

REVIEW ARTICLE

Emerging Antibiotic Resistance in *Mycoplasma* Microorganisms, Designing Effective and Novel Drugs / Therapeutic Targets: Current Knowledge and Futuristic Prospects

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(Received: 02 February 2019; accepted: 06 March 2019)

Citation: Mohd. Iqbal Yatoo, Oveas Raffiq Parray, Riyaz Ahmed Bhat, Muheet, Arumugam Gopalakrishnan, Archana Saxena, Sandip Chakraborty, Ruchi Tiwari, Sandip Kumar Khurana, Shoor Vir Singh and Kuldeep Dhama, Emerging Antibiotic Resistance in *Mycoplasma* Microorganisms, Designing Effective and Novel Drugs / Therapeutic Targets: Current Knowledge and Futuristic Prospects, *J Pure Appl Microbiol.*, 2019; **13**(1):27-44 doi: 10.22207/JPAM.13.1.03

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Abstract

Emerging antibiotic resistance among mycoplasma microorganisms is of major concern in present times as they cause various diseases in both animals and humans. Mycoplasmoses, infections caused by mycoplasma microorganisms have become common in recent past and have gained importance both due to inability to diagnose and difficulty to treat. Respiratory tract infection, mastitis, arthritis, and septicemia caused by *Mycoplasma* in livestock are responsible for causing heavy economic losses. These diseases are frequently reported from countries of Africa and Asia, including India. Antimycoplasma antibiotics are frequently being used as therapeutic agents for the treatment of mycoplasmoses infection in livestock. They include macrolides, tetracyclines, fluoroquinolones, and aminoglycosides which are the main antibiotic classes commonly used against mycoplasma globally. Oxytetracyclines are the commonest antibiotics used for decades followed by enrofloxacin, tylosin, and streptomycin. Danofloxacin, lincomycin, spiramycin, erythromycin, gamithromycin, azithromycin, clarithromycin, gentamicin, doxycycline, and tulathromycin are also used occasionally. Continuous and unregulated use of these antibiotics over prolonged period can lead to menace of antibiotic resistance which is aided by inappropriate doses and uncontrolled use. Resistance to some antibiotics is already emerging. Mycoplasmas have devised different resistance mechanisms for combating antimicrobial action of these drugs. Common mechanisms noted are acquisitions of proteins affecting ribosomal subunits, inhibition of antibiotic efflux, structural changes in the ribosomal subunit, target mutations, expression or production of enzymes. Additional novel mechanisms of resistance still need to be investigated. Strategies for prevention and encountering of this antibiotic resistance are being devised by alternating antibiotics in application, using antimycoplasma antibiotic sensitivity tests, along with evaluation of specific doses and exploration of novel mycoplasma specific class of antibiotics. Novel targets based on various cell structures including cell membrane, organelles, proteins, enzymes or metabolites are being explored for antimycoplasma therapy. These all will help in effective therapeutic management of mycoplasmoses with minimal side effects.

Keywords: Antibiotics, Mycoplasma, Novel drug Targets, antibiotic resistance.

INTRODUCTION

Mycoplasmoses, infections caused by mycoplasma (smallest prokaryotes lacking cell wall) microorganisms have become common in both livestock and humans in recent past which might be due to emergence of these pathogens and secondly due to resistance to antibiotics^{1,2,3,4,5}. For farmers it has vast economic constraints. Mycoplasmoses has gained importance both due to occurrence of frequent outbreaks, inability to diagnose and difficulty to treat^{4,5,6,7,8}. Utilization of inappropriate doses of mycoplasma non specific antibiotics is the routine practice in both medical and veterinary practice^{4,9,10}. Continuous use of antibiotics against other microbial pathogens also flares up mycoplasma growth and hence frequent emergence of outbreaks^{9,10,11,12}. Lack of prophylactic measures adds to the problem^{4,8}. Fastidious nature and special requirements of costly media for mycoplasma along with lack of laboratory facilities renders diagnosis difficult

hence non utilization of mycoplasma specific therapies or control measures for mycoplasma infections usually get unnoticed or ignored or remain undiagnosed^{4,8,13,14}. Prolonged usage of antibiotics against microbial infections in inappropriate doses leads to development of antibiotic resistance which is recent concern of importance^{15,16}.

Even though *Mycoplasma* lack cell wall but many other cellular systems are similar to bacteria. They are cellular parasites and derive their requirements from host^{17,18} and have limited synthetic ability due to lack of machinery and hence parasitise host cells *in vivo* and require specialized media *in vitro*^{17,19,20,21}. These wall less organisms contain capsule, lipoprotein plasma membrane, genetic material (DNA), ribosomes, soluble proteins, metabolites and enzymes^{18,20}. Of these capsule or plasma membrane is believed to be of importance for antigenicity. Despite such limited capability and specific requirements

mycoplasma have enormous reproducibility and cause serious infections and a range of diseases in farm animals and humans^{18,22}. These smallest fastidious bacteria affect respiratory tract, udder, eyes, ear, joints, and reproductive system and cause various diseases in animals as well as in humans.

