Antimicrobial activity of bioactive compounds from plants against a multi-resistant *Mycobacterium abscessus* clinical isolate

Emanuela Marini[1], Gloria Magi[1], Maria Enrica Cimarelli[2], Claudio Piersimoni[3], Bruna Facinelli[1]

[1]Unit of Microbiology, Department of Biomedical Sciences and Public Health, Polytechnic University of Marche, Ancona, Italy
[2]Department of Pneumology, Carlo Urbani Hospital, Jesi (AN), Italy
[3]Regional Reference Mycobacteria Laboratory, Clinical Pathology Laboratory, United Hospitals, Ancona, Italy

INTRODUCTION

The *Mycobacterium abscessus* complex (MABC) includes rapidly growing mycobacteria, ubiquitously present in water and decaying vegetation, and is the second most common cause of nontuberculous mycobacteria lung disease after the *M. avium* complex. [1, 2]. The taxonomy of MABC is complex. Recently, whole genome sequencing data analysis supported the differentiation into three subspecies, i.e. *abscessus, bolletii*, and *massiliense* (**Figure 1**). They infect macrophages of the lungs and skin causing serious lung infections in patients with chronic pulmonary diseases, e.g. cystic fibrosis or bronchiectasis, and are also associated with infections following surgical interventions or tattooing; nosocomial outbreaks have been also described in connection with automated endoscope washers and in hospital water supplies [3].

M. abscessus subsp. *abscessus* (*M. abscessus*) is now recognized as an emerging human pathogen [2, 4]. *M. abscessus* exists as two variants, rough and smooth, rough *M. abscessus* being more virulent than smooth *M. abscessus* [5]. Initial combination therapy with three agents (amikacin, cephoxitin, and clarithromycin) is recommended for severe infections, including pulmonary disease.

The major threat posed by *M. abscessus* is mainly due to its resistance to antibiotics [6]. Intrinsic resistance is attributed to a combination of the permeability barrier of the complex multilayer cell envelope, drug export systems, antibiotic targets with low affinity and enzymes that neutralize antibiotics in the cytoplasm, and the ability to form biofilm; moreover, antibiotics need to penetrate the macrophage reservoir of the organism. To date, acquired resistance has only been observed for aminoglycosides and macrolides, which is conferred by mutations affecting the genes encoding the antibiotic targets. The intrinsic and acquired resistance of *M. abscessus* to commonly used antibiotics limits the chemotherapeutic options for infections caused by these mycobacteria [1]. In consequence, *M. abscessus* infection is a chronic, incurable infection for most patients [7].

The increasing incidence of drug-resistant pathogens, coupled with reduced development of new agents, has led to fears of a "post-antibiotic era" and stimulated the search for inhibitory compounds from plants [8, 9].

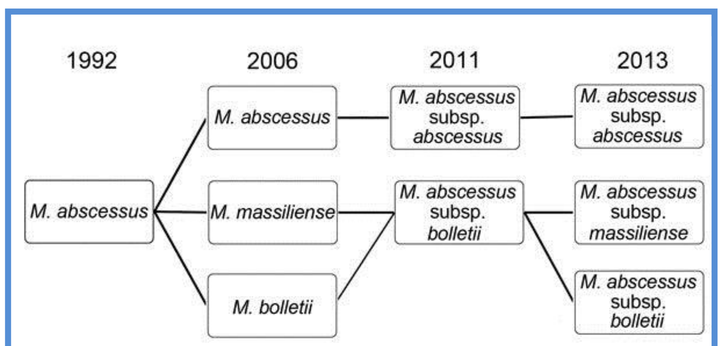

Figure 1. Serial changes in the nomenclature and taxonomic classification of *Mycobacterium abscessus* complex, 1992–2013.

Plants are the basis of both ancient folk remedies and modern pharmacopeia since they produce a variety of secondary metabolites, mostly phenols and terpenes, which possess a wide spectrum of biological activities, including antibacterial effects. A major group of plant antimicrobial compounds is represented by essential oils (EOs), which are complex mixtures of volatile secondary metabolites. Moreover, some plant compounds, at low concentrations, can act in synergy with antibiotics, restoring the antibiotic effectiveness against resistant pathogens [10].

The aim of the present study was to investigate the antimicrobial activity and the synergy with antibiotics of bioactive compounds from plants against a multiresistant *M. abscessus* lung isolate.

