MYCOPLASMOLOGY

Meghan May, Amanda V. Arjoon, Jessica A. Canter, Dylan W. Dunne and **Brittney Terrell**
Towson University, United States of America.

Keywords: Mycoplasma, Ureaplasma, mycoplasmosis, atypical bacteria, pleuropneumonia-like organisms, contagious bovine pleuropneumonia, walking pneumonia, nongonococcal urethritis, synthetic biology, minimal genome concept

Contents

Summary

Members of the genus *Mycoplasma* and the class *Mollicutes* are highly unusual organisms in that they lack a cell wall, are orders of magnitude smaller than most known bacteria, and have minimalist genomes. This lineage of bacteria has undergone intensive degenerate evolution, and as such, all species are obligate parasites that must live in association with other organisms. Most species are commensals or causes of minor infections, but a small number are major pathogens that contribute to substantial economic losses relating to both human and animal infections. Finally, the reduced genome size of *Mollicutes* made them excellent targets for pioneering work in genome sequencing projects, attempts to define the minimal genome required for life, and the creation of the first known self-replicating synthetic cells. This chapter represents an overview of the cellular biology, clinical diseases, and societal impacts of mycoplasmas.

1. Introduction to Mycoplasmology

1.1. History of Mycoplasmology

The first clinically recognized mycoplasmosis, contagious bovine pleuropneumonia (CBPP), was caused by the first mycoplasma to be grown in culture, *Mycoplasma mycoides* subspecies *mycoides* (formerly termed "variant small colony (SC)"). The genus *Mycoplasma* was given its name in 1956, long after the first recognition of mycoplasmosis. This delay in apt nomenclature is highly atypical and stemmed from an inability to resolve many seemingly paradoxical findings. The causative agent of CBPP could pass through filters typically used to remove bacterial cells and could not be viewed by light microscopy.

Conversely, this entity was able to grow in the absence of eukaryotic cells, yet seemed to require several eukaryotic cell components for viability. *Mycoplasma mycoides* displayed characteristics of both bacteria and viruses, and as such it was the first of a short-lived category of pathogens, the pleuropneumonia-like organisms (PPLOs).

Multiple theories surfaced regarding the identity and origin of mycoplasmas. Kleineberger *et al*. suggested that mycoplasmas were L-forms (L-phase variants) (Klieneberger, 1939), a notion that was later refuted by Razin *et al.* in 1969. The contradictory work demonstrated that PPLOs seemed to have smaller genomes and lower guanine+cytosine (G+C) contents than those of L-forms, which retain the traits of their parent species (Razin, 1969). As evidence mounted that mycoplasmas were a unique and simplistic variety of bacteria, the proposition was made that they were the progenitors of more complex bacteria (Wallace and Morowitz, 1973).

The confusion recurred when the causative agent of human primary atypical pneumoniae (PAP) was cultured in chicken embryos and fulfilled Koch's postulates by producing disease in hamsters and rats (Meiklejohn et al, 1945). As the infectious agent was filterable, could not be visualized by light microscopy, and was resistant to penicillin, the authors concluded that it was an unknown virus, and termed it Eaton's agent. Attempts to culture Eaton's agent in PPLO medium were unsuccessful, adding to the notion that it was a viral agent. Subsequent studies showed sensitivity to other antibacterial compounds (Eaton and Liu, 1957; Eaton et al, 1951), and eventually growth in an alternate cell-free medium. Eaton's agent was reclassified and renamed *Mycoplasma pneumoniae* (Chanock, 1963).

The complex and textured nature of the history of mycoplasmas is easily understood in retrospect. It is now clear why these bacteria displayed certain characteristics normally associated with viruses. Two such characteristics, the observations that the agents of CBPP and PAP could pass through filters and could not be visualized by light microscopy, is due to the currently known size of mycoplasmas. Copious studies using electron microscopy to visualize mycoplasmas demonstrated that they are 200-500 nm; making them more comparable in size to viruses than other bacteria.

The observation by Meiklejohn *et al.* that the agent of PAP could grow in chicken embryos in the presence of penicillin despite being bacterial (Meiklejohn et al, 1945) is

also explainable by subsequent data. The antimicrobial mechanism of penicillin involves breaking down the peptidoglycan layer of the bacterial cell wall (Fisher, 1946). It became clear 15 years after the initial description of Eaton's agent that mycoplasmas lack cell walls, and are enclosed only by a sterol-containing cell membrane. Such organisms would obviously have an intrinsic resistance to penicillin.

The final observations used to conclude that the agent of PAP was caused by a virus were the growth of the agent in chicken embryos and lack of viability of the agent in cell-free culture. Culture media used in an attempt to cultivate Eaton's agent included the medium intended to grow PPLOs (developed for growth of *M. mycoides*), which was considerably more rich than typical bacterial growth media. Eaton's agent required eukaryotic cells, or so it seemed.

The cultivation of Eaton's agent by Chanock *et al.* in 1960 (Chanock et al, 1960) in a cell-free medium (PPLO medium supplemented with horse serum and yeast extract, now known as Hayflick's medium) led to the recognition of this agent as a mycoplasma. Though the newly-named *M. pneumoniae* cells were free-living, self-replicating entities, it was clear that they were heavily reliant on eukaryotic components for growth. It was proposed that mycoplasmas, though bacterial rather than viral pathogens, were prone to parasitism.

1.2. Evolution and Taxonomy

Mycoplasmas fall within the class *Mollicutes* (phylum *Tenericutes*). As there are more than 160 distinct *Mycoplasma* species and 8 distinct *Ureaplasma* speciesaccording the Bergey's Manual of Systematic Bacteriology (Brown et al, 2010), further classification of the *Mycoplasmataceae* was undertaken. *Mycoplasma* species are subdivided into groups, and then into clusters. Phylogenitc analysis based on the 16S rRNA gene clearly indicates that the *Mollicutes* belong to the clade of gram-positive bacteria despite lacking a cell wall.

Mollicutes have undergone extensive reductive evolution enabled by living in associated with eukaryotic hosts, and thus have very small genome sizes. Genetic material is lost through obsolescence as bacteria adapt to living in conjunction with a host, either parasitically or mutualistically. At present, the complete genomes of thirty *Mollicutes* (twenty-seven mycoplasmas) have been sequenced (see Table 1).

An examination of these genomes reveals, among many other things, that mycoplasmas lack biosynthetic capabilities, thus explaining their extreme host dependence. In addition to earlier studies contradicting the notion of mycoplasmas being the progenitors of more complex bacteria (Neimark, 1967; Neimark and Pene, 1965; Woese et al, 1980), the complete genomes clearly indicate that these species have evolved degenerately.

***Mollicutes* species**	**Strain**	**Genome Size (MB)**	**%G+C**	**Predicted Open Reading Frames**	**Type Strain[a]**	**Reference**
M. agalactiae	PG2	0.88	29%	742	Yes	Sirand-Pugnet *et al*, 2010
M. alligatoris	A21JP2	0.97	27%	839	Yes	Brown *et al*, 2011
M. arthritidis	158L3-1	0.82	30%	631	No	Dybvig *et al*, 2008
M. bovis[b]	PG45	1.0	29%	868	Yes	Wise *et al*, 2011
M. capricolum sbsp *capricolum*[b]	California kid	1.0	23%	812	Yes	Glass *et al*, 2007
M. capricolum sbsp *capripneumoniae*	M1601	1.01	23%	875	No	Chu *et al*, 2011
M. conjunctivae	HRC/581	0.85	28%	727	Yes	Calderon-Copete *et al*, 2009
M. crocodyli	MP145	0.93	26%	763	Yes	Brown *et al*, 2011
M. fermentans[b]	JER	0.98	27%	835	No	Shu *et al*, 2011
M. gallisepticum[b]	R	0.996	31%	726	No	Papazisi *et al*, 2003
M. genitalium[b]	G37	0.58	31%	475	Yes	Fraser *et al*, 1995
M. haemofelis[b]	Langford 1	1.15	39%	1545	NA	Barker *et al*, 2011
M. hominis	PG21	0.67	27.1%	537	Yes	Pereyre *et al*, 2009
M. hyopneumoniae[b]	J	0.90	28%	657	No	Vasconcelos *et al*, 2005
M. hyorhinis[b]	HUB-1	0.84	26%	654	No	Liu *et al*, 2010
M. leachii	PG50	1.01	24%	925	Yes	Wise *et al*, 2012
M. mobile	163K	0.78	25%	633	Yes	Jaffe *et al*, 2004
M. mycoides sbsp *capri*	GM12	1.09	24%	911	Yes	Wise *et al* 2006
M. mycoides sbsp *mycoides*[b]	PG1	1.21	24%	1016	Yes	Westberg *et al*, 2004
M. ovipneumoniae	SC01	1.02	29%	864	No	Yang *et al*, 2011
M. penetrans	HF-2	1.36	26%	1037	Yes	Sasaki *et al*, 2002
M. pneumoniae[b]	M129	0.82	40%	689	No	Himmelreich *et al*, 1996

M. pulmonis	UAB CTIP	0.96	26%	782	No	Chambaud *et al*, 2001
M. putrefaciens	KS1	0.83	27%	725	Yes	Calcutt and Foecking, 2011
M. suis[b]	Illinois	0.74	31%	783	NA	Messick *et al*, 2011
M. synoviae	53	0.80	28%	659	No	Vasconcelos *et al*, 2005
Acholeplasma laidlawii	PG-8A	1.5	31%	1433	Yes	Lazarev *et al*, 2011
Candidatus Phytoplasma asteris[b]	AYWB	0.71	26%	671	NA	Bai *et al*, 2009
Mesoplasma florum	L1	0.79	27%	687	Yes	Knight *et al*, 2004
Ureaplasma parvum[b]	700970	0.75	25%	653	No	Glass *et al*, 2000
Ureaplasma urealyticum[b]	33699	0.87	26%	695	No	Shrivastava *et al*, 2012

NOTE: This table is current as of this publication. Additional genome sequencing projects are currently ongoing, and thus this table is by its nature likely to be incomplete

[a] NA = not applicable, due to non-culturable status of the species

[b] Data for additional strains or serovars have also been reported

Table 1. Characteristics of Sequenced Mollicutes Genomes

1.3. Metabolism and Cell Biology

Mycoplasma cells are highly pleiomorphic due to the lack of a cell wall, and form colonies with "fried egg" morphology when grown on solid agar. A minority of species exhibit polarity due to internal cytoskeletons (Fig. 1, Fig. 2).

These structures seem to have arisen from multiple distinct evolutionary events based on the dissimilarity of the proteins composing and regulating them, and the function of the polar structure. In several species these structure are associated with cell motility, cytadherence, or both (Balish and Krause, 2006). The cellular machinery used for gliding motility differs between species depending on the composition of the cytoskeleton; similarly, the mechanism(s) for cytadherence and the target of cell attachment vary grately by species.

Mycoplasmas have limited capacity for metabolic function, lacking all anabolic and many metabolic and catabolic pathways. The functions that remain are involved in energy generation rather than biomolecule synthesis, as they have evolved mechanisms to rely on their hosts for the latter. Pathways for energy generation are also minimal, as mycoplasmas lack the machinery necessary for the tricarboxylic acid cycle and other mediators of oxidative phosphorylation (Razin et al, 1998). *Mollicutes* have been

demonstrated to catabolize a limited number of sugars, arginine, organic acids, urea, and alcohols for energy generation.

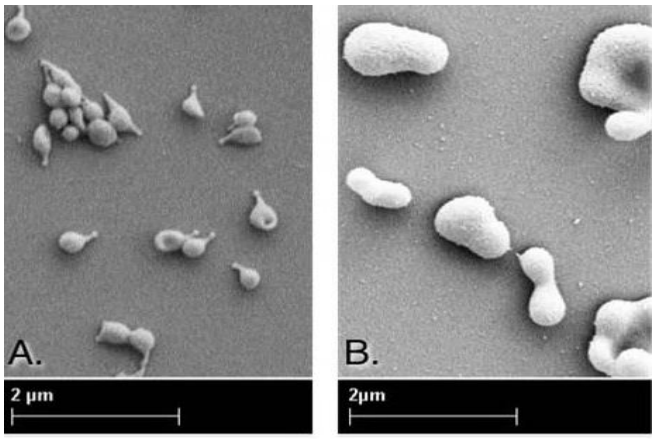

Figure 1. *Mycoplasma* Cellular Morphology. Most *Mycoplasma* cells are highly pleiomorphic due to the lack of a cell wall, and are exemplified here by *Mycoplasma gallinarum* (**A**). A minority of species exhibit polarity due to internal structures, represented by *Mycoplasma genitalium* (**B**). Scanning electron microscopy images are presented courtesy of M. F. Balish.