There are around 32 species of mycoplasma of veterinary importance²³ and around 7 species of mycoplasma that are known to cause disease in humans^{24,25}. Of the important species of mycoplasma, *Mycoplasma bovis*, *M. mycoides*, *M. alkalescens*, *M. ovipneumonia*, *M. capripneumonia*, *M. capricolum*, *M. capri*, *M. gallisepticum*, *M. gallinarium*, *M. agalactiae*, *M. canadense*, *M. californicum*, *M. bovis genitalium*, *M. putrefaciens*, *M. synoviae*, *M. agalactiae*, *M. putrefaciens*, *M. conjunctiva*, *M. arginine* are important causative agents of various diseases affecting respiratory tract, udder, joints, eyes and ears, reproductive tract etc^{17,19,20,21,26}. *M. alkalescens*, *M. ovipneumonia*, *M. capripneumonia*, *M. capricolum*, *M. capri*, *M. gallisepticum*, *M. gallinarium*, mastitis (*Mycoplasma bovis*, *M. agalactiae*, *M. canadense*, *M. californicum*, *M. alkalescens*, *M. bovis genitalium*, *M. putrefaciens*), arthritis (*M. canadense*, *M. synoviae*, *M. agalactiae*, *M. capricolum*, *M. putrefaciens*), abortion (*M. bovis genitalium*), eye and ear infections (*M. conjunctiva*, *M. arginini*), other conditions like lymphadenitis, peritonitis, pericarditis, septicemia, hepatopathy, air sac disease, decrease fertility, hatchability and repeat breeding^{17,19,20,21,27,28,29,30}. These range from mild to severe peracute and highly fatal infections. Pneumonia, mastitis, and arthritis are the common diseases of mycoplasma whereas meningitis, otitis, kerato-conjunctivitis, decubital abscesses, vaginitis and/or abortion are occasional findings^{31,32}. In small ruminants, they commonly cause respiratory diseases, arthritis, eye lesions, genital disease and mastitis^{20,33}. Contagious bovine pleuropneumonia (CBPP), contagious caprine pleuropneumonia (CCPP), contagious agalactia, chronic respiratory disease (CRD), mastitis, arthritis, kerato-conjunctivitis (pink eye), infectious sinusitis are some of the important diseases caused by mycoplasma in livestock and poultry^{4,5,34,35,36}. In humans they cause pneumonia (*M. pneumonia*), inflammatory diseases (*M.*

genitalium), infections of the genitourinary tract, infertility, septic abortion and amnionitis (*M. pneumonia*, *M. hominis*), disorders of the hematopoietic, cardiovascular, central nervous, musculoskeletal, cutaneous and gastrointestinal systems (*M. pneumonia*, *M. hominis*)^{24,25,37,38,39}.

Among the mycoplasmal diseases of livestock, respiratory tract infection, mastitis, arthritis, and septicemia are considered to cause heavy economic losses and are often reported from countries of Africa and Asia, including India^{4,35,36,40,41}. These usually terminate in chronic complications, leading to huge economic losses in the form of diminished production, treatment cost, high mortality and decreased export, and are causing threat to production, creating carriers and constant risk for disease outbreak. Due to lack of vaccines, preventive measures are non specific and usually rarely adopted against mycoplasma. Diagnostic facilities are not well equipped for culture, isolation or detection of mycoplasma. Moreover there is also difficulty in controlling the diseases because of lack in consistency of expression of disease³⁰. Hence the only possible option and current practice of dealing with mycoplasma is utilizing antibiotics for therapeutic purposes. They are usually used for treating mycoplasma affected animals and now a days few have been used for prophylactic purposes for preventing mycoplasma infections in risk herds e.g. oxytetracycline. Treatment of affected flocks with broad acting antibiotics is practiced to control the spread of the infection. These treatments and the antibiotics used however are having fewer efficacies, thus there is a doubt on the efficacy of antibiotic therapy⁴². A very significant instance is the disease contagious caprine pleuropneumonia (CCPP) in India wherein antibiotic therapy (usual) is proven to be a failure ultimately resulting in quick disease spread⁴³. Since Mollicutes lack cell wall, hence the cell wall acting antibiotics, such as betalactam antibiotics, glycopeptides, and fosfomycin, do not affect them and also the biological characteristic features of mycoplasmas results in the ineffectiveness of a number of other antimicrobials (sulfonamides, trimethoprim, rifampin, polymyxin, nalidixic acid, linezolid, and some others)⁴⁴. In addition to this use of inappropriate doses of antibiotics and use at large scale in field conditions endangers to the

risk of antibiotic resistance.

Mycoplasmas have devised a number of resistance mechanisms for combating the antibiotics. Due to lack of cell wall mycoplasma have intrinsic resistance to antibiotic classes beta-lactams and to all antimicrobials which target the cell wall⁴⁵. Target alterations and efflux mechanisms are novel acquired resistance mechanisms of mycoplasma developed through genetic mutation or transfer of new genes⁴⁴. Target alterations, in ribosome and in topoisomerase II genes have been found as resistance mechanisms to macrolide and tetracycline and fluoro-quinolone class of antibiotics, respectively^{44,45}. However other mechanisms of antibiotic resistance in mycoplasma need to be explored⁴⁶.

As there is rise of antibiotic resistance against mycoplasma, ineffectiveness of commonly available and used antibiotics adds to problem hence there is dire need for exploring novel strategies and novel targets for antimycoplasma therapy^{47,48,49}. These include the follow ups for antibiotic sensitivity testing, employing alternative antibiotics, and alternate therapies. Novel targets are based on proteomics^{46,50}, genomics, enzymes⁵² or metabolism⁵³, besides there are numerous other novel targets which are under investigation^{44,47, 48,49} and further exploration can help in identifying specific and effective therapeutic and drug targets in future. All these measures will help in preventing antibiotic resistance in mycoplasma, emergence of resistant species, exploring novel targets, safe and effective therapy of mycoplasma diseases in future.

