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REVIEW

Understanding the bacteria in *Mycobacterium avium* complex (MAC) from a bioinformatic perspective – a review

Anindita Banerjee, Mistu Karmakar, Saubashya Sur*

Postgraduate Department of Botany, Life Sciences Block, Ramananda College, Bishnupur-722122, West Bengal, India

ABSTRACT *Mycobacterium avium* complex (MAC) houses a group of non-tuberculous mycobacteria causing pulmonary and disseminated infections. They are accountable for nodular bronchiectatic and fibrocavitary lung diseases in humans, Johne's disease in ruminants, and respiratory diseases in birds. MAC infections pose challenges, owing to antibiotic resistance, prolonged therapy with antibiotic combinations, side effects, and risk of reinfections. Our objective was to summarize the outcome of computational research on the bacteria in MAC. This aimed to advance our understanding of characteristics, pathogenicity, and transmission dynamics to control infections. We incorporated information from the research on genomes, microbiomes, phylogeny, transcriptomes, proteomes, antibiotic resistance, and vaccine/drug target development to enhance our knowledge. It illuminated the significance of computational studies in distinguishing MAC species/subspecies and recognizing: virulence factors, lineage-specific markers, and transmission clusters. Moreover, it assisted in understanding: genomic diversity, resistance patterns, impact of polymorphisms in disease susceptibility, and taxa-induced dysbiosis in microbiomes. Additionally, this work highlighted the outcome of bioinformatic studies in predicting suitable vaccine epitopes, and novel drug targets to combat MAC infections. Bioinformatic research on bacteria within MAC has contributed to a deeper insight into the pathogens. These would facilitate better diagnosis, improved: therapeutic strategies, patient-specific surveillance, and community-level awareness.

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***Corresponding author**
E-mail: saubashya@gmail.com

Introduction

The genus *Mycobacterium* consists of gram-positive and rod-shaped bacteria incident in soil, water, biofilms, and dust. It is responsible for diseases in humans, livestock, and wildlife (Nishiuchi et al. 2017). They are broadly divided into tuberculous and non-tuberculous mycobacteria (NTM) (Runyon 1959). NTMs consist of more than 200 species (Daley 2017; Johansen et al. 2020) found in soils, water bodies, and municipal water systems (Johnson and Odell 2014). They are responsible for chronic pulmonary disease, disseminated infections, lymphadenopathy, and infections in tissues (Ratnatunga et al. 2020; Sur and Pal 2021). Pulmonary diseases caused by NTMs are increasing globally (Loebinger 2017) owing to human-pathogen interaction, global human mobility, aging populations, and improved diagnosis (Shah et al. 2016). NTM infections are difficult to treat due to increased resistance, prolonged treatment, medication side effects, and lack of effective vaccines (Baldwin et al. 2019).

In recent years, pulmonary NTM diseases mediated by *Mycobacterium avium* complex (MAC) have become predominant (Loebinger 2017). MAC comprises of *M. avium*, *M. intracellulare, M. chimaera*, *M. colombiense*, *M. marseillense*, *M. arosiense*, *M. bouchedurhonense*, *M. timonense*, *M. vulneris,* and *M. yongonense* (Daley 2017). *M. avium* comprises four subspecies: *M. avium* subsp. *avium* (MAA), *M. avium* subsp. *paratuberculosis* (MAP), *M. avium* subsp. *hominissuis* (MAH) and *M. avium* subsp. *silvaticum* (MAS) (Mizzi et al. 2022). MAC has been isolated from soil, dust, bathrooms, tap water, and drinking water systems (Nishiuchi et al. 2017).

MAC infections include pulmonary, disseminated, and lymphadenitis in immunocompromised adults and children. Nodular bronchiectatic, hypersensitive pneumonitis, and fibrocavitary diseases are lung diseases caused by MAC (Hwang et al. 2017; Diel et al. 2018). Fibrocavitary diseases are more severe requiring immediate attention. People suffering from tuberculosis, bronchiectasis, cystic fibrosis, emphysema, or chronic obstructive pulmonary disease are vulnerable (Wu et al. 2019). Low-grade fever, chronic cough, hemoptysis, fatigue, chest pain, and shortness of breath are the common symptoms (Daley 2017;

Loebinger 2017) (Fig. 1). *M. avium* subsp. *hominissuis* is clinically significant, causing chronic pulmonary disease and lymphadenitis in children and animals (Lahiri et al. 2014). Virulence factors contributing to MAC infections are the ability to invade epithelial cells, protein adherence, lipid-rich cell walls, and genetic clustering (Daley 2017). A year-long regimen of a combination of macrolides, ethambutol, rifamycin, and aminoglycosides is prescribed. However, high antibiotic resistance, adverse side effects, and reinfection make it unsuccessful (Kim et al. 2022). Thus, maintaining hygiene, use of alternative drugs, lung resection therapy, and treatment monitoring assume significance (Daley 2017).

In the past two decades, the proliferation of microbial genome projects resulted in a flood of information. Advances in bioinformatics coupled with the development of state-of-the-art computational resources, strengthened our understanding of microorganisms (Sur and Pal 2021). This review summarizes the outcome of computational studies on MAC bacteria, to advance our understanding of the characteristics, pathogenicity, and transmission dynamics, alongside strategies to tackle infection. In line with the aims, we conducted an extensive literature review and searched for articles in PubMed/PubMed Central/Google Scholar until April 2023.

Genomics and comparative genomics research

The early 21st century witnessed genome sequencing of MAC organisms, *M. avium* subsp. *paratuberculosis* strain K-10 and *M. avium* strain 104 (Turenne et al. 2007). While, the former had numerous genes linked to lipid metabolism, and fewer PE/PPE genes associated with virulence, the latter showcased a larger genetically distinct genome (Turenne et al. 2007). This was followed by genome sequencing of different strains of *M. avium*, *M. intracellulare*, *M. chimaera, M. yongonense, M. colombiense,* MAA, MAP, MAH and MAS (Bannantine et al. 2012; Kim et al. 2012; Tateishi et al. 2012; Bannantine et al. 2014; Mac et al. 2015; Möbius et al. 2015; Hasan et al. 2016; Yee et al. 2017; Zhao et al. 2017; Bouso and Planet 2019; Operario et al. 2019; Wibberg et al. 2020). They were sequenced using diverse technologies. These genome sequences opened avenues for an enhanced understanding of pathogenicity, adaptations, transmission, and antibiotic resistance.

The accelerating growth of whole-genome sequences entailed advancement in databases, software, and hardware resources. MyBASE and MycoCAP offered platforms for analysing genome structure, polymorphisms, virulence factor comparison, comparative genomics, and evolution of various mycobacteria, including those within MAC (Zhu et al. 2009; Choo et al. 2015). MetaCyc enabled computational prediction of pathways from sequenced genomes (Caspi et al. 2013). It included information of bacteria within MAC. Visual Gene Developer, SyntTax, and BAGET 2.0 aided easy sequence retrieval, optimization, genome, and synteny analysis (Jung and McDonald

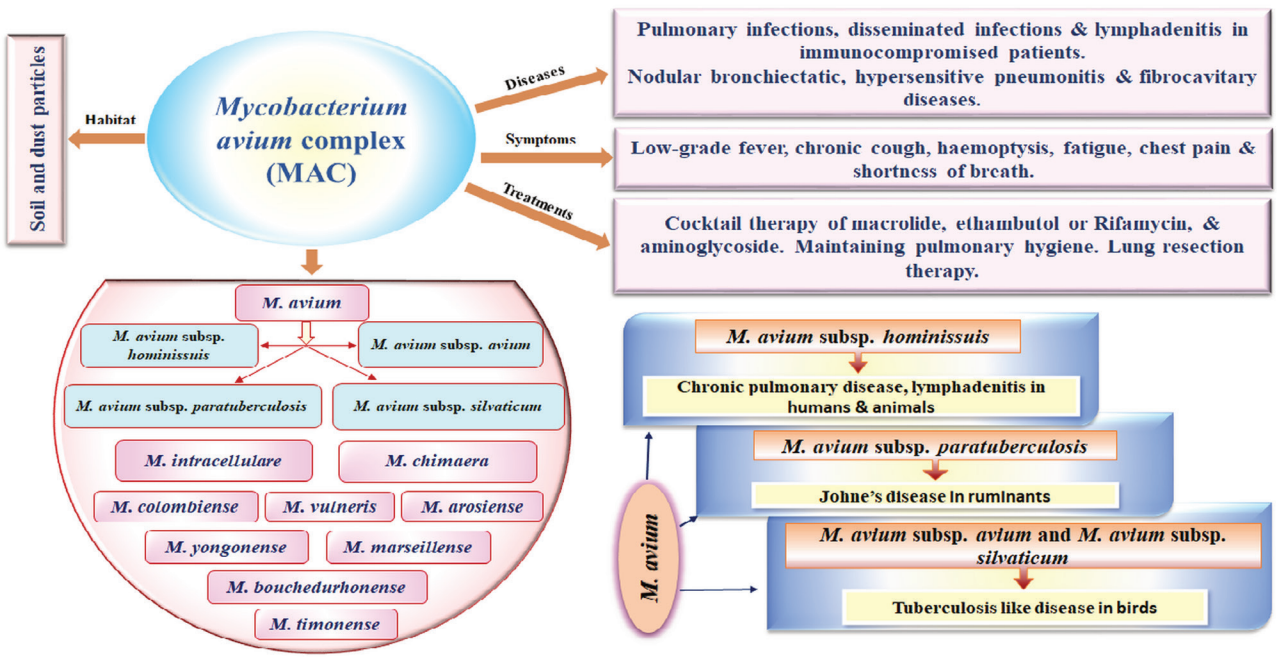

Figure 1. An overview of *Mycobacterium avium* complex (MAC) illustrating the bacteria housed in the complex (Daley et al. 2017; Mizzi et al. 2022), diseases they cause (Lahiri et al. 2014), and the available treatments (Kim et al. 2022).

2011; Oberto 2013; Hepp et al. 2021).

The availability of genome sequences and software laid the foundation for genomic and comparative genomics research. Bioinformatic analysis of ORF-MAP1138c from MAP revealed its potential to obstruct MHC-II Ag processing. This prevented CD4 + T cells from recognizing infected macrophages (Hassan et al. 2014). Genomic comparison of MAP strain JIII-386, MAP-S (Type I/III), MAP-C (Type II), and MAH strains revealed phenotypic differences (Möbius et al. 2015). Comparison of the pan and core genomes identified horizontally transferred genes and differentiated genomic features among MAP strains (Wibberg et al. 2020). The outcome of pan and core phylogeny highlighted the separation of MAP-S, MAP-C, and MAP-B strains (Lim et al. 2021). A comparison of MAP-S genomes from Australia and New Zealand specified the significance of lineage-specific differences (Mizzi et al. 2021) in aiding diagnosis and developing vaccines against Johne's disease.

Comparative analysis of MAH genomes characterized the role of a novel genomic island in virulence. It showcased variation in integrase, *mmpL, mce*, phage, and plasmid-derived genes in the island (Lahiri et al. 2014a). Exploration of 35 *M. avium* clinical isolates portrayed unique genetic characteristics, and affirmed the gain of pathogenesis-related genes during evolution (Uchiya et al. 2013). The MAH strain TH135 harboured virulence-related genes essential for pulmonary disease (Uchiya et al. 2013). A study with 79 *M. avium* strains divulged an open pangenome in MAH, with high genetic diversity at the subspecies level (Uchiya et al. 2017). Transposon sequencing identified 270 essential genes in MAH crucial for infection and survival (Matern et al. 2020). These could be potential drug targets.

High throughput sequencing identified MAC in drinking water. Multivariate analysis correlated water age with MAA abundance (Haig et al. 2018), thereby assessing the risk of waterborne infection. One comparative study specified faster evolution in MAC and closer association with non-pathogenic mycobacteria (Saha et al. 2019). Sequence analysis revealed the role of *M. avium* and *M. colombiense* in pneumonia in an HIV patient (Yu and Jiang 2021). A comparison of *M. intracellulare* and *M. yonogonense* genomes uncovered subspecies diversity driven by *mce* operons. *mce* genes differentiated virulent phenotypes, and hypermutation of *fadE3, fadE33,* and ESX-2 system genes were reported (Tateshi et al. 2021).