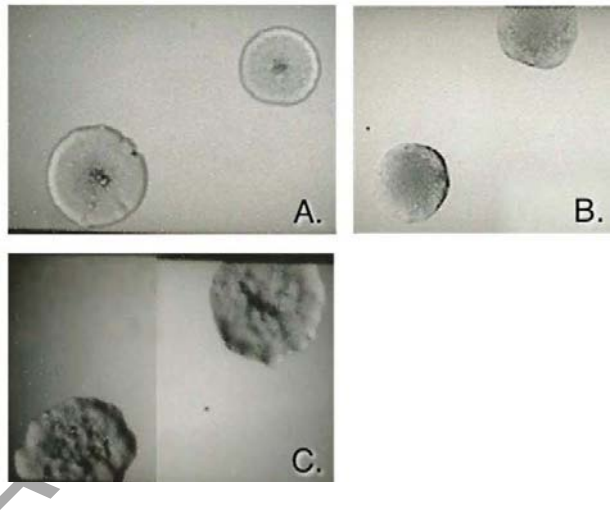

Figure 2. *Mycoplasma* Colony Morphology. The classical appearance of *Mycoplasma* colonies on solid agar are said to resemble fried eggs, as exemplified by *Mycoplasma anatis* (**A**). Some species have an umbonate (*Mycoplasma canis*, **B**) or granular (uncharacterized *Mycoplasma* species, **C**) appearance. Colonies images are presented courtesy of D.R. Brown.

Acid-producing mycoplasmas generate much of their energy from glycolysis primarily via glucose utilization, although additional complex glycans can be catabolized by certain species (Abu-Groun et al, 1994; Dahl and Dahl, 1984; Matsuda et al, 1997; Miles et al, 1986). The byproducts of these pathways result in the accumulation of acidic compounds, which results in the characteristic downward pH shift of the culture

medium of these organisms (May et al, 2008). Some mycoplasmas hydrolyze arginine as a means of energy generation.

This occurs through the arginine dihydrolase pathway, which requires only three enzymes. Hydrolysis of arginine results in the accumulation of ammonia, resulting in the characteristic upward pH shift of the culture medium. Some species, such as *M. fermentans*, are capable of utilizing both sugars and arginine as carbon sources (Brown et al, 2010; Brown et al, 2010). Culture medium pH shifts can be used diagnostically as an adjunct to additional techniques (May et al, 2008).

A relatively small number of mycoplasmas have been shown to catabolize organic acids and alcohols as carbon sources. These less common carbon sources include lactic acid, pyruvic acid, and oxobutyric acid, and ethanol, isopropanol, and glycerol (Khan et al, 2005; Razin et al, 1998; Taylor et al, 1994). The catabolism of glycerol leads to the production of hydrogen peroxide, which is now considered to be a component of the virulence of multiple species (Megid et al, 2001; Niang et al, 1998; Pilo et al, 2005).

Certain *Mollicutes* rely on more complex sources of energy as either a primary or secondary source. The hydrolysis of urea for energy has only been observed for species of *Ureaplasma*. Urease inhibitors are toxic to ureaplasmas, indicating that urea may act as a sole a carbon source (Razin, 1978; Romano et al, 1980; Smith et al, 1993). In addition, the potential exists for sialic acid, hyaluronic acid, N-acetylglucosamine, and glucuronic acid to serve as nutritional supplements in the small number of species with genes for catabolism of these glycans (Brown et al, 2004; May and Brown, 2008; Vasconcelos et al, 2005). Experimental confirmation of this utilization remains an ongoing field of study.

The complete lack of anabolic pathways in mycoplasmas is compensated for by an overrepresentation of transport-associated proteins, most commonly ATP-binding cassette transporters (ABC transporters). These integral membrane proteins are highly promiscuous transporters that facilitate the uptake of biomolecules (Saurin and Dassa, 1996). Sugar phosphotransferase transport systems (PTSs) are also found in fermentative *Mycoplasma* species (Razin et al, 1998). It is likely that multiple other transport mechanisms exist to compensate for the limited biosynthetic capabilities of mycoplasmas.

1.4. Molecular Biology of Mycoplasmas

A major hurdle that has yet to be overcome is the limited ability at present to genetically manipulate mycoplasmas. With notable exceptions, mycoplasmas do not appear to harbor naturally occurring self-replicating plasmids, and attempts to transform them with commercial cloning vectors have failed. Success with targeted disruption of genes in order to determine their function has been rare for all species but *Mycoplasma genitalium*. Gene and protein function in mycoplasmas, much like any other species, is most often determined by examining deletion mutants. Mutants are typically obtained by random insertion of transposons such as *Tn*916 or *Tn*4001 (Dybvig and Cassell, 1987; Mahairas and Minion, 1989). Several modifications (Table 2) have since been

made to *Tn*4001 (Hahn et al, 1999; Knudtson and Minion, 1993; Pour-El et al, 2002), rendering it one of the most widely used molecular tools in mycoplasmology.

Transposon	Origin	Resistance Marker	Reporter Gene	Notes/Distinctions
*Tn*916	*Streptococcus*	Tetracycline	None	Large; inefficient; first mycoplasma transpositions
*Tn*4001	*Staphylococcus*	Gentamycin	None	Efficient; more widely used; replaced *Tn*916
*Tn*4001*mod*	*Staphylococcus Tn*4001	Gentamycin	None	Multiple cloning site; gene delivery and expression
*Tn*4001*cat*	*Staphylococcus Tn*4001	Chloramphenicol	None	Multiple cloning site; alternative resistance
Mini-*Tn*4001*tet*	*Staphylococcus Tn*4001	Tetracycline	None	Multiple cloning site; alternative resistance; limited mobility
*Tn*4001*lac*	*Staphylococcus Tn*4001	Gentamycin	β-galactosidase	Promoterless *lacZ*; assessment of promoter strengths

Table 2. Characteristics of Commonly Used Transposons in *Mycoplasma* spp.

Because mycoplasmas do not successfully carry commercial plasmid vectors, artificial plasmids containing the chromosomal origin of replication have been developed and are termed OriC plasmids (Renaudin and Lartigue, 2005). Distinct vectors must be generated for each species. Successful expression of genes has been described for *Spiroplasma citri*, *Mycoplasma pulmonis*, *Mycoplasma mycoides* subspecies *capri*, *M. mycoides* subspecies *mycoides*, *M. capricolum*, *M. gallisepticum*, and *M. agalactiae* (Chopra-Dewasthaly et al, 2005; Cordova et al, 2002; Renaudin and Lartigue, 2005; Renaudin et al, 1995; Ye et al, 1994). In addition, artificial plasmid vectors have been made based on sequences of integrative conjugal elements (ICE), which are found naturally occurring both intra- and extrachromosomally in certain *Mycoplasma* species (Brown et al, 2010). A small number of *Mollicutes* species (*Mycoplasma arthritidis*, *Mycoplasma pulmonis*, *Mycoplasma fermentans*, and multiple *Spiroplasma* species) are hosts to bacteriophage (Dybvig and Cassell, 1987; Röske et al, 2004; Voelker et al, 1995). None of the bacteriophage infecting mycoplasmas has been successfully used to deliver genes via transduction. Only the virus SpV1, that infects *Spiroplasma citri*, has been successful (Marais et al, 1993; Stamburski et al, 1991). A factor complicating the cloning and expression of mycoplasmal genes in other bacteria is the usage of the UGA codon to encode tryptophan, making it difficult to express their genes in other bacteria without altering the gene first. Strides have been made in recent years toward expanding the repertoire of molecular tools available for use in mycoplasmas. Commonly used molecular tools are presented in Table 3.

Though the isolation of the first PPLO, much later named *M. mycoides*, occurred fairly early in the discipline of medical microbiology, the implications of the findings were not appreciated until much later. The eventual realization that these pathogens, which blurred the contemporary line between bacteriology and virology, were minimalist bacteria led to the powerful suggestion that they preceded complex life. The observation

that mycoplasmas were parasitic naturally begged the question of how they could possibly have been progenitors. Such queries led to the notion that bacteria can adapt to their niches in a reductive fashion, an important concept in pathogen evolution.

Tool	Description	Uses	Features
Transposons	See Table 2	Gene disruption, gene expression	Random insertion, *in trans* expression
OriC Plasmids	Commercial vector + species origin of replication	Gene expression, homologous recombination	Direct cassette insertion, *in cis* expression
ICE vectors	Commercial vector + mycoplasmal ICE element	Gene expression, homologous recombination	Direct cassette insertion, *in cis* expression
psPuro	*Tn*4001 mini Tet with *pac* gene	Gene disruption, gene expression	Random insertion, *in trans* expression, puromycin selection
Genome Synthesis	*In vitro* genesis of designed chromosome	Generation of artificial chromosome	Specific genome design
Genome Transplantation	Insertion of chromosome into an existing membrane	Expression of artificial or heterologous chromosome	Synthetic cell generation, species reassignment

Table 3. Molecular Tools Used in Mycoplasmology

2. Clinical Manifestations of Mycoplasmosis

Infections with *Mycoplasma* species exhibit a spectrum of clinical manifestations ranging from asymptomatic states to fulminant inflammatory diseases with high mortality rates. Despite these extremes, the classical manifestation (without clinical intervention) is a chronic inflammatory illness that is not typically fatal, though this is the normal state for a small number of mycoplasma such as *M. alligatoris* and *M. mycoides* subsp. *mycoides*. Often these infections are compounded by secondary pathogens, resulting in more severe disease with higher rates of morbidity and mortality. The primary sites of most mycoplasmal infections are mucosal surfaces. The establishment of infection is mediated by attachment to the epithelium, where they exist as surface pathogens. Additionally, mounting evidence suggests that multiple species have invasive capabilities and that intracellular phases may be part of the infectious process (Andreev et al, 1995; Dallo and Baseman, 2000; Díaz-García et al, 2006; Jensen et al, 1994; Tarshis et al, 2004; Taylor-Robinson et al, 1991; Winner et al, 2000; Yavlovich et al, 2004). As highly adapted parasites, mycoplasmas tend to exhibit host specificity and tissue tropism, although exceptions to both rules have been extensively documented. The number of distinct *Mycoplasma* species is large, and as such, detailed clinical descriptions of the disease state of each are not provided. Tables 4-6 describe mycoplasmosis of humans and animals.

2.1. Human Disease

Community-acquired pneumonia and other manifestations of M. pneumoniae

Clinical manifestations of upper respiratory tract infections by *M. pneumoniae* most commonly include pharyngitis and laryngitis, and those of the lower respiratory tract include tracheitis and interstitial pneumonia. The latter is clinically referred to as "community-acquired pneumonia" in addition to PAP, and commonly referred to as "walking pneumonia". Symptoms include low-grade fever, non-productive cough, and chest pain. Community-acquired pneumonia outbreaks are frequently associated with close quarters and stress, and as such are often associated with military barracks or student dormitories. Post-infectious complications following community-acquired pneumonia disease can include the development or exacerbation of asthma and secondary ear infections, and rarely Guillain-Barré syndrome, Stevens-Johnson syndrome, Bell's Palsy, optic neuritis, or demyelinating disorders. Atypical manifestations of *M. pneumoniae* infections are depicted in Figure 3.

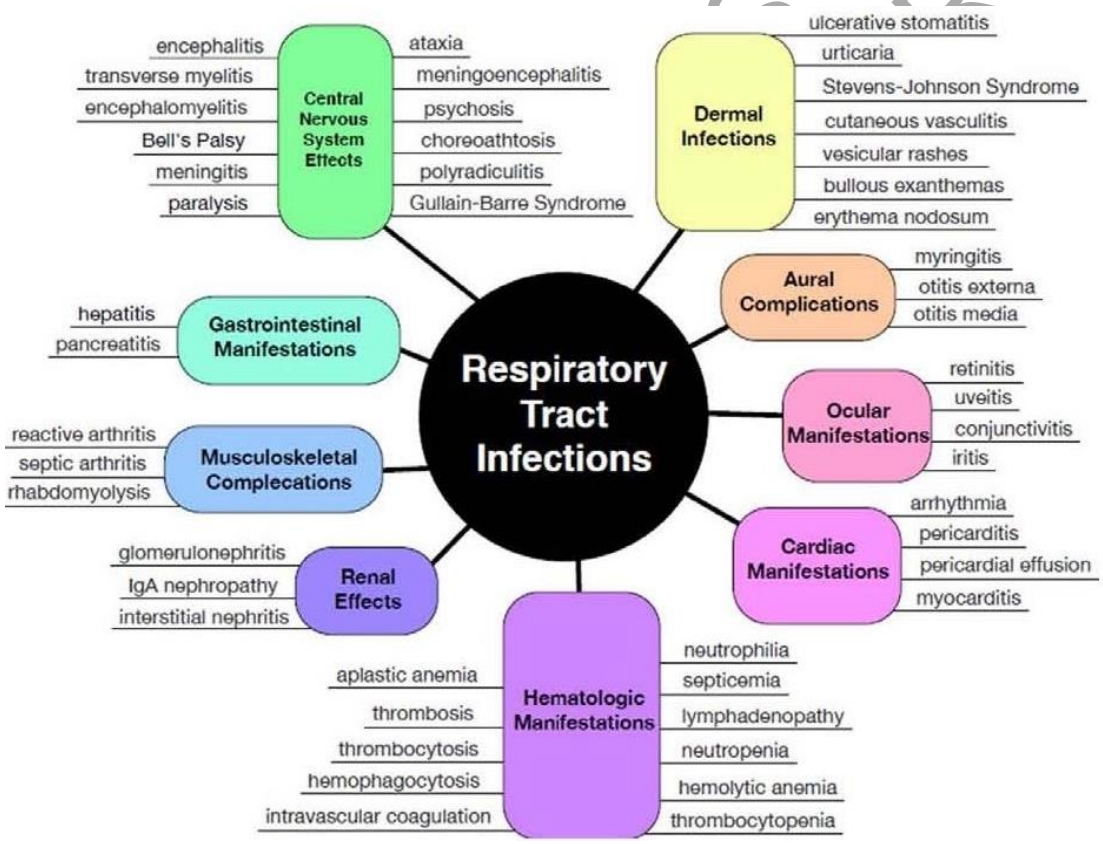

Figure 3. Atypical Manifestations of *Mycoplasma pneumoniae* Infection. The primary clinical presentation of *M. pneumoniae* infection is primary atypical pneumonia (community-acquired pneumonia); however, many additional manifestations have been described.