MATERIALS AND METHODS

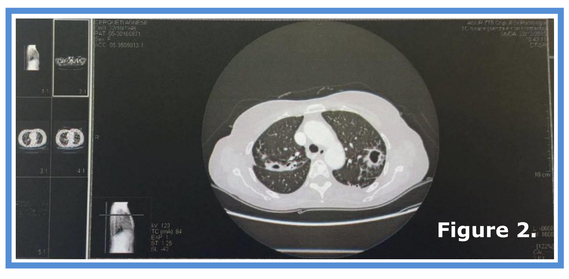

Figure 2.

Case report. In 2012, a 66-year old woman with a long history of pulmonary disease and suffering of a persistent cough and mild hemoptysis was admitted at the Department of Pneumology of Jesi (An) for suspected tubercolosis. The patient was 165 cm tall, weighed 48 kg and she was a non-smoker. Chest TAC revealed the presence of cavities of both upper lung lobes and numerous bilateral bronchiectasis (**Figure 2**). A rapidly growing mycobacterium (strain 29904) was isolated at the Regional Reference Mycobacteria Laboratory, Clinical Pathology Laboratory of Ancona Regional Hospital and identified as *M. abscessus* subsp. *ascessus* by a line-probe reverse hybridization assay (GenoType CM, Hain Lifescience, Nehren, Germany) and by conventional biochemical and cultural tests [11]. **Figure 3** shows colony morphology of *M. abscessus* strain 29904.

Antimicrobials and susceptibility tests. Antibiotics, EOs, and bioactive plant compounds were used (**Table 1**). MICs were determined by a broth microdilution method, as recommended by CLSI guidelines [12]. Inoculated microdilution plates were covered with adhesive seals and incubated at 30°C for 4/5 days before growth was assessed using a rapid p-iodonitrotetrazolium chloride (INT) colorimetric assay [13].

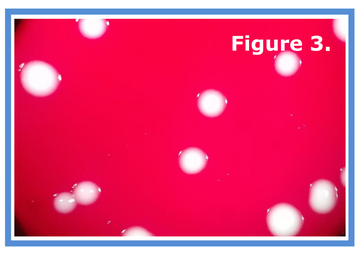

Figure 3.

Checkerboard test. Synergy with antibiotics was evaluated by the checkerboard assay and by calculating the Fractional Inhibitory Concentration Index (FICI). FICI values were interpreted accordingly to Odds [14]: synergy (FICI ≤0.5), antagonism (FICI >4.0), and no interaction (FICI >0.5–4.0). Test results were also represented by isobolograms constructed by plotting synergistic concentrations of curcumin and antibiotics [15].

RESULTS

M. abscessus strain 29904 was tested for susceptibility to seven antibiotics, 12 EOs and six bioactive plant compounds (**Table 1**).

It was found to be resistant to ciprofloxacin (MIC 8-16 µg/mL), sulfamethoxazole (MIC >128 µg/mL), meropenem (MIC 32 µg/mL), and clarithromycin (MIC 64-128 µg/mL); intermediate to amikacin (MIC 32 µg/mL) and cefoxitin (MIC 32 µg/mL); MIC of tigecycline was 8 µg/mL, but there are no established breakpoints for mycobacteria.

The MICs of EOs in broth ranged from 32 to 128 µg/mL (**Table 1**), whereas those in agar were higher (data not shown), probably for an inhibitory effect of the vapors during the five days of incubation in the sealed microtiter plates. Other MICs ranged from 128 to >2048 µg/mL (**Table 1**), curcumin and carvacrol exhibiting the lowest MICs (128 µg/mL and 256 µg/mL, respectively). Curcumin, the most active compound, was used in synergy experiments.

In the checkerboard assay, different combinations of curcumin with antibiotics (amikacin, clarithromycin, ciprofloxacin, cefoxitin, and meropenem), ranging from several dilutions below the MIC to twice the MIC, were tested. FICI values were calculated by considering all combinations of curcumin and antibiotics in which there was no visible growth (**Table 2**).

Table 1. Susceptibility (MIC, µg/mL) of *M. abscessus* strain 29904 to antibiotics, EOs and plant compounds.

Antibiotics	MIC	Essential oils	MIC	Plant compounds	MIC
Ciprofloxacin	8-16 (R)	*Allium sativum*	32	α-pinene	>2048
Sulfamethoxazole	>128 (R)	*Cannabis sativa**	64	β-pinene	>2048
Meropenem	32 (R)	*Cymbopogon citratus*	128	Capsaicin	>2048
Clarithromycin	64-128(R)	*Helichrysum italicum**	64	Curcumin	128
Tigecycline	8	*Lavandula angustifolia*	64	Carvacrol	256
Amikacin	32 (I)	*Melaleuca alternifolia*	128	Thymol	512
Cefoxitin	32 (I)	*Mentha piperita*	64		
		Origanum vulgare	64		
		Rosmarinus officinalis	64		
		Salvia officinalis	64		
		*Satureja montana**	64		
		Thymus vulgaris	64		

All purchased from Sigma-Aldrich except *, kindly provided by APPO (Associazione Produttori Piante Officinali, Regione Marche)

FICI values of synergistic combinations (ranging from 0.19 to 0.5) between curcumin and amikacin, clarithromycin, and ciprofloxacin (**Table 2**) were detected; antagonism was never observed. Isobolographic analysis confirmed a synergistic effect against strains with FIC Index ≤0.5 (**Figure 4**) and no interaction against those with FIC Index >0.5 (data not shown).

