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Difference in drug susceptibility distribution and clinical characteristics between *Mycobacterium avium* and *Mycobacterium intracellulare* lung diseases in Shanghai, China

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Abstract

Introduction. *Mycobacterium avium* complex (MAC) has been reported as the most common aetiology of lung disease involving nontuberculous mycobacteria.

Hypothesis. Antimicrobial susceptibility and clinical characteristics may differ between *Mycobacterium avium* and *Mycobacterium intracellulare*.

Aim. We aimed to evaluate the differences in antimicrobial susceptibility profiles between two major MAC species (*Mycobacterium avium* and *Mycobacterium intracellulare*) from patients with pulmonary infections and to provide epidemiologic data with minimum inhibitory concentration (MIC) distributions.

Methodology. Between January 2019 and May 2020, 45 *M. avium* and 242 *M. intracellulare* isolates were obtained from Shanghai Pulmonary Hospital. The demographic and clinical characteristics of patients were obtained from their medical records. The MICs of 13 antimicrobials were determined for the MAC isolates using commercial Sensititre SLOWMYCO MIC plates and the broth microdilution method, as recommended by the Clinical and Laboratory Standards Institute (CLSI; Standards M24-A2). MIC_{50} and MIC_{90} values were derived from the MIC distributions.

Results. *M. intracellulare* had higher resistance rates than *M. avium* for most tested antimicrobials except clarithromycin, ethambutol, and ciprofloxacin. Clarithromycin was the most effective antimicrobial against both the *M. avium* (88.89%) and *M. intracellulare* (91.32%) isolates, with no significant difference between the species (P=0.601). The MIC_{90} of clarithromycin was higher for *M. avium* (32 µg ml^{-1}) than *M. intracellulare* (8 µg ml^{-1}). The MIC_{50} of rifabutin was more than four times higher for *M. intracellulare* (1 µg ml^{-1}) than *M. avium* (≤0.25 µg ml^{-1}). The percentages of patients aged >60 years and patients with sputum, cough, and cavitary lesions were significantly higher than among patients with *M. intracellulare* infection than *M. avium* infections.

Conclusions. The pulmonary disease caused by distinct MAC species had different antimicrobial susceptibility, symptoms, and radiographic findings.

BACKGROUND

Nontuberculous mycobacteria (NTM) are widely distributed in soil, water, and animals [1]. The incidence of NTM pulmonary diseases has been increasing in many industrialized countries, such as Canada [2], Australia [3], Netherlands [4], and United States [5]. Furthermore, the proportion of NTM among all mycobacterial isolates has increased from 11.1–22.9% in China [6, 7]. *Mycobacterium avium* and *M. intracellulare* are the major *Mycobacterium avium* complex (MAC) species, which is an important group among NTM and involves slow-growing mycobacteria [8]. MAC has been reported as the most common aetiology of lung disease

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Keywords: clinical characteristics; infection; *M. avium*; *M. intracellulare*; MIC distributions.

Abbreviations: AN, amikacin; CLSI, Clinical and Laboratory Standards Institute; CYC, cycloserine; EMB, ethambutol; ETH, ethionamide; KAN, kanamycin; MICs, minimum inhibitory concentrations INH: isoniazid; MXF, moxifloxacin; OFX, ofloxacin; PAS, pyrazinamide; RFB, rifabutin; RIF, rifampin; SM, streptomycin.

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Table 1. Antimicrobial susceptibilities and minimum inhibitory concentrations (MICs) of *Mycobacterium avium* and *Mycobacterium intracellulare* isolates