Sexually-acquired mycoplasmosis

Urogenital infection with *M. genitalium*, *M. hominis*, and *Ureaplasma* species can be detected in asymptomatic patients; however, numerous studies have explored the correlation of patient infection with several urogenital lesions. The clinical states

associated with *Ureaplasma* spp. and *M. hominis* appear to be strain- or patient-specific, and can include nongonococcal urethritis (*Ureaplasma* spp.), bacterial vaginosis (*M. hominis*), spontaneous abortion, or preterm labor (both organisms) (Waites and Talkington, 2005). The role of *M. genitalium* in nongonococcal urethritis, pelvic inflammatory disease, spontaneous abortion, and infertility is much less ambiguous, and it is consequently considered an emerging urogenital pathogen (McGowin and Anderson-Smits, 2011). Infection with *M. genitalium* has been associated with increased shedding of human immunodeficiency virus (HIV) particles in one study (Manhart et al, 2008), but did not appear to influence shedding rates in another (Gatski et al, 2011). Because of the enormous public health consequences involved, the notion that *M. genitalium* may facilitate HIV transmission requires further study.

Infection of pregnant women with *Ureaplasma* spp. has been associated with preterm labor and premature rupture of membranes. Disruption of the physical barrier between organisms present in the vagina and a perinatal infant can result in infection of the respiratory tract or central nervous system of the infant. Congenital and neonatal pneumonia is a grave concern of preterm infants, and *Ureaplasma* spp. have been included as causal agents. Chronic lung conditions can persist in these infants following the resolution of infection. *Ureaplasma* and *M. hominis* have also been implicating in neonatal meningitis following premature rupture of the membranes (Washburn et al, 1980).

-

-

-

Bibliography

1. Abu-Groun, E.A., Taylor, R.R., Varsani, H., Wadher, B.J., Leach, R.H., and Miles, R.J. (1994) Biochemical diversity within the "Mycoplasma mycoides" cluster. *Microbiology* 140 (Pt 8), 2033-2042 [This article describes phenotypic diversity within the Mycoides Cluster and may be helpful with diagnostics] .

2. Al-Daccak, R., Mehindate, K., Hébert, J., Rink, L., Mecheri, S., and Mourad, W. (1994) Mycoplasma arthritidis-derived superantigen induces proinflammatory monokine gene expression in the THP-1 human monocytic cell line. *Infect Immun* 62, 2409-2416 [This article describes immunopathology associated with mycoplasmal superantigens].

3. Aldridge, K.E., Cole, B.C., and Ward, J.R. (1977) Mycoplasma-dependent activation of normal lymphocytes: induction of a lymphocyte-mediated cytotoxicity for allogeneic and syngeneic mouse target cells. *Infect Immun* 18, 377-385 [This article describes immunopathology associated with mycoplasmosis].

4. Almeida, R.A., Wannemuehler, M.J., and Rosenbusch, R.F. (1992) Interaction of Mycoplasma dispar with bovine alveolar macrophages. *Infect Immun* 60, 2914-2919 [This article describes attachment and adherence of *M. dispar*].

5. Alvarez, R.A., Blaylock, M.W., and Baseman, J.B. (2003) Surface localized glyceraldehyde-3-phosphate dehydrogenase of Mycoplasma genitalium binds mucin. *Mol Microbiol* 48, 1417-1425 [This article describes attachment and adherence of *M. genitalium* to the glycoprotein mucin, which may aid in immune evasion].

6. Andreev, J., Borovsky, Z., Rosenshine, I., and Rottem, S. (1995) Invasion of HeLa cells by Mycoplasma penetrans and the induction of tyrosine phosphorylation of a 145-kDa host cell protein. *FEMS Microbiol Lett* 132, 189-194 [This article describes attachment and invasion of *M. penetrans* to cultured cells].

7. Asai, T., Okada, M., Yokomizo, Y., Sato, S., and Mori, Y. (1996) Suppressive effect of bronchoalveolar lavage fluid from pigs infected with Mycoplasma hyopneumoniae on chemiluminescence of porcine peripheral neutrophils. *Vet Immunol Immunopathol* 51, 325-331 [This article describes immunopathology associated with porcine enzootic pneumonia].

8. Athanasopoulos, A.N., Economopoulou, M., Orlova, V.V., Sobke, A., Schneider, D., Weber, H., Augustin, H.G., Eming, S.A., Schubert, U., Linn, T., Nawroth, P.P., Hussain, M., Hammes, H.P., Herrmann, M., Preissner, K.T., and Chavakis, T. (2006) The extracellular adherence protein (Eap) of Staphylococcus aureus inhibits wound healing by interfering with host defense and repair mechanisms. *Blood* 107, 2720-2727 [This article describes the inhibition of innate immune and repair mechanisms by *S. aureus*].

9. Aurora, A.B., Baluk, P., Zhang, D., Sidhu, S.S., Dolganov, G.M., Basbaum, C., McDonald, D.M., and Killeen, N. (2005) Immune complex-dependent remodeling of the airway vasculature in response to a chronic bacterial infection. *J Immunol* 175, 6319-6326 [This article describes immunopathology associated with chronic, community-acquired pneumonia].

10. Balish, M., and Krause, D. (2006) Mycoplasmas: a distinct cytoskeleton for wall-less bacteria. *J Mol Microbiol Biotechnol* 11, 244-255 [This review article discusses the components, function, and evolution of the mycoplasmal cytoskeleton. Cytoskeletons are features normally seen in Eukaryotes, and rarely seen in bacteria].

11. Baranowski, E., Guiral, S., Sagné, E., Skapski, A., and Citti, C. (2010) Critical role of dispensable genes in Mycoplasma agalactiae interaction with mammalian cells. *Infect Immun* 78, 1542-1551 [This article describes a broad survey for genes involved in host-pathogen interaction of *M. agalactiae*, and focuses attention on genes required for these interaction that are not known to be essential for growth in broth culture].

12. Barile, M.F., Chandler, D.K., Yoshida, H., Grabowski, M.W., and Razin, S. (1988) Hamster challenge potency assay for evaluation of Mycoplasma pneumoniae vaccines. *Infect Immun* 56, 2450-2457 [This article describes an animal model trial of a candidate vaccine against *M. pneumoniae*-associated community acquired pneumonia].

13. Bébéar, C.M., and Kempf, I.. (2005) Antimicrobial Therapy and Antimicrobial Resistance. In A. Blanchard, and G. Browning (Eds.), *Mycoplasmas: Molecular Biology, Pathogenicity, and Strategies for Control* (pp. 439-483). Norfolk, UK: Horizon Bioscience [This textbook chapter is a broad review of know patterns of successful antibiotic therapies against human and animal mycoplasmosis, and patterns and mechanisms of intrinsic resistance].

14. Behrens, A., Heller, M., Kirchhoff, H., Yogev, D., and Rosengarten, R. (1994) A family of phase- and size-variant membrane surface lipoprotein antigens (Vsps) of Mycoplasma bovis. *Infect Immun* 62, 5075-5084 [This article describes a multigene family associated with immune evasion in *M. bovis*].

15. Biberfeld, G. (1979) Autoimmune reactions associated with Mycoplasma pneumoniae infection. *Zentralbl Bakteriol Orig A* 245, 144-149[This review article describes immunopathology associated with *M. pneumoniae* infection due to induced anti-self reactions].

16. Binder, A., and Kirchhoff, H. (1991) Effects of active and passive immunization in the Mycoplasma arthritidis infection of rats. *Zentralbl Bakteriol* 275, 241-247 [This article describes vaccine strategies (inducing immunity vs. transferring antibodies) effective against the rodent pathogen *M. arthiritidis*].

17. Bower, K., Djordjevic, S.P., Andronicos, N.M., and Ranson, M. (2003) Cell surface antigens of Mycoplasma species bovine group 7 bind to and activate plasminogen. *Infect Immun* 71, 4823-4827 [This article describes attachment and modulation of the host clotting factor plasminogen, the inappropriate activation of which has implications for wound healing, tissue repair, and clot formation, by *M. leachii*].

18. Brown, D. R., May, M., Bradbury, J. M., Balish, M. F., Calcutt, M. J., Glass, J. I., Tasker, S., Messick, J.B., Johansson, K., and Neimark, H. (2010) Genus I. *Mycoplasma*. In N. R. Krieg, J. T. Staley, B. Hedlund, B. J. Paster, N. Ward, W. Ludwig, and W. B. Whitman (Eds.), *Bergey's Manual of Systematic Bacteriology* (Vol. 4) (2nd ed.). New York, NY: Springer [This textbook chapter is the validated, legitimate description of the genus *Mycoplasma* and all of the member species. This work carries the taxonomic authority of the Bergey's Trust, and is consistent with the rulings of the International Committee for the Systematics of Prokaryotes].

19. Brown, D. R., May, M., Bradbury, J. M., and Johansson, K. (2010) The *Mollicutes*. In N. R. Krieg, J. T. Staley, B. Hedlund, B. J. Paster, N. Ward, W. Ludwig, and W. B. Whitman (Eds.), *Bergey's Manual of Systematic Bacteriology* (Vol. 4) (2nd ed.). New York, NY: Springer [This textbook chapter is the validated, legitimate description of the class *Mollicutes* and all of the member orders, families, genera, and species. This work carries the taxonomic authority of the Bergey's Trust, and is consistent with the rulings of the International Committee for the Systematics of Prokaryotes].

20. Brown, D.R., Zacher, L.A., and Farmerie, W.G. (2004) Spreading factors of Mycoplasma alligatoris, a flesh-eating mycoplasma. *J Bacteriol* 186, 3922-3927 [This article describes the presence of the glycosidases sialidase and hyaluronidase in the unusually virulent *M. alligatoris*].

21. Carpenter, T.E., Howitt, R., McCapes, R., Yamamoto, R., and Riemann, H.P. (1981) Formulating a control program against Mycoplasma meleagridis using economic decision analysis. *Avian Dis* 25, 260-271[This article describes the development of strategies to minimize the economic impact of the turkey pathogen *M. meleagridis*].

22. Cartner, S.C., Lindsey, J.R., Gibbs-Erwin, J., Cassell, G.H., and Simecka, J.W. (1998) Roles of innate and adaptive immunity in respiratory mycoplasmosis. *Infect Immun* 66, 3485-3491 [This article describes the immune responses that are both protective and pathological to the respiratory tract during mycoplasmosis of the airway].

23. Chambaud, I., Wróblewski, H., and Blanchard, A. (1999) Interactions between mycoplasma lipoproteins and the host immune system. *Trends Microbiol* 7, 493-499 [This article describes the potential immunostimulatory effect of mycoplasmal lipoproteins and their potential connection to pattern recognition receptors].

24. Chanock, R.M. (1963) Mycoplasma pneumoniae: proposed nomenclature for atypical pneumonia organism (Eaton agent). *Science* 140, 662[This article is the initial legitimate species description of *Mycoplasma pneumoniae*].

25. Chanock, R.M., Fox, H.H., James, W.D., Bloom, H.H., and Mufson, M.A. (1960) Growth of laboratory and naturally occurring strains of Eaton agent in monkey kidney tissue culture. *Proc Soc Exp Biol Med* 105, 371-375 [This article describes early culture conditions of *M. pneumoniae* that predates axenic culture methods].

26. Chopra-Dewasthaly, R., Marenda, M., Rosengarten, R., Jechlinger, W., and Citti, C. (2005) Construction of the first shuttle vectors for gene cloning and homologous recombination in Mycoplasma agalactiae. *FEMS Microbiol Lett* 253, 89-94[This article describes the development of molecular tools for genetic manipulation of *M. agalactiae*, and the unexpected finding of genomic integration of the vector].