The present review highlights the situation of emerging antibiotic resistance in *Mycoplasma* microorganisms, and designing effective and novel drugs / therapeutic targets to counter these pathogens for safeguarding health of animals and humans.

Antimycoplasma therapy

Commonly used therapeutics in antimycoplasma therapy include antibiotics and rarely anti-inflammatory, antipyretic, analgesic and antiallergic agents. The antibiotics used against mycoplasma are usually of class tetracycline, fluoroquinolones, macrolides, aminoglycosides, and cephalosporins. Among these oxytetracycline is commonly used followed by enrofloxacin, tylosin^{5,8,54}, streptomycin, sometimes danofloxacin,

tiamulin, and azithromycin^{55,56}. In humans, commonly used antibiotics for mycoplasma belong to tetracyclines, fluoroquinolones, and macrolides besides commonly used ones include lincosamides, streptogramins, and ketolides⁵⁷. The antimycoplasma activity of triclosan against species of the entity (*Mycoplasma*) which are both sensitive as well as resistant to fluoroquinolones has been well documented⁵⁸. Spectinomycin therapy has been documented against urethritis caused by *Mycoplasma genitalium* (macrolide resistant) in human⁵⁹. A list of antibiotics used against various species of mycoplasma is given in Table 1. Prolonged use of these antibiotics in inappropriate doses in common herds may lead to adverse effects including abortion and teratogenicity (tetracyclines), tooth decay and bone weakness (fluoroquinolones), ototoxicity (amino-glycosides) besides predisposing to menace of antibiotic resistance^{5,8}.

Novel, specific and effective antimycoplasma antibiotics need to be explored for better therapeutic results. *In vitro* testing⁵⁴, alternating antibiotics^{81,84,85}, long acting antibiotics⁸⁶, novel macrolide, tetracyclines, aminoglycosides or quinolones^{81,84}, pleuro-mutilin antibiotics like valnemulin and tiamulin^{87,88} and advanced antimicrobials^{89,90} are the future prospects with possible promising results for making antibiotic therapy of mycoplasma safe and effective and combating antibiotic resistance⁴⁴.

Antimycoplasma antibiotic resistance

Antimycoplasma antibiotic resistance is the current issue of emergence and requires timely intervention for proper resolution. Since decades mycoplasmosis is being treated mainly with antimicrobials with varying degree of success, as vaccination is either lacking or has not been effective^{49,91}. Antimicrobials like tetracyclines, macrolides, aminoglycosides, fluoroquinolones, and cephalosporins are common agents used in animals and humans against mycoplasmosis^{34,57,92,93,94}. Other antibiotics used are lincosamides, streptogramins, and ketolides⁵⁷. Since mycoplasmas are among the fastest evolving bacteria, with high mutation rates hence resistance to antibiotics has developed⁴⁴. Also due to indiscriminate use of antimicrobials that too in inappropriate doses drug resistance has developed^{91,95}. It has been also observed that

in Mycoplasmas there is development of intrinsic resistance to β -lactams and other antimicrobials targeting the bacterial cell wall⁹⁶.

It is interesting to note that the antibiotics to which the entities are usually sensitive may prove to be ineffective due to development of resistance⁹⁷. Recently a number of studies have been carried out for evaluating antibiotic sensitivity of mycoplasma species against antimycoplasma antibiotics^{98,99,100,101,102}. The antibiotics under focus included enrofloxacin, marbofloxacin, melittin, gramicidin D, spiramycin, tulathromycin, erythromycin, tylosin, florfenicol, oxytetra-cycline, doxycycline, tilmicosin, lincomycin, tiamulin, gamithromycin, tildipirosin and valnemulin^{98,99,100,101,102}. These studies predict decrease of sensitivity and rise of resistance to antibiotics. Increased resistance of *Mycoplasma bovis* to antibiotics like spectinomycin and tilmicosin is also quite noteworthy¹⁰³.

Antibiotic resistance in microbes is an emergent issue, which poses a serious concern throughout the world^{3,104,105}. In the initial stages of discovery, most pathogenic organisms were susceptible to antibiotics and hence were cured successfully but with the passage of time resistance developed and effectiveness of antibiotics decreased¹⁰⁶. In the present times commonly tetracyclines, macrolides, fluoroquinolones and aminoglycosides are being used against mycoplasma. Generally, macrolides such as tylosin is considered as the drug of choice and is extensively used in practice for the treatment of caprine, bovine and avian mycoplasmosis. Enrofloxacin was considered as the most efficacious among the antimicrobial agents against mycoplasma infection^{93,107,108}. In many European countries, antimicrobial resistance of *Mycoplasma* spp. has been reported against tylosin, oxytetracycline and spectinomycin¹⁰⁹. Resistance to tetracyclines, quinolones, and macrolides in mycoplasma develops in a similar manner as observed in other bacteria. However in some mycoplasmas such mechanisms are yet to be known⁵⁷ indicating unexplored mechanisms of resistance or adaptations in mycoplasma⁴⁴. Resistance to oxytetracycline is suspected in Mccp^{5,8}.