Agent	MIC range (μg ml^{-1})	Critical concentrations			*M. intracellulare*			*M. avium*			X^2	*P*
		R	I	S	MIC_{50}	MIC_{90}	R (%)	MIC_{50}	MIC_{90}	R (%)		
AN	1–64	≥64	32	≤16	32	>64	31.82	16	64	11.11	7.972	0.004
CIP	0.12–16	≥4	2	≤1	>16	>16	99.59	16	>16	100	0.187	0.665
CLR	0.06–64	≥32	16	≤8	4	8	8.68	4	32	11.11	0.273	0.601
DO	0.12–16	≥8	2–4	≤1	>16	>16	99.17	>16	>16	97.78	0.715	0.398
EMB	0.5–16	≥8	4	≤2	8	>16	81.82	16	16	95.56	5.320	0.021
LNZ	1–64	≥32	16	≤8	32	>64	82.23	64	>64	77.78	0.500	0.480
MXF	0.12–8	≥4	2	≤1	4	>8	82.23	4	>8	80.00	0.127	0.722
RIF	0.12–8	≥2	–	≤1	>8	>8	98.35	4	>8	88.89	11.175	0.001
RFB	0.25–8	≥4	–	≤2	1	4	24.79	≤0.25	4	15.56	1.809	0.179
SXT	0.12–8	≥4/76	–	≤2/38	4	>8	77.27	4	>8	64.44	3.354	0.067
SM	0.5–64				>64	>64		64	>64			
ETH	0.3–20				>20	>20		>20	>20			
INH	0.25–8				>8	>8		>8	>8			

RIF: rifampicin, RFB: rifabutin, INH: isoniazid, EMB: ethambutol, ETH: ethionamide, CLR: clarithromycin, MXF: moxifloxacin, CIP: ciprofloxacin, AN: amikacin, SM: streptomycin, LNZ: linezolid, SXT: sulfamethoxazole, DO: doxycycline.
*No breakpoints.

involving NTM [9]. The species involved in MAC infections vary geographically [10, 11]. In the USA and Europe, *M. avium* was the most frequent isolate among MAC species [12, 13]. Furthermore, a Japanese study found that pulmonary diseases caused by *M. avium* were more prevalent in the north and east of Japan, while those caused by *M. intracellulare* were more prevalent in the south and west of Japan [14]. In addition, antimicrobial susceptibility profiles and treatment outcomes were different between cases of *M. avium* lung disease and cases of *M. intracellulare* lung disease [15, 16]. Thus, precise identification on the MAC species isolated from patients is becoming important due to differences in the treatment outcomes and epidemiological implications [17, 18].

Information regarding antimicrobial susceptibility are regarded as essential for effective and appropriate treatment of NTM diseases [1]. Standard treatment regimens for MAC infections involve macrolides (such as clarithromycin [CLR] and azithromycin [AZM]), ethambutol (EMB), and either rifamycin (RIF) or rifabutin (RFB). If the patient requires more aggressive therapy, an injectable aminoglycoside (such as amikacin [AN]) may be added to this combination [11, 19]. Macrolides are the only antimicrobials that have been shown to exhibit a correlation between *in vitro* susceptibility results and clinical responses in patients with MAC lung disease [20, 21]. The isolation of macrolide-resistant MAC is associated with poor treatment outcomes and increased mortality [22, 23]. The minimal inhibitory concentration (MIC) is used to evaluate differences in antimicrobial susceptibility, though MIC breakpoints for many antimicrobials are not well established. Thus, it is critical to monitor the level of resistance among MAC isolates to multiple antimicrobials. However, data on the differences in antimicrobial susceptibility profiles among MAC isolates remain limited. In this study, we aimed to evaluate the differences in clinical characteristics and antimicrobial susceptibility profiles of MAC isolates from patients with pulmonary infections and to provide epidemiologic data with MIC distributions.

METHODS

Isolate collection, identification, and ethics approval

MAC isolates (287 isolates) were collected from Shanghai Pulmonary Hospital between January 2019 and May 2020. The demographic and clinical characteristics of the patients were obtained from their medical records. The NTM isolates were grown on either liquid medium (BACTEC MGIT 960) and solid medium (Löwenstein–Jensen). All the isolates were identified as NTM by the conventional method with para nitrobenzoic acid (PNB) and thiophene-2-carboxylic acid hydrazide (TCH) in solid media [24]. Additionally, all isolates were identified using a commercially available line probe assay (ZEESAN, Xiamen, China). Ethical approval for acquiring the patient information from the medical records and for obtaining and assessing the NTM isolates was granted by the ethical committee of Shanghai Pulmonary Hospital, which is affiliated with Tongji University School of Medicine. Informed consent was obtained from all patients involved.

