that *Mycoplasma pneumoniae* is able to use efficiently is consistent with other studies. However, maltitol showing as much growth as glucose and glycerol is a new factor that may have to be taken into account in future studies of a similar nature. Maltitol as a carbon source that is able to be utilized by *Mycoplasma pneumoniae* is new data that is not been explored in other studies involving the bacteria. Common in most chewing gums, maltitol as a carbon source that *Mycoplasma pneumoniae* may be able to use efficiently is still new information (93). While its growth was unprecedented, the statistical analysis states that it is not a statistically significant carbon source for *Mycoplasma pneumoniae*. Further studies can be done to look into the how this is able to occur. A carbon source pathway for maltitol can't be accurately defined at the moment, which is a step that needs to be looked into future studies into carbon sources. The enzymes and mechanisms for this carbon source to be used by *Mycoplasma pneumoniae* are perhaps there, albeit not discovered fully yet.

Mannitol as a carbon source seems to be present in *Mycoplasma pneumoniae*, even though the substrate cannot be used by the bacteria (24). Glycerol showing positive growth is comparable to other studies that show that glycerol is one of the few carbon sources to already be known to utilized by *Mycoplasma pneumoniae* (25). It uses glycerol metabolism, which its first component is the glycerol facilitator encoded by *glpF* gene. The importance of glycolysis for most mollicutes, such as *Mycoplasma pneumoniae*, is underlined by the lack of the oxidative part of the pentose phosphate pathway and of the citric acid cycle. *Mycoplasma pneumoniae* grows the fastest with glucose; though, with the abundance of glycerol and glycerol-containing molecules in the bacterium's environment and the role of glycerol as the trigger of one of the few regulatory proteins indicate that glycerol might be the preferred carbon source (86).

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Reductive Evolution & Statistical Analysis

The role of reductive evolution in the utilization of carbon sources by *Mycoplasma pneumoniae* may have been seen in this study. While most of the carbon sources were able to be utilized by in the well plate tests, not all of the carbon sources were not able to be grown in the growth curve tests. This can be hypothesized as a result of reductive evolution, as even though the paths for those carbon sources are there for them to be used, the redundancy of those metabolic paths are not beneficial for *Mycoplasma pneumoniae*. Certain pathways that are available for most bacteria seem to not be advantageous for use, resulting in fewer carbon sources being able to be utilized by *Mycoplasma pneumoniae* better, if not at all.

The significance of the statistical analysis in this study show some notable data of the carbon sources. With the ranges of the carbon source fluctuating in different ways, especially on different days on the six-day growth curves, the findings varied per day. Xylitol, for example, grew at an increased rate for the first 4 days of the cycle, the decreasing by day 6. This could be because of two notions; by day 6, *Mycoplasma pneumoniae* no longer can use this carbon source, or the carbon source was no longer effective and slowly degraded over time. With this shifting range of data, the graphs with data points showing the carbon sources by days was devised to show the growth of the carbon sources in a more comparable approach. Both approaches to graphs in this study have its advantages and disadvantages, both showing the data in new light so it can be used to come to new conclusions.

Glycerol is one of the known carbon sources that is able to be utilized fully by *Mycoplasma pneumoniae*. The complex process of being synthesized for use in the bacteria has a variety of ways for it to happen. The outcome could either result in glycerol phosphate that can be used in other processes, or it can take a longer path to eventually used for the biosynthesis of

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phospholipids. No matter what, glycerol is clearly a significant carbon source for *Mycoplasma pneumoniae* that is allows in thrive in the human lung.

The hypothesis in this study was shown to be not supported. While other carbon sources were able to be utilized by *Mycoplasma pneumoniae*, they were not as efficient as glucose. A two-way ANOVA that was done testing No Carbon Source against other carbon sources showed that glucose was the only carbon source that was significant. A two-way ANOVA of glucose against other carbon sources showed they were all significant, except for maltitol. It can be assumed with the data that hexose sugars, pentose sugars, and sugar alcohols that are similar to glucose and glycerol can be utilized by *Mycoplasma pneumoniae*. Theoretically, these carbon sources can be used to develop alternate treatments to treat afflictions that infect the lungs. These carbon sources can be possibly be used to counter diseases and difficulties caused by *Mycoplasma pneumoniae*. This could especially be beneficial for patients that inadvertently acquire symptoms in research and healthcare settings in being able to diagnose and treat this before it gets too severe.

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MATERIALS & METHODS

Analysis of carbon sources and their effect on Mycoplasma pneumoniae

All procedures that are to be used in this experiment are adapted and based on scholarly studies by mentor Dr. Sebastian R. Schmidl, as well as direct suggestions from him (12, 25, 82). *Mycoplasma pneumoniae* will be stored in -80°C freezer in cryotubes to be used for these tests. Cryotubes were prepared beforehand by taking samples acquired at placing them at a temperature where they would not degrade over time. *M. pneumoniae* was tested in well plates to with different carbon source concentrations to measure their growth. Optical density was also calculated to be able to add accurate amounts of the mycoplasma to the carbon sources. The use of optical density was to see the cell growth of different carbon sources in a 6-8-day cycle. Growth curves are also made to test optical density of an eight-day period. *Mycoplasma pneumoniae* will be tested with carbon sources to see how they affect their growth. Growth curves containing no carbon sources will be tested as a control. A ThermoScientific Herasafe KS, Class II safety cabinet will be the main equipment used to do experiments.

M. pneumoniae cells will be harvested in 1.5 ml phosphate-buffered saline (PBS) pH 7.4. The cells will then be spun down in a centrifuge for 5 minutes at 10,000 x g and 4°C and the supernatant is then to be discarded. The cells will then be washed again in 1 ml PBS pH 7.4 and centrifuged for another 5 minutes at 10,000 x g and 4C. The pellet then needs to be resuspended in 1 ml and 800 µl water of the solution will be used for determination of optical density at 550 nm, which is the best wavelength to test for density of this bacteria.