27. Citti, C., Browning, G. F., and Rosengarten. R. (2005) Phenotypic Diversity and Cell Invasion in Host Subversion by Pathogenic Mycoplasmas. In A. Blanchard, and G. Browning (Eds.), *Mycoplasmas: Molecular Biology, Pathogenicity, and Strategies for Control* (pp. 439-483). Norfolk, UK: Horizon Bioscience [This textbook chapter is a broad review of known multigene families and their mechanisms of genetic variation, and of known mechanisms of host cell invasion. The broader theme of the chapter is evasion of the host immune system by mycoplasmas].

28. Citti, C., Kim, M.F., and Wise, K.S. (1997) Elongated versions of Vlp surface lipoproteins protect Mycoplasma hyorhinis escape variants from growth-inhibiting host antibodies. *Infect Immun* 65, 1773-1785 [This article describes the protective mechanism used by members of a multigene family to evade the immune system by *M. hyorhinis*].

29. Clark, H.W., Coker-Vann, M.R., Bailey, J.S., and Brown, T.M. (1988) Detection of mycoplasmal antigens in immune complexes from rheumatoid arthritis synovial fluids. *Ann Allergy* 60, 394-398 [This article describes the coincident finding of human antibody-mycoplasmal antigen complexes in the joints of arthritis patients, and suggests a causal role of mycoplasmosis in the development of autoimmune arthritis].

30. Cole, B.C., Mu, H.H., and Sawitzke, A.D. (2000) The mycoplasma superantigen MAM: role in arthritis and immune-mediated disease. *Int J Med Microbiol* 290, 489-490 [This article describes immunopathology associated with mycoplasmal superantigens].

31. Cole, B.C., and Thorpe, R.N. (1983) Induction of human gamma interferons by a mitogen derived form Mycoplasma arthritidis and by Phytohemagglutinin: differential inhibition with monoclonal anti-HLA.DR antibodies. *J Immunol* 131, 2392-2396 [This article describes a potential mechanism for immunopathology associated with mycoplasmal superantigens by examining cytokine secretion].

32. Cordova, C.M., Lartigue, C., Sirand-Pugnet, P., Renaudin, J., Cunha, R.A., and Blanchard, A. (2002) Identification of the origin of replication of the Mycoplasma pulmonis chromosome and its use in oriC replicative plasmids. *J Bacteriol* 184, 5426-5435 [This article describes the development of molecular tools for genetic manipulation of *M. pulmonis*].

33. Corfield, T. (1992) Bacterial sialidases--roles in pathogenicity and nutrition. *Glycobiology* 2, 509-521 [This review article discussion the duel function of sialic acid cleavage during bacterial infection. Host desialylation is associated with additional energy sources and with increased invasiveness for bacterial pathogens].

34. Czifra, G., Kleven, S.H., Engström, B., and Stipkovits, L. (1995) Detection of specific antibodies directed against a consistently expressed surface antigen of Mycoplasma gallisepticum using a monoclonal blocking enzyme-linked immunosorbent assay. *Avian Dis* 39, 28-31 [This article describes the development of a diagnostic tool for the detection of *M. gallisepticum*].

35. Dahl, C.E., and Dahl, J.S. (1984) Phospholipids as acyl donors to membrane proteins of Mycoplasma capricolum. *J Biol Chem* 259, 10771-10776 [This article describes the utilization of host phospholipids for membrane generation by *M. capricolum*].

36. Dallo, S.F., and Baseman, J.B. (2000) Intracellular DNA replication and long-term survival of pathogenic mycoplasmas. *Microb Pathog* 29, 301-309 [This article describes the demonstration of intracellular survival and replication by human mycoplasmas. These findings have implications for antimicrobial treatment regiments, because intracellular survival necessitates longer treatment times].

37. Dallo, S.F., Kannan, T.R., Blaylock, M.W., and Baseman, J.B. (2002) Elongation factor Tu and E1 beta subunit of pyruvate dehydrogenase complex act as fibronectin binding proteins in Mycoplasma pneumoniae. *Mol Microbiol* 46, 1041-1051[This article describes attachment and adherence of *M. pneumoniae* to the glycoprotein fibronectin, which may aid in both cytadherence and immune evasion].

38. Davis, J.K., Simecka, J.W., Williamson, J.S., Ross, S.E., Juliana, M.M., Thorp, R.B., and Cassell, G.H. (1985) Nonspecific lymphocyte responses in F344 and LEW rats: susceptibility to murine respiratory mycoplasmosis and examination of cellular basis for strain differences. *Infect Immun* 49, 152-158[This article describes differing immune responses during mycoplasmosis by two rat strains. These findings suggest an explanation for why some infected humans may recover and have a protective immune response, while others develop a pathologic immune response].

39. Deiters, U., and Mühlradt, P.F. (1999) Mycoplasmal lipopeptide MALP-2 induces the chemoattractant proteins macrophage inflammatory protein 1alpha (MIP-1alpha), monocyte chemoattractant protein 1, and MIP-2 and promotes leukocyte infiltration in mice. *Infect Immun* 67, 3390-3398[This article describes the immunostimulatory mechanism of a specific mycoplasmal lipoprotein].

40. Delaye, L., and Moya, A. (2010) *Bioessays* 32, 281-287[This article explores the fallacy of the minimal genome concept, describing the differing genome content of reductively evolved bacteria from different biological niches (*e.g.*, parasites versus symbionts)].

41. Denison, A.M., Clapper, B., and Dybvig, K. (2005) Avoidance of the host immune system through phase variation in Mycoplasma pulmonis. *Infect Immun* 73, 2033-2039[This article describes a protein that undergoes "on/off switching" in *M. pulmonis*, which is thought to help the organism evade the immune system].

42. Díaz-García, F.J., Herrera-Mendoza, A.P., Giono-Cerezo, S., and Guerra-Infante, F.M. (2006) Mycoplasma hominis attaches to and locates intracellularly in human spermatozoa. *Hum Reprod* 21, 1591-1598[This article describes the interaction between human spermatozoa and *M. hominis* cells. This finding may explain the observations of elevated rates of infertility in some *M. hominis*-infected individuals].

43. Dinkla, K., Rohde, M., Jansen, W.T., Carapetis, J.R., Chhatwal, G.S., and Talay, S.R. (2003) Streptococcus pyogenes recruits collagen via surface-bound fibronectin: a novel colonization and immune evasion mechanism. *Mol Microbiol* 47, 861-869[This article describes the adherence of *S. pyogenes* to the host glycoprotein fibronectin, which then binds to the host glycoprotein collagen. This interaction may shield the bacteria from the host immune system by "hiding" their surface antigens behind host proteins].

44. Dybvig, K., and Cassell, G.H. (1987) Transposition of gram-positive transposon Tn916 in Acholeplasma laidlawii and Mycoplasma pulmonis. *Science* 235, 1392-1394[This article describes the initial use of transposons as molecular tools in mycoplasmology].

45. Eaton, M.D., and Liu, C. (1957) Studies on sensitivity to streptomycin of the atypical pneumonia agent. *J Bacteriol* 74, 784-787[This article describes early explorations into antimicrobial therapy for "Eaton's Agent" (*Mycoplasma pneumoniae*). Sensitivity to streptomycin is consistent with later fidings including the absence of a cell wall].

46. Eaton, M.D., Perry, M.E., and Gocke, I.M. (1951) Effect of nitro-compounds and aldehyde semicarbazones on virus of primary atypical pneumonia. *Proc Soc Exp Biol Med* 77, 422-425[This article describes early explorations into antimicrobial therapy for "Eaton's Agent" (*Mycoplasma pneumoniae*)].

47. Epel, B.L. (2009) Plant viruses spread by diffusion on ER-associated movement-protein-rafts through plasmodesmata gated by viral induced host beta-1,3-glucanases. *Semin Cell Dev Biol* 20, 1074-1081[This article describes the ability of glycosidases (glucanases, in this instance) to confer invasiveness onto many types of pathogens].

48. Eterpi, M., McDonnell, G., and Thomas, V. (2011) Decontamination efficacy against Mycoplasma. *Lett Appl Microbiol* 52, 150-155[[This article describes continuing explorations into antimicrobial strategies against mycoplasmas].

49. Evengård, B., Sandstedt, K., Bölske, G., Feinstein, R., Riesenfelt-Orn, I., and Smith, C.I. (1994) Intranasal inoculation of Mycoplasma pulmonis in mice with severe combined immunodeficiency (SCID) causes a wasting disease with grave arthritis. *Clin Exp Immunol* 98, 388-394[This article describes infection of mice without adaptive immune systems with *M. pulmonis*. The induced disease indicates that pathogenicity of *M. pulmonis* is not exclusively immune-mediated].

50. Feizi, T., and Loveless, R.W. (1996) Carbohydrate recognition by Mycoplasma pneumoniae and pathologic consequences. *Am J Respir Crit Care Med* 154, S133-136[This review article describes the interaction of *M. pneumoniae* with host cell ligands (primarily sialic acid), and the resultant autoantibodies this complex generates].

51. Fisher, A.M. (1946) A study on the mechanism of action of penicillin as shown by its effect on bacterial morphology. *J Bacteriol* 52, 539-554[This article describes early explorations into antimicrobial effects of β-lactam antibiotics, including a lack of effect on "Eaton's Agent" (*Mycoplasma pneumoniae*)].

52. Fraser, C.M., Gocayne, J.D., White, O., Adams, M.D., Clayton, R.A., Fleischmann, R.D., Bult, C.J., Kerlavage, A.R., Sutton, G., Kelley, J.M., Fritchman, R.D., Weidman, J.F., Small, K.V., Sandusky, M., Fuhrmann, J., Nguyen, D., Utterback, T.R., Saudek, D.M., Phillips, C.A., Merrick, J.M., Tomb, J.F., Dougherty, B.A., Bott, K.F., Hu, P.C., Lucier, T.S., Peterson, S.N., Smith, H.O., Hutchison, C.A., and Venter, J.C. (1995) The minimal gene complement of Mycoplasma genitalium. *Science* 270, 397-403[This article describes the complete genome of *M. genitalium*, the second cellular genome ever to be sequenced following the publication of the *Haemophilus influenzae* genome earlier in 1995].

53. García-Castillo, M., Morosini, M.I., Gálvez, M., Baquero, F., del Campo, R., and Meseguer, M.A. (2008) Differences in biofilm development and antibiotic susceptibility among clinical Ureaplasma urealyticum and Ureaplasma parvum isolates. *J Antimicrob Chemother* 62, 1027-1030[This article describes the connection between biofilm formation and antibiotic resistance in *Ureaplasma* species, and diversity among strains of these clinically-relevant phenotypes. This may explain differing rates of recrudescence among patients following treatment].

54. Gatski, M., Martin, D.H., Theall, K., Amedee, A., Clark, R.A., Dumestre, J., Chhabra, P., Schmidt, N., and Kissinger, P. (2011) Mycoplasma genitalium infection among HIV-positive women: prevalence, risk factors and association with vaginal shedding. *Int J STD AIDS* 22, 155-159[This article describes epidemiological connections between *M. genitalium* and HIV, and explores a possible contribution of *M. genitalium* to increased ability to shed and transmit HIV. This would have profound public health implications].

55. Gibson, D.G., Glass, J.I., Lartigue, C., Noskov, V.N., Chuang, R.Y., Algire, M.A., Benders, G.A., Montague, M.G., Ma, L., Moodie, M.M., Merryman, C., Vashee, S., Krishnakumar, R., Assad-Garcia, N., Andrews-Pfannkoch, C., Denisova, E.A., Young, L., Qi, Z.Q., Segall-Shapiro, T.H., Calvey, C.H., Parmar, P.P., Hutchison, C.A., Smith, H.O., and Venter, J.C. (2010) Creation of a bacterial cell controlled by a chemically synthesized genome. *Science* 329, 52-56[This landmark article describes the first generation of a synthetic living cell, and serves as proof-of-concept for future studies on human-designed and generated organisms].

56. Girón, J.A., Lange, M., and Baseman, J.B. (1996) Adherence, fibronectin binding, and induction of cytoskeleton reorganization in cultured human cells by Mycoplasma penetrans. *Infect Immun* 64, 197-208[This article describes attachment, adherence, and a mechanism for host cell invasion of *M. penetrans*].

57. Glasgow, L.R., and Hill, R.L. (1980) Interaction of Mycoplasma gallisepticum with sialyl glycoproteins. *Infect Immun* 30, 353-361[This article describes attachment and adherence of *M. gallisepticum* to a particular host cell ligand].

58. Glass, J.I., Assad-Garcia, N., Alperovich, N., Yooseph, S., Lewis, M.R., Maruf, M., Hutchison, C.A., Smith, H.O., and Venter, J.C. (2006) Essential genes of a minimal bacterium. *Proc Natl Acad Sci U S A* 103, 425-430[This article describes a confirmatory study attempting to disrupt all genes not essential for *M. genitlaium* viability, with the ultimate goal of describing a minimum set of genes necessary for life].

59. Güner, E.S. (1996) Complement evasion by the Lyme disease spirochete Borrelia burgdorferi grown in host-derived tissue co-cultures: role of fibronectin in complement-resistance. *Experientia* 52, 364-372[This article describes attachment, adherence, and immune evasion by *B. burgdorferi*].