Of the various antimicrobials used against mycoplasma, tetracyclines are the most commonly

and widely in humans and farm animals^{57,110,111}. Their mechanisms of action involves inhibition of protein synthesis by affecting 30S subunit of ribosome through blocking the attachment of charged aminoacyl-tRNA to the A site on the 30S subunit ribosome¹¹². Thus, it prevents introduction of new amino acids to the nascent peptide chain¹¹³. Because of prolonged and continuous use of tetracyclines against microbial infections the problem of antibiotic resistance has developed. The different mechanisms involved in resistance are active cellular efflux, expression of protective proteins, antibiotic degradation with enzymes, interfering drug entry, and target modification^{112,114}. These are considered to be the main mechanisms of tetracyclines resistance in classic bacteria. The development of tetracycline resistance in mycoplasmas in some cases is associated with the acquisition of tet(M) determinants located at the Tn916 transposon¹¹⁵. The transposon encodes the TetM protein, protecting ribosomes from the effects of tetracyclines. This protein is homologous to the eF-Tu and eF-G elongation factors. It can cause conformational changes in the 30S ribosomal subunit, preventing it from binding to tetracyclines. TetM determinant when present is found to be associated with a greater degree of tetracycline resistance. This is actually responsible for mycoplasmal cross resistance to other members of tetracycline group of antibiotics^{44,57}. Also, resistance mechanisms in mycoplasma are also believed to be due to mutations in the tetracycline-binding unit of 16S rRNA^{116,117}. Preliminary studies suggest that Mccp may have developed resistance to oxytetracycline^{5,8}.

Macrolides are recommended as drug of choice against mycoplasma and are also being applied in few areas. The mechanism of action of macrolides is by inhibition of bacterial protein biosynthesis, by preventing peptidyltransferase from adding the growing peptide attached to tRNA to the next amino acid¹¹⁸. Resistance to macrolides is noted in both classical bacteria and mycoplasma (e.g. *Mycoplasma pneumoniae*)^{119,120,121}. Target modification, alteration in drug efflux, and degradation of antibiotics by enzyme are the three mechanisms of macrolide resistance^{122,123}. Development of resistance to macrolides in mycoplasma is on rise and is linked to inhibition of efflux of antibiotic and changes in structure of

50S ribosomal sub unit⁵⁷. The alteration in the central loop of domain V of 23S rRNA can also cause resistance in mycoplasmas⁵⁷. Mutation can also lead to resistance to macrolides in mycoplasma⁴⁴. It has been observed that due to point mutations at various sites in the 23S rRNA domain V there may be development of resistance to macrolides^{124,125, 126,127}. It is to be noted that resistance of greater intensity to macrolides (14- as well as 15-membered) is associated with mutation A2058G¹²⁸. *In vivo* mutation has been reported in certain instances in the L4 as well as L22 proteins (ribosomal)^{12,120,129}. Rise of new mutants can also infer resistance⁹⁸.

Tylosin is considered as the drug of choice and is widely used in practice for the treatment of caprine, bovine and avian mycoplasmosis¹³⁰. But¹³¹ Shahet al. has reported that tylosin showed resistance in clinical cases of CCPP and attributed it to indiscriminate use of the antimicrobial drug.

Fluoroquinolones though constitute the most popular group of drugs in human medicine against mycoplasma^{57,132,133} but are occasionally used in animal mycoplasma diseases^{134,135}. These antimicrobials act by interfering DNA synthesis targeting DNA gyrase and topoisomerase IV¹³⁶. The resistance mechanisms involve either alterations in the drug target or alterations in permeability^{135, 134,136,137}. Quinolone-modifying or degrading enzymes have not been identified yet for resistance mechanism in bacteria but in some fungi¹³⁸. There may be development of mutations in the genes coding for particular enzyme significantly and that depends on the antibiotic. For instance resistance to ofloxacin, trova-floxacin and ciprofloxacin has been observed in *Mycoplasma hominis*. This is due to topo-isomerase IV gene mutation. Due to mutation of the gene coding for DNA gyrase, sparfloxacin resistance is observed^{44,139}.

Main mechanisms of fluoroquinolone resistance in bacteria include target modifications due to mutations in the quinolone resistance-determining region (QRDR) of the target genes *gyrA* (DNA gyrase subunit A), *gyrB* (DNA gyrase subunit B), *parC* (topoisomerase IV subunit A), *parE* (topoisomerase IV subunit B), reduced drug assimilation by inhibiting influx and increasing efflux and acquiring resistance through genes¹⁴⁰. In case of mycoplasmas, development of drug resistance is most probably associated with

mutations in the QRDR region of the target genes (DNA gyrase and topoisomerase IV)⁵⁷. Fluoroquinolones show cross-resistance among members of this group which is related to mutations and their location^{57,137,141}.

Aminoglycosides are the other mainly used antibiotic class against mycoplasma. They inhibit protein synthesis by interfering with 30S subunit of ribosome and contain an amino-modified glycoside (sugar)¹⁴². Due to prolonged and large scale use, emergence of resistant strains decreased the therapeutic potential of aminoglycosides¹⁴³. Resistance mechanisms include alteration of the ribosomal binding sites in case of streptomycin, reduced uptake of drug and expression of drug inactivating enzymes^{144, 145,146}.

In case of mycoplasma species, mutational resistance has been recorded to aminoglycosides¹⁴⁷. In case of Gram-negative and Gram-positive bacteria three types of antibiotic modifying enzymes are found viz., phosphotransferases, acetyltransferases and nucleotransferases¹⁴⁸ but none of these enzymes have been revealed in mycoplasma. Experimentally, one transposon encoding for gentamicin resistance was introduced by transformation into mycoplasma strains, which expressed them^{149,150}.