DNA methylation analysis has facilitated the understanding of bacterial pathogenicity, disease progression, and prognosis (Oh et al. 2021). Analysis of DNA methylation datasets from lung disease patients illustrated differences in methylation profiles based on prognosis (Oh et al. 2021). It identified *TGFBr1* and *HLA-DR* as treatment response biomarkers. Comparison of DNA methylation patterns in tissues of MAP-infected cows demonstrated potential biomarkers for the management of Johne's disease (Ibeagha-Awemu et al. 2021).

Microbiome profiling of diseases caused by bacteria in MAC

Of late, comprehending the interrelationship between lung microbiome and NTMs assumed significance, given increased prevalence and antibiotic resistance (Thornton et al. 2021). Comprehensive analysis of the lung microbiome data from cystic fibrosis patients, non-cystic fibrosis patients, and those with lung diseases portrayed an association between these bacteria and inflammation (Huang et al. 2016; Caverly et al. 2021; Sulaiman et al. 2018; Iwasaki et al. 2021). Microbiome analysis of NTM-affected women with breast cancer history showed the involvement of MAC in lung disease. It highlighted patient-specific surveillance for effective therapies (Philley et al. 2019). Analysis of nasopharyngeal microbiome data from COVID-19 patients in India, specified co-infections by *M. avium* and opportunistic pathogens (Prasad et al. 2022).

Increasing incidence of Johne's and Crohn's diseases along with antibiotic resistance in MAP (Agrawal et al. 2021), necessitated gastrointestinal microbiome analysis of humans and ruminants. Comparison of fecal microbiota from healthy individuals and those with Crohn's disease divulged an overrepresentation of *E. coli*, *Enterococcus faecium*, and proteobacterial species in diseased individuals (Mondot et al. 2011). Microbiome analysis of MAP-infected patients in Sudan demonstrated distinctive differences, emphasizing the need for community-level awareness (Elmagzoub et al. 2022). Examination of MAP-infected microbiomes from ruminants, uncovered enrichment of *Arthrobacter, Proteobacteria*, *Enterococcus*, *Psychrobacter,* and changes in metabolites (Matthews et al. 2021). This was corroborated by microbiome analysis of MAP-infected cattle in South Korea (Lee et al. 2023). These studies may assist in detecting biomarkers.

Genetic and genomic variation studies

Advancements in high-throughput technologies and efficient software facilitated the identification and characterization of genetic variation. It also catalysed the investigation of genetic variation in mycobacteria, for a deeper understanding of outbreaks, thereby aiding surveillance (Wilson 2017). MyBASE provided a resource for housing information on mycobacterial genomic polymorphisms (Zhu et al. 2009).

Statistical analysis of the data from 3020 Dutch cows, highlighted variations causing susceptibility to paratuberculosis infection (Koets et al. 2000). Statistical investigation applying a bivariate mixed animal model

from 11535 Danish Holstein cows, showed the relation between genetic variability and heritability. The outcome portrayed the heritability of MAP antibody production (Mortensen et al. 2004). In silico and statistical analysis of genetic association data from European Holstein-Friesian cows, identified two single nucleotide polymorphisms (SNPs) in the *SLC111A* gene (Ruiz-Larrañaga et al. 2010) linked to MAP susceptibility. A genome-wide association study in Italian cattle suffering from Johne's disease identified a genetic locus linked with antibody response to MAP (Minozzi et al. 2010). Investigation of the whole genomes of MAP and MAA from various sources illustrated variability like SNPs between the two. Additionally, the resemblance of MAP isolates from human and dairy sources indicated a similar source of infection (Hsu et al. 2011). Comparative genomics identified an SNP in MAP strain 1025, changing the amino acid at residue 28 in the 17A12 epitope (Bannantine et al. 2011). This SNP was specific for the 17A12 antibody. The exclusive binding of this antibody to MAP ushered new avenues for pathogen detection (Bannantine et al. 2011). Genome-wide analysis of MAP isolates from camels in Saudi Arabia, divulged numerous single nucleotide polymorphisms (SNPs) defining host adaptation of MAP (Ghosh et al. 2012). These camel isolates were a sub-lineage of sheep isolates of MAP and the sharing of SNPs between them laid the basis for comprehending disease transmission among them (Ghosh et al. 2012). These studies underlined the application of genetic tools to control paratuberculosis infection.

Comparative studies of *M. avium* subspecies using DNA microarray unveiled 14 sequence polymorphisms differentiating MAA from MAP. The outcome displayed considerable diversity among MAC bacteria (Semret et al. 2004). Investigation of DNA microarray data and genomic examination of MAC isolates from various sources identified large sequence polymorphisms (LSP) distinguishing MAA from MAP. LSP8 was absent in MAP isolates and they housed 7 specific and 10 non-specific LSP regions (Semret et al. 2005). Comparative genomic studies using microarray and PCR identified LSPs, that could distinguish host-associated variants in MAC isolates (Semret et al. 2006). The work highlighted the absence of LSP17 in bird isolates of *M. avium.* Furthermore, cow and sheep isolates of MAP lacked LSP8, LSP4-11, LSP18; and LSP8, LSP20 respectively. These outcomes emphasized the relevance of polymorphisms in diagnostics.

One study stressed the role of a variable 3' region of the *hsp65* gene as a marker for differentiating MAP isolates from cow, sheep, and MAA isolates from birds (Turenne et al. 2006). Sequence analysis of *gyrA* and *gyrB* genes using MEGA 3.1 and BLAST, illustrated distinctive single nucleotide polymorphisms for types I, II, and III of MAP (Castellanos et al. 2007). Analysis of AFLP and 16S rDNA data of MAC isolates, from patient and environmental origin portrayed differences. It reinforced the epidemiological investigation of MAC isolates from various sources for identifying the source of infection (Pfaller et al. 2007).

Whole genome analysis was carried out to investigate the PE and PPE gene families associated with virulence in MAC. Identification of numerous distinctive PPE proteins in MAH and MAP alongside polymorphisms, in PE and PPE gene families could pave the way for better diagnostics (Mackenzie et al. 2009). Exploration of the whole genomes from 41 MAH isolates in Germany, identified a hypervariable genomic island. It highlighted the implication of cross-species transfer of virulent genes through this genomic island in future outbreaks (Sanchini et al. 2016). Phenotype microarray data analysis of the clinical and environmental isolates from MAH disclosed intra-species diversity owing to different metabolic patterns (Sanchini et al. 2017). Environmental isolates had a higher metabolic rate than clinical isolates.

In silico analysis of tandem repeats and VNTR-MIRU typing data, exemplified variation VNTR-MIRU loci repetitions in MAC isolates (Romano et al. 2005). Moreover, variations in gene expression and pathogenicity between *M. avium* strain 104 and MAP strain K10, were due to a polymorphism in the VNTR-MIRU loci. An Argentinian study examined the MIRU-VNTR typing data of MAC isolates from different hosts. It described the genetic diversity of MAH and MAP isolates and recognized six new MAH patterns (Imperiale et al. 2017).

Exploration of genetic population, homologous recombination, and gene content datasets from global and Japanese isolates of MAH revealed five predominant MAH lineages (Yano et al. 2017). Furthermore, mutual homologous recombination between the MAH lineages implied adaptation (Yano et al. 2017). One Norwegian research divulged the impact of SNPs in sequence variation among MAC isolates from humans. This variation and differential host responses were attributed to a high rate of mutation of *M. avium,* alongside adaptation in chronically infected individuals (Kannan et al. 2019). Examination of differential genotyping data of MAC species and subspecies signified interconnections between epidemiology and source of infection (Shin et al. 2020). This would aid in the advancement of procedures for preventing MAC infection.

Multiple factors fashioned the phylogeny of MAC bacteria

Over the years, the identification of numerous mycobacterial species, coupled with their sequencing formed the basis for research on mycobacterial evolution (Behra 2022). Molecular phylogenetic analysis of the clinical isolates of

MAC, using 16S-23S rDNA internal transcribed spacers revealed distinctive differences. The pulmonary-source MAC isolated displayed more diversity compared to others (Frothingham and Wilson 1994). Phylogenetic analysis of the insertion sequence IS*1245,* from *M. avium* encoding a transposase, was performed using the GCG package and PHYLIP 3.5. It demonstrated 64% and 50% amino acid similarities with IS*1081* and IS*6120,* of *M. bovis* and *M. smegmatis* (Guerrero et al. 1995). One study inferred a phylogenetic relationship in *M. avium* strains from different serotypes, based on *gtfB* and *rtfA–mtfC* genes linked to glycopeptidolipid biosynthesis (Krzywinska et al. 2004). It illustrated multiple origins of serotypes from AIDS patients and the polyphyletic origin of serotype 1 strains. Sequencing and phylogenetic studies of the variable 3" region of *hsp65* gene using MEGA 3.1, differentiated the species and subspecies of MAC (Turenne et al. 2006). There were distinct sequevars for *M. avium* subsp. *avium*, *M. avium* subsp. *hominissuis*, and *M. avium* subsp. *paratuberculosis*. Furthermore, bovine and ovine strains of *M. avium* subsp. *paratuberculosis* varied (Turenne et al. 2006).

The 1990s and early 2000s saw interest among microbiologists regarding the inclusion of undescribed mycobacteria in MAC (Tortoli et al. 2004). Examination of the 16S rRNA and 16S–23S rDNA internal transcribed spacer, illustrated a MAC-A variant phylogenetically distant from *M. avium* and *M. intracellulare*. Following further characterization, it was elevated to *M. chimaera* sp. nov. and included within MAC (Tortoli et al. 2004). Another study of 16S and 16S-23S internal transcribed spacer sequences using MEGA 4.0 software, elevated the MAC-Q sequevar to *M. vulneris* sp. nov. within MAC (van Ingen et al. 2009). Sequence analysis of *hsp65* and *rpoB* genes too revealed strong similarities with *M. avium* and *M. colombiense*. For defining MAC members, phylogenetic studies using full 16S rRNA, partial 5' 16S rRNA, *hsp65, rpoB* genes, concatenated analyses of >2500 bp region comprising of 16S rRNA, *hsp65* and *rpoB* genes were carried out against *M. avium* ATCC 25291^T or *M. intracellulare* ATCC 13950^T (van Ingen et al. 2018). Besides, average nucleotide identity analyses recognized 12 valid species in MAC.

In one study, Tandem Repeat Finder software was used to search for VNTR loci from genomic data of *M. intracellulare* ATCC 13950. It revealed 16 loci. Examination of the discriminatory power of these loci in 74 isolates of MAC-infected patients, followed by phylogenetic analysis differentiated the isolates into 49 genotypes in 17 clusters (Ogawa 2013). A cross-species in silico analysis specified the conservation of tryptic peptides amongst mycobacterial species including MAC (Rajaonarifara 2013).

Phylogenomic analysis of 141 MAP isolates from ruminants and humans with Crohn's disease, selected from 17 countries, disclosed two major lineages in line with earlier observations (Bryant et al. 2016). While Type I and Type III strain groups were subtypes of Type S, Type B strains were a subtype of Type C. However, the latter was not limited to *Bison* sp. The result underscored whole genome sequencing of more isolates, for better interpretation of the evolution, epidemiology, and population architecture of MAP (Bryant et al. 2016). The outcome of a phylogenetic study based on single nucleotide variants of 79 *M. avium* strains, divulged lineage-specific genetic elements acquired by horizontal gene transfer (HGT) (Uchiya et al. 2017). These strains were categorized into clusters I, II, and III. Additionally, each cluster consisted of a distinct subcluster of strains (cluster Ia, cluster IIb, or cluster IIIb) with varying genetic distances. MAH, MAP, MAA, and MAS existed in clusters I-III, cluster IIIb, and cluster IIb respectively (Uchiya et al. 2017). While MAH showed the highest sequence diversity, MAP revealed the least.

Population genomics and phylogenetic investigation of 364 MAC isolates from cystic fibrosis patients in the USA, stressed the significance of surveillance and epidemiologic follow-up to restrict MAC infections (Hasan et al. 2021). The work highlighted evidence of shared MAC strains among patients with cystic fibrosis. Several clusters of highly similar isolates were identified in cystic fibrosis patients, sharing cystic fibrosis care centers. A similar study in 996 MAC isolates from cystic fibrosis and non-cystic fibrosis patients, in London, revealed lineages in numerous patients (van Tonder et al. 2023). Interestingly, the work pinpointed transmission clusters at the local, national, and global levels. Also, the study divulged the origin of an already circulating heater-cooler unit associated with *M. chimaera* lineage causing respiratory illness (van Tonder et al. 2023).