60. Hahn, T.W., Mothershed, E.A., Waldo, R.H., and Krause, D.C. (1999) Construction and analysis of a modified Tn4001 conferring chloramphenicol resistance in Mycoplasma pneumoniae. *Plasmid* 41, 120-124[This article describes a novel molecular tool for genetic manipulation in mycoplasmas].

61. Hames, C., Halbedel, S., Hoppert, M., Frey, J., and Stülke, J. (2009) Glycerol metabolism is important for cytotoxicity of Mycoplasma pneumoniae. *J Bacteriol* 191, 747-753[This article describes the contribution of the toxic metabolite H_2O_2 to *M. pneumoniae* pathogenesis].

62. Hardy, R.D., Coalson, J.J., Peters, J., Chaparro, A., Techasaensiri, C., Cantwell, A.M., Kannan, T.R., Baseman, J.B., and Dube, P.H. (2009) Analysis of pulmonary inflammation and function in the mouse and baboon after exposure to Mycoplasma pneumoniae CARDS toxin. *PLoS One* 4, e7562[This article describes the contribution of purified community-acquired respiratory disease syndrome (CARDS) toxin to respiratory lesions, and thus to *M. pneumoniae* pathogenesis].

63. Hayatsu, E., Kawakubo, Y., Yayoshi, M., Araake, M., Yoshioka, M., and Nishiyama, Y. (1980) Role of humoral antibodies in resistance to Mycoplasma pneumoniae pneumonia in hamsters. *Microbiol Immunol* 24, 585-593[This article describes an important correlate of immunity to *M. pneumoniae* infection].

64. Henry, C., Overbeek, R., and Stevens, R.L. (2010) Building the blueprint of life. *Biotechnol J* 5, 695-704[This commentary discusses the impact of the publication of the first synthetic cell (see reference 55)].

65. Himpsl, S.D., Lockatell, C.V., Hebel, J.R., Johnson, D.E., and Mobley, H.L. (2008) Identification of virulence determinants in uropathogenic Proteus mirabilis using signature-tagged mutagenesis. *J Med Microbiol* 57, 1068-1078[This article describes a global method for finding new virulence factors using the bacterial species *P. mirabilis*].

66. Horton, M.R., Burdick, M.D., Strieter, R.M., Bao, C., and Noble, P.W. (1998) Regulation of hyaluronan-induced chemokine gene expression by IL-10 and IFN-gamma in mouse macrophages. *J Immunol* 160, 3023-3030[This article describes the contribution of fragmentation of the host protein hyaluronan to the induction of "sterile" inflammation. Pathogens can mediate this by producing the enzyme hyaluronidase].

67. Horton, M.R., Shapiro, S., Bao, C., Lowenstein, C.J., and Noble, P.W. (1999) Induction and regulation of macrophage metalloelastase by hyaluronan fragments in mouse macrophages. *J Immunol* 162, 4171-4176[This article describes the contribution of fragmentation of the host protein hyaluronan to the induction of "sterile" inflammation. Pathogens can mediate this by producing the enzyme hyaluronidase].

68. Hudson, P., Gorton, T.S., Papazisi, L., Cecchini, K., Frasca, S., and Geary, S.J. (2006) Identification of a virulence-associated determinant, dihydrolipoamide dehydrogenase (lpd), in Mycoplasma gallisepticum through in vivo screening of transposon mutants. *Infect Immun* 74, 931-939[This article describes a global method for finding new virulence factors using the bacterial species *M. gallisepticum*, and follows up the initial screening with proof of principle studies showing the effect of *lpd* on *M. gallisepticum* virulence].

69. Hunt, M.E., and Brown, D.R. (2007) Role of sialidase in Mycoplasma alligatoris-induced pulmonary fibroblast apoptosis. *Vet Microbiol* 121, 73-82[This article demonstrates that *M. alligatoris* induces apoptosis in cultured cells, and that a sialidase inhibitor blocks the effect].

70. Hutchison, C.A., Peterson, S.N., Gill, S.R., Cline, R.T., White, O., Fraser, C.M., Smith, H.O., and Venter, J.C. (1999) Global transposon mutagenesis and a minimal Mycoplasma genome. *Science* 286, 2165-2169[This article describes an attempt to disrupt all genes not essential for *M. genitlaium* viability, with the ultimate goal of describing a minimum set of genes necessary for life].

71. Jenkins, C., Samudrala, R., Geary, S.J., and Djordjevic, S.P. (2008) Structural and functional characterization of an organic hydroperoxide resistance protein from Mycoplasma gallisepticum. *J Bacteriol* 190, 2206-2216[This article describes a component of the virulence of *M. gallisepticum*; namely, that is provides itself protection from the toxic effects of the H_2O_2 it produces].

72. Jenkins, C., Wilton, J.L., Minion, F.C., Falconer, L., Walker, M.J., and Djordjevic, S.P. (2006) Two domains within the Mycoplasma hyopneumoniae cilium adhesin bind heparin. *Infect Immun* 74, 481-487[This article describes a novel attachment mechanism of *M. hyopneumoniae*].

73. Jensen, J.S., Blom, J., and Lind, K. (1994) Intracellular location of Mycoplasma genitalium in cultured Vero cells as demonstrated by electron microscopy. *Int J Exp Pathol* 75, 91-98[This article describes the potential for *M. genitalium* to invade host cells, potentially explaining the tendency of short-term antibiotic therapy to fail].

74. Johnson, D.C., Emory, W.H., Kleven, S.H., and Stallknecht, D.E. (1981) A Mycoplasma gallisepticum epornitic in turkeys: its epidemiology and eradication. *Avian Dis* 25, 1047-1052[This article describes features of a particular outbreak of *M. gallisepticum* in turkeys].

75. Jones, H.P., Tabor, L., Sun, X., Woolard, M.D., and Simecka, J.W. (2002) Depletion of CD8+ T cells exacerbates CD4+ Th cell-associated inflammatory lesions during murine mycoplasma respiratory disease. *J Immunol* 168, 3493-3501[This article describes components of the immunopathologic response of mice to *M. pulmonis*].

76. Kahane, I., Banai, M., Razin, S., and Feldner, J. (1982) Attachment of mycoplasmas to host cell membranes. *Rev Infect Dis 4 Suppl*, S185-192[This review article describes various mechanisms of attachment used by mycoplasmas to adhere to host cells].

77. Kannan, T.R., Coalson, J.J., Cagle, M., Musatovova, O., Hardy, R.D., and Baseman, J.B. (2011) Synthesis and Distribution of CARDS Toxin During Mycoplasma pneumoniae Infection in a Murine Model. *J Infect Dis* 204, 1596-1604[This article describes the physical location and spatial association with lesions of community-acquired respiratory disease syndrome (CARDS) toxin during *M. pneumoniae* infection].

78. Kannan, T.R., Provenzano, D., Wright, J.R., and Baseman, J.B. (2005) Identification and characterization of human surfactant protein A binding protein of Mycoplasma pneumoniae. *Infect Immun* 73, 2828-2834[This article describes the ability of *M. pneumoniae* to adsorb a host component for toxic and immunomodulatory purposes].

79. Kaufmann, A., Mühlradt, P.F., Gemsa, D., and Sprenger, H. (1999) Induction of cytokines and chemokines in human monocytes by Mycoplasma fermentans-derived lipoprotein MALP-2. *Infect Immun* 67, 6303-6308[This article describes components of the immunopathologic response of human immune cells to *M. fermentans*].

80. Keystone, E.C., Cunningham, A.J., Metcalfe, A., Kennedy, M., and Quinn, P.A. (1982) Role of antibody in the protection of mice from arthritis induced by Mycoplasma pulmonis. *Clin Exp Immunol* 47, 253-259[This article describes an important correlate of immunity to *M. pulmonis* infection].

81. Keystone, E.C., Taylor-Robinson, D., Osborn, M.F., Ling, L., Pope, C., and Fornasier, V. (1980) Effect of T-cell deficiency on the chronicity of arthritis induced in mice by Mycoplasma pulmonis. *Infect Immun* 27, 192-196[This article describes an important correlate of immunity to *M. pulmonis* infection].

82. Khan, L.A., Loria, G.R., Ramirez, A.S., Nicholas, R.A., Miles, R.J., and Fielder, M.D. (2005) Biochemical characterisation of some non fermenting, non arginine hydrolysing mycoplasmas of ruminants. *Vet Microbiol* 109, 129-134[This article describes the ability of certain mycoplasmas to utilize organic acids and alcohols for growth, in hopes of using these features diagnostically].

83. King, S.J., Hippe, K.R., and Weiser, J.N. (2006) Deglycosylation of human glycoconjugates by the sequential activities of exoglycosidases expressed by Streptococcus pneumoniae. *Mol Microbiol* 59, 961-974[This article describes the systematic removal of sugars from the host extracellular matrix down to the cell surface by a collective action of a suite of glycosidases by *S. pneumoniae*].

84. Kirchner, H., Bauer, A., Moritz, T., and Herbst, F. (1986) Lymphocyte activation and induction of interferon gamma in human leucocyte cultures by the mitogen in Mycoplasma arthritidis supernatant (MAS). *Scand J Immunol* 24, 609-613[This article is an early description of the only known mycoplasmal superantigen, MAM].

85. Klieneberger, E. (1939) Studies on pleuropneumonia-like organisms: the L4 organism as the cause of Woglom's "Pyogenic Virus". *J Hyg (Lond)* 39, 260-265[This article describes the suspicion that pleuropneumonia-like organisms were bacterial cells rather than viral agents].

86. Knudson, W., Casey, B., Nishida, Y., Eger, W., Kuettner, K.E., and Knudson, C.B. (2000) Hyaluronan oligosaccharides perturb cartilage matrix homeostasis and induce chondrocytic chondrolysis. *Arthritis Rheum* 43, 1165-1174[This article describes the detrimental effect of disrupting the extracellular matrix of cells composing cartilage on their structure and function].

87. Knudtson, K.L., and Minion, F.C. (1993) Construction of Tn4001lac derivatives to be used as promoter probe vectors in mycoplasmas. *Gene* 137, 217-222[This article describes a novel molecular tool for genetic manipulation in mycoplasmas].

88. Lancaster, J.E., and Fabricant, J. (1988) The history of avian medicine in the United States. IX. Events in the history of avian mycoplasmosis 1905-70. *Avian Dis* 32, 607-623[This review article describes important events and findings in avian mycoplasmosis in the United States].

89. Lartigue, C., Vashee, S., Algire, M.A., Chuang, R.Y., Benders, G.A., Ma, L., Noskov, V.N., Denisova, E.A., Gibson, D.G., Assad-Garcia, N., Alperovich, N., Thomas, D.W., Merryman, C., Hutchison, C.A., Smith, H.O., Venter, J.C., and Glass, J.I. (2009) Creating bacterial strains from genomes that have been cloned and engineered in yeast. *Science* 325, 1693-1696[This landmark publication describes the first generation of a living synthetic cell, wherein the chromosome was synthetically generated and transplanted into a heterologous cell membrane. This technology opens the door for the design of bacteriological organisms to perform specific functions such as produce biofuels, deliver vaccine antigens, or detoxify contaminants].

90. Limoli, D.H., Sladek, J.A., Fuller, L.A., Singh, A.K., and King, S.J. (2011) BgaA acts as an adhesin to mediate attachment of some pneumococcal strains to human epithelial cells. *Microbiology* 157, 2369-2381[This article describes the dual action of the enzyme β-galactosidase as both a glycosidase and an adherence mechanism].

91. Lo, S.C., Hayes, M.M., Tully, J.G., Wang, R.Y., Kotani, H., Pierce, P.F., Rose, D.L., and Shih, J.W. (1992) Mycoplasma penetrans sp. nov., from the urogenital tract of patients with AIDS. *Int J Syst Bacteriol* 42, 357-64[This article is the initial legitimate species description of *M. penetrans*].

92. Luo, W., Yu, H., Cao, Z., Schoeb, T., Marron, M., and Dybvig, K. (2008) Association of Mycoplasma arthritidis mitogen with lethal toxicity but not with arthritis in mice. *Infect Immun* 76, 4989-4998[This article describes the clinical impact of MAM in mice].

93. Machado, C.X., Pinto, P.M., Zaha, A., and Ferreira, H.B. (2009) A peroxiredoxin from Mycoplasma hyopneumoniae with a possible role in H2O2 detoxification. *Microbiology* 155, 3411-3419[This article describes a component of the virulence of *M. hyopneumoniae*; namely, that is provides itself protection from the toxic effects of the H_2O_2 it produces].

94. Mahairas, G.G., and Minion, F.C. (1989) Random insertion of the gentamicin resistance transposon Tn4001 in Mycoplasma pulmonis. *Plasmid* 21, 43-47[This article describes the initial use of Tn4001. Derivatives of this transposon as among the most commonly used molecular tools in mycoplasmology].

95. Manchee, R.J., and Taylor-Robinson, D. (1969) Studies on the nature of receptors involved in attachment of tissue culture cells to mycoplasmas. *Br J Exp Pathol* 50, 66-75[This article describes attachment and adherence of *M. gallisepticum* to a particular host cell ligand].