The other resistance mechanisms to these classes of drugs which have been noted in other classical microbes are yet to be deciphered in mycoplasma. The resistance mechanisms to other occasionally used antibiotics are also under investigation. Further resistance mechanisms in various species and subspecies of mycoplasma causing severe diseases in humans and animals are also not known. Proper evaluation of antibiotic resistance mechanisms in mycoplasma will help in devising better future therapeutics.

Combating antibiotic resistance

The regulation of antimycoplasma antibiotic therapy is an important aspect of prevention of resistance to antibiotics besides making antibiotic therapy effective. Antibiotic usage needs to be routinely checked in livestock rearing areas. Farmers should be made aware about disadvantages and side effects of prolonged usage of single type of antibiotics in animals. Proper dose of antibiotic is essential as subnormal doses can lead to ineffectiveness and hence resistance. Alternating two or more antibiotics

periodically can help in preventing antibiotic resistance. Though novel, specific and effective antimycoplasmal antibiotics are needed of the hour but better is to develop prophylactic measures as need for the use of antibiotics should not arise. Better managemental practices along with prophylactics can help in preventing mycoplasma diseases and hence limiting use of antibiotics.

Antibiotic sensitivity testing (AST) is one of the most commonly used and convenient method for evaluating sensitivity of mycoplasma to antibiotics^{99,100}. It can predict decrease of sensitivity and rise of resistance⁹⁹. Regulation of antibiotic application in mycoplasmosis based on AST will be helpful in preventing antibiotic resistance. The antibiotics showing better sensitivity can be employed in mycoplasmosis. Recently a number of studies have been carried out for evaluating antibiotic sensitivity of mycoplasma species against antimycoplasmal antibiotics^{99,100,101,102}. The antibiotics under focus included enrofloxacin, marbofloxacin, spiramycin, tulathromycin, erythromycin, tylosin, florfenicol, oxytetracycline, doxycycline, tilmicosin, lincomycin, tiamulin, gamithromycin, tildipirosin and valnemulin. These studies predict decrease of sensitivity and rise of resistance to antibiotics. Novel methods of AST are being employed for sensitivity testing¹⁵¹.

In vitro testing will help in determining sensitivity and standardizing appropriate dose, long acting antibiotics will help in minimizing frequent use of antibiotics, novel members of commonly used classes of antibiotics, and advanced antimicrobials like CRISPER designed antibiotics will be resistance free. Antibiotic alternatives are also being evaluated for effective therapy and prevention of rise of antibiotic resistance¹⁵². The use of antibiotic alternatives along with immunomodulators (systemic) viz., corticosteroids or immunoglobulins intra-venously are found to be efficacious to treat infections due to macrolide-resistant *Mycoplasma pneumoniae* (MRMP)^{153,154,155}. Identifying the novel drug targets for designing effective anti-mycoplasma antibiotics has future prospects for exploration.

Novel targets for antimycoplasmal therapy

The rise of resistance against commonly available and used antimycoplasmal antibiotics ensues exploration of novel targets of mycoplasma for therapeutic purposes. This can also be helpful

in designing effective prophylactic measures. As the mycoplasma consists of cell membranes and genetic material hence molecular structures of these organelles can be targeted besides the mechanisms in which these are involved like adhesion, invasion, pathogenesis, transcription and translation (DNA and protein synthesis)^{48,49}. Capsular lipopoly-saccharides (LPS) of mycoplasma have been investigated¹⁵⁶. The main components of mycoplasma cell membranes are lipoproteins and immediate targets can be lipids or proteins of membrane¹⁵⁷. Proper exploration of biological membranes of mycoplasma for various molecular components can enable better evaluation of molecular targets. Such studies are lacking. Other possible targets are enzymes, proteins, metabolites, metabolic or cycle pathways^{47,48,49}. Besides therapeutics based on these various targets, having different mechanisms of action can also be helpful in preventing antibiotic resistance as mutations of molecular targets can be overpowered. Exploration of novel antigenic structures can be exploited for raising antibodies or serum for therapeutic use or developing vaccines for prophylactic use. For all these novel approaches, exploration of molecular structures of mycoplasma is must. Common structures of mycoplasma include DNA, ribosome, membrane lipoproteins, soluble proteins, enzymes and metabolites. Fig. 1 depicts the common structural components of mycoplasma cell. Among these plasma membrane¹⁵⁷, proteins¹⁷⁴, toxic molecules, metabolic pathways, and attachment and motility⁴⁹, peptidoglycan synthesis or ribosomal activity⁴⁸, glycolipids¹⁷⁵, genomics, proteomics, and metabolic pathways⁴⁷ are being evaluated as novel targets of antimycoplasmal therapy.

Rise of antimycoplasmal antibiotic resistance and continuous use of commonly available antibiotics which are usually effective against mycoplasma further aggravates resistance situation. Hence exploration of novel targets for antimycoplasmal antibiotics is essential^{47,48,49}. These novel targets are being investigated and are usually based on protein^{46,50}, gene^{47,51}, enzymes⁵² or metabolism⁵³. In addition a number of novel targets are being elucidated^{44,47,48,49} and future research will help in identifying specific and effective therapeutic targets in future.