A phylogenetic study of 109 *M. avium* core genomes, underlined environment-specific adaptation. It exemplified variation in HGT in isolates from natural environments, animals, human pulmonary infections, and human disseminated infections (Keen et al. 2021). Furthermore, *M. intracellulare* housed more foreign DNA compared to *M. avium* and *M. colombiense*. Moreover, animal isolates portrayed diminished HGT, especially in MAP. Global phylogenetic analysis of *M. avium* isolates, underscored MAH as most diverse and MAA and MAP, as pathogenic sub-clones having specific environmental adaptation (Mizzi et al. 2022). This adaptation was aided by substantial mutations.

The diversity and evolution of *M. avium* subspecies were shaped by the existence of insertion sequences, deletions, duplications, and mutations in chromosome loci and genes (Rindi and Garzelli 2014). That, NGS workflow and phylogenomics facilitated in-depth identification of *M. avium* subtypes from metagenomic data, holds promise for the future (Schildkraut et al. 2023).

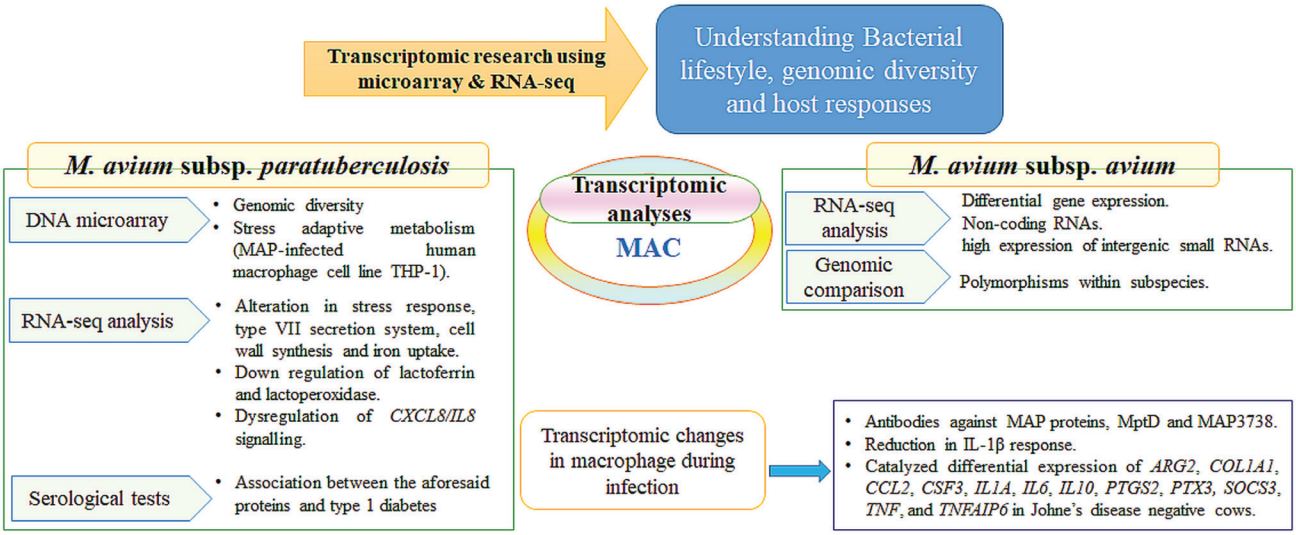

Figure 2. Illustration of the outcome of DNA microarray studies (Cossu 2011; Cossu et al. 2012), RNA-seq analysis in MAP (Alonso-Hearn et al. 2019; Park et al. 2022), key findings from transcriptomic analyses in MAA (Kim et al. 2021), and transcriptomic changes taking place in the macrophage during infection (Ariel et al. 2020).

Transcriptomic research delivers insights into host-pathogen interaction and heralds potential for improved diagnosis

Technological advancements in functional genomics in the 21st century, utilizing microarray (Pribylova et al. 2009) and RNA-seq have revolutionized our understanding of transcription. It aided comprehensive analysis of pathogenic bacterial lifestyle, genomic diversity, and host responses (Bannantine and Talaat 2010). Whole genome DNA microarray data analysis specified the role of divergent ORF clusters, in differentiating MAP from other MAC bacteria (Paustian et al. 2005). One study using DNA microarray illustrated genomic diversity among the isolates of the veterinary pathogen MAP (Bannantine and Talaat 2010). Comparison of *M. avium* strain 724R transcriptomes in lung tissues of resistant and susceptible mice, specified differential expression of genes. The upregulation of genes linked to cell wall properties, anaerobic nitrate respiration, fatty acid degradation, and biosynthesis of mycobactin in resistant mice implied the transition of *M. avium* to latency within the host (Ignatov et al. 2010). Assessment of RNA-seq data from the non-coding transcriptome of MAA strain TMC724 revealed numerous non-coding RNAs. Additionally, 6 intergenic small RNAs showed very high expression (Ignatov et al. 2013). These may be explored for comprehending their role in virulence. Moreover, a genomic comparison of MAA strain TMC724 with MAH strain 104 exhibited, 25 large sequence polymorphisms in the latter (Ignatov et al. 2013).

The investigation of macrophage transcriptomes during the course of infection has gained importance. One study investigated the MAP transcriptome in infected human macrophages. It revealed the existence of antibodies against MAP proteins, MptD, and MAP3738. Interestingly, serological examination of patients suffering from type 1 diabetes, demonstrated a positive association between the aforesaid proteins and type 1 diabetes (Cossu 2011) (Fig. 2). DNA microarray analysis of MAP-infected human macrophage cell line THP-1, under simulated intraphagosomal situations, illustrated stress adaptive metabolism (Cossu et al. 2012). Upregulation of genes linked to oxidative stress accompanied by peptidoglycan spoliation and increased anabolism in lipid membranes highlighted mimicry-based interaction between MAP and host cell (Cossu et al. 2012). RNA-seq analysis of MAP-infected human THP-1 cells exhibited alteration to stress response and metabolism in MAP and host. Upregulation of two-component systems and sigma factors, including alteration of type VII secretion system, cell wall synthesis, and iron uptake genes occurred in the macrophages (Park et al. 2022). Some researchers underscored the application of dual RNA-seq on *M. avium* infected human monocyte-derived macrophages. This would aid in a better understanding of virulence in intracellular mycobacteria (Schildkraut et al. 2021). Expression studies of macrophages/monocytes in MAC lung disease patients revealed reduced IL-1β response. This along with polymorphisms in the *NLRP3* and *TLR2* genes increased susceptibility (Wu et al. 2019).

Transcriptomic studies of MAP-infected bovine macrophages using RNA-seq, portrayed altered expression in

monocyte-derived macrophages (Switzenberg et al. 2013) A similar work identified 245 and 574 differentially expressed genes, in MAP-infected and non-infected samples after 2 and 6 hrs of infection (Casey et al. 2015). It revealed the hitherto unknown function of these genes in MAP-infected host cells. Another study indicated no impact of MAP on macrophages of Johne's disease-positive cows compared to Johne's disease-negative cows. Furthermore, MAP catalyzed differential expression of *ARG2, COL1A1, CCL2, CSF3, IL1A, IL6, IL10, PTGS2, PTX3, SOCS3, TNF*, and *TNFAIP6* in macrophages of Johne's disease negative cows (Ariel et al. 2020) (Fig. 2). This impacted host signaling pathway and highlighted the function of genes in the host's response to MAP infection (Ariel et al. 2020). Investigation of mRNA and circRNA from MAP-infected bovine monocyte macrophages, showcased differences in multiple genes and cell signaling pathways (Bao et al. 2022). The outcome will assist in comprehending the immune escape of MAP and improve diagnosis.

RNA-seq technology was applied to examine the ileocecal valve and peripheral blood from MAP-infected cattle. It divulged transcriptomic differences in infected cows compared to controls, and dysregulation of CXCL8/IL8 signaling (Fig. 2) Moreover, it recognized differentially expressed genes and metabolic pathways, that are biomarkers for diagnostics, therapeutics, and vaccines (Alonso-Hearn et al. 2019). Transcriptomic analysis of *M. avium* infected canine peripheral blood mononuclear cells (PBMCs) displayed contrasting immune responses. Genes linked to activation of Th1 and Th17 responses were highly expressed, while those associated with apoptosis were inhibited (Kim et al. 2021).

Exploration of the transcriptome of MAP-exposed salivary glands of cattle depicted the downregulation of lactoferrin and lactoperoxidase in saliva. This induced disease susceptibility since these genes have antimicrobial and immunoregulatory properties (Mallikarjuappa et al. 2019).

All these studies specified the significance of expression studies in controlling infections caused by *M. avium.* It can assist in characterizing the interactions between bacteria and host, improve diagnosis, and develop biomarkers (van den Esker and Koets 2019).

Proteomics research on MAC bacteria

Mycobacterial proteins secreted from the cell envelope, are crucial for survival, replication, and modulation of immune responses within the host macrophage (Cáceres et al. 2011). The postgenomic era witnessed the application of different proteomics approaches, to fathom the pathobiology of mycobacteria (Gcebe et al. 2016; Nicholson et al. 2021). Ever increasing data necessitated the development of databases and software for secretome analysis. MycoSec provided information on Sec type, Lipotype, twin type, and TAT type signal peptides in mycobacteria including those within MAC (Roy et al. 2013). Pncs-Hub was developed for comprehensive annotation and exploration of non-classical secretory proteins in bacteria (Dai et al. 2022).

Computational analysis of 2D-PAGE and MALDI-TOF data from MAP detected ten upregulated proteins that expedited natural infection in sheep (Hughes et al. 2007). A similar analysis coupled with serological investigations identified eleven MAP-specific proteins from paratuberculosis-infected sheep. These were prospective diagnostic antigens (Hughes et al. 2008). Extensive postgenomic and immunoproteomic investigation of the secretome of MAP revealed the vaccine potential of MAP0586c and MAP4308c proteins (Roupie et al. 2008). Proteomic studies of MAP strain K10 and MAP determined different proteins from the cell wall, outer membrane, and membrane vesicles (He and De Buck 2010; Rana et al. 2014; Martin 2016). These are novel avenues for serodiagnosis, therapeutic strategies, and vaccine design against diseases caused by mycobacteria (He and De Buck 2010; Rana et al. 2014; Martin 2016). Proteomic analysis of the secretome of MAP, facilitated better serological and immunoglobulin A-based diagnosis of Johne's disease in MAP-infected cattle (Facciuolo 2015). Detailed sequence analysis of six secretome genes from MAP-infected goats in India, highlighted the effect of insertions, and deletions on virulence, and host responses (Chaubey et al. 2018).

Expression studies of the membrane and cytosolic proteins of MAP strains K-10 and 187, accompanied by SDS-PAGE, high-performance chromatography, and tandem mass spectroscopic data analysis revealed substantial differences. While AtpC and RpoA were upregulated in MAP 187, AhpC and those linked to nitrogen metabolism were highly expressed in MAP K-10 (Radosevich et al. 2007). This was significant since MAP K-10 was a laboratory-adapted strain while MAP 187 was isolated from a cow with Johne's disease. Computational exploration of 2D DIGE, MALDI-TOF-MS, and nUPLC-ESI Q-TOF-MS/MS data from MAP, demonstrated four differentially expressed membrane-associated proteins (Weigoldt et al. 2011). They influenced the pathogenesis of Johne's disease. That MAH strain 104 proteins expressed differently in exponential and stationary phases, signified adaptation to metabolic and environmental stress (Enany et al. 2021).

Proteome analysis of different stages of host macrophage infection with MAH and MAP, improved our understanding of pathogen infection and dissemination (McNamara et al. 2012; Phillips et al. 2021). The detection of different surface-exposed proteins and integrins can assist in the development of potent treatments.