Table 2. Clinical characteristics of 297 patients with *Mycobacterium avium* or *Mycobacterium intracellulare* lung disease

Characteristic	*M. avium* (*n*=45)	*M. intracellulare* (*n*=242)	*P*
Male	16 (35.56%)	100 (41.32%)	
Female	29 (64.44%)	142 (58.68%)	0.469
Age (years)			
≤40	6 (13.33%)	19 (7.85%)	0.231
41–50	7 (15.56%)	28 (11.57%)	0.453
51–60	13 (28.89%)	52 (21.49%)	0.276
>60	19 (42.225)	143 (59.09%)	0.036
Symptoms			
Fever	4 (8.89%)	52 (21.49%)	0.050
Cough	30 (66.67%)	196 (80.99%)	0.031
Sputum	24 (53.33)	168 (69.42%)	0.035
Hemoptysis	10 (22.22%)	66 (27.27%)	0.481
Chest pain	3 (6.67%)	15 (6.20%)	0.906
Comorbidities			
COPD	6 (13.33%)	4 0(16.53%)	0.592
Bronchiectasis	28 (62.22%)	127 (52.48%)	0.229
Radiographic findings			
Bronchiectatis	21 (46.67%)	84 (34.71%)	0.126
Nodules	34 (75.56%)	178 (73.55%)	0.779
Cavitation	8 (17.78%)	81 (33.47%)	0.037
Fibrosis	25 (55.56%)	145 (59.92%)	0.585
Pulmonary shadow	38 (84.45)	203 (83.88%)	0.924
Treatment success	7 (15.56%)	38 (15.70)	0.975

COPD, chronic obstructive pulmonary disease.

Antimicrobial susceptibility profiles of clinical MAC isolates

Antimicrobial susceptibility testing was performed using a SLOWMYCO Sensititre MIC Plate (Trek Diagnostic System, Thermo Fisher, USA) with the microdilution method according to Clinical and Laboratory Standards Institute (CLSI) Standard M24-A2 [25]. The *M. avium* and *M. intracellulare* isolates were tested using 13 antimicrobials: rifampicin (RIF), rifabutin (RFB), isoniazid (INH), ethambutol (EMB), ethionamide (ETH), clarithromycin (CLR), moxifloxacin (MXF), ciprofloxacin (CIP), amikacin (AN), streptomycin (S), linezolid (LNZ), sulfamethoxazole (SXT), and doxycycline (DO). The MIC range of each antimicrobial for *M. avium* and *M. intracellulare* are shown in Table 1. In brief, the bacteria in the culture media were transferred to Middlebrook 7H9 Broth (Becton, Dickinson Company, USA) supplemented with 10% (vol/vol) oleic acid–albumin dextrose–catalase (OADC; Thermo, USA). The suspension was then diluted to the density of a 0.5 McFarland standard. Subsequently, 50 µl of the suspension was transferred to 11 ml of 7H9 Broth. Thereafter, the inoculum solution was transferred to the wells (100 µl per well) of a 96-well microtiter plate containing lyophilized antimicrobials. According to the manufacturer's instructions (Thermo Fisher Scientific), the incubation time was a maximum of 8 days at 37 °C. *M. avium* ATCC700898 was used as quality control. The breakpoints of the antimicrobials are listed in Table 1.

Statistical analysis

Statistical analyses were performed using SPSS 24.0 (SPSS Inc. USA). Comparisons were made between cases involving *M. avium* and *M. intracellulare* isolates using the chi-square test. A P value <0.05 (two-tailed) was considered statistically significant. Variables with a P <0.20 in the univariate analysis were introduced into the multivariate analysis.