For these growth experiments, the number of cells corresponding to different OD_{550} units in a final volume of 1 ml is going to be used. The cells are to be pelleted by centrifugation at 10,000 x g for 5 minutes at 4°C and the supernatant then discarded. The washed cells then are going to be

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resuspended in MP medium 900 µl. One ml of MP medium is to be plated into the wells of a sterile 24-well cell culture plate. The OD_{550} added to the wells are different according to the carbon source that is to be tested in that trial. Cells will be incubated for 3-4 days at 37°C.

When the incubation period is done, the well plates will be taken out of the incubator and observed to see a change in color. A lighter change in color, such as an orange or yellow, will signify that *Mycoplasma pneumoniae* in the wells were able to grow in the different carbon source solutions that will be presented in the wells.

Measuring optical density

Carbon source preparation procedures that are to be used are adapted and based on scholarly studies by mentor Dr. Sebastian R. Schmidl, as well as direct suggestions from him and discussion with fellow master students in the lab (65, 94, 95). Similar to glucose, hexoses and pentoses – such as fructose, galactose, mannose, rhamnose, sorbose, fucose, sorbose, arabinose, xylose, and ribose – and glycerol, sugar alcohols – such as dulcitol, inositol, mannitol, sorbitol, xylitol, and maltitol.

Mycoplasma pneumoniae is to be placed into 50-milliliter tube. The tube was then filled with a solution of MP medium – Beef Heart Infusion Broth, Yeast extract, Deoxyribonucleic acid, Phenol red, and Agar – at a final pH of 7.8 (25°C). horse serum, penicillin, and the specific carbon source that will be tested in the trials. The calculations – (Number of microliters in tube) (Starting OD) times X x (OD x 2) – are based on the optical density of the *Mycoplasma pneumoniae* cells to be recorded from testing will help determine the appropriate amount of the cells to place into the flask. The starting optical density of all trials is to be 0.001.

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Mycoplasma pneumoniae cells are to be thawed from their cryotubes in -80°C freezer by putting them on ice. The tubes are then to be mixed gently and pipetted 400 µl into sterile 1.5 ml microcentrifuge tubes. These cells will be centrifuged for 5 minutes at 10,000 x g and 4°C and the supernatant will be discarded. The pellet that remains will be resuspended in 800 µl of PBS pH 7.4 and the cells will be centrifuged for 5 minutes at 10,000 x g and 4°C for a second time. After discarding the supernatant, the pellet will be resuspended in 400 µl PBS pH 7.4.

The pellet resuspended in 400 µl PBS pH 7.4 will then be placed into a spectrophotometer cuvette and an additional 400 µl PBS pH 7.4 will be added to the cuvette, for a total of 800 µl. Another 800 µl PBS pH 7.4 will be placed in a reference blank cuvette. This reference blank will be used as a control to be referenced against carbon sources to be tested. Specific cell culture volumes to be used are based the OD_{550} and will be inoculated into the modified MP medium. This medium will consist of 20% horse serum, 300 µl penicillin, and the specific carbon source volume will be added to these mediums. The MP medium is prepared because it is the best solution for carbon sources to be tested in. Three empty cell culture flasks – labeled Day 2, Day 4, and Day 6 – are to be prepared. The 3 cell culture flasks will each have 10 ml of Hayflick medium containing the appropriate carbon source. Incubation of these flasks will be for 6 days at 37°C and checked and recorded every 2 days. Every 2 days, the cells with be harvested according to the correct day flask by using a cell scraper and scraping the growing cells from side of the flask. They are to be harvested in 1 ml PBS pH 7.4 and placed in a sterile 2 ml microcentrifuge tube. The cells will then be centrifuged for 5 minutes at 10,000 x g and 4°C and supernatant is to then be discarded. The pellet will be resuspended in 1 ml PBS pH 7.4 and 800 µl of this will be transferred into a cell suspension in a spectrophotometer cuvette. The OD_{550} will be measured alongside an 800 ul PBS pH 7.4 reference blank. The OD_{550} will recorded for the specific carbon source. The same

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procedure will be then done for Day 4 and Day 6 for all carbon sources tested in the current cycle. All data will then be put together for use in growth curves and statistical analysis.

Statistical Analysis

Statistical analysis was done to find if the carbon sources were significant compared to no carbon source and glucose. Using the program GraphPad Prism, these tests were able to be done once data was added for comparisons. A Dunnett's test was done to compare a number of treatments with a single control to see that difference in the groups. When a Two-Way ANOVA test has significant findings, it doesn't show which pairs are significant compared to other pairs. A Dunnett's test can be used after an ANOVA test run the pairs to find the significance, if any can be found.

A Two-Way ANOVA test was done for no carbon source vs. carbon sources and glucose vs. carbon sources. A Dunnett's test was done afterwards to see if there is any significance in the carbon sources tested and compare these multiple comparisons.

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REFERENCES

1. Citti, C., & Blanchard, A. 2013. Mycoplasmas and their host: Emerging and re-emerging minimal pathogens. Trends in Microbiology*, 21*(4), 196-203

2. Blötz, C., Lartigue, C., Timana, Y. V., Ruiz, E., Paetzold, B., Busse, J., & Stülke, J. 2018. Development of a replicating plasmid based on the native oriC in *Mycoplasma pneumoniae.* Microbiology,*164*(11), 1372-1382. doi:10.1099/mic.0.000711

3. Kashif, M., Dudekula, R. A., & Khaja, M. 2017. A Rare Case of Cavitary Lesion of the Lung Caused by *Mycoplasma pneumoniae* in an Immunocompetent Patient. Case Reports in Medicine*,2017*, 1-4. doi:10.1155/2017/9602432

4. Waites, K. B., Balish, M. F., Atkinson, T. P. 2009. New insights into the pathogenesis and detection of *Mycoplasma pneumoniae* infections. Future Microbiol, 3(6), 635-648.

