REVIEW ARTICLE

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The *Mycobacterium avium* complex – an underestimated threat to humans and animals

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Abstract

Introduction and objective. The *Mycobacterium avium* complex (MAC) is a group of acid-resistant bacteria within the *Mycobacteriaceae*. Their cell walls have a specific structure impervious to many disinfectants. Mycobacteria are widespread in the environment and can also be found in food. This aim of the article is to review the current state of knowledge about the sources of infection, symptoms and treatment of MAC diseases in humans and animals, and summarizes the available methods for identifying the bacteria. It pays a special attention to the zoonotic potential of MAC bacteria and possible routes of transmission between humans and animals, including possible food-borne routes.

Brief description of the state of knowledge. MAC bacterial infections occur both in immunocompetent people and those with functional predispositions and compromised immunity, particularly during HIV infection or immunosuppressive treatment. The incidence of MAC infections in humans is growing, with the most common form of infection being pulmonary disease (MTC-PD); however, there are conflicting reports on the role of *Mycobacterium avium* paratuberculosis (MAP) in the development of Crohn's disease. MAC bacteria can also attack livestock, household pets, and wild animals. Unfortunately, treatment is lengthy and often fails due to microbiological relapse; there is also increasing evidence of MAC bacteria are developing multi-drug resistance.

Conclusions. Although new antibiotics are being created to inhibit the growth and division of *Mycobacterium avium*, there is clearly a need for further research into the virulence factors associated with MAC bacteria. Further studies should also examine the role of MAP in the etiopathogenesis of Crohn's disease.

Key words

biofilm, Crohn's disease, MAC, multi-drug resistance, MAP, MOTT, Mycobacteriosis, pets

INTRODUCTION AND OBJECTIVE

The *Mycobacterium avium* complex (MAC) is a group of slow-growing bacteria of the *Mycobacteriaceae*, classified as MOTT, i.e. Mycobacteria Other Than Tuberculosis. MOTT are found globally and are widespread in the environment; they are also responsible for opportunistic infections in humans, livestock and wild animals. Such non-tuberculous *mycobacterial* diseases present a major threat to public health, especially in developed countries [1]. The rapid increase in MAC infections worldwide is associated with the growing number of immunocompromised patients [2]. In addition, humans and animals are subject to a continuous increase in environ and milk, as well as in heat-treated animal products [4, 5]. In humans, skin contact with MAC bacteria, consumption of contaminated food, or even aerosols, can result in a range of symptoms, such as lymphadenitis, lung infections, and infections of the skin and soft tissue [6].

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OBJECTIVE

The aim of this review article is to present the current state of knowledge about MAC infections in humans and animals, the virulence factors of MAC bacteria and potential sources of infection, as well as future research on new drugs. The review describes the etiological factors of mycobacteriosis, prevalence of these diseases, ways of identification, pathogenesis and immunology of infection, clinical signs and treatment in both humans and animals. A summary of the most important information and current trends in this area is also presented.

STATE OF KNOWLEDGE

Etiological factor. Mycobacteria are gram-positive acid-resistant bacilli capable of surviving in host phagolysosomes. The *Mycobacterium* genus demonstrate hydrophobicity, impermeability, and slow growth; they are also resistant to disinfectants and antibiotics, which has been attributed to the presence of a lipid-rich outer membrane enriched with long-chain mycolic acids [7]. Over 180 species of mycobacteria have been identified to-date [8]. The genus *Mycobacterium* includes *Mycobacterium tuberculosis* complex (MTC), *M. leprae* and no-tuberculous mycobacteria (NTM) [9],

including MAC, which are widespread in the environment. Historically, the MAC consisted of two species: *M. avium* and *M. intracellulare*. This division was based on pathogenicity in birds, with *M. avium* being pathogenic and *M. intracellulare* non-virulent. However, in September 2018, a new taxonomic division was proposed, comprising *Mycobacterium avium*, *M. intracellulare*, *M. bouchedurhonense*, *M. chimaera*, *M. colombiense*, *M. ituriense*, *M. lepraemurium*, *M. marseillense*, *M. paraintracellulare*, *M. scrofulaceum*, *M. timonense*, *M. lukis*, and *M. yongonense* [10].