RESULTS

Demographic and clinical characteristics of patients

In total, 287 MAC isolates were assessed, comprising 45 (15.68%) *M. avium* isolates and 242 (84.32%) *M. intracellulare* isolates. The demographic and clinical characteristics of the patients with *M. avium* and *M. intracellulare* lung diseases are presented in Tables 2 and 3. The median age was 58 years (range, 34–79 years) in patients with *M. avium* infection and 63 years (range, 22–84 years) in those with *M. intracellulare* infection. The percentage of patients aged >60 years was significantly higher among patients with *M. intracellulare* infection compared to those with *M. avium* infection (P=0.036). However, no significant differences in the other demographic characteristics were found between patients with *M. avium* or *M. intracellulare* lung disease.

The percentages of patients with cough and sputum were significantly higher among patients with *M. intracellulare* lung disease rather than *M. avium* lung disease (P=0.031 and P=0.035, respectively). Furthermore, radiological analysis revealed that pulmonary shadow was the dominant pattern in both patients with *M. avium* infection (84.45%) and *M. intracellulare* infection (83.88%), followed by nodular form in both those with *M. avium* infection (75.56%) and *M. intracellulare* infection (73.55%). The cavitary form was found among 33.47% of patients with *M. avium* infection and 17.78% of patients with *M. intracellulare* infection, and the difference was significant (P=0.037). However, there were no significant differences in other symptoms (fever, hemoptysis, and chest pain), other radiographic features, or comorbidities between cases of *M. avium* and *M. intracellulare* infection (P >0.05).

Table 3. Demographic and clinical characteristics of patients with *Mycobacterium avium* or *Mycobacterium intracellulare* lung disease

Characteristic	Univariate analysis			Multivariate analysis		
	HR	95% CI	*P*	HR	95% CI	*P*
Male	1.273	0.656–2.468	0.476			
Female						
Age (years)	0.762	0.565–1.027	0.075	0.728	0.528–1.004	0.053
Symptoms						
Fever	0.355	0.121–1.035	0.058	0.403	0.134–1.212	0.106
Cough	0.495	0.243–1.007	0.052	0.814	0.266–2.493	0.718
Sputum	0.553	0.278–1.020	0.057	0.512	0.183–1.433	0.202
Hemoptysis	0.758	0.355–1.616	0.473			
Chest pain	1.071	0.297–3.864	0.916			
Comorbidities						
COPD	0.928	0.526–1.792				
Bronchiectasis	1.600	0.826–3.098	0.163	2.018	0.926–4.397	0.077
Radiographic findings						
Bronchiectatis	1.635	0.860–3.110	0.134	1.491	0.730–3.045	0.273
Nodules	1.094	0.523–2.289	0.811			
Cavitation	0.427	0.190–0.960	0.039	0.495	0.213–1.149	0.273
Fibrosis	0.828	0.435–1.573	0.563			
Pulmonary shadow	1.016	0.423–1.444	0.971			

Antimicrobial susceptibility profiles

The MIC range of each antimicrobial for the 45 clinical *M. avium* isolates and the 242 *M. intracellulare* isolates along with the breakpoints are shown in Table 1. The MIC distributions of the MAC isolates are shown in Fig. 1) . The results indicate the high diversity regarding antimicrobial resistance among the 13 antimicrobials.

CLR was the most effective antimicrobial against both *M. avium* (88.89%) and *M. intracellulare* (91.32%) isolates, with no significant difference between the species (*P*=0.601). The MIC_{90} values were 32 μg ml^{-1} and 8 μg ml^{-1}, respectively. RFB was also highly active against *M. avium* (84.44%) and *M. intracellulare* (75.44%) isolates, with MIC_{50} values ≤0.25 and 1 μg ml^{-1}, respectively. Among the *M. intracellulare* isolates, 37.60% were susceptible to AN, 30.58% had intermediate susceptibility, and 31.82% were resistant. Among the *M. avium* isolates, 71.11% were susceptible to AN, 17.78% had intermediate susceptibility, and 11.11% were resistant.