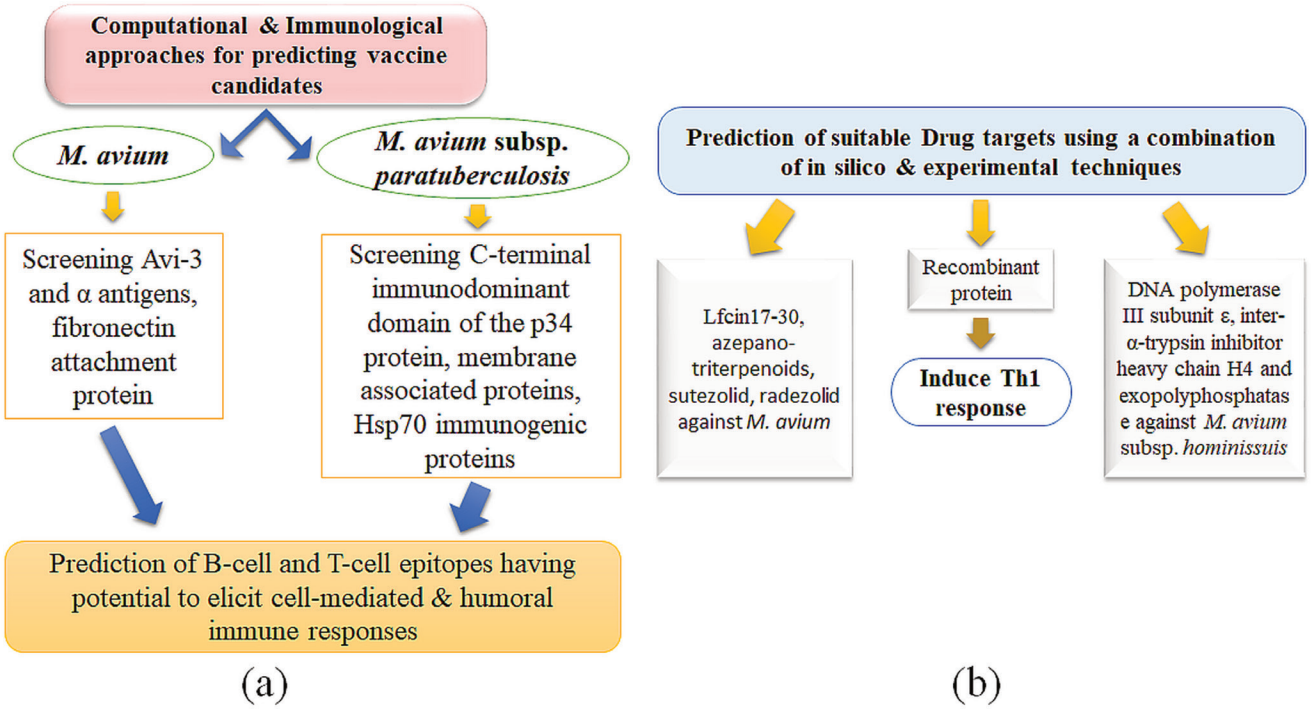

Figure 3. Prediction of immune response eliciting epitopes from suitable proteins (a) (Santema et al. 2011), and drug targets that may combat infection by members of MAC (b) (Low et al. 2017; Kazakova et al. 2021).

Exosome proteins released from MAP-infected resting macrophages displayed differential expression compared to uninfected macrophages (Wang et al. 2014). Differentially expressed guanine nucleotide-binding protein β-1, cofilin-1, peptidyl-prolyl cis-trans isomerase A and actin isoforms were recognized by MALDI-TOF/TOF mass spectrometric data analysis (Wang et al. 2014). MALDI-TOF-MS differentiated MAP genotypes based on SSRs in Canadian dairy samples, paving the way for its use in molecular epidemiology (Ahlstrom et al. 2014). Application of MALDI-TOF-MS, sequencing of ITS and *hsp65* genes, and molecular techniques successfully identified *M. chimaera* strains from MAC isolates (Lecorche et al. 2018).

An examination of the secreted proteome of MAH identified unique proteins in the ESX-3 region. Metal concentration within the phagosome stimulated the secretion of these proteins and impacted virulence (Chinison et al. 2016). Mass spectroscopic data analysis of breast cancer patients portrayed that the secreted proteins, ECM1, APOC4, APN, and AZPG1 enhanced vulnerability to MAC infections (Philley et al. 2017).

Proteomic investigation of Type I and Type II MAP isolated from ruminants in the UK, divulged significant differences and polymorphisms influencing protein expression (Hughes et al. 2012). Comparison of the proteomes of the wild type of *Mycobacterium avium* strain 104 and *lysX* mutant, indicated enhanced β-oxidation of fatty acids, and concentration of lipid inclusions in the latter (Kirubakar et al. 2018). This mutant influenced the metabolism and virulence of the bacterium.

Analysis of proteomic data also provided an understanding of the impact of antibiotics and varying environmental conditions on MAH strain 104. Analysis of tandem mass tag mass spectrometric data revealed potential metabolic pathways that could be targeted for limiting bacterial tolerance to amikacin, and clarithromycin in aerobic, anaerobic, and biofilm conditions (Rojony et al. 2019). This may prevent prolonged antibiotic therapy. Laser microdissection studies of lung tissues coupled with bioinformatic analysis of mass spectrometric data illustrated distinct variations in the lung granulomatous lesions caused by tuberculosis and MAC (Seto et al. 2020). The abundance of proteins linked to antimicrobial and metabolic activities varied. Proteomic analysis can advance our understanding of the pathogenesis and facilitate novel serodiagnosis and therapeutic strategies.

Antibiotic resistance mechanism research

Pulmonary infections caused by the members of MAC are difficult to treat since they are resistant to the majority of antibiotics like clarithromycin, azithromycin, rifamycin, and clofazimine (Falkinham 2007; Pasipanodya et al. 2017; Cushman et al. 2021). Multiple drug resistance in MAC bacteria has been attributed to, modification in

outer polysaccharide layers, cell wall impermeability, increased persistence in biofilms and granulomas, mutations, antibiotic modifying enzymes, escalated drug efflux, and limited drug sequestration (Falkinham 2007; Saxena et al. 2021). The information from the genomic data of MAC members facilitated an in-depth understanding of antibiotic resistance (Saxena et al. 2021).

Sequence and structure-based studies revealed that resistance of *M. avium* to clarithromycin and azithromycin was a consequence of mutations in the 23S rRNA gene (Nash and Inderlied 1995). The mutations altered rRNA folding patterns and structural conformations, resulting in resistance (Nash and Inderlied 1995). The incidence of point mutation at positions 2058 and 2059 in the 23S rRNA gene caused macrolide resistance (Moon et al. 2016). It hampered the treatment of lung diseases due to MAC in South Korea. Mutation in the *rpoB* gene of MAP was accountable for resistance to rifabutin and rifampicin (Beckler et al. 2008). It negatively affected Crohn's disease therapy. Application of Mycobacterial IDentification and Drug Resistance Screen (MID-DRS) assay, and analysis of DNA sequencing data aided faster identification of antibiotic-resistant *M. tuberculosis* and MAC strains (Pérez-Osorio et al. 2012). One study described that *M. avium* and *M. intracellulare* efflux pumps caused a progressive increase in azithromycin resistance during the course of therapy (Schmalstieg et al. 2012). It identified the genes encoding efflux pumps, determined their secondary structure, and reported their conserved nature across pathogenic mycobacteria. In silico analysis applying population pharmacokinetics and Monte Carlo simulations, successfully detected azithromycin susceptibility breakpoint beyond which therapy failed (Deshpande et al. 2016).

Multi-locus sequencing and insertion sequence analysis of MAH, isolated from Korean patients with lung disease, confirmed the involvement of IS*Mav6* in conferring resistance to moxifloxacin (Kim et al. 2016). Sequencing and SNP analysis of *gyrA*, *gyrB,* and *rpsL* genes from *M. avium* isolates in Chinese patients divulged resistance to fluoroquinolone and aminoglycoside (Pang et al. 2018). A study revealed the association of the *lysX* gene from MAH with cationic antimicrobial resistance (Kirubakar et al. 2020). Genome-wide transposon screening detected 193 *M. avium* mutants, showing tolerance and altered susceptibility to clarithromycin, rifabutin, moxifloxacin, and ethambutol (Matern et al. 2021). Interestingly, this study pin-pointed antibiotic tolerant genes that could be targeted for designing novel drugs to combat *M. avium* infections.

Tandem mass tag mass spectrometry sequencing, evaluated MAH proteins exposed to amikacin and clarithromycin, in aerobic, anaerobic, and biofilm environments (Parker et al. 2020) Substantial synthesis of nitrate, nitrate transporter, and nitrate reductive enzymes occurred in MAH in anaerobic and biofilm environments (Parker et al. 2020). It highlighted metabolic changes linked to MAC resistance.

Analyzing antibiotic resistance of MAC using in silico and in vitro tools (Oliveira de Sousa 2020), can go a long way in developing novel drugs for controlling infections. Nevertheless, research on antibiotic resistance in MAC using computational techniques remains inadequate.

Research on prediction of vaccine candidates and drug targets

The infections caused by bacteria in MAC in humans and animals are persistent and hard to control (Rivoire et al. 1989; Abdellrazeq et al. 2020). Moreover, the incidence of MAC infections especially MAP, in animals often resulted in prevalence in humans (Abdellrazeq et al. 2020). This warranted efforts in developing vaccines, novel drugs, and targeted drug delivery (de Steenwinkel 2007). A preliminary study recognized T-cell epitopes from the fibronectin attachment protein of *M. avium*. The immune response elicited by the T-cell epitopes in mice formed the basis for developing subunit vaccines (Holsti et al. 1998).

Application of immunological and computational techniques detected sequential and conformational B-cell epitopes, from the C-terminal immunodominant domain of the p34 protein in MAP (Ostrowski et al. 2003). The outcome created a platform for investigating humoral response, during the course of MAP infection in cows. Another study identified MAP Hsp70-specific T cell epitopes in cows, using a combination of immunological and in silico tools. It underlined the prospect of developing a MAP Hsp70 subunit vaccine for controlling paratuberculosis in cattle (Hoek et al. 2010). Hsp70 vaccination is significant, since antibodies induced by vaccination in ruminants, were able to identify two B-cell epitopes in MAP cell walls (Santema et al. 2011) (Fig. 3). These antibodies protect against bovine paratuberculosis.

Codon optimization using experimental and bioinformatic approaches underlined better expression of MAP-specific antigens in *Lactobacillus salivarius* (Johnston et al. 2013; Johnston et al. 2014). The outcome established the feasibility of *L. salivarius* as a vaccine to control Johne's disease (Johnston et al. 2014).

Utilization of immunoinformatic tools predicted antigenic proteins from MAP. This resulted in the identification of numerous T-cell, B-cell, and conformational B-cell epitopes from upregulated proteins under stress (Gurung et al. 2012), membrane-associated proteins (Carlos et al. 2015), and other immunogenic proteins (Swathi et al. 2020). The epitopes had the capability to elicit cell

and humoral responses. T-cell epitopes showed a binding affinity for MHC class I and II alleles. The outcome of in silico (Gurung et al. 2012; Carlos et al. 2015; Swathi et al. 2020) and in vitro (Carlos et al. 2015) investigation of the antigenic proteins from MAP, underscored their potential in vaccine development and immunodiagnostics. Experimental evaluation of these epitopes may pave the way for developing effective vaccines. Given the prevalence of Johne's disease on a global scale, a multi-institutional consortium was created for monitoring trials and assessing next-generation vaccines against MAP (Bannantine et al. 2014a).

Combining experimental and in silico approaches, a recombinant protein was constructed (Eraghi et al. 2017). This recombinant protein had the capability to induce Th1 response against MAP. Screening of active compounds revealed high hit rates of sutezolid, radezolid, and synthesized intermediate of radezolid against *M. avium* (Low et al. 2017) (Fig. 3). An *in vitro* study revealed the efficacy of a computationally predicted antimicrobial peptide Lfcin17-30, against an axenically grown strain of *M. avium* (Oliveira de Sousa 2020).

Implementation of subtractive genomics technique using different software, ascertained novel drug targets against MAH strains TH135, OCU466, and A5 (Uddin et al. 2020). Notable amongst them were DNA polymerase III subunit ε, inter-α-trypsin inhibitor heavy chain H4 and exopolyphosphatase of MAH-TH135, MAH-OCU466, and MAH-A5 strains, respectively (Uddin et al. 2020). A blend of molecular docking, machine learning, and experimental techniques revealed the antimycobacterial activity of seventeen azepano-triterpenoids against *M. avium*. Furthermore, the compound 14 displayed similar MIC as rifampicin (Kazakova et al. 2021) (Fig. 3). The outcome portrayed the suitability of these candidates for drug design. Comprehensive in silico analysis, recognized eight molecules from the DrugBank database, successfully inhibiting the MAP proteins viz. katG, rpoB, and narH. It highlighted the evaluation of these eight molecules in treating MAP-associated autoimmune diseases viz. Crohn's disease, type 1 diabetes, and multiple sclerosis (Garg et al. 2021). The utilization of robust computational techniques for designing epitopes and precise identification of drug targets can assist experimental analysis by saving costs and minimizing efforts.