The most clinically-important subspecies of *M. avium* are believed to be *M. avium* subsp. *hominissuis* (MAH), *M. avium* subsp. *paratuberculosis* (MAP), *M. avium* subsp. *avium* (MAA) and *M. avium* subsp. *silvaticum* (MAS), and *Mycobacterium avium intracellulare* (MAI). Of these, MAH, a ubiquitous environmental saprophyte, causes chronic lung disease and is an etiological agent of lymphadenitis in pigs. MAH also has a wide range of hosts: cases of disseminated MAH infection have been described in other mammals, including dogs, cats, cattle, goats, domestic rabbits, cervidae and horses [11, 12, 13, 14]. MAA and MAS are most often isolated from birds, in which their symptoms and course resemble tuberculosis [3]. MAP is responsible for Johne's disease, which mainly affects ruminants. *M. chimaera* has also been isolated from patients after heart valve surgery, where it caused infections at the site of surgery, as well as endocarditis, both with a high mortality rate [15].

Prevalence. The majority of MAC species are found in natural waters, water supply systems, and soils [16, 17, 18], as well as in raw [19], cooked and fermented meat products [4], pasteurized milk [20] or other dairy products [21]. Fresh or frozen fruit and vegetables can also be a source of infection [22]. NTMs are oligotrophs that are capable of growing in low carbon environments, and thus surviving in nutrient-poor environments. In addition, thanks to their oligotrophicity and capacity for biofilm production, the bacteria can grow in drinking water distribution systems and water supply networks [7]. This can pose a serious threat: humans can become infected by inhaling aerosols containing NTMs in the shower or in the swimmimg pool [23]. Interestingly, MAC can persist in amoebas: wild amoebas can provide an ideal environment for bacteria to multiply, and can promote their persistence in macrophages [3, 24, 25].

Identification. Microbiological identification involves mainly isolation and culture of the bacteria on selective media in conventional or automatic systems. Conventional approaches include the use of Löwenstein-Jensen (with malachite green), Ogawa (with sodium glutamate) and Stonebrink (sodium pyruvate) media. Bacterial growth lasts from four to twelve weeks and the results are based on visual evaluation [26]. Automatic systems (Bactec MGIT 960, Bactec 460 Tb) [27, 28] automatically record the growth of mycobacteria using a liquid media with known composition. The presence of bacteria is typically confirmed after around five to twelve days of culture. After multiplication of the bacteria on the media, a common identification method is Ziehl-Neelsen staining [29]. The obtained strains can also be used in further, more advanced molecular identification methods.

Serological diagnostics are most commonly performed by detecting specific antibodies using ELISA (enzyme-linked immunosorbent assay). This approach is mainly used to detect anti-MAP antibodies [30]. The test is fast and inexpensive, has a relatively high sensitivity and specificity, and serum or milk samples are easy to obtain [31]. *Mycobacterium* infection can also be detected by the agar gel immunodiffusion test (AGID) [32]: the antigen and antibodies diffuse through a semi-solid agar medium, forming a precipitation where they interact. Typically, a positive result would be indicated by the presence of one or more visible lines in the gel. The AGID has many advantages: it is inexpensive, easy to perform and does not require any specialized auxiliary equipment; however its main disadvantage is its relatively low sensitivity [33].

When identifying *Mycobacterium* species, molecular techniques have a higher sensitivity than serological methods and the results are typically available within 24–48 hours. Molecular methods also offer the further advantage that they can be used to directly identify a species or subspecies, and to detect drug resistance. Polymerase C*hain Reaction (*PCR) is a fast and practical identification technique that can be found in a range of variant, such as Ligase Chain Reaction (LCR), Strand Displacement Amplification (SDA), Nucleic Acid Sequence – Based Amplification (NASBA) [34], and Polymerase C*hain Reaction – R*estriction Fragments Length Polymorphism PCR-RFLP [35]. Commercial linear probe assays (LPA) are also used to identify the most commonly-occurring NTM strains at species or subspecies level [36]. Several commercial hybridization probes are available, including AccuProbe (Genprobe, San Diego, California), INNO-LiPA (Innogenetics, Ghent, Belgium) or GenoType *Mycobacterium* assay (Hain, Lifescience, Germany) [6].