The majority of the MAC isolates showed no susceptibility to the other antimicrobials. Almost all the MAC isolates were resistant to CIP and DO. Lower rates of resistance to LNZ, MXF, RIF, and SXT were observed aomng *M. avium* isolates than the *M. intracellulare* isolates, but there were no significant differences between the species. The rate of EMB resistance was significantly lower for *M. intracellulare* (81.82%) than *M. avium* (95.56%; *P*=0.021).

DISCUSSION

MAC species are the most common pathogens associated with NTM lung disease [26, 27]. *M. avium* and *M. intracellulare* infections have been demonstrated to have different sources of environmental exposure [28], various degrees of pathogenicity [29], and even differences in treatment outcomes [12, 30]. MAC treatment is prone to fail due to the intrinsic resistance of MAC isolates and the predisposition of the bacteria to developing acquired resistance during treatment. Therefore, antimicrobial susceptibility testing is essential for the effective treatment of NTM diseases.

In this study, we investigated the antimicrobial susceptibility profiles of clinical *M. avium* and *M. intracellulare* isolates of 13 antimicrobials. The American Thoracic Society recommends that standard therapy for MAC infections should consist of a combination of a macrolide with EMB and RIF. Aminoglycosides are also used as second-line antimicrobials. Consistent with previous studies [15, 31], CLR showed the best *in vitro* activity against MAC isolates among the 13 tested antimicrobials. The MIC_{90} of CLR was