5. Lluch-Senar, M., Cozzuto, L., Cano, J., Delgado, J., Llórens-Rico, V., Pereyre, S., … Serrano, L. 2015. Comparative "-omics" in *Mycoplasma pneumoniae* Clinical Isolates Reveals Key Virulence Factors. *PLoS ONE*, *10*(9), e0137354.

6. Kaler, J., Mukhtar, O., Khan, B., Shrestha, B., Kaler, R., Ting, B., & Khalid, M. 2018. Rhabdomyolysis: An Unusual Presentation of *Mycoplasma pneumoniae* Infection in an Adult—A Case Report and Literature Review. Case Reports in Medicine,*2018*, 1-4. doi:10.1155/2018/6897975

7. Matsumoto, M., Nagaoka, K., Suzuki, M., Konno, S., Takahashi, K., Takashina, T., . . . Nishimura, M. 2019. An adult case of severe life-threatening *Mycoplasma pneumoniae* pneumonia due to a macrolide-resistant strain, Japan: A case report. BMC Infectious *Diseases,19*(1). doi:10.1186/s12879-019-3846-1

8. Kuźma-Mroczkowska, E., Pańczyk-Tomaszewska, M., Szmigielska, A., Szymanik-Grzelak, H., & Roszkowska-Blaim, M. 2015. *Mycoplasma pneumoniae* as a trigger for Henoch-Schönlein purpura in children. Central European Journal of *Immunology,4*, 489-492. doi:10.5114/ceji.2015.56976

9. D'Alonzo, R., Mencaroni, E., Genova, L. D., Laino, D., Principi, N., & Esposito, S. 2018. Pathogenesis and Treatment of Neurologic Diseases Associated with *Mycoplasma pneumoniae* Infection. Frontiers in Microbiology*,9*. doi:10.3389/fmicb.2018.02751

10. Razin, S., Yogev, D., & Naot, Y. 1998. Molecular Biology and Pathogenicity of Mycoplasmas. Microbiology and Molecular Biology *Reviews, 62*(4), 1096-1156

11. Rosengarten, R., Citti, C., Glew, M., Lischewski, A., Droeße, M., Much, P., . . . Spergser, J. 2000. Host-pathogen interactions in mycoplasma pathogenesis: Virulence and survival strategies of minimalist prokaryotes. International Journal of Medical *Microbiology, 290*(1), 15-25

المنارة للاستشارات
www.manaraa.com

12. Schmidl, S. R., Gronau, K., Pietack, N., Hecker, M., Becher, D., & Stülke, J. 2010. The Phosphoproteome of the Minimal Bacterium *Mycoplasma pneumoniae*: Analysis of the complete known SER/THR kinome suggests the existence of novel kinases. Molecular & Cellular Proteomics*: MCP*, *9*(6), 1228–1242. doi.org/10.1074/mcp.M900267-MCP200

13. Yimenicioğlu, S., Yakut, A., Ekici, A., Bora Carman, K., & Cagrı Dinleyici, E. 2014. *Mycoplasma pneumoniae* Infection with Neurologic Complications.Iranian Journal of Pediatrics, *24*(5), 647–651.

14. Xiao, L., Ptacek, T., Osborne, J. D., Crabb, D. M., Simmons, W. L., Lefkowitz, E. J., … Dybvig, K. 2015. Comparative genome analysis of *Mycoplasma pneumoniae*. BMC Genomics, *16*(1), 610.

15. Liong, T., Lee, K., Poon, Y., Lam, S., Kwok, K., Ng, W., . . . Law, K. 2015. Extrapulmonary involvement associated with *Mycoplasma pneumoniae* infection. Hong Kong Medical Journal*,*569-572. doi:10.12809/hkmj144403

16. Song, W. J., Kang, B., Lee, H. P., Cho, J., Lee, H. J., & Choe, Y. H. 2017. Pediatric *Mycoplasma pneumoniae* Infection Presenting with Acute Cholestatic Hepatitis and Other Extrapulmonary Manifestations in the Absence of Pneumonia. Pediatric Gastroenterology, Hepatology & Nutrition*,20*(2), 124. doi:10.5223/pghn.2017.20.2.124

17. Benzaquen, M., Lebowitz, D., Belenotti, P., Durand, J., & Serratrice, J. 2016. Acute pancreatitis and pneumonia due to *Mycoplasma pneumoniae*: A case report. BMC Research Notes*,9*(1). doi:10.1186/s13104-016-2196-y

18. Jin, X., Zou, Y., Zhai, J., Liu, J., & Huang, B. 2018. Refractory *Mycoplasma pneumoniae* pneumonia with concomitant acute cerebral infarction in a child. Medicine*,97*(13). doi:10.1097/md.0000000000010103

19. Dhawan, B., & Kokkayil, P. 2015. Ureaplasma: Current perspectives. Indian Journal of Medical Microbiology*,33*(2), 205. doi:10.4103/0255-0857.154850

20. Combaz-Söhnchen, N., & Kuhn, A. 2017. A Systematic Review of Mycoplasma and Ureaplasma in Urogynaecology. Geburtshilfe Und Frauenheilkunde*,77*(12), 1299-1303. doi:10.1055/s-0043-119687

21. Balish, M. F. 2014. *Mycoplasma pneumoniae*, an Underutilized Model for Bacterial Cell Biology. Journal of Bacteriology, *196*(21), 3675–3682.