Conclusions

The ever-increasing incidence of MAC infections in humans, livestock, and birds has posed challenges in uncovering effective control measures. The tsunami of information generated from sequencing projects in the postgenomic era has bolstered myriad bioinformatic analysis and functional research on MAC. The valuable knowledge garnered from these studies laid the foundation for applicability by clinicians. Utilization of advanced tools for investigating whole genomes, and comparing genomes, yielded precise identification of the factors causing genomic diversity among MAC species/subspecies. To this end, lineage-specific markers were detected for better diagnosis and drug development. Furthermore, several essential and virulence genes necessary for a pathogenic lifestyle of the bacteria in MAC were identified. Analysis of microbiome and DNA methylation revealed the stark contrast between diseased and healthy hosts and ascertained biomarkers for disease management. Genomic variation studies divulged intra-species diversity of the bacteria in MAC and signified the importance of polymorphisms in diagnosis. Phylogenetic analysis specified the association of numerous factors, in effecting diversity among MAC species/subspecies. The outcome of proteomics, and antibiotic resistance research, improved the interpretation of pathogenic metabolism, stress adaptations, resistance patterns, and disease transmission. These would boost effective serodiagnosis and superior drug discovery. Transcriptomic analysis emphasized the involvement of differentially expressed genes in shaping virulence, bypassing immunity, and relationship with infected hosts. Vaccine epitopes and drug molecules designed using bioinformatic tools have provided a cost-effective platform for experimental validation by pharmaceutical researchers. We have tried our best to integrate the findings from various bioinformatic studies. We underline the importance of amalgamating computational and experimental studies on a global scale, for enhanced understanding of the bacteria in MAC. Future investigations should accommodate machine learning and AI techniques, for precise identification of strains, phenotype-genotype associations; tailormade diagnosis, categorizing virulence, and drug discovery.

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References

Abdellrazeq GS, Fry LM, Elnaggar MM, Bannantine JP, Schneider DA, Chamberlin WM, Mahmoud AHA, Park KT, Hulubei V, Davis WC (2020) Simultaneous cognate epitope recognition by bovine CD4 and CD8 T cells

is essential for primary expansion of antigen-specific cytotoxic T-cells following ex vivo stimulation with a candidate *Mycobacterium avium* subsp. *paratuberculosis* peptide vaccine. Vaccine 38(8):2016-2025.

Agrawal G, Aitken J, Hamblin H, Collins M, Borody TJ (2021) Putting Crohn's on the MAP: Five common questions on the contribution of *Mycobacterium avium* subspecies *paratuberculosis* to the pathophysiology of Crohn's disease. Dig Dis Sci 66(2):348-358.

Ahlstrom C, Barkema HW, De Buck J (2014) Improved short-sequence-repeat genotyping of *Mycobacterium avium* subsp. *paratuberculosis* by using matrix-assisted laser desorption ionization-time of flight mass spectrometry. Appl Environ Microbiol 80(2):534-539.

Alonso-Hearn M, Canive M, Blanco-Vazquez C, Torremocha R, Balseiro A, Amado J, Varela-Martinez E, Ramos R, Jugo BM, Casais R (2019) RNA-Seq analysis of ileocecal valve and peripheral blood from Holstein cattle infected with *Mycobacterium avium* subsp. *paratuberculosis* revealed dysregulation of the CXCL8/IL8 signaling pathway. Sci Rep 9(1):14845.

Ariel O, Gendron D, Dudemaine PL, Gévry N, Ibeagha-Awemu EM, Bissonnette N (2020) Transcriptome profiling of bovine macrophages infected by *Mycobacterium avium* spp. *paratuberculosis* depicts foam cell and innate immune tolerance phenotypes. Front Immunol 10:2874.

Baldwin SL, Larsen SE, Ordway D, Cassell G, Coler RN (2019) The complexities and challenges of preventing and treating nontuberculous mycobacterial diseases. PLoS Negl Trop Dis 13(2): e0007083.

Bannantine JP, Bayles DO, Robbe-Austerman S, Burrell AM, Stabel JR (2014) Draft genome sequence of a *Mycobacterium avium* complex isolate from a broadbill bird. Genome Announc 2(1):e01268-13.

Bannantine JP, Hines ME 2nd, Bermudez LE, Talaat AM, Sreevatsan S, Stabel JR, Chang YF, Coussens PM, Barletta RG, Davis WC, Collins DM, Gröhn YT, Kapur V (2014) A rational framework for evaluating the next generation of vaccines against *Mycobacterium avium* subspecies *paratuberculosis*. Front Cell Infect Microbiol 4:126.

Bannantine JP, Stabel JR, Lamont EA, Briggs RE, Sreevatsan, S (2011) Monoclonal antibodies bind a SNP-sensitive epitope that is present uniquely in *Mycobacterium avium* subspecies *paratuberculosis. Front Microbiol* 2:163.

Bannantine JP, Talaat AM (2010) Genomic and transcriptomic studies in *Mycobacterium avium* subspecies *paratuberculosis*. Vet Immunol Immunopathol 138(4):303-11.

Bannantine JP, Wu CW, Hsu C, Zhou S, Schwartz DC, Bayles DO, Paustian ML, Alt DP, Sreevatsan S, Kapur V, Talaat AM (2012) Genome sequencing of ovine isolates of *Mycobacterium avium* subspecies *paratuberculosis* offers insights into host association. BMC Genom 13:89.

Bao Y, Yao Y, Wang Z, Wu S, Jiang X, Ma H (2022) Analysis of mRNA and circRNA expression profiles of bovine monocyte-derived macrophages infected with *Mycobacterium avium* subsp. *paratuberculosis*. Front Microbiol 12:796922.

Beckler DR, Elwasila S, Ghobrial G, Valentine JF, Naser SA (2008) Correlation between *rpoB* gene mutation in *Mycobacterium avium* subspecies *paratuberculosis* and clinical rifabutin and rifampicin resistance for treatment of Crohn's disease. World J Gastroenterol 14(17):2723-30.

Behra PRK (2022) Comparative genomics of the genus *Mycobacterium*: Genome evolution, phylogeny and diversity. PhD Thesis, Uppsala University, Uppsala, Sweden.

Bouso JM, Planet PJ (2019) Complete nontuberculous mycobacteria whole genomes using an optimized DNA extraction protocol for long-read sequencing. BMC Genomics 20(1):793.

Bryant JM, Thibault VC, Smith DG, McLuckie J, Heron I, Sevilla IA, Biet F, Harris SR, Maskell DJ, Bentley SD, Parkhill J, Stevenson K (2016) Phylogenomic exploration of the relationships between strains of *Mycobacterium avium* subspecies *paratuberculosis*. BMC Genomics 17:79.

Cáceres SM, Ocampo M, Arévalo-Pinzón G, Jimenez RA, Patarroyo ME, Patarroyo MA (2011) The *Mycobacterium tuberculosis* membrane protein Rv0180c: Evaluation of peptide sequences implicated in mycobacterial invasion of two human cell lines. Peptides 32(1):1-10.

Carlos P, Roupie V, Holbert S, Ascencio F, Huygen K, Gomez-Anduro G, Branger M, Reyes-Becerril M, Angulo C (2015) In silico epitope analysis of unique and membrane associated proteins from *Mycobacterium avium* subsp. *paratuberculosis* for immunogenicity and vaccine evaluation. J Theor Biol 384:1-9.

Casey ME, Meade KG, Nalpas NC, Taraktsoglou M, Browne JA, Killick KE, Park SD, Gormley E, Hokamp K, Magee DA, MacHugh DE (2015) Analysis of the bovine monocyte-derived macrophage response to *Mycobacterium avium* subspecies *paratuberculosis* infection using RNA-seq. Front Immunol 6:23.

Caspi R, Billington R, Ferrer L, Foerster H, Fulcher CA, Keseler IM, Kothari A, Krummenacker M, Latendresse M, Mueller LA, Ong Q, Paley S, Subhraveti P, Weaver DS, Karp PD (2016) The MetaCyc database of metabolic pathways and enzymes and the BioCyc collection of pathway/genome databases. Nucleic Acids Res 44(D1): D471-80.

Castellanos E, Aranaz A, Romero B, de Juan L, Alvarez J, Bezos J, Rodríguez S, Stevenson K, Mateos A, Domínguez L (2007) Polymorphisms in *gyrA* and *gyrB* genes among *Mycobacterium avium* subsp. *paratuberculosis* type I, II, and III isolates. J Clin Microbiol 45(10):3439-42.

Caverly LJ, Zimbric M, Azar M, Opron K, LiPuma JJ (2021) Cystic fibrosis airway microbiota associated with outcomes of nontuberculous mycobacterial infection. ERJ

Open Res 7(2):00578-2020.
Chaubey KK, Kumaresan G, Gupta RD, Bhatia AK, Rathore AS (2018) Molecular diversity and homology in six CFPs genes in the novel bio-type, 'Indian Bison Type' of *Mycobacterium avium* subspecies *paratuberculosis* of goat origin vs. other biotypes. Mycobact Dis 8:267.
Chinison JJ, Danelishvili L, Gupta R, Rose SJ, Babrak LM, Bermudez LE (2016) Identification of *Mycobacterium avium* subsp. *hominissuis* secreted proteins using an *in vitro* system mimicking the phagosomal environment. BMC Microbiol 16(1):270.
Choo SW, Ang MY, Dutta A, Tan SY, Siow CC, Heydari H, Mutha NV, Wee WY, Wong GJ (2015) MycoCAP - *Mycobacterium* comparative analysis platform. Sci Rep 5:18227.
Cossu A (2011) Functional transcriptome of *Mycobacterium avium* subsp. *paratuberculosis* in human macrophage infection and immunological relevance of its specific antigens in type I diabetes mellitus. PhD Thesis, Universita' degli studi di Sassari, Sassari, Italy.
Cossu A, Sechi LA, Zanetti S, Rosu V (2012) Gene expression profiling of *Mycobacterium avium* subsp. *paratuberculosis* in simulated multi-stress conditions and within THP-1 cells reveals a new kind of interactive intramacrophage behaviour. BMC Microbiol 12:87.
Cushman J, Freeman E, McCallister S, Schumann A, Hutchison KW, Molloy SD (2021) Increased whiB7 expression and antibiotic resistance in *Mycobacterium chelonae* carrying two prophages. BMC Microbiol 21(1):176.
Dai W, Li J, Li Q, Cai J, Su J, Stubenrauch C, Wang J (2022) PncsHub: a platform for annotating and analyzing non-classically secreted proteins in gram-positive bacteria. Nucleic Acids Res 50(D1): D848-D857.
Daley CL (2017) *Mycobacterium avium* complex disease. Microbiol Spectr 5(2).
de Steenwinkel JE, van Vianen W, Ten Kate MT, Verbrugh HA, van Agtmael MA, Schiffelers RM, Bakker-Woudenberg IA (2007) Targeted drug delivery to enhance efficacy and shorten treatment duration in disseminated *Mycobacterium avium* infection in mice. J Antimicrob Chemother 60(5):1064-73.
Deshpande D, Pasipanodya JG, Gumbo T (2016) Azithromycin dose to maximize efficacy and suppress acquired drug resistance in pulmonary *Mycobacterium avium* disease. Antimicrob Agents Chemother 60(4):2157-63.
Diel R, Lipman M, Hoefsloot W (2018) High mortality in patients with *Mycobacterium avium* complex lung disease: a systematic review. BMC Infect Dis 18(1):206.
Elmagzoub WA, Idris SM, Isameldin M, Arabi N, Abdo A, Ibrahim M, Khan MAA, Tanneberger F, Bakhiet SM, Okuni JB, Ojok L, Gameel AA, Abd El Wahed A, Bekaert M, Mukhtar ME, Amanzada A, Eltom KH, Eltayeb E (2022) *Mycobacterium avium* subsp. *paratuberculosis* and microbiome profile of patients in a referral gastrointestinal diseases centre in the Sudan. PLoS One 17(4): e0266533.
Enany S, Ato M, Matsumoto S (2021) Differential protein expression in exponential and stationary growth phases of *Mycobacterium avium* subsp. *hominissuis* 104. Molecules 26(2):305.
Eraghi V, Derakhshandeh A, Hosseini A, Motamedi-Boroojeni A (2017) In silico design and expression of a novel fusion protein of HBHA and high antigenic region of FAP-P of *Mycobacterium avium* subsp. *paratuberculosis* in *Pichia pastoris*. Mol Biol Res Commun 2017 6(4):161-168.
Facciuolo A (2015) Proteomic and immunological investigation of *Mycobacterium avium* subspecies *paratuberculosis* secreted proteins. PhD Thesis, The University of Guelph, Guelph, Canada.
Falkinham JO (2007) Growth in catheter biofilms and antibiotic resistance of *Mycobacterium avium*. J Med Microbiol 56(Pt 2):250-254.
Frothingham R, Wilson KH (1994) Molecular phylogeny of the *Mycobacterium avium* complex demonstrates clinically meaningful divisions. J Infect Dis 169(2):305-12.
Garg A, Singhal N, Kumar M (2021) Discerning novel drug targets for treating *Mycobacterium avium* ss. *paratuberculosis*-associated autoimmune disorders: an in silico approach. Brief Bioinform 22(3): bbaa195.
Gcebe N, Michel A, Gey van Pittius NC, Rutten V (2016) Comparative genomics and proteomic analysis of four non-tuberculous *Mycobacterium* species and *Mycobacterium tuberculosis* complex: Occurrence of Shared Immunogenic Proteins. Front Microbiol 7:795.
Ghosh P, Hsu C, Alyamani EJ, Shehata MM, Al-Dubaib MA, Al-Naeem A, Hashad M, Mahmoud OM, Alharbi KB, Al-Busadah K, Al-Swailem AM, Talaat AM (2012) Genome-wide analysis of the emerging infection with *Mycobacterium avium* subspecies *paratuberculosis* in the Arabian camels (*Camelus dromedarius*). PLoS One 7(2): e31947.
Guerrero C, Bernasconi C, Burki D, Bodmer T, Telenti A (1995) A novel insertion element from *Mycobacterium avium*, IS1245, is a specific target for analysis of strain relatedness. J Clin Microbiol 33(2):304-7.
Gurung RB, Purdie AC, Begg DJ, Whittington RJ (2012) In silico identification of epitopes in *Mycobacterium avium* subsp. *paratuberculosis* proteins that were upregulated under stress conditions. Clin Vaccine Immunol 19(6):855-64.
Haig SJ, Kotlarz N, LiPuma JJ, Raskin L (2018) A high-throughput approach for identification of nontuberculous mycobacteria in drinking water reveals relationship between water age and *Mycobacterium avium*. Mbio 9(1): e02354-17.
Hasan NA, Davidson RM, Epperson LE, Kammlade SM, Beagle S, Levin AR, de Moura VC, Hunkins JJ, Weakly N, Sagel SD, Martiniano SL, Salfinger M, Daley CL,