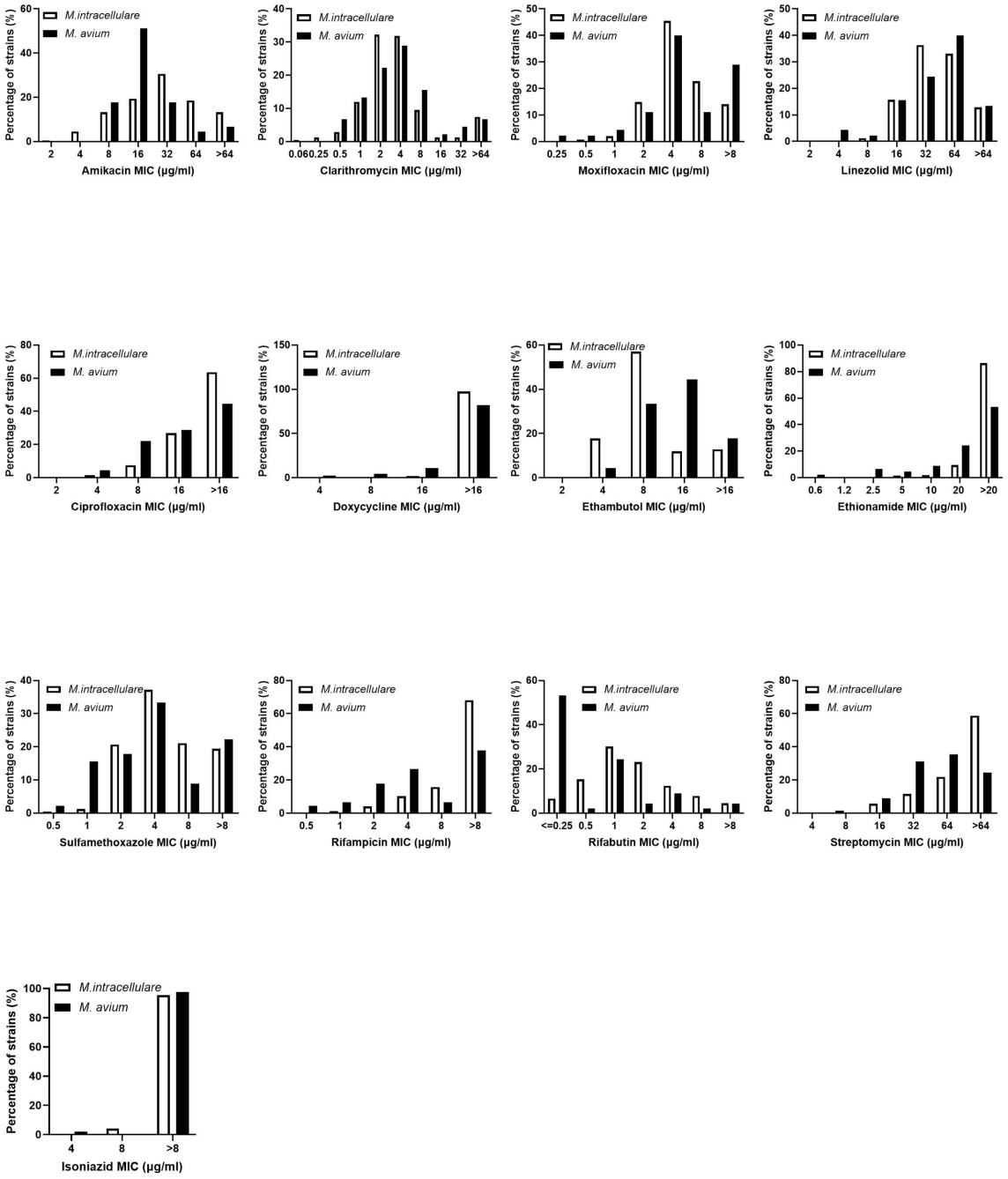

Fig. 1. MIC distributions for *Mycobacterium avium* and *Mycobacterium intracellulare*.

higher for *M. avium* (32 μg ml^{-1}) than for *M. intracellulare* (8 μg ml^{-1}), which was similar to previous results [16]. However, several studies have found that the MIC_{90} of CLR was the same between the two species [15, 32, 33]. In addition, the EMB and RIF resistance rates among the *M. avium* and *M. intracellulare* isolates were high, which may be the cause of the reported poor clinical outcome. We found that *M. intracellulare* was significantly less likely to be resistant to EMB than *M. avium*, which was similar to the findings of Guthertz *et al.* [34]. In contrast to these findings, Zhang *et al.* reported that *M. intracellulare* isolates had a higher EMB resistance rate than *M. avium* isolates [15].

Interestingly, our study showed that RFB was more active than RIF against the two MAC species. The RFB resistance rate was not significantly different between *M. avium* and *M. intracellulare* (P=0.179). Previous studies on RFB resistance reported that the MIC value was truncated towards the lower end of the tested range of antimicrobial concentrations [35, 36]. The MIC_{90} values of RFB for the two species were both 4 μg ml^{-1},

while the MIC_{50} value for *M. avium* (≤0.25 μg ml^{-1}) was more than four times lower than that for *M. intracellulare* (1 μg ml^{-1}), indicating that the RFB resistance rate may be lower for *M. avium*. Furthermore, a pharmacokinetic/pharmacodynamic study on the treatment of MAC lung disease found that RFB increased the serum concentration of macrolides, especially regarding azithromycin, whereas RIF strongly lowered the serum concentration of CLR [11]. Thus, RIF could be replaced with RFB in the treatment of MAC infection.

Most studies have reported low AN resistance rates among both *M. avium* and *M. intracellulare* isolates [15, 16, 32]. However, we observed a significantly higher rate of AN resistance among *M. intracellulare* isolates (31.82%) compared to *M. avium* isolates (11.11%), which may be due to using different methods or breakpoints. Cho *et al.* found that 35.5% (292 of 823) of *M. intracellulare* isolates exhibited intermediate susceptibility to AN, which was similar to our finding (30.58%, 74 of 242) [33]. Whether the CLSI breakpoints should be changed still requires a large amount of further research. Although EMB, RIF, RFB, and S are useful clinically, the breakpoints for determining susceptibility and resistance have not been established. Due to a lack of data on the breakpoints for most antimicrobials, more studies on the MIC distribution of each antimicrobial should be performed.