96. Manhart, L.E., Mostad, S.B., Baeten, J.M., Astete, S.G., Mandaliya, K., and Totten, P.A. (2008) High Mycoplasma genitalium organism burden is associated with shedding of HIV-1 DNA from the cervix. *J Infect Dis* 197, 733-736[This article describes epidemiological connections between *M. genitalium* and HIV, and explores a possible contribution of *M. genitalium* to increased ability to shed and transmit HIV. This would have profound public health implications].

97. Manuel, S.G., Ragunath, C., Sait, H.B., Izano, E.A., Kaplan, J.B., and Ramasubbu, N. (2007) Role of active-site residues of dispersin B, a biofilm-releasing beta-hexosaminidase from a periodontal pathogen, in substrate hydrolysis. *FEBS J* 274, 5987-5999[This article describes the connection between hexose-degrading enzymes and additional cellular function such as carbohydrate catabolism, in addition to its role in biofilm escape. This illustrates the often dual role that glycosidases play in the biology of pathogens].

98. Marais, A., Bove, J.M., Dallo, S.F., Baseman, J.B., and Renaudin, J. (1993) Expression in Spiroplasma citri of an epitope carried on the G fragment of the cytadhesin P1 gene from Mycoplasma pneumoniae. *J Bacteriol* 175, 2783-2787[This article is a description of the expression of a fragment of the primary attachment protein of *M. pneumoniae*].

99. March, J.B., Kerr, K., and Lema, B. (2003) Rapid detection of contagious bovine pleuropneumonia by a Mycoplasma mycoides subsp. mycoides SC capsular polysaccharide-specific antigen detection latex agglutination test. *Clin Diagn Lab Immunol* 10, 233-240[This article describes a novel diagnostic strategy to detect the agent of contagious bovine pleuropneumonia (CBPP) by detecting bacterial capsule material. Rapid diagnosis of CBPP is crucial for control of outbreaks].

100. March, J., Gammack, C., and Nicholas, R. (2000) Rapid detection of contagious caprine pleuropneumonia using a Mycoplasma capricolum subsp. capripneumoniae capsular polysaccharide-specific antigen detection latex agglutination test. *J Clin Microbiol* 38, 4152-4159[This article describes a novel diagnostic strategy to detect the agent of contagious caprine pleuropneumonia (CCPP) by detecting bacterial capsule material. Rapid diagnosis of CCPP is crucial for control of outbreaks].

101. Markham, P.F., Glew, M.D., Browning, G.F., Whithear, K.G., and Walker, I.D. (1998) Expression of two members of the pMGA gene family of Mycoplasma gallisepticum oscillates and is influenced by pMGA-specific antibodies. *Infect Immun* 66, 2845-2853[This article is the initial description of *vlhA*, a family of antigenically variable proteins in avian mycoplasmas].

102. Matsuda, K., Li, J.L., Harasawa, R., and Yamamoto, N. (1997) Phosphocholine-containing glycoglycerolipids (GGPL-I and GGPL-III) are species-specific major immunodeterminants of Mycoplasma fermentans. *Biochem Biophys Res Commun* 233, 644-649[This article describes the proteins that humans raise the most potent antibody response to during *M. fermentans* infection].

103. Matsushita, O., and Okabe, A. (2001) Clostridial hydrolytic enzymes degrading extracellular components. *Toxicon* 39, 1769-1780[This article describes glycosidases of *C. perfringens*].

104. May, M., and Brown, D.R. (2008) Genetic variation in sialidase and linkage to N-acetylneuraminate catabolism in Mycoplasma synoviae. *Microb Pathog* 45, 38-44[This article describes sequence diversity in the sialidase gene of *M. synoviae*, and concludes that sialic acid is unlikely to play a nutritional role in the ecology of the organism].

105. May, M., Whitcomb, R. F., and Brown, D. R. (2008) Mycoplasmas and Related Organisms. In E. Goldman (Ed.), *The Practical Handbook of Microbiology*. Boca Raton, FL: Taylor and Francis Group[This textbook chapter describes the taxonomy of the *Mollicutes*, and treatment and control measures applied to agents of human and veterinary mycoplasmosis].

106. May, M., and Brown, D.R. (2011) Diversity of expressed vlhA adhesin sequences and intermediate hemagglutination phenotypes in Mycoplasma synoviae. *J Bacteriol* 193, 2116-2121[This article describes sequence diversity in the VlhA adhesins of *M. synoviae*, and concludes that the adherence capacity is correlated and functionally balanced with sialidase].

107. May, M., Kleven, S.H., and Brown, D.R. (2007) Sialidase activity in Mycoplasma synoviae. *Avian Dis* 51, 829-833[This article is the initial description of sialidase activity in *M, synoviae*].

108. May, M., Papazisi, L., Gorton, T.S., and Geary, S.J. (2006) Identification of fibronectin-binding proteins in Mycoplasma gallisepticum strain R. *Infect Immun* 74, 1777-1785[This article describes attachment and adherence of *M. gallisepticum* to host fibronectin, and describes an atypical transmembrane domain].

109. McAuliffe, L., Ellis, R., Miles, K., Ayling, R., and Nicholas, R. (2006) Biofilm formation by mycoplasma species and its role in environmental persistence and survival. *Microbiology* 152, 913-922[This article describes a survey of several ruminant mycoplasmas for biofilm formation, which aids the organisms in extended survival on surfaces and increased protection from antimicrobials].

110. McGowin, C.L., and Anderson-Smits, C. (2011) Mycoplasma genitalium: an emerging cause of sexually transmitted disease in women. *PLoS Pathog* 7, e1001324[This article describes a retrospective study that assimilates numerous epidemiological surveys reporting *M. genitalium* rates].

111. Megid, R., Nicholas, R.A., and Miles, R.J. (2001) Biochemical characterization of Mycoplasma bovirhinis, Mycoplasma dispar and recent bovine isolates of Mycoplasma canis. *Vet Res Commun* 25, 1-12[This article describes the biochemical features of bovine mycoplasmas that can be used diagnostically].

112. Meiklejohn, G., Eaton, M.D., and van Herick, W. (1945) A clinical report on cases of primary atypical pneumonia caused by a new virus. *J Clin Invest* 24, 241-250[This case report represents one of the earliest case reports of primary atypical pneumonia].

113. Miles, R.J., Beezer, A.E., and Lee, D.H. (1986) Growth of Mycoplasma mycoides subspecies mycoides on media containing various sugars and amino sugars: an ampoule microcalorimetric study. *Microbios* 45, 7-19[This article describes an extensive characterization of the metabolic capacity of *M. mycoides* subsp. *mycoides*].

114. Miller, J. (2010) A step to artificial life: Manmade DNA powers cell. In: Ethics Illustrated: Associated Press[This press report is a commentary on the ethics and issues associated with synthetic cells].

115. Minion, F.C., and Goguen, J.D. (1985) Trypsin-sensitive, bovine serum albumin-dependent hemolysis activity in Mycoplasma pulmonis. *Infect Immun* 49, 440-442[This article describes a protein toxin that destroys erythrocytes made by *M. pulmonis*].

116. Minion, F.C., and Jarvill-Taylor, K. (1994) Membrane-associated hemolysin activities in mycoplasmas. *FEMS Microbiol Lett* 116, 101-106[This article describes a survey of twenty-nine mycoplasmas for erythrocyte toxicity].

117. Mizutani, H. (1984) Circulating immune complexes in patients with mycoplasmal pneumonia. *Am Rev Respir Dis* 130, 627-629[This article describes autoreactive antibodies as a source of immunopathology associated with mycoplasmosis in humans].

118. Mizutani, H. (1986) Immunoglobulin M rheumatoid factor in patients with mycoplasmal pneumonia. *Am Rev Respir Dis* 134, 1237-1240[This article describes the detection of rheumatoid factor associated with mycoplasmosis in humans, and suggests it as a source of immunopathology].

119. Mohammed, H.O., Carpenter, T.E., and Yamamoto, R. (1987) Economic impact of Mycoplasma gallisepticum and M. synoviae in commercial layer flocks. *Avian Dis* 31, 477-482[This article reports economic losses associated with avian mycoplasmosis in the United States].

120. Mohammed, H.O., Carpenter, T.E., and Yamamoto, R. (1987) Evaluation of factors associated with infection of commercial layers with Mycoplasma gallisepticum and M. synoviae. *Avian Dis* 31, 470-476[This article identifies risk factors such as hygiene, flock dynamics, and lack of vaccination associated with increased probability of outbreaks of avian mycopalsmosis].

121. Mu, H.H., Sawitzke, A.D., and Cole, B.C. (2000) Modulation of cytokine profiles by the Mycoplasma superantigen Mycoplasma arthritidis mitogen parallels susceptibility to arthritis induced by *M. arthritidis*. *Infect Immun* 68, 1142-1149[This article describes a potential mechanism for immunopathology associated with mycoplasmal superantigens by examining cytokine secretion as an analogy to the cytokine changes seen in *M. arthitidis* arthritis].

122. Muir, M.T., Cohn, S.M., Louden, C., Kannan, T.R., and Baseman, J.B. (2011) Novel toxin assays implicate Mycoplasma pneumoniae in prolonged ventilator course and hypoxemia. *Chest* 139, 305-310[This article describes the detection of community-acquired respiratory disease syndrome (CARDS) toxin as a method for associating *M. pneumoniae* with prolonged respiratory infection, indicating that more broad-spectrum treatment regimens should be employed].

123. Mühlradt, P.F., Kiess, M., Meyer, H., Süssmuth, R., and Jung, G. (1997) Isolation, structure elucidation, and synthesis of a macrophage stimulatory lipopeptide from Mycoplasma fermentans acting

at picomolar concentration. *J Exp Med* 185, 1951-1958[This article describes Toll-like receptor agonists made by *M. fermentans* that stimulate innate immune responses].

124. Nagayama, Y., and Sakurai, N. (1991) Clinical observations on lower respiratory tract infections with special reference to serum IgE levels. *Pediatr Pulmonol* 11, 44-48[This article describes the detection hypersensitivity-associated immune responses during respiratory tract infections including those by *M. pneumoniae* in pediatric patients].

125. Neimark, H. (1967) Heterogeneity among the mycoplasma and relationships to bacteria. *Ann N Y Acad Sci* 143, 31-37[This review article describes the biological characteristics of mycoplasmas in the context of how they fit into the bacterial tree].

126. Neimark, H.C., and Pene, J.J. (1965) Characterization of pleuropneumonia-like organisms by deoxyribonucleic acid composition. *Proc Soc Exp Biol Med* 118, 517-519[This article describes an early classification of mycoplasmas based on DNA content].

127. Niang, M., Rosenbusch, R.F., DeBey, M.C., Niyo, Y., Andrews, J.J., and Kaeberle, M.L. (1998) Field isolates of Mycoplasma ovipneumoniae exhibit distinct cytopathic effects in ovine tracheal organ cultures. *Zentralbl Veterinarmed A* 45, 29-40[This article describes an *in vitro* assessment of variation among different strains of *M. ovipneumoniae* in their ability to damage tracheal cells].

128. Noormohammadi, A.H., Markham, P.F., Markham, J.F., Whithear, K.G., and Browning, G.F. (1999) Mycoplasma synoviae surface protein MSPB as a recombinant antigen in an indirect ELISA. *Microbiology* 145 (Pt 8), 2087-2094[This article describes a novel diagnostic method for *M. synoviae*].

129. Pereyre, S., Gonzalez, P., De Barbeyrac, B., Darnige, A., Renaudin, H., Charron, A., Raherison, S., Bébéar, C., and Bébéar, C.M. (2002) Mutations in 23S rRNA account for intrinsic resistance to macrolides in Mycoplasma hominis and Mycoplasma fermentans and for acquired resistance to macrolides in M. hominis. *Antimicrob Agents Chemother* 46, 3142-3150[This article describes the specific mechanism by which the human pathogens *M. hominis* and *M. fermentans* exhibit resistance to an entire class of antibiotics].

130. Peters, J., Singh, H., Brooks, E. G., Diaz, J., Kannan, T. R., Coalson, J. J., Baseman, J. G., Cagle, M., and Baseman, J. B. (2011) Persistence of community acquired respiratory distress syndrome toxin-producing *Mycoplasma pneumoniae* in refractory asthma. *CHEST*, 140 (2), 401-407[This article describes an association between the presence of toxigenic *M. pneumoniae* and post-infectious asthma].

131. Pilo, P., Vilei, E.M., Peterhans, E., Bonvin-Klotz, L., Stoffel, M.H., Dobbelaere, D., and Frey, J. (2005) A metabolic enzyme as a primary virulence factor of Mycoplasma mycoides subsp. mycoides small colony. *J Bacteriol* 187, 6824-6831[This article describes the effect of the bi-product H_2O_2 on bovine cells, and attributes elevated virulence of *M. mycoides mycoides* to this capability].