Nick JA, Strong M (2021) Population genomics and inference of *Mycobacterium avium* complex clusters in cystic fibrosis care centers, United States. Emerg Infect Dis 27(11):2836-2846.

Hasan NA, Honda JR, Davidson RM, Epperson LE, Bankowski MJ, Chan ED, Strong M (2016) Complete genome sequence of *Mycobacterium chimaera* strain AH16. Genome Announc 4(6): e01276-16.

Hassan SA, Hasnain SE, Halawani SM (2014) In silico characterization of a putative ORF-MAP1138c of *Mycobacterium avium* subspecies paratuberculosis (MAP) with its implications in virulence. BMC Genomics 15(Suppl 2): P14.

He Z, De Buck J (2010) Localization of proteins in the cell wall of *Mycobacterium avium* subsp. *paratuberculosis* K10 by proteomic analysis. Proteome Sci 8:21.

Hepp B, Da Cunha V, Lorieux F, Oberto J (2021) BAGET 2.0: an updated web tool for the effortless retrieval of prokaryotic gene context and sequence. Bioinformatics 37(17):2750-2752.

Hoek A, Rutten VP, van der Zee R, Davies CJ, Koets AP. Epitopes of *Mycobacterium avium* ssp. *paratuberculosis* 70kDa heat-shock protein activate bovine helper T cells in outbred cattle. Vaccine 28(36):5910-9.

Holsti MA, Schorey JS, Brown EJ, Allen PM (1998) Identification of epitopes of fibronectin attachment protein (FAP-A) of *Mycobacterium avium* which stimulate strong T-cell responses in mice. Infect Immun 66(3):1261-4.

Hsu CY, Wu CW, Talaat AM (2011) Genome-wide sequence variation among *Mycobacterium avium* subspecies *paratuberculosis* Isolates: A better understanding of Johne's disease transmission dynamics. Front Microbiol 2:236.

Huang YJ, LiPuma JJ (2016) The microbiome in cystic fibrosis. Clin Chest Med 37(1):59-67.

Hughes V, Bannantine JP, Denham S, Smith S, Garcia-Sanchez A, Sales J, Paustian ML, Mclean K, Stevenson K (2008) Immunogenicity of proteome-determined *Mycobacterium avium* subsp. *paratuberculosis*-specific proteins in sheep with paratuberculosis. Clin Vaccine Immunol 15(12):1824-33.

Hughes V, Garcia-Sanchez A, Smith S, Mclean K, Lainson A, Nath M, Stevenson K (2012) Proteome-determined type-specific proteins of *Mycobacterium avium* subspecies *paratuberculosis*. Vet Microbiol 158(1-2):153-62.

Hughes V, Smith S, Garcia-Sanchez A, Sales J, Stevenson K (2007) Proteomic comparison of *Mycobacterium avium* subspecies *paratuberculosis* grown in vitro and isolated from clinical cases of ovine paratuberculosis. Microbiology (Reading) 153(Pt 1):196-205.

Hwang JA, Kim S, Jo KW, Shim TS (2017) Natural history of *Mycobacterium avium* complex lung disease in untreated patients with stable course. Eur Respir J 49(3):1600537.

Ibeagha-Awemu EM, Bissonnette N, Bhattarai S, Wang M, Dudemaine PL, McKay S, Zhao X (2021) Whole genome methylation analysis reveals role of DNA methylation in Cow's ileal and ileal lymph node responses to *Mycobacterium avium* subsp. *paratuberculosis* infection. Front Genet 12:797490.

Ignatov D, Malakho S, Majorov K, Skvortsov T, Apt A, Azhikina T (2013) RNA-Seq analysis of *Mycobacterium avium* non-coding transcriptome. PLoS One 8(9): e74209.

Ignatov DV, Skvortsov TA, Majorov KB, Apt AS, Azhikina TL (2010) Adaptive changes in *Mycobacterium avium* gene expression profile following infection of genetically susceptible and resistant mice. Acta Naturae 2(3):78-83.

Imperiale BR, Moyano RD, DI Giulio AB, Romero MA, Alvarado Pinedo MF, Santangelo MP, Travería GE, Morcillo NS, Romano MI (2017) Genetic diversity of *Mycobacterium avium* complex strains isolated in Argentina by MIRU-VNTR. Epidemiol Infect 145(7):1382-1391.

Iwasaki K, Matsuzawa Y, Wakabayashi H, Shioya M, Hayakawa S, Tatsuno I (2021) Lower airway microbiota in patients with clinically suspected *Mycobacterium avium* complex lung disease. Heliyon 7(6): e07283.

Johansen MD, Herrmann JL, Kremer L (2020) Non-tuberculous mycobacteria and the rise of *Mycobacterium abscessus*. Nat Rev Microbiol 18(7):392-407.

Johnson MM, Odell JA (2014) Nontuberculous mycobacterial pulmonary infections. J Thorac Dis 6(3):210-20.

Johnston C, Douarre PE, Soulimane T, Pletzer D, Weingart H, MacSharry J, Coffey A, Sleator RD, O'Mahony J (2013) Codon optimisation to improve expression of a *Mycobacterium avium* ssp. *paratuberculosis*-specific membrane-associated antigen by *Lactobacillus salivarius*. Pathog Dis 68(1):27-38.

Johnston CD, Bannantine JP, Govender R, Endersen L, Pletzer D, Weingart H, Coffey A, O'Mahony J, Sleator RD (2014) Enhanced expression of codon optimized *Mycobacterium avium* subsp. *paratuberculosis* antigens in *Lactobacillus salivarius*. Front Cell Infect Microbiol 4:120.

Jung SK, McDonald K (2011) Visual gene developer: a fully programmable bioinformatics software for synthetic gene optimization. BMC Bioinformatics 12:340.

Kannan N, Lai YP, Haug M, Lilleness MK, Bakke SS, Marstad A, Hov H, Naustdal T, Afset JE, Ioerger TR, Flo TH, Steigedal M (2019) Genetic variation/evolution and differential host responses resulting from in-patient adaptation of *Mycobacterium avium*. Infect Immun 87(4): e00323-18.

Kazakova O, Racoviceanu R, Petrova A, Mioc M, Militaru A, Udrescu L, Udrescu M, Voicu A, Cummings J, Robertson G, Ordway DJ, Slayden RA, Șoica C (2021) New investigations with lupane type A-ring azepane triterpenoids for antimycobacterial drug candidate design. Int J Mol Sci 22(22):12542.

Keen EC, Choi J, Wallace MA, Azar M, Mejia-Chew CR, Mehta SB, Bailey TC, Caverly LJ, Burnham CD, Dantas

G (2021) Comparative genomics of *Mycobacterium avium* complex reveals signatures of environment-specific adaptation and community acquisition. mSystems 6(5): e0119421.

Kim BG, Jhun BW, Kim H, Kwon OJ (2022) Treatment outcomes of *Mycobacterium avium* complex pulmonary disease according to disease severity. Sci Rep 2(1):1970.

Kim BJ, Choi BS, Lim JS, Choi IY, Lee JH, Chun J, Kook YH, Kim BJ (2012) Complete genome sequence of *Mycobacterium intracellulare* strain ATCC 13950(T). J Bacteriol 194(10):2750.

Kim BJ, Kim BR, Lee SY, Seok SH, Kook YH, Kim BJ (2013) Whole-genome sequence of a novel species, *Mycobacterium yongonense* DSM 45126T. Genome Announc 1(4): e00604-13.

Kim S, Park HE, Park WB, Kim SY, Park HT, Yoo HS (2021) *Mycobacterium avium* modulates the protective immune response in canine peripheral blood mononuclear cells. Front Cell Infect Microbiol 10:609712.

Kim SY, Jeong BH, Park HY, Jeon K, Han SJ, Shin SJ, Koh WJ (2016) Association of ISMav6 with the pattern of antibiotic resistance in Korean *Mycobacterium avium* clinical isolates but no relevance between their genotypes and clinical features. PLoS One 11(2): e0148917.

Kirubakar G, Murugaiyan J, Schaudinn C, Dematheis F, Holland G, Eravci M, Weise C, Roesler U, Lewin A (2018) Proteome analysis of a *M. avium* mutant exposes a novel role of the bifunctional protein LysX in the regulation of metabolic activity. J Infect Dis 218(2):291-299.

Kirubakar G, Schäfer H, Rickerts V, Schwarz C, Lewin A (2020) Mutation on lysX from *Mycobacterium avium hominissuis* impacts the host-pathogen interaction and virulence phenotype. Virulence 11(1):132-144.

Koets AP, Adugna G, Janss LL, van Weering HJ, Kalis CH, Wentink GH, Rutten VP, Schukken YH (2000) Genetic variation of susceptibility to *Mycobacterium avium* subsp. *paratuberculosis* infection in dairy cattle. J Dairy Sci 83(11):2702-8.

Krzywinska E, Krzywinski J, Schorey JS (2004) Phylogeny of *Mycobacterium avium* strains inferred from glycopeptidolipid biosynthesis pathway genes. Microbiology (Reading) 150(Pt 6):1699-1706.

Lahiri A, Kneisel J, Kloster I, Kamal E, Lewin A (2014) Abundance of *Mycobacterium avium* ssp. *hominissuis* in soil and dust in Germany - implications for the infection route. Lett Appl Microbiol 59(1):65-70.

Lahiri A, Sanchini A, Semmler T, Schäfer H, Lewin A (2014a) Identification and comparative analysis of a genomic island in *Mycobacterium avium* subsp. *hominissuis*. FEBS Lett 588(21):3906-11.

Lecorche E, Haenn S, Mougari F, Kumanski S, Veziris N, Benmansour H, Raskine L, Moulin L, Cambau E, CNR-MyRMA (2018) Comparison of methods available for identification of *Mycobacterium chimaera*. Clin Microbiol Infect 24(4):409-413.

Lee SM, Park HT, Park S, Lee JH, Kim D, Yoo HS, Kim D (2023) A machine learning approach reveals a microbiota signature for infection with *Mycobacterium avium* subsp. *paratuberculosis* in cattle. Microbiol Spectr 11(1): e0313422.