The CLSI Standard M24-A2 suggests tentative breakpoints for MXF and LNZ. In previous studies, MXF resistance was found in 10.8–49.7% of *M. avium* strains [15, 33, 37] and 1.6–64.0% of *M. intracellulare* strains [15, 33, 38]. However, we found high rates of MXF resistance (80.00% of the 45 *M. avium* isolates and 82.23% of the 242 *M. intracellulare* isolates) and high MIC_{50} and MIC_{90} values (4 μg ml^{-1} and >8 μg ml^{-1}, respectively, for both *M. avium* and *M. intracellulare*). The MIC distribution of MXF is similar to that reported in a German study [35]. Zhao *et al.* observed that infections involving clustered *M. intracellulare* strains were significantly associated with MXF resistance, which may be related to the pathogenicity and host preference of *M. intracellulare* [38]. Our results provide information regarding the candidate antimicrobials to use against *M. avium* and *M. intracellulare*. However, there is an urgent need for further comprehensive research on the antimicrobial susceptibility profiles of MAC species to establish optimal treatment regimens.

Prince *et al.* showed that MAC infection was more likely to occur in the older population groups [39]. Han *et al.* found that *M. intracellulare* was more pathogenic and tends to infect postmenopausal women (aged ≥50 years) [40]. We found that the percentage of patients aged >60 years was significantly higher among patients with *M. intracellulare* infection than *M. avium* infection, while the gender distribution of the infected patients was no different between the two species.

Zhang *et al.* found a strong association between chronic obstructive pulmonary disease (COPD) and *M. intracellulare* infection [15]. Also, Prevots *et al.* found that bronchiectasis and COPD, associated with immunosuppression, increase the risk of colonization and infection by *M. avium* [9]. However, we found no associations between COPD and the MAC species. The reasons may be that our study had a retrospective design and was conducted at a single facility, and selection bias might have occurred due to the characteristics of the patients visiting our hospital.

Consistent with the indications of *M. abscessus* lung disease, the presence of cavitary lesions has been reported to be an independent factor related to treatment failure in MAC lung disease [41]. We found that cavitary lesions were more strongly associated with *M. intracellulare* infection than *M. avium* infection. The initiation of aggressive, guideline-recommended treatments should be considered before the disease progresses to cavitary lesions. Furthermore, patients with cavitary lesions might need to be considered for surgical resection [41]. A study of 100 randomly selected patients with bronchiectasis on chest computed tomography (CT) scans reported that the constellation of bronchiectasis plus peripheral parenchymal nodules was 80% sensitive and 87% specific for the diagnosis of MAC pulmonary disease.

In conclusion, our data demonstrated differences in antimicrobial susceptibility profiles between *M. avium* and *M. intracellulare*, with different MIC distributions for the various antimicrobials between the two species. CLR, AN, and RFB exhibited strong antimicrobial activity against both MAC species. However, *M. intracellulare* was more resistant to AN and RFB, and *M. avium* was more resistant to EMB. In addition, the percentages of patients aged >60 years and patients with sputum, cough, and cavitation were significantly higher among patients with *M. avium* than among patients with *M. intracellulare*. Pulmonary disease caused by distinct MAC species had different symptoms and radiographic findings. Monitoring the local prevalence and antimicrobial susceptibility of these species among patients with MAC pulmonary disease is crucial to promote efficacious treatment.

Availability of data and materials

The datasets used during the current study are available from the corresponding author upon reasonable request.

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Author contributions
W. W., J. Y., X. W., B. W. and H. W., designed the work and analysed and interpreted data for the work. F. Y. and Y. G., drafted the work and revised it critically for important intellectual content. F. Y., provided approval for publication of the content. W. W., J. Y. and B. W., participated in the experimental design and data analysis. F. Y., agreed to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. All authors read and approved the final manuscript.

Conflicts of interest
The authors declare that there are no conflicts of interest.

Ethical statement

The Shanghai Pulmonary Hospital Affiliated to Tongji University School of Medicine Ethics Committee approved the research protocols. The informed consent that was both written and informed was obtained from each patient who was treated in accordance with the Helsinki Declaration on the participation of human subjects in medical research.

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