22. Szczepanek, S. M., Boccaccio, M., Pflaum, K., Liao, X., & Geary, S. J. 2014. Hydrogen Peroxide Production from Glycerol Metabolism Is Dispensable for Virulence of *Mycoplasma gallisepticum* in the Tracheas of Chickens. Infection and Immunity, *82*(12), 4915–4920.

المنارة للاستشارات
www.manaraa.com

23. Manikandan, K., Sabareesh, V., Singh, N., Saigal, K., Mechold, U., & Sinha, K. 2014. Two-Step Synthesis and Hydrolysis of Cyclic di-AMP in *Mycobacterium tuberculosis*. PLoS ONE*, 9*(1).

24. Hames, C., Halbedel, S., Hoppert, M., Frey, J., & Stülke, J. 2009. Glycerol Metabolism Is Important for Cytotoxicity of *Mycoplasma pneumoniae*. Journal of Bacteriology, *191*(3), 747–753.

25. Großhennig, S., Schmidl, S. R., Schmeisky, G., Busse, J., & Stülke, J. 2013. Implication of Glycerol and Phospholipid Transporters in *Mycoplasma pneumoniae* Growth and Virulence. Infection and Immunity, *81*(3), 896–904.

26. Fadiel, A. 2007. Mycoplasma genomics: tailoring the genome for minimal life requirements through reductive evolution. Frontiers in Bioscience, *12*(1), 2020. doi: 10.2741/2207

27. Maselli, D. J., Medina, J. L., Brooks, E. G., Coalson, J. J., Kannan, T. R., Winter, V. T., … Peters, J. I. 2018. The Immunopathologic Effects of *Mycoplasma pneumoniae* and Community-acquired Respiratory Distress Syndrome Toxin. A Primate Model. American Journal of Respiratory Cell and Molecular Biology, *58*(2), 253–260. doi: 10.1165/rcmb.2017-0006oc

28. Lee, H., Moon, K. C., & Kim, S. 2015. Cutaneous vasculitis and renal involvement in *Mycoplasma pneumoniae* infection. The Korean Journal of Internal *Medicine*, *30*(3), 402–405.

29. Bowling, M., Schmutzler, T., & Glick, S. 2018. *Mycoplasma pneumoniae*-induced mucositis without rash in an 11-year-old boy. Clinical Case Reports*,6*(3), 551-552. doi:10.1002/ccr3.1400

30. Zão, I., Ribeiro, F., Rocha, V., Neto, P., Matias, C., & Jesus, G. 2018. *Mycoplasma pneumoniae*-associated *Mucositis*: A Recently Described Entity. European Journal of Case Reports in Internal Medicine*,5*(11), 1. doi:10.12890/2018_000977

31. Yin, S., Ma, F., & Gao, X. 2017. Association of *Mycoplasma pneumoniae* infection with increased risk of asthma in children. Experimental and Therapeutic Medicine*,13*(5), 1813-1819. doi:10.3892/etm.2017.4219

32. Tanaka, H. 2016. Correlation between Radiological and Pathological Findings in Patients with *Mycoplasma pneumoniae* Pneumonia. Frontiers in Microbiology*,7*. doi:10.3389/fmicb.2016.00695

33. Søndergaard, M. J., Friis, M. B., Hansen, D. S., & Jørgensen, I. M. 2018. Clinical manifestations in infants and children with *Mycoplasma pneumoniae* infection. Plos One*,13*(4). doi:10.1371/journal.pone.0195288

المنارة للاستشارات
www.manaraa.com

34. Shimizu, T. 2016. Inflammation-inducing Factors of *Mycoplasma pneumoniae*. Frontiers in Microbiology,*7*. doi:10.3389/fmicb.2016.00414

35. Kutty, P. K., Jain, S., Taylor, T. H., Bramley, A. M., Diaz, M. H., Ampofo, K., . . . Hicks, L. A. 2018. *Mycoplasma pneumoniae* Among Children Hospitalized With Community-acquired Pneumonia. Clinical Infectious Diseases. doi:10.1093/cid/ciy419

36. Yang, M., Meng, F., Wang, K., Gao, M., Lu, R., Li, M., . . . Wang, X. 2017. Interleukin 17A as a good predictor of the severity of *Mycoplasma pneumoniae* pneumonia in children. Scientific Reports,*7*(1). doi:10.1038/s41598-017-13292-5

37. Saraya, T., Ohkuma, K., Tsukahara, Y., Watanabe, T., Kurai, D., Ishii, H., . . . Takizawa, H. 2018. Correlation between clinical features, high-resolution computed tomography findings, and a visual scoring system in patients with pneumonia due to *Mycoplasma pneumoniae*. Respiratory Investigation,*56*(4), 320-325. doi: 10.1016/j.resinv.2018.03.001

38. Lee, Y. H., Seo, H., Cha, S. I., Kim, C. H., & Lee, J. 2019. A case of pseudomembranous tracheitis caused by *Mycoplasma pneumoniae* in an immunocompetent patient. Annals of Translational Medicine,*7*(9), 205-205. doi:10.21037/atm.2019.03.70

39. Bajantri, B., Venkatram, S., & Diaz-Fuentes, G. 2018. *Mycoplasma pneumoniae*: A Potentially Severe Infection. Journal of Clinical Medicine Research,*10*(7), 535-544. doi:10.14740/jocmr3421w

40. Wang, L., Feng, Z., Zhao, M., Yang, S., Yan, X., Guo, W., . . . Li, G. 2017. A comparison study between GeXP-based multiplex-PCR and serology assay for *Mycoplasma pneumoniae* detection in children with community acquired pneumonia. BMC Infectious Diseases,*17*(1). doi:10.1186/s12879-017-2614-3