132. Polissi, A., Pontiggia, A., Feger, G., Altieri, M., Mottl, H., Ferrari, L., and Simon, D. (1998) Large-scale identification of virulence genes from Streptococcus pneumoniae. *Infect Immun* 66, 5620-5629[This article describes a global approach to the identification of previously unrecognized facets of *S. pneumoniae* virulence].

133. Pour-El, I., Adams, C., and Minion, F.C. (2002) Construction of mini-Tn4001tet and its use in Mycoplasma gallisepticum. *Plasmid* 47, 129-137[This article describes the "mini tet" transposon, a molecular tool that has facilitated many mutagenesis studies in mycoplasmology].

134. Pritchard, R.E., Mokhnatkin, J.V., Hatchel, J.M., and Balish, M.F. (2010) Potential Role of a Streptolysin S-like Molecule in Mycoplasma amphoriforme Pathogenicity. International Organization for Mycoplasmology 18th Congress. Chianciano Terme, Siena, Italy[This platform presentation reported the presence and activity of a true hemolysin in the putative human pathogen *M. amphoriforme*].

135. Razin, S. (1969) Structure and function in mycoplasma. *Annu Rev Microbiol* 23, 317-356[This review article describes basic cellular functionality as applied to *Mollicutes*].

136. Razin, S. (1978) The mycoplasmas. *Microbiol Rev* 42, 414-470[This review article describes the cellular biology, morphology, virulence, and genetics of mycoplasmas and related organisms circa 1978].

137. Razin, S., Yogev, D., and Naot, Y. (1998) Molecular biology and pathogenicity of mycoplasmas. *Microbiol Mol Biol Rev* 62, 1094-1156[This review article describes the cellular biology, morphology, virulence, and genetics of mycoplasmas and related organisms circa 1998, and is a companion to Dr. Razin's chapter in the third edition of The Prokaryotes].

138. Renaudin, J., and Lartigue, C. (2005) OriC Plasmids as Gene Vectors for Mollicutes. In A. Blanchard, and G. Browning (Eds.), *Mycoplasmas: Molecular Biology, Pathogenicity, and Strategies for Control* (pp. 439-483). Norfolk, UK: Horizon Bioscience[This textbook chapter describes the development, generation, and functionality of artificial plasmids using mycoplasmal and spiroplasmal origins of replication as molecular tools in mycoplasmology].

139. Renaudin, J., Marais, A., Verdin, E., Duret, S., Foissac, X., Laigret, F., and Bové, J.M. (1995) Integrative and free Spiroplasma citri oriC plasmids: expression of the Spiroplasma phoeniceum spiralin in Spiroplasma citri. *J Bacteriol* 177, 2870-2877[This article describes the development, generation, and functionality of an artificial plasmid using the *S. citri* origin of replication as a delivery system for an exogenous gene].

140. Rink, L., Nicklas, W., Luhm, J., Kruse, R., and Kirchner, H. (1996) Induction of a proinflammatory cytokine network by Mycoplasma arthritidis-derived superantigen (MAS). *J Interferon Cytokine Res* 16, 861-868[This article describes the signaling mechanics of massive immunological response generate by the superantigen MAM].

141. Romano, N., Tolone, G., Ajello, F., and La Licata, R. (1980) Adenosine 5'-triphosphate synthesis induced by urea hydrolysis in Ureaplasma urealyticum. *J Bacteriol* 144, 830-832[This article describes mechanistically how urea is used metabolically by *U. urealyticum*].

142. Rosendal, S., Lumsden, J.H., Viel, L., and Physick-Sheard, P.W. (1987) Phagocytic function of equine neutrophils exposed to Mycoplasma felis in vitro and in vivo. *Am J Vet Res* 48, 758-762[This article describes the capacity of professional phagocytes from horses to engulf *M. felis*, and exhibits a difference when the neutrophils were collected from infected horses as opposed to uninfected horses and then exposed in a laboratory].

143. Rosengarten, R., and Wise, K.S. (1990) Phenotypic switching in mycoplasmas: phase variation of diverse surface lipoproteins. *Science* 247, 315-318[This landmark article is the first demonstration of antigenic variation in *Mollicutes*, which we now understand to be a major mechanism of immune evasion for many species].

144. Rosengarten, R., and Wise, K.S. (1991) The Vlp system of Mycoplasma hyorhinis: combinatorial expression of distinct size variant lipoproteins generating high-frequency surface antigenic variation. *J Bacteriol* 173, 4782-4793[This article describes the mechanism associated with antigenic variation in *M. hyorhinis*].

145. Röske, K., Calcutt, M.J., and Wise, K.S. (2004) The Mycoplasma fermentans prophage phiMFV1: genome organization, mobility and variable expression of an encoded surface protein. *Mol Microbiol* 52, 1703-1720[This article describes an bacteriophage virus integrated into the genome of *M. fermentans*, and the gene it contributes encoding an antigenic protein on the bacterial surface].

146. Saurin, W., and Dassa, E. (1996) In the search of Mycoplasma genitalium lost substrate-binding proteins: sequence divergence could be the result of a broader substrate specificity. *Mol Microbiol* 22, 389-390[This article describes attempts to uncover the nature of differential avidities in binding proteins in *M. genitalium*. Later work by Iverson-Cabral *et al.* specifies a mechanism].

147. Schmidl, S.R., Otto, A., Lluch-Senar, M., Piñol, J., Busse, J., Becher, D., and Stülke, J. (2011) A Trigger Enzyme in Mycoplasma pneumoniae: Impact of the Glycerophosphodiesterase GlpQ on Virulence and Gene Expression. *PLoS Pathog* 7, e1002263[This article describes the expression and regulatory control of an enzyme associated with glycerol metabolism. The bi-product of glycerol metabolism, H_2O_2, is associated with tissue damage and virulence of many bacterial species].

148. Seggev, J.S., Lis, I., Siman-Tov, R., Gutman, R., Abu-Samara, H., Schey, G., and Naot, Y. (1986) Mycoplasma pneumoniae is a frequent cause of exacerbation of bronchial asthma in adults. *Ann Allergy* 57, 263-265[This article is one of the early reports detailing clinical associations between *M. pneumoniae* infection and asthma development or exacerbation].

149. Seggev, J.S., Sedmak, G.V., and Kurup, V.P. (1996) Isotype-specific antibody responses to acute Mycoplasma pneumoniae infection. *Ann Allergy Asthma Immunol* 77, 67-73[This article describes immune correlates of protection or pathology during *M. pneumoniae* infection].

150. Sekiguchi, K., & Hakomori, S. (1980) Functional domain structure of fibronectin. *Proceedings of the National Academy of Sciences*, 77 (5), 2661-2665[This article describes the different structures and functional binding portions of the extracellular matrix protein fibronectin].

151. Seleem, M.N., Boyle, S.M., and Sriranganathan, N. (2008) Brucella: a pathogen without classic virulence genes. *Vet Microbiol* 129, 1-14[This review article describes aspects of the virulence of *B. abortis*, which does not involve toxins, capsules, or other so-called classical virulence factors. This in many ways is akin to mycopalsmal virulence].

152. Seymour, L.M., Deutscher, A.T., Jenkins, C., Kuit, T.A., Falconer, L., Minion, F.C., Crossett, B., Padula, M., Dixon, N.E., Djordjevic, S.P., and Walker, M.J. (2010) A processed multidomain mycoplasma hyopneumoniae adhesin binds fibronectin, plasminogen, and swine respiratory cilia. *J Biol Chem* 285, 33971-33978[This article describes the *M. hyopneumoniae* adhesin, which is posttranslationally cleaved into many different sub-types which in turn bind many host cells ligands].

153. Simecka, J.W. (2005) Immune Responses Following Mycoplasma Infection. In A. Blanchard and G. Browning (Eds*.). Mycoplasmas: Molecular Biology, Pathogenicity, and Strategies for Control* (485-534). Norfolk, UK: Horizon Bioscience[This textbook chapter gives a broad overview of mycoplasmal immunology, including correlates of protection an mechanisms of immunopathology].

154. Simecka, J.W., Davis, J.K., and Cassell, G.H. (1987) Specific vs. nonspecific immune responses in murine respiratory mycoplasmosis. *Isr J Med Sci* 23, 485-489[This article describes differentiation between protection and non-protective and potentially pathogenic immune responses or mice to *M. pulmonis*].

155. Simmons, W.L., Bolland, J.R., Daubenspeck, J.M., and Dybvig, K. (2007) A stochastic mechanism for biofilm formation by Mycoplasma pulmonis. *J Bacteriol* 189, 1905-1913[This article characterizes the formation and regulation of *M. pulmonis* biofilm formation, and represents the first and most extensive characterization of this process in the *Mollicutes*].

156. Simmons, W.L., Denison, A.M., and Dybvig, K. (2004) Resistance of Mycoplasma pulmonis to complement lysis is dependent on the number of Vsa tandem repeats: shield hypothesis. *Infect Immun* 72, 6846-6851[This article describes the dual role in immune evasion by Vsa proteins of *M. pulmonis*: antigenic variation and complement resistance].

157. Simmons, W.L., and Dybvig, K. (2003) The Vsa proteins modulate susceptibility of Mycoplasma pulmonis to complement killing, hemadsorption, and adherence to polystyrene. *Infect Immun* 71, 5733-5738[This article is the basis for the findings in reference 156, and demonstrates that Vsa proteins modulate cytadherence and provide resistance to complement-mediated lysis].

158. Simmons, W.L., and Dybvig, K. (2009) Mycoplasma biofilms ex vivo and in vivo. *FEMS Microbiol Lett* 295, 77-81[This article describes the formation of *M. pulmonis* biofilms on tracheal explants cultures, indicating that the observed *in vitro* biofilm formation described in reference 2007 has relevance during infection].

159. Smith, D.G., Russell, W.C., Ingledew, W.J., and Thirkell, D. (1993) Hydrolysis of urea by Ureaplasma urealyticum generates a transmembrane potential with resultant ATP synthesis. *J Bacteriol* 175, 3253-3258[This article describes the contribution of electrochemical gradients generated by urea processing aids in energy generation for *U. urealyticum*].

160. Stamburski, C., Renaudin, J., and Bove, J.M. (1991) First step toward a virus-derived vector for gene cloning and expression in spiroplasmas, organisms which read UGA as a tryptophan codon: synthesis of chloramphenicol acetyltransferase in Spiroplasma citri. *J Bacteriol* 173, 2225-2230[This article describes the transference of the *cat* gene into *S. citri* using a bacteriophage, which initiated a push to develop more molecular tools for *Mollicutes*].

161. Starr, C.R., and Engleberg, N.C. (2006) Role of hyaluronidase in subcutaneous spread and growth of group A streptococcus. *Infect Immun* 74, 40-48[This article describes the contribution to the glycosidase hyaluronidase to tissue destruction by *S. pyogenes* and related organisms].

162. Stipkovits, L., and Kempf, I. (1996) Mycoplasmoses in poultry. *Rev Sci Tech* 15, 1495-1525[This review article describes the disease states associated with *M. gallisepticum*, *M. synoviae*, *M. iowae*, *M. meleagridis*, and other species in poultry. Also discussed are treatment and control strategies.

163. Suits, M.D., Zhu, Y., Taylor, E.J., Walton, J., Zechel, D.L., Gilbert, H.J., and Davies, G.J. (2010) Structure and kinetic investigation of Streptococcus pyogenes family GH38 alpha-mannosidase. *PLoS One* 5, e9006[This article describes mannose cleavage by *S. pyogenes*].

164. Synthetic Genomics. (n.d). The Global Challenge: Sustainably meeting the increasing demand for critical resources. Retrieved from http:// www.syntheticgenomics.com /index.html. [The company

Synthetic Genomics' web site describes some of the potential benefits to designed, synthetically created organisms including biofuel production].

165. Szczepanek, S.M., Frasca, S., Schumacher, V.L., Liao, X., Padula, M., Djordjevic, S.P., and Geary, S.J. (2010) Identification of lipoprotein MslA as a neoteric virulence factor of Mycoplasma gallisepticum. *Infect Immun* 78, 3475-3483[This article describes attenuation of *M. gallisepticum* disease by a strain lacking MslA, an uncharacterized lipoprotein. This protein seems to be an element of virulence, but its function is not currently understood].

166. Tajima, M., Yagihashi, T., and Miki, Y. (1982) Capsular material of Mycoplasma gallisepticum and its possible relevance to the pathogenic process. *Infect Immun* 36, 830-833[This article describes the observation of putative exopolysaccharides associated with *M. gallisepticum*].

167. Takii, T., Hamasaki, S., Hirano, K., Abe, C., and Onozaki, K. (2005) Simple fibroblast-based assay to test the pyrazinamide susceptibility of Mycobacterium tuberculosis. *Antimicrob Agents Chemother* 49, 804-807[This article describes a method to assess susceptibility of an intracellular pathogen to an antimicrobial agents].

168. Tarshis, M., Yavlovich, A., Katzenell, A., Ginsburg, I., and Rottem, S. (2004) Intracellular location and survival of Mycoplasma penetrans within HeLa cells. *Curr Microbiol* 49, 136-140[This article describes invasion of cultured human cells by *M. penetrans*. Intracellular location is often associated with failure of antimicrobial therapy to completely resolve infection, resulting in recrudescence].