Targetable metabolic pathways include

ABC transport pathway, phosphotransferase system, secretory pathway system, fatty acid and glycerophospholipid metabolism, pentose phosphate pathway, glycolysis, carbohydrate metabolism, amino acid metabolism, terpenoid backbone biosynthesis, carotenoid biosynthesis, purine and pyrimidine metabolism^{44,47}. Targetable genetic pathways include recombination and repair, replication, transcription, translation, RNA degradation, chaperons and folding^{44,47}.

Some of the targetable proteins and enzymes are 50S ribosomal protein L10, phenylalanyl-trnasynthetase subunit beta, cytidylate kinase, 50S ribosomal protein L32, putative nicotinate-nucleotide adenyl transferase, DNA polymerase III subunit beta, DNA polymerase I, thymidylate kinase, DNA-directed RNA polymerase subunit alpha, fructose-bisphosphatealdolase, putative 2, 3-bisphosphoglycerate-independent phosphoglycerate-mutase, ribose-5-phosphate isomerase, ATP synthase subunit C, acetate kinase, purine-nucleoside phosphorylase, and putative nucleoside phosphorylase^{44,47}.

Some of the novel therapeutics approved and/or under experimental trials that target these pathways, enzymes or proteins have been highlighted previously. They target glycolysis/gluconeogenesis, pentose phosphate pathway, fructose and mannose metabolism (phosphoglycolohydroxamic acid), cysteine and methionine metabolism, biosynthesis of amino acids (adenine),

pyrimidine metabolism (P1-(52 -AdeNosyl)P5-(52 -Thymidyl) Pentaphosphate), nicotinateand nicotinamide metabolism (deamido-nad+, citric acid), pyrimidine metabolism (cytosine arabinose-5'-Phosphate, 2',3'-Dideoxycytidine-5'-Monophosphate Cytidine-5'-Monophosphate, Cytidine-5'-diphosphate), purine metabolism, RNA polymerase (rifabutin, Myxopyronin B, Methyl carbamate), oxidative phosphorylation (N-Formylmethionine, Nonan-1-Ol), **biosynthesis of secondary metabolites** (guanosine, cyclouridine, cysteinesulfonic acid, 4-phospho-D-erythronate), glycolysis/gluconeogenesis, glycine, serine and threonine metabolism, methane metabolism (2-Phosphoglyceric Acid, 3-Phosphoglyceric Acid, formic acid), pyruvate metabolism, propanoate metabolism, pentose phosphate pathway, nucleotide excision repair, homologous recombination (aAzelaic Acid, B-2-octylglucoside), aminoacyl-tRNA biosynthesis (1-{3-[(4-pyridin-2-yl)piperazin-1-14yl] sulfonyl} phenyl)-3-(1,3-thiazol-2-yl)urea), DNA replication, mismatch repair, homologous recombination [(5R)-5-(2,3-dibromo-5-ethoxy-4-hydroxybenzyl)-4-oxo-2-thioxo-1,3-thiazolidin-3-yl]acetic acid)], **ribosome** (roxithromycin, clindamycin, clarithromycin, quinupristin, lincomycin, troleandomycin)^{44,47}.

Recently glycolytic enzymes e.g. pyruvate dehydrogenases A to C (PdhA-C), glyceraldehyde-3-phosphate dehydrogenase (GapA), lactate