41. Medjo, B., Atanaskovic-Markovic, M., Radic, S., Nikolic, D., Lukac, M., & Djukic, S. 2014. *Mycoplasma pneumoniae* as a causative agent of community-acquired pneumonia in children: Clinical features and laboratory diagnosis. Italian Journal of Pediatrics,*40*(1). doi:10.1186/s13052-014-0104-4

42. Busaidi, I. A., Al-Amin, M., Ibrahim, S., Balkhair, A., & Gaifer, Z. 2017. Multi-system manifestations of *Mycoplasma pneumoniae* infection in a young patient. JMM Case Reports,*4*(9). doi:10.1099/jmmcr.0.005117

43. Rajah, F. B., Zheng, R., Zhou, Y., & Xuan, K. 2017. Association of *Mycoplasma pneumoniae* antibody titer level with clinical severity of pneumonia in children. Journal of Pediatric Diseases,1, 24. doi:10.24294/jpedd. v1i0.105

44. Li, S., Xue, G., Zhao, H., Feng, Y., Yan, C., Cui, J., & Sun, H. 2018. The *Mycoplasma* pneumoniae HapE alters the cytokine profile and growth of human bronchial epithelial cells. Bioscience Reports,*39*(1). doi:10.1042/bsr20182201

المنارة للاستشارات
www.manaraa.com

45. Waites, K. B., Xiao, L., Liu, Y., Balish, M. F., & Atkinson, T. P. 2017. *Mycoplasma pneumoniae* from the Respiratory Tract and Beyond. Clinical Microbiology Reviews,*30*(3), 747-809. doi:10.1128/cmr.00114-16

46. Diaz, M. H., Benitez, A. J., Cross, K. E., Hicks, L. A., Kutty, P., Bramley, A. M., … Winchell, J. M. 2015. Molecular Detection and Characterization of *Mycoplasma pneumoniae* Among Patients Hospitalized with Community-Acquired Pneumonia in the United States. Open Forum Infectious Diseases,*2*(3), ofv106.

47. Kashyap, S., & Sarkar, M. 2010. *Mycoplasma pneumonia*: Clinical features and management. Lung India*,* 75-75. Retrieved 2015.

48. Kishaba, T. 2016. Community-Acquired Pneumonia Caused by *Mycoplasma pneumoniae*: How Physical and Radiological Examination Contribute to Successful Diagnosis. Frontiers in Medicine,*3*. doi:10.3389/fmed.2016.00028

49. Segovia, J. A., Chang, T., Winter, V. T., Coalson, J. J., Cagle, M. P., Pandranki, L., . . . Kannan, T. R. 2017. NLRP3 Is a Critical Regulator of Inflammation and Innate Immune Cell Response during *Mycoplasma pneumoniae* Infection. Infection and Immunity,*86*(1). doi:10.1128/iai.00548-17

50. Shi, S., Zhang, X., Zhou, Y., Tang, H., Zhao, D., & Liu, F. 2019. Immunosuppression Reduces Lung Injury Caused by *Mycoplasma pneumoniae* Infection. Scientific Reports,*9*(1). doi:10.1038/s41598-019-43451-9

51. Gao, M., Wang, K., Yang, M., Meng, F., Lu, R., Zhuang, H., . . . Wang, X. 2018. Transcriptome Analysis of Bronchoalveolar Lavage Fluid from Children With *Mycoplasma pneumoniae* Pneumonia Reveals Natural Killer and T Cell-Proliferation Responses. Frontiers in Immunology,*9*. doi:10.3389/fimmu.2018.01403

52. Wagner, K., Imkamp, F., Pires, V., & Keller, P. 2019. Evaluation of Lightmix Mycoplasma macrolide assay for detection of macrolide-resistant *Mycoplasma* pneumoniae in pneumonia patients. Clinical Microbiology and Infection,*25*(3). doi: 10.1016/j.cmi.2018.10.006

53. Christie, J. 2013. Diagnosing *Mycoplasma pneumoniae* in Children and Adolescents with Community-Acquired Pneumonia. *AJN,* American Journal of Nursing,*113*(5), 65. doi: 10.1097/01.naj.0000430240.42261.16

54. Gao, J., Yue, B., Li, H., Chen, R., Wu, C., & Xiao, M. 2015. Epidemiology and clinical features of segmental/lobar pattern *Mycoplasma pneumoniae* pneumonia: A ten-year retrospective clinical study. Experimental and Therapeutic Medicine,*10*(6), 2337-2344. doi:10.3892/etm.2015.2818

المنارة للاستشارات

www.manaraa.com

55. Chaudhry, R., Ghosh, A., & Chandolia, A. 2016. Pathogenesis of *Mycoplasma pneumoniae*: An update. Indian Journal of Medical Microbiology,*34*(1), 7. doi:10.4103/0255-0857.174112

56. Kawakami, N., Namkoong, H., Ohata, T., Sakaguchi, S., Saito, F., & Yuki, H. 2018. A Fulminant Case of Acute Respiratory Distress Syndrome Associated with *Mycoplasma pneumonia* Treated with Nasal High-Flow Oxygen Therapy. Case Reports in Critical Care,*2018*, 1-3. doi:10.1155/2018/1067593

57. Miyashita, N., Kawai, Y., Akaike, H., Ouchi, K., Hayashi, T., Kurihara, T., & Okimoto, N. 2012. Macrolide-resistant *Mycoplasma pneumoniae* in adolescents with community-acquired pneumonia. BMC Infectious Diseases,*12*(1). doi:10.1186/1471-2334-12-126