Lim J, Park HT, Ko S, Park HE, Lee G, Kim S, Shin MK, Yoo HS, Kim D (2021) Genomic diversity of *Mycobacterium avium* subsp. *paratuberculosis*: pangenomic approach for highlighting unique genomic features with newly constructed complete genomes. Vet Res 52(1):46.

Loebinger MR (2017) *Mycobacterium avium* complex infection: phenotypes and outcomes. Eur Respir J 50(3):1701380.

Low JL, Wu ML, Aziz DB, Laleu B, Dick T (2017) Screening of TB Actives for activity against nontuberculous mycobacteria delivers high hit rates. Front Microbiol 8:1539.

Mac Aogáin M, Roycroft E, Raftery P, Mok S, Fitzgibbon M, Rogers TR (2015) Draft genome sequences of three *Mycobacterium chimaera* respiratory isolates. Genome Announc 3(6): e01409-15.

Mackenzie N, Alexander DC, Turenne CY, Behr MA, De Buck JM (2009) Genomic comparison of PE and PPE genes in the *Mycobacterium avium* complex. J Clin Microbiol 47(4):1002-11.

Mallikarjunappa S, Adnane M, Cormican P, Karrow NA, Meade KG (2019) Characterization of the bovine salivary gland transcriptome associated with *Mycobacterium avium* subsp. *paratuberculosis* experimental challenge. BMC Genomics 20(1):491.

Martin WS (2016) The isolation and proteomic analysis of *Mycobacterium avium* subspecies *paratuberculosis* membrane vesicles. Master's Thesis, The University of Guelph, Guelph, Canada.

Matern WM, Jenquin RL, Bader JS, Karakousis PC (2020) Identifying the essential genes of *Mycobacterium avium* subsp. *hominissuis* with Tn-Seq using a rank-based filter procedure. Sci Rep 10(1):1095.

Matern WM, Parker H, Danchik C, Hoover L, Bader JS, Karakousis PC (2021) Genetic determinants of intrinsic antibiotic tolerance in *Mycobacterium avium*. Microbiol Spectr 9(2): e0024621.

Matthews C, Cotter PD, O' Mahony J (2021) MAP, Johne's disease and the microbiome; current knowledge and future considerations. Anim Microbiome 3(1):34.

McNamara M, Tzeng SC, Maier C, Zhang L, Bermudez LE (2012) Surface proteome of "*Mycobacterium avium* subsp. *hominissuis*" during the early stages of macrophage infection. Infect Immun 80(5):1868-80.

Minozzi G, Buggiotti L, Stella A, Strozzi F, Luini M, Williams JL (2010) Genetic loci involved in antibody response to *Mycobacterium avium* ssp. *paratuberculosis* in cattle. PLoS One 5(6):e11117.

Mizzi R, Plain KM, Whittington R, Timms VJ (2022) Global phylogeny of *Mycobacterium avium* and identification of mutation hotspots during niche adaptation. Front Microbiol 13:892333.

Mizzi R, Timms VJ, Price-Carter ML, Gautam M, Whittington R, Heuer C, Biggs PJ, Plain KM (2021) Comparative genomics of *Mycobacterium avium* subspecies *paratuberculosis* sheep strains. Front Vet Sci 8:637637.

Möbius P, Hölzer M, Felder M, Nordsiek G, Groth M, Köhler H, Reichwald K, Platzer M, Marz M (2015) Comprehensive insights in the *Mycobacterium avium* subsp. *paratuberculosis* genome using new WGS data of sheep strain JIII-386 from Germany. Genome Biol Evol 7(9):2585-2601.

Mondot S, Kang S, Furet JP, Aguirre de Carcer D, McSweeney C, Morrison M, Marteau P, Doré J, Leclerc M (2011) Highlighting new phylogenetic specificities of Crohn's disease microbiota. Inflamm Bowel Dis 17(1):185-92.

Moon SM, Park HY, Kim SY, Jhun BW, Lee H, Jeon K, Kim DH, Huh HJ, Ki CS, Lee NY, Kim HK, Choi YS, Kim J, Lee SH, Kim CK, Shin SJ, Daley CL, Koh WJ (2016) Clinical characteristics, treatment outcomes, and resistance mutations associated with macrolide-resistant *Mycobacterium avium* complex lung disease. Antimicrob Agents Chemother 60(11):6758-6765.

Mortensen H, Nielsen SS, Berg P (2004) Genetic variation and heritability of the antibody response to *Mycobacterium avium* subspecies *paratuberculosis* in Danish Holstein cows. J Dairy Sci 87(7):2108-13.

Nash KA, Inderlied CB (1995) Genetic basis of macrolide resistance in *Mycobacterium avium* isolated from patients with disseminated disease. Antimicrob Agents Chemother 39(12):2625-30.

Nicholson KR, Mousseau CB, Champion MM, Champion PA (2021) The genetic proteome: Using genetics to inform the proteome of mycobacterial pathogens. PLoS Pathog 17(1): e1009124.

Nishiuchi Y, Iwamoto T, Maruyama F (2017) Infection sources of a common non-tuberculous mycobacterial pathogen, *Mycobacterium avium* complex. Front Med (Lausanne) 4:27.

Oberto J (2013) SyntTax: a web server linking synteny to prokaryotic taxonomy. BMC Bioinformatics 14:4.

Ogawa K (2013) Genetic research about *Mycobacterium avium* complex. Kekkaku 88(1):17-22.

Oh JY, Ko YK, Gim JA (2021) DNA methylation profiling for the diagnosis and prognosis of patients with nontuberculous *Mycobacterium* lung disease. Curr Issues Mol Biol 43(2):501-512.

Oliveira de Sousa JG (2020) Exploring novel approaches to tackle *Mycobacterium avium.* Master's Thesis, Universidade do Porto, Porto, Portugal.

Operario DJ, Pholwat S, Koeppel AF, Prorock A, Bao Y, Sol-Church K, Scheurenbrand M, Poulter M, Turner S, Parikh HI, Mathers A, Houpt ER (2019) *Mycobacterium avium* complex diversity within lung disease, as revealed by whole-genome sequencing. Am J Respir Crit Care Med 200(3):393-396.

Ostrowski M, Mundo SL, Harris NB, Barletta RG, Lopez OJ (2003) B-cell epitopes in the immunodominant p34 antigen of *Mycobacterium avium* ssp. *paratuberculosis* recognized by antibodies from infected cattle. Scand J Immunol 58(5):511-21.

Pang H, Wan K, Wei L (2018) Single-nucleotide polymorphisms related to fluoroquinolone and aminoglycoside resistance in *Mycobacterium avium* isolates. Infect Drug Resist 11:515-521.

Park HT, Lee SM, Ko S, Kim S, Park HE, Shin MK, Kim D, Yoo HS (2022) Delineating transcriptional crosstalk between *Mycobacterium avium* subsp. *paratuberculosis* and human THP-1 cells at the early stage of infection via dual RNA-seq analysis. Vet Res 53(1):71.

Parker H, Lorenc R, Ruelas Castillo J, Karakousis PC (2020) Mechanisms of antibiotic tolerance in *Mycobacterium avium* complex: lessons from related mycobacteria. Front Microbiol 11:573983.

Pasipanodya JG, Ogbonna D, Deshpande D, Srivastava S, Gumbo T (2017) Meta-analyses and the evidence base for microbial outcomes in the treatment of pulmonary *Mycobacterium avium-intracellulare* complex disease. J Antimicrob Chemother 72(suppl_2): i3-i19.

Paustian ML, Kapur V, Bannantine JP (2005) Comparative genomic hybridizations reveal genetic regions within the *Mycobacterium avium* complex that are divergent from *Mycobacterium avium* subsp. *paratuberculosis* isolates. J Bacteriol 187(7):2406-15.

Pérez-Osorio AC, Boyle DS, Ingham ZK, Ostash A, Gautom RK, Colombel C, Houze Y, Leader BT (2012) Rapid identification of mycobacteria and drug-resistant *Mycobacterium tuberculosis* by use of a single multiplex PCR and DNA sequencing. J Clin Microbiol 50(2):326-36.

Pfaller SL, Aronson TW, Holtzman AE, Covert TC (2007) Amplified fragment length polymorphism analysis of *Mycobacterium avium* complex isolates recovered from southern California. J Med Microbiol 56(Pt 9):1152-1160.

Philley JV, Kannan A, Griffith DE, Devine MS, Benwill JL, Wallace RJ Jr, Brown-Elliott BA, Thakkar F, Taskar V, Fox JG, Alqaid A, Bains H, Gupta S, Dasgupta S (2017) Exosome secretome and mediated signaling in breast cancer patients with nontuberculous mycobacterial disease. Oncotarget 8(11):18070-18081.

Philley JV, Kannan A, Olusola P, McGaha P, Singh KP, Samten B, Griffith DE, Dasgupta S (2019) Microbiome diversity in sputum of nontuberculous mycobacteria infected women with a history of breast cancer. Cell Physiol Biochem 52(2):263-279.

Phillips IL, Danelishvili L, Bermudez LE (2021) Macrophage proteome analysis at different stages of *Mycobacterium avium* subspecies paratuberculosis infection reveals a mechanism of pathogen dissemination. Proteomes 9(2):20.

Prasad P, Mahapatra S, Mishra R, Murmu KC, Aggarwal S, Sethi M, Mohapatra P, Ghosh A, Yadav R, Dodia H, Ansari SA, De S, Singh D, Suryawanshi A, Dash R, Senapati S, Beuria TK, Chattopadhyay S, Syed GH, Swain R, Raghav SK, Parida A (2022) Long-read 16S-seq reveals nasopharynx microbial dysbiosis and enrichment of *Mycobacterium* and *Mycoplasma* in COVID-19 patients: a potential source of co-infection. Mol Omics 18(6):490-505.

Pribylova R, Kralik P, Pavlik I (2009) Oligonucleotide microarray technology and its application to *Mycobacterium avium* subsp. *paratuberculosis* research: a review. Mol Biotechnol 42(1):30-40.

Radosevich TJ, Reinhardt TA, Lippolis JD, Bannantine JP, Stabel JR (2007) Proteome and differential expression analysis of membrane and cytosolic proteins from *Mycobacterium avium* subsp. *paratuberculosis* strains K-10 and 187. J Bacteriol 189(3):1109-17.

Rajaonarifara E (2013) A bioinformatic study on the feasibility of a cross-species proteomics analyses of mycobacteria. Master's Thesis, University of Capetown, Capetown, South Africa.

Rana A, Rub A, Akhter Y (2014) Proteome-scale identification of outer membrane proteins in *Mycobacterium avium* subspecies paratuberculosis using a structure based combined hierarchical approach. Mol Biosyst 10(9):2329-37.

Ratnatunga CN, Lutzky VP, Kupz A, Doolan DL, Reid DW, Field M, Bell SC, Thomson RM, Miles JJ (2020) The rise of non-tuberculosis mycobacterial lung disease. Front Immunol 11:303.

Rindi L, Garzelli C (2014) Genetic diversity and phylogeny of *Mycobacterium avium*. Infect Genet Evol 21:375-83.

Rivoire B, Ranchoff BJ, Chatterjee D, Gaylord H, Tsang AY, Kolk AH, Aspinall GO, Brennan PJ (1989) Generation of monoclonal antibodies to the specific sugar epitopes of *Mycobacterium avium* complex serovars. Infect Immun 57(10):3147-58.

Rojony R, Martin M, Campeau A, Wozniak JM, Gonzalez DJ, Jaiswal P, Danelishvili L, Bermudez LE (2019) Quantitative analysis of *Mycobacterium avium* subsp. *hominissuis* proteome in response to antibiotics and during exposure to different environmental conditions. Clin Proteomics 16:39.

Romano MI, Amadio A, Bigi F, Klepp L, Etchechoury I, Llana MN, Morsella C, Paolicchi F, Pavlik I, Bartos M, Leão SC, Cataldi A (2005) Further analysis of VNTR and MIRU in the genome of *Mycobacterium avium* complex, and application to molecular epidemiology of isolates from South America. Vet Microbiol. 110(3-4):221-37.

Roupie V, Leroy B, Rosseels V, Piersoel V, Noël-Georis I, Romano M, Govaerts M, Letesson JJ, Wattiez R, Huygen K (2008) Immunogenicity and protective efficacy of DNA vaccines encoding MAP0586c and MAP4308c of *Mycobacterium avium* subsp. *paratuberculosis* secretome. Vaccine 26(37):4783-94.