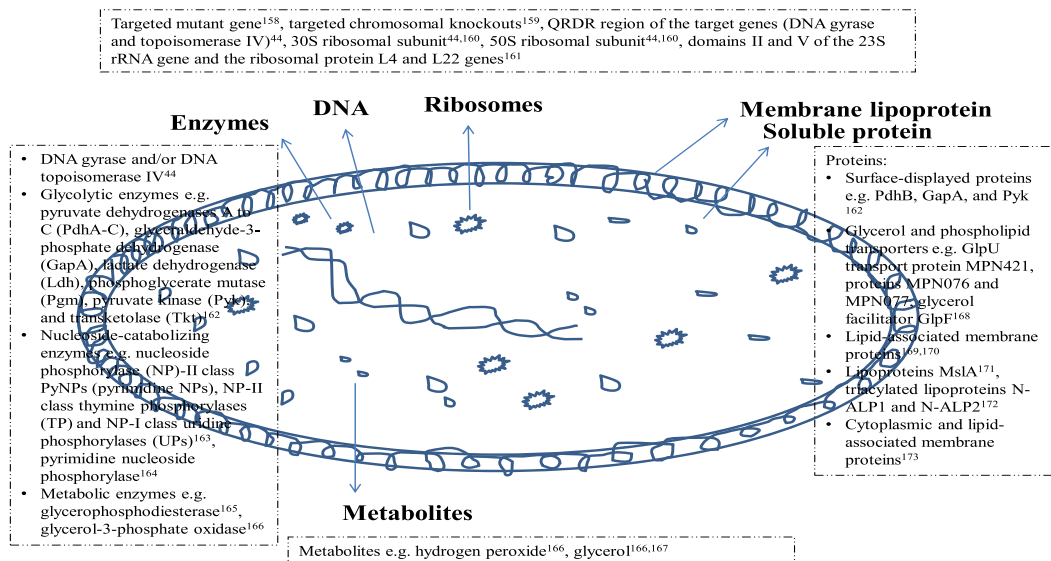


Fig. 1. Mycoplasma cellular structure and antimycoplasmal targets

dehydrogenase (Ldh), phosphoglyceratemutase (Pgm), pyruvate kinase (Pyk), and transketolase (Tkt)¹⁶², nucleoside-catabolizing enzymes e.g. nucleoside phosphorylase (NP)-II class PyNPs (pyrimidine NPs), NP-II class thymine phosphorylases (TP) and NP-I class uridinephosphorylases (UPs)¹⁶³, pyrimidine nucleoside phosphorylase¹⁶⁴, metabolic enzymes e.g. glycerophospho-diesterase¹⁶⁵, glycerol-3-phosphate oxidase¹⁶⁶, metabolites e.g. hydrogen peroxide¹⁶⁶, glycerol^{166,167}, surface-displayed proteins e.g. PdhB, GapA, and Pyk¹⁶², glycerol and phospholipid transporters e.g. GlpU transport protein MPN421, proteins MPN076 and MPN077, glycerol facilitator GlpF¹⁶⁸, lipid-associated membrane proteins^{169,170,173}, lipoproteins MslA¹⁷¹, triacylated lipoproteins N-ALP1 and N-ALP2¹⁷², and cytoplasmic proteins¹⁷³, targeted mutant gene¹⁵⁸, targeted chromosomal knockouts¹⁵⁹, QRDR region of the target genes (DNA gyrase and topoisomerase IV)⁴⁴, components of 30S ribosomal subunit⁴⁴ and 50S ribosomal subunit^{44,160}, domains II and V of the 23S rRNA gene and the ribosomal protein L4 and L22 genes¹⁷³ of mycoplasma have been explored for therapeutic and/or prophylactic possibilities.

It has also been observed that for the purpose of designing therapeutics in order to treat infections caused by *Mycoplasma pneumoniae*, community-acquired respiratory distress syndrome (CARDS) toxin acts as a target (promising) as well as a significant candidate. The NLRP3 inflammasome is activated by the toxin intracellularly which leads to vigorous inflammation in association with infection due to *M. pneumoniae*^{49,176}. CARDS is a significant virulent factor generating antibody titers at high level^{177,178,179}.

Novel drugs/therapies against mycoplasma infections

Considering the rise of resistance against main antimycoplasmal antibiotics like tetracyclines, macrolides or fluoroquinolones, novel drugs or therapies are being explored. Josamycin, pristinamycin, marbofloxacin, solithromycin, lefamulin, sitafloxacin, zoliflodacin, tigecycline, spectinomycin and moxifloxacin are newer antimycoplasmal antibiotics that have shown promising results in antimycoplasmal therapy^{181,182}. Herbal medicines^{182,183}, traditional medicine¹⁸⁴, or their combination¹⁸⁵ have been elucidated for

mycoplasma infections. Novel technologies for safeguarding health of humans and animals are being evaluated¹⁸⁶. Phage therapy^{187,188} has proven effective in some antimycoplasmal therapies. Immunotherapy¹⁸⁹, cytokine therapy^{190,191} and immunomodulation¹⁹² have potent biomedical applications and are being applied in mycoplasma infections also^{189,190}. Immuno-modulators which help in boosting immunity¹⁹³ can be helpful in mycoplasma therapy. Nano therapeutics¹⁹⁴ involving nanomedicines¹⁹⁵ are advanced therapeutics in biomedicine and pharmacotherapy and can help in effective targeting and accurate drug delivery¹⁹⁶. These novel and emerging therapies can overcome resistance problems in mycoplasma infections. Still many aspects of these drugs or therapies are yet to be explored however they hold a great promise for future of antimycoplasmal therapies.

CONCLUSION AND FUTURE PROSPECTS

Antibiotics are frequently being used as antimicrobial agents against mycoplasma and as first line of treatment for the therapeutic management of mycoplasma infection. Commonly macrolides, tetracyclines, fluoroquinolones, and aminoglycosides are the main classes of antibiotics being employed worldwide. Among these, oxytetracycline has been the most commonly used antibiotic for a prolonged period followed by enrofloxacin, tylosin, streptomycin and rarely danofloxacin, lincomycin, spiramycin, erythromycin, gamithromycin, azithromycin, clarithromycin, gentamicin, doxycycline, tulathromycin have been employed. Due to emergence of drug resistant strains of Mycoplasma the effectiveness of these antibiotics is dramatically reduced. Frequent prolonged usage of single type of antibiotics and inappropriate doses can lead to menace of antibiotic resistance. Different microbial mechanisms have been developed by mycoplasma for resistance to antibiotics. This usually includes acquisition of proteins affecting ribosomal subunits, inhibition of antibiotic efflux, structural changes in the ribosomal subunit, target mutations, expression or production of enzymes. Still the exploration of several novel mechanisms to counter Mycoplasma infections is under evaluation. Besides alternating anti-biotics, antimycoplasmal antibiotic sensitivity