58. Mubarik, A., Jupalli, A., Iqbal, A. M., Mohammed, S. K., Muddassir, S., & Abdulmagid, E. 2019. *Mycoplasma pneumonia* with hydropneumothorax: A case report. Respiratory Medicine Case Reports,*26*, 299-300. doi: 10.1016/j.rmcr.2019.02.018

59. He, J., Qu, H., & Gao, C. 2019. Association between inflammation factors and *Mycoplasma pneumoniae* in children. Medicine,*98*(15). doi:10.1097/md.0000000000015118

60. Namkoong, H., Yamazaki, M., Ishizaki, M., Endo, I., Harada, N., Aramaki, M., . . . Mitamura, K. 2018. Clinical Evaluation of the Immunochromatographic System Using Silver Amplification for the Rapid Detection of *Mycoplasma pneumoniae*. Scientific Reports,*8*(1). doi:10.1038/s41598-018-19734-y

61. Chang, H., Chang, L., Shao, P., Lee, P., Chen, J., Lee, C., . . . Huang, L. 2014. Comparison of real-time polymerase chain reaction and serological tests for the confirmation of *Mycoplasma pneumoniae* infection in children with clinical diagnosis of atypical pneumonia. Journal of Microbiology, Immunology and Infection,*47*(2), 137-144. doi: 10.1016/j.jmii.2013.03.015

62. Saraya, T., Kurai, D., Nakagaki, K., Sasaki, Y., Niwa, S., Tsukagoshi, H., . . . Goto, H. 2014. Novel aspects on the pathogenesis of *Mycoplasma pneumoniae* pneumonia and therapeutic implications. Frontiers in Microbiology,*5*. doi:10.3389/fmicb.2014.00410

63. Xue, G., Li, M., Wang, N., Zhao, J., Wang, B., Ren, Z., . . . Sun, H. 2018. Comparison of the molecular characteristics of *Mycoplasma pneumoniae* from children across different regions of China. Plos One,*13*(8). doi: 10.1371/journal.pone.0198557

64. Ha, S. G., Oh, K. J., Ko, K., Sun, Y. H., Ryoo, E., Tchah, H., . . . Cho, H. 2018. Therapeutic Efficacy and Safety of Prolonged Macrolide, Corticosteroid, Doxycycline, and Levofloxacin against Macrolide-Unresponsive *Mycoplasma pneumoniae* Pneumonia in Children. Journal of Korean Medical Science,*33*(43). doi:10.3346/jkms.2018.33. e268

المنارة للاستشارات

www.manaraa.com

65. Dumke, R., & Jacobs, E. 2014. Evaluation of Five Real-Time PCR Assays for Detection of *Mycoplasma pneumoniae*. Journal of Clinical Microbiology, *52*(11), 4078–4081.

66. Youn, Y., Lee, S., Rhim, J., Shin, M., Kang, J., & Lee, K. 2014. Early Additional Immune-Modulators for *Mycoplasma pneumoniae* Pneumonia in Children: An Observation Study. Infection & Chemotherapy,*46*(4), 239. doi:10.3947/ic.2014.46.4.239

67. Akashi, Y., Hayashi, D., Suzuki, H., Shiigai, M., Kanemoto, K., Notake, S., . . . Imai, H. 2018. Clinical features and seasonal variations in the prevalence of macrolide-resistant *Mycoplasma pneumoniae*. Journal of General and Family Medicine,*19*(6), 191-197. doi:10.1002/jgf2.201

68. Guo, S., Bao, L., Qu, T., Mao, X., Gao, Y., Xu, Y., & Cui, X. 2018. Ameliorative Effects of Infantile Feire Kechuan Oral Solution on *Mycoplasma pneumoniae* Pneumonia in Infant Mouse and Rat Models. Evidence-Based Complementary and Alternative Medicine,*2018*, 1-10. doi:10.1155/2018/8139040

69. Bao, Y., Li, X., Wang, K., Zhao, C., Ji, X., & Jiang, M. 2016. Central retinal artery occlusion and cerebral infarction associated with *Mycoplasma pneumoniae* infection in children. BMC Pediatrics,*16*(1). doi:10.1186/s12887-016-0750-3

70. Cao, B., Qu, J., Yin, Y., & Eldere, J. V. 2015. Overview of antimicrobial options for *Mycoplasma pneumoniae* pneumonia: Focus on macrolide resistance. The Clinical Respiratory Journal,*11*(4), 419-429. doi:10.1111/crj.12379

71. Al-Zaidy, S. A., Macgregor, D., Mahant, S., Richardson, S. E., & Bitnun, A. 2015. Neurological Complications of PCR-Proven *M. pneumoniae* Infections in Children: Prodromal Illness Duration May Reflect Pathogenetic Mechanism. Clinical Infectious Diseases,*61*(7), 1092-1098. doi:10.1093/cid/civ473

72. Li, Q., Wu, Y., Sun, H., Gu, W., Zhang, X., Wang, M., . . . Chen, Z. 2019. The role of miR-29c/B7-H3/Th17 axis in children with *Mycoplasma pneumoniae* pneumonia. Italian Journal of Pediatrics,*45*(1). doi:10.1186/s13052-019-0655-5

73. Bajantri, B., Danial, S., Duncalf, R., & Khaja, M. 2017. Rare complicated parapneumonic effusion, *Mycoplasma pneumoniae* with new-onset lupus flare: Case report and literature review. Respiratory Medicine Case *Reports,22*, 287-291. doi:10.1016/j.rmcr.2017.10.010

74. Ito, Y., Iwashima, S., Hayano, S., Nishio, T., Shiozawa, R., Yata, S., . . . Uemura, K. 2018. Rapid Detection of the Macrolide Sensitivity of Pneumonia-Causing *Mycoplasma pneumoniae* Using Quenching Probe Polymerase Chain Reaction (GENECUBE®). Molecular Diagnosis & Therapy,*22*(6), 737-747. doi:10.1007/s40291-018-0360-x