Roy A, Bhattacharya S, Bothra AK, Sen A (2013) A database for *Mycobacterium* secretome analysis: 'MycoSec' to accelerate global health research. OMICS 17(10):502-9.

Ruiz-Larrañaga O, Garrido JM, Manzano C, Iriondo M, Molina E, Gil A, Koets AP, Rutten VP, Juste RA, Estonba A (2010) Identification of single nucleotide polymorphisms in the bovine solute carrier family 11 member 1 (*SLC11A1*) gene and their association with infection by *Mycobacterium avium* subspecies *paratuberculosis*. J Dairy Sci 93(4):1713-21.

Runyon EH (1959) Anonymous mycobacteria in pulmonary disease. Med Clin North Am 43(1):273-90.

Saha MS, Pal S, Sarkar I, Roy A, Das Mohapatra PK, Sen A (2019) Comparative genomics of *Mycobacterium* reveals evolutionary trends of *M. avium* complex. Genomics 111(3):426-435.

Sanchini A, Dematheis F, Semmler T, Lewin A (2017) Metabolic phenotype of clinical and environmental *Mycobacterium avium* subsp. *hominissuis* isolates. Peer J 5: e2833.

Sanchini A, Semmler T, Mao L, Kumar N, Dematheis F, Tandon K, Peddireddy V, Ahmed N, Lewin A (2016) A hypervariable genomic island identified in clinical and environmental *Mycobacterium avium* subsp. *hominissuis* isolates from Germany. Int J Med Microbiol 306(7):495-503.

Santema W, van Kooten P, Hoek A, Leeflang M, Overdijk M, Rutten V, Koets A (2011) Hsp70 vaccination-induced antibodies recognize B cell epitopes in the cell wall of *Mycobacterium avium* subspecies *paratuberculosis*. Vaccine 29(7):1364-73.

Saxena S, Spaink HP, Forn-Cuní G (2021) Drug resistance in nontuberculous mycobacteria: mechanisms and models. Biology (Basel) 10(2):96.

Schildkraut JA, Koeken VACM, Coolen JPM, van Crevel R, van Ingen J (2021) An optimized protocol for dual RNA-Seq of human macrophages infected with *Mycobacterium avium,* bioRxiv doi: https://doi.org/10.1101/2021.05.20.443437.

Schildkraut JA, Coolen JPM, Severin H, Koenraad E, Aalders N, Melchers WJG, Hoefsloot W, Wertheim HFL, van Ingen J (2023) MGIT enriched shotgun metagenomics for routine identification of nontuberculous mycobacteria: a route to personalized health care. J Clin Microbiol 61(3):e0131822.

Schmalstieg AM, Srivastava S, Belkaya S, Deshpande D, Meek C, Leff R, van Oers NS, Gumbo T (2012) The anti-

biotic resistance arrow of time: efflux pump induction is a general first step in the evolution of mycobacterial drug resistance. Antimicrob Agents Chemother 56(9):4806-15.

Semret M, Alexander DC, Turenne CY, de Haas P, Overduin P, van Soolingen D, Cousins D, Behr MA (2005) Genomic polymorphisms for *Mycobacterium avium* subsp. *paratuberculosis* diagnostics. J Clin Microbiol 43(8):3704-12.

Semret M, Turenne CY, de Haas P, Collins DM, Behr MA (2006) Differentiating host-associated variants of *Mycobacterium avium* by PCR for detection of large sequence polymorphisms. J Clin Microbiol 44(3):881-7.

Semret M, Zhai G, Mostowy S, Cleto C, Alexander D, Cangelosi G, Cousins D, Collins DM, van Soolingen D, Behr MA (2004) Extensive genomic polymorphism within *Mycobacterium avium*. J Bacteriol 186(18):6332-4.

Seto S, Morimoto K, Yoshida T, Hiramatsu M, Hijikata M, Nagata T, Kikuchi F, Shiraishi Y, Kurashima A, Keicho N (2020) Proteomic profiling reveals the architecture of granulomatous lesions caused by tuberculosis and *Mycobacterium avium* complex lung disease. Front Microbiol 10:3081.

Shah NM, Davidson JA, Anderson LF, Lalor MK, Kim J, Thomas HL, Lipman M, Abubakar I (2016) Pulmonary *Mycobacterium avium-intracellulare* is the main driver of the rise in non-tuberculous mycobacteria incidence in England, Wales and Northern Ireland, 2007-2012. BMC Infect Dis 16:195.

Shin JI, Shin SJ, Shin MK (2020) Differential genotyping of *Mycobacterium avium* complex and its implications in clinical and environmental epidemiology. Microorganisms 8(1):98.

Sulaiman I, Wu BG, Li Y, Scott AS, Malecha P, Scaglione B, Wang J, Basavaraj A, Chung S, Bantis K, Carpenito J, Clemente JC, Shen N, Bessich J, Rafeq S, Michaud G, Donington J, Naidoo C, Theron G, Schattner G, Garofano S, Condos R, Kamelhar D, Addrizzo-Harris D, Segal LN (2018) Evaluation of the airway microbiome in nontuberculous mycobacteria disease. Eur Respir J 52(4):1800810.

Sur S, Pal B (2021) Comprehensive review of *Mycobacterium ulcerans* and Buruli ulcer from a bioinformatics perspective – what have we learnt? Acta Biol Szeged 65(2):233-45.

Swathi D, Saranya S, Raja A, Vijayarani K, Kumanan, K (2020) In silico prediction of the epitopes for the immunogenic proteins present in *Mycobacterium avium* subsp. *paratuberculosis.* Indian J Anim Sci 90:156–160.

Switzenberg J, Kabara E, Sommer S, Coussens P (2013) Transcriptomic studies of *Mycobacterium avium* subsp. *paratuberculosis* infected monocyte-derived macrophages from individual dairy cows (P6095). J Immunol 190(1_Supplement): 141.12.

Tateishi Y, Kitada S, Miki K, Maekura R, Ogura Y, Ozeki Y, Nishiuchi Y, Niki M, Hayashi T, Hirata K, Kobayashi K, Matsumoto S (2012) Whole-genome sequence of the hypervirulent clinical strain *Mycobacterium intracellulare* M.i.198. J Bacteriol 194(22):6336.

Tateishi Y, Ozeki Y, Nishiyama A, Miki M, Maekura R, Fukushima Y, Nakajima C, Suzuki Y, Matsumoto S (2021) Comparative genomic analysis of *Mycobacterium intracellulare:* implications for clinical taxonomic classification in pulmonary *Mycobacterium avium-intracellulare* complex disease. BMC Microbiol 21(1):103.

Thornton CS, Mellett M, Jarand J, Barss L, Field SK, Fisher DA (2021) The respiratory microbiome and nontuberculous mycobacteria: an emerging concern in human health. Eur Respir Rev 30(160):200299.

Tortoli E, Rindi L, Garcia MJ, Chiaradonna P, Dei R, Garzelli C, Kroppenstedt RM, Lari N, Mattei R, Mariottini A, Mazzarelli G, Murcia MI, Nanetti A, Piccoli P, Scarparo C (2004) Proposal to elevate the genetic variant MAC-A, included in the *Mycobacterium avium* complex, to species rank as *Mycobacterium chimaera* sp. nov. Int J Syst Evol Microbiol 54(Pt 4):1277-1285.

Turenne CY, Semret M, Cousins DV, Collins DM, Behr MA (2006) Sequencing of *hsp65* distinguishes among subsets of the *Mycobacterium avium* complex. J Clin Microbiol 44(2):433-40.

Turenne CY, Wallace R Jr, Behr MA (2007) *Mycobacterium avium* in the postgenomic era. Clin Microbiol Rev 20(2):205-29.

Uchiya K, Takahashi H, Yagi T, Moriyama M, Inagaki T, Ichikawa K, Nakagawa T, Nikai T, Ogawa K (2013) Comparative genome analysis of *Mycobacterium avium* revealed genetic diversity in strains that cause pulmonary and disseminated disease. PLoS One 8(8): e71831.

Uchiya KI, Tomida S, Nakagawa T, Asahi S, Nikai T, Ogawa K (2017) Comparative genome analyses of *Mycobacterium avium* reveal genomic features of its subspecies and strains that cause progression of pulmonary disease. Sci Rep 7:39750.

Uddin R, Siraj B, Rashid M, Khan A, Ahsan Halim S, Al-Harrasi A (2020) Genome subtraction and comparison for the identification of novel drug targets against *Mycobacterium avium* subsp. *hominissuis*. Pathogens 9(5):368.

van den Esker MH, Koets AP (2019) Application of transcriptomics to enhance early diagnostics of mycobacterial infections, with an emphasis on *Mycobacterium avium* ssp. *paratuberculosis*. Vet Sci 6(3):59.

van Ingen J, Boeree MJ, Kösters K, Wieland A, Tortoli E, Dekhuijzen PN, van Soolingen D (2009) Proposal to elevate *Mycobacterium avium* complex ITS sequevar MAC-Q to *Mycobacterium vulneris* sp. nov. Int J Syst Evol Microbiol 59(Pt 9):2277-82.

van Ingen J, Turenne CY, Tortoli E, Wallace RJ Jr, Brown-Elliott BA (2018) A definition of the *Mycobacterium avium* complex for taxonomical and clinical purposes, a review.

Int J Syst Evol Microbiol 68(11):3666-3677.
van Tonder AJ, Ellis HC, Churchward CP, Kumar K, Ramadan N, Benson S, Parkhill J, Moffatt MF, Loebinger MR, Cookson WOC (2023) *Mycobacterium avium* complex genomics and transmission in a London hospital. Eur Respir J 61(4):2201237.
Wang JJ, Chen C, Xie PF, Pan Y, Tan YH, Tang LJ (2014) Proteomic analysis and immune properties of exosomes released by macrophages infected with *Mycobacterium avium*. Microbes Infect 16(4):283-91.
Weigoldt M, Meens J, Doll K, Fritsch I, Möbius P, Goethe R, Gerlach GF (2011) Differential proteome analysis of *Mycobacterium avium* subsp. *paratuberculosis* grown *in vitro* and isolated from cases of clinical Johne's disease. Microbiology (Reading) 157(Pt 2):557-565.
Wibberg D, Price-Carter M, Rückert C, Blom J, Möbius P (2020) Complete genome sequence of ovine *Mycobacterium avium* subsp. *paratuberculosis* Strain JIII-386 (MAP-S/type III) and its comparison to MAP-S/type I, MAP-C, and *M. avium* complex genomes. Microorganisms 9(1):70.
Wilson SA (2017) Genetic variation of rapid-growing and slow-growing mycobacteria. Master's Thesis, North Carolina A&T State University, USA
Wu MF, Shu CC, Wang JY, Yan BS, Lai HC, Chiang BL, Wu LS, Yu CJ (2019) NLRP3 inflammasome is attenuated in patients with *Mycobacterium avium* complex lung disease and correlated with decreased interleukin-1β response and host susceptibility. Sci Rep 9(1):12534.
Yano H, Iwamoto T, Nishiuchi Y, Nakajima C, Starkova DA, Mokrousov I, Narvskaya O, Yoshida S, Arikawa K, Nakanishi N, Osaki K, Nakagawa I, Ato M, Suzuki Y, Maruyama F (2017) Population structure and local adaptation of MAC lung disease agent *Mycobacterium avium* subsp. *hominissuis*. Genome Biol Evol 9(9):2403-2417.
Yee M, Klinzing D, Wei JR, Gengenbacher M, Rubin EJ, Chien JY, Hsueh PR, Dick T (2017) Draft genome sequence of *Mycobacterium avium* 11. Genome Announc 5(32): e00766-17.
Yu X, Jiang W (2021) *Mycobacterium colombiense* and *Mycobacterium avium* complex causing severe pneumonia in a patient with HIV identified by a novel molecular-based method. Infect Drug Resist 14:11-16.
Zhao X, Epperson LE, Hasan NA, Honda JR, Chan ED, Strong M, Walter ND, Davidson RM (2017) Complete genome sequence of *Mycobacterium avium* subsp. *hominissuis* strain H87 isolated from an indoor water sample. Genome Announc 5(16): e00189-17.
Zhu X, Chang S, Fang K, Cui S, Liu J, Wu Z, Yu X, Gao GF, Yang H, Zhu B, Wang J (2009) MyBASE: a database for genome polymorphism and gene function studies of *Mycobacterium*. BMC Microbiol 9:40.