Table 1. Antimycoplasma antibiotics used in various mycoplasma diseases

Name of Antibiotic	Class	Dose used/ Recommended	Used against Mycoplasma / Mycoplasma disease	Reference
Tylosin	Macrolide	10 mg/kg/day	<i>Mycoplasma capricolum capripneumoniae</i> , <i>M. agalactiae</i> , <i>Mycoplasma californicum</i>	34,60,61,62, 63,64,65,66
Oxytetracycline	Tetracycline	14-15 mg/kg	<i>Mycoplasma capricolum</i> <i>M. capripneumoniae</i> , <i>M. agalactiae</i> , <i>M. californicum</i>	34,60,61,62,6 3,66,67
Lincomycin	Lincosamide	5 mg/kg IM	<i>Mycoplasma ovipneumoniae</i>	34,68
Oxytetracycline	Tetracycline	20 mg/kg	<i>Mycoplasma ovipneumoniae</i>	34,68
Spiramycin	Macrolide	100 mg or 200 mg/dose	<i>Mycoplasma gallisepticum</i>	55
Enrofloxacin	Fluoroquinolone	15 mg/kg	<i>Mycoplasma gallisepticum</i>	69
Erythromycin	Macrolide	25 mg/kg	<i>M. agalactiae</i> ,	64,65
Gamithromycin	Macrolide	6 mg/kg	<i>Mycoplasma bovis</i>	70
Azithromycin	Macrolide	500mg/dose	<i>M. pneumonia</i>	6
Clarithromycin	Macrolide	15mg/kg/day	<i>M. pneumonia</i>	6
Gentamicin	Aminoglycoside	Topical application of 0.5 % gentamycin (100 mg/ml)	<i>Mycoplasma conjunctivae</i>	41
Doxycycline	Tetracycline	10 mg/kg/day (oral)	<i>Mycoplasma haemofelis</i>	71
Enrofloxacin	fluoroquinolones	5 mg/kg	<i>Mycoplasma haemofelis</i>	71
Spiramycin	Macrolide	100mg/dose	<i>Mycoplasma gallisepticum</i>	55
Tulathromycin	Macrolide	2.5/mg/kg	<i>Mycoplasma ovipneumoniae</i>	72
Doxycycline	Tetracycline	200mg	<i>Mycoplasma genitalium</i>	73
Doxycycline	Tetracycline	0.03125-16 mg/L	<i>Mycoplasma gallisepticum</i>	74
Tulathromycin	Macrolide	2.5 mg/kg SC	<i>Mycoplasma mycoides</i>	34
Florfenicol	chloramphenicol	20 mg/kg q48 IM	<i>Mycoplasma mycoides</i>	34
Tilmicosin	Macrolide	10 mg/kg SC as single dose	<i>Mycoplasma mycoides</i>	34
Gamithromycin	Macrolide	6 mg/kg SC as single dose	<i>Mycoplasma mycoides</i>	34
Danofloxacin	Fluoroquinolone	2.5 mg/kg q24h SC	<i>Mycoplasma mycoides</i>	34
Oxytetracycline	Tetracycline	10 mg/kg IM q24 for at least 4 days	<i>Mycoplasma mycoides</i>	34
Danofloxacin	Fluoroquinolone	danofloxacin (6 mg/kg subcutaneously	<i>Mycoplasma capricolum</i> subsp. <i>capripneumoniae</i>	61
Marbofloxacin	Fluoroquinolone	2 mg/kg BW for 3 days	<i>Mycoplasma capricolum</i> subsp. <i>capripneumoniae</i> <i>Mycoplasma haemofelis</i>	63,75
Tilmicosin	Macrolide		<i>Mycoplasma capricolum</i> subsp. <i>capripneumoniae</i>	56,76
Tylosin	Macrolide		<i>Mycoplasma gallisepticum</i> <i>Mycoplasma synoviae</i>	77
Gamithromycin	Macrolide	6mg/ kg subcutaneously	<i>Mycoplasma bovis</i>	70,78
Spectinomycin	It falls in aminocyclitol class; has close relation with aminoglycosides	20 mg/kg i/m in three repeated doses.	<i>Mycoplasma bovis</i>	79
Minocycline	Tetracycline		Macrolide resistant <i>M. pneumoniae</i> (MRMP)	80,81,82,83,84
Tosufloxacin	Fluroquinolone		Macrolide resistant <i>M. pneumoniae</i> (MRMP)	81

evaluation should be undertaken on routine basis for checking the emerging drug resistance. Evaluation of specific doses and novel mycoplasma specific class of antibiotics need to be explored. Exploring novel therapeutic and drug targets in mycoplasma for effective antibiotic therapy is the recent area of research with promising results.

The rise of antibiotic resistance and ineffectiveness of conventional antibiotic therapy in mycoplasmosis has led to devising strategies and exploring possibilities for combating antimycoplasma resistance and treating mycoplasmosis effectively. It cannot be denied that changes in genomic, transcriptomic, secretomic as well as proteomic profiles of the microbes (including *Mycoplasma*) have influenced the phenomenon of antimicrobial resistance significantly. Regulating antibiotic use, alternating therapies along with appropriate doses of antibiotics, antibiotic sensitivity testing and exploring novel antimycoplasma antibiotics could solve the problem. Novel therapeutic targets are being explored in mycoplasma for antibiotic action which can help in safe and effective treatment of mycoplasmosis. These targets may be toxins, toxic metabolites, enzymes, proteins, metabolic pathways, metabolites, cell division, nucleotides, or cellular structural parts of mycoplasma. However to be successful, these measures need to be explored after proper experimental evaluation followed by application in clinical cases. Future of antibiotic therapy in mycoplasmosis relies solely on devising safe and effective antibiotics with mycoplasma specific targets or mechanism of action and less prone to development of resistance.

ACKNOWLEDGEMENTS

The financial support by Science and Engineering Research Board, Department of Science and Technology, Government of India to the first author through SERB-DST Project (EMR/2016/004929) for this study is highly acknowledged.

CONFLICT OF INTERESTS

The authors declare that there is no conflict of interest.

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