المنارة للاستشارات

www.manaraa.com

75. Komatsu, H., Tsunoda, T., Inui, A., Sogo, T., & Fujisawa, T. 2014. Characteristics of hospitalized children infected with macrolide-resistant *Mycoplasma pneumoniae*. The Brazilian Journal of Infectious Diseases,*18*(3), 294-299. doi: 10.1016/j.bjid.2013.09.004

76. Yin, Y., Wang, R., Zhuo, C., Wang, H., Wang, M., Xie, C., . . . Liu, Y. 2017. Macrolide-resistant *Mycoplasma pneumoniae* prevalence and clinical aspects in adult patients with community-acquired pneumonia in China: A prospective multicenter surveillance study. Journal of Thoracic Disease,*9*(10), 3774-3781. doi:10.21037/jtd.2017.09.75

77. Schmidl, S. R., Otto, A., Lluch-Senar, M., Piñol, J., Busse, J., Becher, D., & Stülke, J. 2011. A Trigger Enzyme in *Mycoplasma pneumoniae*: Impact of the Glycerophosphodiesterase GlpQ on Virulence and Gene Expression. PLoS Pathogens, *7*(9), e1002263.

78. Saraya, T., Nakata, K., Nakagaki, K., Motoi, N., Iihara, K., Fujioka, Y., . . . Goto, H. 2011. Identification of a mechanism for lung inflammation caused by *Mycoplasma pneumoniae* using a novel mouse model. Results in Immunology,*1*(1), 76-87. doi: 10.1016/j.rinim.2011.11.001

79. Hon, K. L., Leung, A. S., Cheung, K. L., Fu, A. C., Chu, W. C., Ip, M., & Chan, P. K. 2014. Typical or atypical pneumonia and severe acute respiratory symptoms in PICU. The Clinical Respiratory Journal,*9*(3), 366-371. doi:10.1111/crj.12149

80. Spuesens, E. B., Fraaij, P. L., Visser, E. G., Hoogenboezem, T., Hop, W. C., Adrichem, L. N., . . . Rossum, A. M. 2013. Carriage of *Mycoplasma pneumoniae* in the Upper Respiratory Tract of Symptomatic and Asymptomatic Children: An Observational Study. PLoS Medicine,*10*(5). doi: 10.1371/journal.pmed.1001444

81. Großhennig, S., Ischebeck, T., Gibhardt, J., Busse, J., Feussner, I., & Stülke, J. 2016. Hydrogen sulfide is a novel potential virulence factor of *Mycoplasma pneumoniae*: Characterization of the unusual cysteine desulfurase/desulfhydrase HapE. Molecular Microbiology,*100*(1), 42-54. doi:10.1111/mmi.13300

82. Dutow, P., Schmidl, S., Ridderbusch, M., & Stülke, J. 2010. Interactions between Glycolytic Enzymes of *Mycoplasma pneumoniae*. J Mol Microbiol Biotechnol Journal of Molecular Microbiology and Biotechnology*,* 134-139. Retrieved September 29, 2015

83. Alrousan, E., & Fan, X. 2015. Glycerophosphorylcholine regulates *Haemophilus influenzae* glpQ gene expression. FEMS Microbiology Letters. Retrieved September 29, 2015

84. Chuang, Y.-P., Peng, Z.-R., Tseng, S.-F., Lin, Y.-C., Sytwu, H.-K., & Hsieh, Y.-C. 2015. Impact of the *glpQ2* Gene on Virulence in a *Streptococcus pneumoniae* Serotype 19A Sequence Type 320 Strain. Infection and Immunity,*83*(2), 682–692.

المنارة للاستشارات

www.manaraa.com

85. Yus, E., Maier, T.… 2009. Impact of Genome Reduction on Bacterial Metabolism and its Regulation, Science*, 326*, 1263.

86. Wodke, J. A. H., Puchałka, J., Lluch-Senar, M., Marcos, J., Yus, E., Godinho, M., … Maier, T. 2013. Dissecting the energy metabolism in *Mycoplasma pneumoniae* through genome-scale metabolic modeling. Molecular Systems Biology, *9*, 653.

87. Chuang, Y.-P., Peng, Z.-R., Tseng, S.-F., Lin, Y.-C., Sytwu, H.-K., & Hsieh, Y.-C. 2015. Impact of the *glpQ2* Gene on Virulence in a *Streptococcus pneumoniae* Serotype 19A Sequence Type 320 Strain. Infection and Immunity,*83*(2), 682–692.

88. Blötz, C., & Stülke, J. 2017. Glycerol metabolism and its implication in virulence in *Mycoplasma*. FEMS Microbiology *Reviews,41*(5), 640-652.

89. Klement, M. L. R., Öjemyr, L., Tagscherer, K. E., Widmalm, G., & Wieslander, Å. 2007. A processive lipid glycosyltransferase in the small human pathogen *Mycoplasma pneumoniae*: involvement in host immune response. Molecular Microbiology, *65*(6), 1444–1457. doi: 10.1111/j.1365-2958.2007.05865.x

90. Grembecka, M. 2015. Erratum to: Sugar alcohols—their role in the modern world of sweeteners: a review. European Food Research and Technology, *241*(1), 15–16. doi: 10.1007/s00217-015-2458-2

91. Ruiz-Ojeda, F. J., Plaza-Díaz, J., Sáez-Lara, M. J., & Gil, A. 2019. Effects of Sweeteners on the Gut Microbiota: A Review of Experimental Studies and Clinical Trials. Advances in Nutrition, *10*(suppl_1). doi: 10.1093/advances/nmy037