In 2012, a reference genotyping technique was introduced, known as MIRU-VNTR (Mycobacterial Interspersed Repetitive Units – Variable Numbers of Tandem Repeat), based on the analysis of a variable number of tandem repetitions, [37]. The *Mycobacterium* genome contains repeated sequences of several to several dozen base pairs, the number of which varies between strains of the same species. The largest group of VNTR motifs comprises 46–100 nucleotide MIRU sequences. Of the 41 loci that have been identified so far, the 15 with the highest variability are used in mycobacteria genotyping. Briefly, in MIRU-VNTR analysis, individual loci are amplified using appropriate oligonucleotide starter sequences, and the amplicons are then separated in agarose gel. The number of MIRU motif repetitions calculated for each locus allows the results to be catalogued as a 15-digit MIRU-VNTR code [38]. Nowadays, MALDI-TOF MS (Matrix-Assisted Laser Desorption Ionization) is gaining significance in MAC identification: a mass spectrometry-based approach which enables identification of microorganisms by comparing their protein content with reference spectra in a database [39, 40].

Pathogenesis and immunology of infection. The exact pathogenesis of MAC infections and their virulence factors are not yet fully understood. However, evidence suggests that after passing through the oral cavity, the bacteria interact with the gastrointestinal mucosa: *in vitro* studies have confirmed that MAC are able to bind to enterocytes [41]. Such binding may be facilitated by the presence of adhesion proteins on the surface of the bacteria. After passing through the mechanical barriers, mycobacteria are recognized by mononuclear macrophages. This interaction leads to phagocytosis and the release of reactive metabolites, resulting in the initiation of intracellular signaling and the release of cytokines and

chemokines; these are responsible for further activation of the host immune response and chemotaxis of immune cells. MACs employ several mechanisms to survive in adverse conditions inside macrophages: they produce agents that inhibit the mechanisms associated with an oxidative burst (e.g. superoxide dismutase or heat shock proteins), or inhibit the fusion of phagosome and lysosome [42].

The recognition of MAC by macrophages mainly acts through toll-like receptor 2 (TLR2) [43]; this leads to the production of pro-inflammatory cytokines, such as interleukins IL-1β, IL-12, IL-18, tumour necrosis factor α (TNFα), as well as chemokines such as the C-X-C motif chemokine **10 (CXCL-10)** [42]. Chemokines and TNFα cause taxis of inflammatory cells such as lymphocytes, macrophages and dendritic cells, to the inflammation site. The activated macrophages, together with the living mycobacteria, migrate to lymph nodes; here, the antigen is presented to T helper cells by the major histo-compatibility complex class II (MHC II). This mechanism induces a specific immune response in the host [42]. The ability of macrophages to kill mycobacteria is enhanced by the presence of Th1 lymphocytes, which secrete IFN-γ and IL-2. In addition, mycobacteria can also be recognized by the MHC II independent pathway thanks to cluster of differentiation 1 (CD1) [42]. Such recognition is specific to γδT lymphocytes, which are highly reactive towards mycobacteria and capable of killing them [42].

The immune response develops for four to six weeks after infection. Eventually, the bacteria stop multiplying and become trapped in granulomas formed as a result of the host immune response, thus isolating the pathogen. However, this isolation provides the mycobacteria with a niche in which they can survive for a long time by modulating the immune response. The granuloma consists mainly of blood-derived macrophages, epithelial cells (differentiated macrophages) and multinuclear giant cells (also known as Langhans giant cells), which are surrounded by T lymphocytes and fibroblasts [6]. In the middle of the granuloma, a caseous necrosis can occur, in which the decayed cells are located.

Pathogenesis and clinical signs in humans. MAC infections occur mainly in people with functional predispositions or compromised immunity [44]. NTM infections are most commonly associated with secondary immunodeficiency caused by Human Immunodeficiency Virus and TNF-α antagonist therapy, as well as by pulmonary diseases, such as cystic fibrosis and chronic obstructive pulmonary disease, and by immunosuppressive treatment after transplantation or other systemic diseases [6, 45]. Gastro-oesophageal reflux disease (GERD), vitamin D deficiency, rheumatoid arthritis or low body mass index are also risk factors for NTM infection [2]. However, the disease may also develop in immunocompetent people [8, 46, 47].

The most frequently detected NTM causing infection in humans is MAH [6]. Infection usually occurs by inhalation, via contaminated aerosols, or through injured skin [6]. The most common form of infection caused by MAC complex worldwide is MAC-PD pulmonary disease [48,49], which has two clinical forms: fibrous-cavernous and nodular. The former is usually associated with lung diseases already developing in the body, such as lung tuberculosis or chronic obstructive pulmonary disease (COPD); this variant often affects older men and demonstrates rapid progression [8]. The nodular form is characterized by bilateral bronchial dilatation with numerous nodules (Lady Windermere syndrome). It usually occurs in non-smoking post-menopausal women and is characterized by a slow progression [2, 18]. Clinical indications of NTM-PD may be indistinguishable from those of tuberculosis or other respiratory diseases, including lung cancer. The general symptoms are fatigue, fever, and weight loss, while the respiratory symptoms are coughing, haemoptysis and dyspnea [49].