169. Taylor, G., and Taylor-Robinson, D. (1976) Effects of active and passive immunization on Mycoplasma pulmonis-induced pneumonia in mice. *Immunology* 30, 611-618[This article describes the differences between attempting to develop protective immunity against *M. pulmonis* by administration of a vaccine (active immunization) as opposed to protecting mice by transferring antibodies (passive immunization). Such studies provide insight into correlates of protection and immunopathology].

170. Taylor, R.R., Varsani, H., and Miles, R.J. (1994) Alternatives to arginine as energy sources for the non-fermentative Mycoplasma gallinarum. *FEMS Microbiol Lett* 115, 163-167[This article describes catabolism of organic acids by *M. gallinarum*, and recommends this features as a diagnostic indicator differentiating it from *M. meleagridis*].

171. Taylor-Robinson, D., Davies, H.A., Sarathchandra, P., and Furr, P.M. (1991) Intracellular location of mycoplasmas in cultured cells demonstrated by immunocytochemistry and electron microscopy. *Int J Exp Pathol* 72, 705-714[This article describes invasion of cultured human cells by *M. fermentans*. Intracellular location is often associated with failure of antimicrobial therapy to completely resolve infection, resulting in recrudescence].

172. Termeer, C., Benedix, F., Sleeman, J., Fieber, C., Voith, U., Ahrens, T., Miyake, K., Freudenberg, M., Galanos, C., and Simon, J.C. (2002) Oligosaccharides of Hyaluronan activate dendritic cells via toll-like receptor 4. *J Exp Med* 195, 99-111[This article describes the mechanism of immune activation from fragments of the host glycan hyaluronan. This often occurs during infection with hyaluronidase-producing organisms, but can also occur following tissue injury. This "injured-self" immune activation is called "sterile inflammation"].

173. Thirkill, C.E., and Gregerson, D.S. (1982) Mycoplasma arthritidis-induced ocular inflammatory disease. *Infect Immun* 36, 775-781[This article describes experimental infection of mice with *M. arthritidis* that unexpectedly resulted in conjunctivitis].

174. Thomas, C.B., Van Ess, P., Wolfgram, L.J., Riebe, J., Sharp, P., and Schultz, R.D. (1991) Adherence to bovine neutrophils and suppression of neutrophil chemiluminescence by Mycoplasma bovis. *Vet Immunol Immunopathol* 27, 365-381[This article describes the attachment of *M. bovis* to immune system cells and the modulation of at least one function].

175. Thomsen, A.C., and Heron, I. (1979) Effect of mycoplasmas on phagocytosis and immunocompetence in rats. *Acta Pathol Microbiol Scand* C 87C, 67-71[This article describes the impact of multiple mycoplasmas and ureaplasmas on neutrophils, B cells, and T cells, and shows disruption of their normal functions].

176. Uphoff, C.C., and Drexler, H.G. (2002) Comparative antibiotic eradication of mycoplasma infections from continuous cell lines. *In Vitro Cell Dev Biol Anim* 38, 86-89[This article describes strategies to eliminate mycoplasma contamination from cell lines. This is particularly important for newly generated cell lines or those containing a precious mutation].

177. van Ham, R.C., Kamerbeek, J., Palacios, C., Rausell, C., Abascal, F., Bastolla, U., Fernández, J.M., Jiménez, L., Postigo, M., Silva, F.J., Tamames, J., Viguera, E., Latorre, A., Valencia, A., Morán, F., and Moya, A. (2003) Reductive genome evolution in Buchnera aphidicola. *Proc Natl Acad Sci U S A* 100, 581-586[This article describes genome sequencing of *B. aphidicola* and identifies those genes that were lost during adaptation to permanent cohabitation with an animal host].

178. Vasconcelos, A.T., Ferreira, H.B., Bizarro, C.V., Bonatto, S.L., Carvalho, M.O., Pinto, P.M., Almeida, D.F., Almeida, L.G., Almeida, R., Alves-Filho, L., Assunção, E.N., Azevedo, V.A., Bogo, M.R., Brigido, M.M., Brocchi, M., Burity, H.A., Camargo, A.A., Camargo, S.S., Carepo, M.S., Carraro, D.M., de Mattos Cascardo, J.C., Castro, L.A., Cavalcanti, G., Chemale, G., Collevatti, R.G., Cunha, C.W., Dallagiovanna, B., Dambrós, B.P., Dellagostin, O.A., Falcão, C., Fantinatti-Garboggini, F., Felipe, M.S., Fiorentin, L., Franco, G.R., Freitas, N.S., Frías, D., Grangeiro, T.B., Grisard, E.C., Guimarães, C.T., Hungria, M., Jardim, S.N., Krieger, M.A., Laurino, J.P., Lima, L.F., Lopes, M.I., Loreto, E.L., Madeira, H.M., Manfio, G.P., Maranhão, A.Q., Martinkovics, C.T., Medeiros, S.R., Moreira, M.A., Neiva, M., Ramalho-Neto, C.E., Nicolás, M.F., Oliveira, S.C., Paixão, R.F., Pedrosa, F.O., Pena, S.D., Pereira, M., Pereira-Ferrari, L., Piffer, I., Pinto, L.S., Potrich, D.P., Salim, A.C., Santos, F.R., Schmitt, R., Schneider, M.P., Schrank, A., Schrank, I.S., Schuck, A.F., Seuanez, H.N., Silva, D.W., Silva, R., Silva, S.C., Soares, C.M., Souza, K.R., Souza, R.C., Staats, C.C., Steffens, M.B., Teixeira, S.M., Urmenyi, T.P., Vainstein, M.H., Zuccherato, L.W., Simpson, A.J., and Zaha, A. (2005) Swine and poultry pathogens: the complete genome sequences of two strains of Mycoplasma hyopneumoniae and a strain of Mycoplasma synoviae. *J Bacteriol* 187, 5568-5577[This article describes the complete genomes of a field strain of *M. synoviae*, and two strains (one field, one laboratory) of *M. hyopneumoniae*. A third strain (232) of *M. hyopneumoniae* was sequenced by Minion *et al.*].

179. Vilei, E.M., Correia, I., Ferronha, M.H., Bischof, D.F., and Frey, J. (2007) Beta-D-glucoside utilization by Mycoplasma mycoides subsp. mycoides SC: possible involvement in the control of cytotoxicity towards bovine lung cells. *BMC Microbiol* 7, 31[This article describes toxicity and cytopathic effect on bovine epithelial cells by *M. mycoides mycoides* in relation to β-glucosidase. The authors conclude that the enzyme likely aids in virulence by providing a nutritional advantage in the bovine host].

180. Vimr, E., and Lichtensteiger, C. (2002) To sialylate, or not to sialylate: that is the question. *Trends Microbiol* 10, 254-257[This review article discusses the addition of sialic acid modification to the cell surface of bacterial pathogens, and how these pathogens balance evasive modifications with nutritional needs].

181. Voelker, L.L., Weaver, K.E., Ehle, L.J., and Washburn, L.R. (1995) Association of lysogenic bacteriophage MAV1 with virulence of Mycoplasma arthritidis. *Infect Immun* 63, 4016-4023[This article describes a bacteriophage virus integrated into the genome of *M. arthritidis*, and associates its presence or absence with virulence].

182. Waite, E.R., and March, J.B. (2002) Capsular polysaccharide conjugate vaccines against contagious bovine pleuropneumonia: Immune responses and protection in mice. *J Comp Pathol* 126, 171-182[This article describes the development of a subunit vaccine against *M. mycoides mycoides* utilizing the exopolysaccharide layer as the antigen].

183. Waites, K.B., and Talkington, D.F. (2005) New Developments in Human Diseases due to Mycoplasmas. In A. Blanchard, and G. Browning (Eds.), *Mycoplasmas: Molecular Biology, Pathogenicity, and Strategies for Control* (pp. 439-483). Norfolk, UK: Horizon Bioscience[This textbook chapter describes common and rare manifestations of mycoplasmosis in humans].

184. Wallace, D.C., and Morowitz, H.J. (1973) Genome size and evolution. *Chromosoma* 40, 121-126[This article attempts to describe evolutionary history of bacterial species by genome size. Genome sequencing has rendered this approach largely antiquated].

185. Wang, H.H. (2010) Synthetic genomes for synthetic biology. *J Mol Cell Biol* 2, 178-179[This commentary discusses the positive potential contributions that the generation of synthetic genomes and synthetic cells to biological science and to society].

186. Washburn L. R., and Cole B.C. (2002) Mycoplasma Arthritidis Pathogenicity: Membranes, MAM, and MAV1. In S. Razin (Ed.), *Molecular Biology and Pathogenicity of Mycoplasmas* (pp. 473-489). London, UK: Academic/Plenum Publishers[This textbook chapter describes the unique virulence-associated features of *M. arthritidis* including the bacteriophage MAV1 and the superantigen MAM].

187. Washburn, L.R., Cole, B.C., and Ward, J.R. (1980) Chronic arthritis of rabbits induced by mycoplasmas. II. Antibody response and the deposition of immune complexes. *Arthritis Rheum* 23, 837-845[This article describes the possible autoimmune mechanism of the arthritis associated with *M. arthritidis*].

188. Winner, F., Rosengarten, R., and Citti, C. (2000) In vitro cell invasion of Mycoplasma gallisepticum. *Infect Immun* 68, 4238-4244[This article describes invasion of cultured human cells by *M. gallisepticum*. Intracellular location is often associated with failure of antimicrobial therapy to completely resolve infection, resulting in recrudescence].

189. Woese, C.R., Maniloff, J., and Zablen, L.B. (1980) Phylogenetic analysis of the mycoplasmas. *Proc Natl Acad Sci U S A* 77, 494-498[This article is the first demonstration that the *Mollicutes* are descended from low G + C gram positive bacteria and evolved by degenerate evolution, as evidenced by their 16S rRNA sequence].

190. Yano, T., Ichikawa, Y., Komatu, S., Arai, S., and Oizumi, K. (1994) Association of Mycoplasma pneumoniae antigen with initial onset of bronchial asthma. *Am J Respir Crit Care Med* 149, 1348-1353[This article associated *M. pneumoniae* with not only the exacerbation of asthma, but with its development in previously non-asthmatic individuals].

191. Yavlovich, A., Tarshis, M., and Rottem, S. (2004) Internalization and intracellular survival of Mycoplasma pneumoniae by non-phagocytic cells. *FEMS Microbiol Lett* 233, 241-246[This article describes invasion of cultured human cells by *M. pneumoniae*. Intracellular location is often associated with failure of antimicrobial therapy to completely resolve infection, resulting in recrudescence].

192. Ye, F., Renaudin, J., Bové, J.M., and Laigret, F. (1994) Cloning and sequencing of the replication origin (oriC) of the Spiroplasma citri chromosome and construction of autonomously replicating artificial plasmids. *Curr Microbiol* 29, 23-29[This article describes the development, generation, and functionality of artificial plasmids using the *S. citri* origin of replication, setting up the discovery of a class of molecular tools used in mycoplasmology]..

193. Zhang, Q., Young, T.F., and Ross, R.F. (1994) Microtiter plate adherence assay and receptor analogs for Mycoplasma hyopneumoniae. *Infect Immun* 62, 1616-1622[This article describes a novel technique for characterizing *M. hyopneumoniae*-host cell ligand interations. Reporting receptor analogs also suggests methods for interference with host cell ligands].

Biographical Sketches

Meghan Anne May was born in 1979. In 2001 she graduated from the University of New Hampshire's Department of Microbiology and went on to earn Master of Science (2004) and Doctor of Philosophy (2006) degrees from the University of Connecticut's Department of Pathobiology and Veterinary Science for her work on avian mycoplasmosis. She then completed postdoctoral work at the University of Florida College of Veterinary Medicine's Department of Infectious Diseases and Pathology focusing on wildlife and agricultural mycoplasmosis. In 2010 Meghan May was appointed to the faculty of the Department of Biological Sciences at Towson University as the expert in Microbiology and Animal Diseases, and currently hold the Fisher Endowed Chair of Biological Sciences. Also in 2010, she was elected to the International Committee for the Systematics of Prokaryotes subcommittee for the taxonomy of *Mollicutes*. Effective July 2012, she will be the chair of the Molecular Genetics Team of the International Research Programme for Comparative Mycoplasmology. She is the author of several peer-reviewed articles and a growing number of textbook chapters, most notably in Bergey's Manual of Systematic Bacteriology. Her scientific interests include the evolution of virulence as modeled by mycoplasmas and novel approaches to treatment and control of livestock mycoplasmosis as a means to combating poverty and malnutrition.

Amanda Victoria Arjoon, **Jessica Ann Canter**, **Dylan William Dunne**, and **Brittney Terrell** are currently enrolled students in the Fisher College of Science and Mathematics and/or the College of Health Professions at Towson University. These early-career scientists are the authors of five presentations at national and international meetings to date.