92. Ruskoné-Fourmestraux, A., Attar, A., Chassard, D., Coffin, B., Bornet, F., & Bouhnik, Y. 2003. A digestive tolerance study of maltitol after occasional and regular consumption in healthy humans. European Journal of Clinical Nutrition, *57*(1), 26–30. doi: 10.1038/sj.ejcn.1601516

93. Keijser, B. J. F., Broek, T. J. V. D., Slot, D. E., Twillert, L. V., Kool, J., Thabuis, C., Montijn, R. C. 2018. The Impact of Maltitol-Sweetened Chewing Gum on the Dental Plaque Biofilm Microbiota Composition. Frontiers in Microbiology, *9*. doi: 10.3389/fmicb.2018.00381

94. Fan, X., Pericone, C., Lysenko, E., Goldfine, H., & Weiser, J. 2003. Multiple mechanisms for choline transport and utilization in *Haemophilus influenzae*. Molecular Microbiology*, 50*(2), 537-548. doi:10.1046/j.1365-2958.2003. 03703.x

95. Salman, M., & Rottem, S. 1995. The cell membrane of *Mycoplasma penetrans*: Lipid composition and phospholipase A1 activity. Biochimica Et Biophysica Acta (BBA) - Biomembranes*, 1235*(2), 369-377. doi:doi:10.1016/0005-2736(95)80026-C

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FIGURES & DATA [APPENDIXES]

Day	Fructose 1	Fructose 2	Fructose 3	Mannose 1	Mannose 2	Mannose 3	Rhamnose 1	Rhamnose 2	Rhamnose 3
0	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001
2	0.00150	0.00160	0.00130	0.00140	0.00120	0.00170	0.0035	0.0028	0.0027
4	0.00470	0.00810	0.00390	0.00640	0.00760	0.00710	0.0030	0.0042	0.0040
6	0.00128	0.00231	0.00176	0.00119	0.00173	0.00150	0.0032	0.0038	0.0034

Day	Sorbose 1	Sorbose 2	Sorbose 3	Galactose 1	Galactose 2	Galactose 3	Fucose 1	Fucose 2	Fucose 3
0	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001
2	0.0040	0.0026	0.0029	0.0018	0.0023	0.0044	0.0031	0.0028	0.0041
4	0.0068	0.0070	0.0064	0.0053	0.0039	0.0072	0.0042	0.0046	0.0080
6	0.0070	0.0072	0.0069	0.0060	0.0067	0.0070	0.0053	0.0058	0.0044

Day	Glucose 1	Glucose 2	Glucose 3	No Carbon Source 1	No Carbon Source 2	No Carbon Source 3
0	0.001	0.001	0.001	0.001	0.001	0.001
2	0.0030	0.0021	0.0013	0.0013	0.0027	0.0019
4	0.0752	0.0524	0.0494	0.0027	0.0044	0.0037
6	0.1245	0.0737	0.0813	0.0051	0.0053	0.0049

S1: List of trials and the numerical data for the Hexose sugars tested at OD_{550} in Figure 5.

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Day	Arabinose 1	Arabinose 2	Arabinose 3	Xylose 1	Xylose 2	Xylose 3
0	0.001	0.001	0.001	0.001	0.001	0.001
2	0.0018	0.0022	0.0015	0.0010	0.0010	0.0040
4	0.0053	0.0048	0.0050	0.0030	0.0040	0.0060
6	0.0075	0.0074	0.0070	0.0090	0.0050	0.0090

Day	Ribose 1	Ribose 2	Ribose 3	No Carbon Source 1	No Carbon Source 2	No Carbon Source 3
0	0.001	0.001	0.001	0.001	0.001	0.001
2	0.0015	0.0047	0.0045	0.0013	0.0027	0.0019
4	0.0032	0.0069	0.0072	0.0027	0.0044	0.0037
6	0.0039	0.0084	0.0082	0.0051	0.0053	0.0049

S2: List of trials and numerical data for Pentose sugars tested at OD_{550} in Figure 6.

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Day	Dulcitol 1	Dulcitol 2	Dulcitol 3	Inositol 1	Inositol 2	Inositol 3	Maltitol 1	Maltitol 2	Maltitol 3
0	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001
2	0.01070	0.00730	0.00190	0.00890	0.00280	0.00250	0.0042	0.0079	0.0031
4	0.01170	0.00730	0.00600	0.00550	0.00170	0.00590	0.0092	0.0216	0.0183
6	0.00860	0.00710	0.00910	0.00174	0.01410	0.00850	0.0218	0.0470	0.0475

Day	Mannitol 1	Mannitol 2	Mannitol 3	Sorbitol 1	Sorbitol 2	Sorbitol 3	Xylitol 1	Xylitol 2	Xylitol 3
0	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001
2	0.0078	0.0061	0.0025	0.0055	0.0054	0.0033	0.0023	0.0018	0.0023
4	0.0090	0.0076	0.0079	0.0079	0.0082	0.0076	0.0021	0.0048	0.0070
6	0.0064	0.0079	0.0102	0.0058	0.0040	0.0095	0.0018	0.0032	0.0060

Day	Glycerol 1	Glycerol 2	Glycerol 3	Glucose 1	Glucose 2	Glucose 3	No Carbon Source 1	No Carbon Source 2	No Carbon Source 3
0	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001
2	0.00550	0.00640	0.00660	0.0030	0.0021	0.0013	0.0013	0.0027	0.0019
4	0.01560	0.01580	0.01550	0.0752	0.0524	0.0494	0.0027	0.0044	0.0037
6	0.01820	0.01750	0.01740	0.1245	0.0737	0.0813	0.0051	0.0053	0.0049

S3: List of trials and numerical data for Alcohol sugars tested at OD_{550} in Figure 7.

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