MAC is also known to be responsible for a lung disease resembling hypersensitivity pneumonitis. It was first described at the end of the last century in patients who had used rehabilitation pools or spa baths before the symptoms occurred. Initially, it is accompanied by flu-like symptoms, followed by coughing, dyspnea, fever and night sweats [6]. Another relatively common form of disease caused by MAC, particularly among children, is peripheral lymphadenopathy. The infection most likely occurs through the digestive tract [6]. In humans, MAC can also cause gastrointestinal, skin and soft tissue infection [2, 50].

There are conflicting reports on the role of MAP in the pathogenesis of Crohn's disease. The fact that Crohn's disease follows a similar course to Johne's disease, and MAP have been isolated from peripheral blood mononuclear cells in 50–100% of patients with Crohn's disease [51] suggests that MAP may play a role in the origin of the disease [51, 52, 53, 54]. Crohn's disease can attack any part of the gastrointestinal tract from the mouth to the anus and is often manifested by abdominal pain, loss of energy and weight, mouth ulcers and joint pain. It is commonly associated with diarrhea interspersed with mucus, pus or blood, and about 40% of patients need an ileostomy or colostomy [18].

Pathogenesis and clinical signs in animals. MAC bacteria are also known to cause disease in many animal species, such as dogs [55], cats [56], pigs [42], cattle [57], horses [12] and birds [58]. MAC infections are rarely diagnosed in dogs,; however, the presence of the disease is generally regarded to be associated with immunodeficiency. Some breeds are more susceptible to MAC infection, particularly miniature schnauzers [59] and bassets [60]. In miniature schnauzers, this MAC susceptibility has been attributed to a recessive inherited defect in CARD9 adaptive protein [59, 61]. However, most of the described cases of canine mycobacteriosis concern MAP infections, which can cause long-lasting diarrhea and vomiting [62, 63]. Indeed, MAP-specific DNA was found in intestinal biopsy in 19% of dogs with chronic vomiting and diarrhea, while in dogs without gastrointestinal diseases, MAP DNA was not detected [63]. Occasionally, *M. avium* may cause skin lesions in dogs [64]. Given the zoonotic potential of a MAC-infected dog, it can pose a health risk to humans [59], especially to immunocompromised owners.

In cats, infections with MA complex bacteria occur as infrequently as in dogs. The most common clinical signs include weight loss, lethargy and anorexia [56, 65]. However, one study reported a case of CNS symptoms in a cat during empyema-complicated meningitis caused by MAH [13].

In pigs, MAH is the most frequently-isolated pathogen, while MAA is rarely detected. Although MAH infection usually has a subclinical course in pigs, it is nevertheless an economic problem: the pathological lesions are usually located in the mesenteric or mandibular lymph nodes [66], and these are rejected as unfit for human and animal consumption during *post-mortem* examination, together

with other infected tissues. New studies show that *M. avium* complex can occur in lymph nodes that demonstrate no visible changes during the veterinary sanitary examination, and such carcasses are a potential source of human infection [67]; this is particularly the case for minced meat, which can contain lymph nodes [4]. *M. avium* isolates of human origin have been found to closely resemble those of pig origin [68], suggesting the existence of epidemiological links between infections in pigs and infections in humans, or of infections from common sources [68]. Pigs commonly become infected from sources in the external environment, such as litter, feed, water or soil, following contamination with the faeces of wild birds or small land mammals [6]; infection typically occurs through the alimentary route [42, 69].

The tissue changes caused by MAC bacteria in cattle are indistinguishable from those caused by MTC bacteria, which may make diagnosis difficult. Granulomatous lesions are mainly located in the lymph nodes of the gastrointestinal and respiratory systems, although there have been cases of systemic disease [57, 70, 71]. Ruminants are most often infected by MAP, resulting in chronic inflammation of the intestines, called Johne's disease or paratuberculosis. Johne's disease is a progressive intestinal disease that impairs nutrient absorption due to thickening the intestinal wall [72, 73]. Infected individuals are exhausted by diarrhea, which can lead to the death of the animal. MAP infections hence lead to big economic losses, especially in dairy herds [72, 73].

Horses are relatively resistant to MAC infections, with documented cases referring more to MAA [74] and MAH [12] infections. In the course of the disease, horses often suffer from diarrhea, mastitis and neck stiffness, as well as dyspnea and chronic cough. However, since these symptoms can occur in many other diseases, a diagnosis of mycobacterial infection is difficult: diagnostics require a biopsy of the rectum or distal part of the colon, followed by staining for acid-resistant mycobacteria and bacteriological culture of granulomatous lesions [74].

The main etiological factor of mycobacteria in birds is MAA. In such cases, the bird usually becomes sick first, and then acts as the main reservoir of bacteria. Mycobacterioses are a common problem in poultry as well as domestic birds [58, 75]. The disease is rare in intensive poultry breeding due to improved breeding practices. Transmission usually occurs by the oral route, and airborne infections are less common [76]. Symptoms in birds include emaciation, apathy and diarrhea, along with a distinct atrophy of the chest muscles. *M. avium* infection initially involves the intestine, and then spreads to the liver, spleen, bone marrow, lungs, air sacs, and gonads [77]. Later stages of the disease are characterized by the appearance of non-calcified nodules [77]. In some cases, skin lesions can also be observed [76]. So far, no evidence of direct transmission of atypical mycobacteria between birds and humans has been shown, but it cannot be excluded that diseased animals may be a source of infection for humans in their environment [75]. Studies conducted in the Murcia region, Spain, confirm that *M. avium* plaatstudy was regular contact between children and hens [78].

Treatment. The main treatment options for diseases caused by MAC bacteria in humans and animals are macrolide antibiotics such as clarithromycin or azithromycin [79, 80, 81]. The 2007 American Thoracic Society/Infectious Diseases Society of America (ATS/IDSA) guidelines recommend that treatment should be based on macrolides in combination with rifampicin and ethambutol. Additionally, streptomycin or parenteral amikacin can be administered [8]. Recently suggested alternatives include bedaquiline, which has shown high efficacy against *M. avium*, and Liposomal Amikacin for Inhalation [82]. In humans and animals, treatment of an MAC-caused disease requires prolonged antibiotic therapy, i.e. continuing for at least 12 months after negative cultures with continuous drug use, and even after successful completion, recurrence has been found to recur in 32% – 48% of cases, usually due to MAC-reinfection [3]. A similar treatment protocol based on a combination of ethambutol with multiple antimicrobial agents, including rifampicin, clarithromycin, moxyfloxacin and doxycycline, is used in companion animals [79]; however, cats tend to respond better than dogs [79].

Due to the growing incidence of multidrug resistance among MAC bacteria [6,75], there is a constant need for new antibiotics. Studies have been conducted on the inhibitory effects of $Ga(NO_3)_3$, $GaCl_3$, gallium meso tetraphenylporphyrine (GaTP) and gallium nanoparticles (GaNP) on intra- and extra-cellular MAC bacteria [83]. MAC bacteria also show resistance to chemical disinfectants and ultraviolet radiation [18], mainly due to the structure of their cell wall and the impermeability of their cell membrane [6]. In addition, MAC bacteria are capable of producing enzymes that break down or inactivate antimicrobial agents [82]. Another resistance mechanism observed in *M. avium* is the development of biofilms in the environment, water distribution systems and the human respiratory tract, especially in people with cystic fibrosis or bronchial dilatation, which further reduces the effectiveness of antimicrobial agents [84]. Moreover, the biofilm allows *M. avium* to survive traditional disinfection procedures and confers resistance against chlorine and acidic pH [82]. Biofilm production allows MAC to reduce its interaction with the drug by generating an impermeable biofilm layer. In addition, mutations in the bacterial genome cause primary or induced resistance to therapeutic preparations such as macrolides or rifampicin [6].

CONCLUSIONS

M. avium is one of the most commonly-isolated NTM species worldwide and a potential zoonotic agent. Although the transmission of MAC bacteria from animals to humans has not been confirmed, the number of infections caused by MAC in both humans and animals has been increasing. Such infections are difficult to treat due to the production of various resistance factors that protect the bacteria from antibiotics. The search continues for new drugs that will be effective against MAC infections; however, there is a need to identify all the virulence factors associated with infection to better understand its mechanism. In addition, further research on the etiopathogenesis of Crohn's disease is needed to determine whether MAP plays a role in its development.

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