Table 2. Synergic combinations of curcumin with antibiotics against *M. abscessus* strain 29904.

Combination curcumin/antibiotic	Best combination (µg/mL)	FIC	FIC Index	Synergy
Curcumin/Amikacin	16/2	0.1250/0.0625	0.1875	Yes
Curcumin/ Ciprofloxacin	32/1	0.2500/0.1250	0.3750	Yes
Curcumin/ Clarithromycin	16/16	0.1250/0.1250	0.2500	Yes
Curcumin/Cefoxitin	64/0.25	0.5000/0.0078	0.5078	No
Curcumin/Meropenem	64/0.25	0.5000/0.0019	0.5019	No

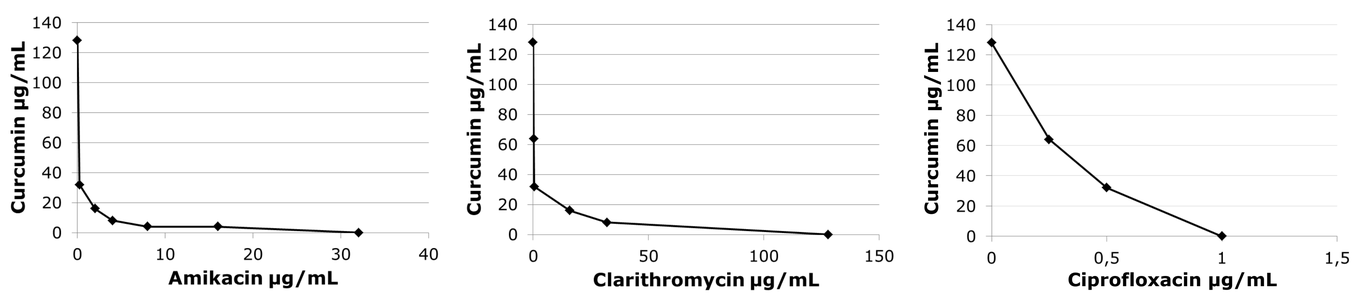

Figure 4. Isobolograms of synergistic combinations between Curcumin and Amikacin/Clarithromycin/Ciprofloxacin.

CONCLUSIONS

- This study represents the first evaluation of the activity of bioactive compounds from plants against *M. abscessus*.
- *M. abscessus* strain 29904, a multiresistant clinical isolate, showed an high susceptibility to all tested EOs and to carvacrol and curcumin. Noticeable, curcumin exhibits antimicrobial activity against a wide range of microorganisms [16], including *M. tubercolosis* [17].
- Remarkably, curcumin demonstrated a synergistic effect with amikacin, clarithromycin, and ciprofloxacin, suggesting a possible re-use of antibiotics against antibiotic-resistant *M. abscessus*. No synergistic interactions occurred between curcumin and meropenem or cephoxitin, as previously reported for rifampicin against *M. tubercolosis* [17].
- Curcumin, a natural component of the rhizome of *Curcuma longa* used in traditional medicine, is pharmacologically safe and has antitumor, anti-inflammatory, and antioxidant effects.
- Further evaluation is required to determine whether the present findings, in particular the synergy of curcumin with antibiotics, can be exploited in treating *M. abscessus* infections.

REFERENCES

1. **El Helou G**, et al. Rapidly growing mycobacterial bloodstream infections. 2013. *Lancet Infect Dis.* 13(2):166-74.
2. **Medjahed H**, et al. *Mycobacterium abscessus*: a new player in the mycobacterial field. 2010. *Trends Microbiol.* 18(3):117-23.
3. **Meng-Rui Lee**, et al. Mycobacterium abscessus Complex Infections in Humans. 2015. *Emerg Infect Dis.* 21(9): 1638–46.
4. **Hauser AR**, et al. Clinical significance of microbial infection and adaptation in cystic fibrosis. 2011. *Clin Microbiol Rev.* 24(1):29-70.
5. **Bernut A**, et al. *Mycobacterium abscessus* cording prevents phagocytosis and promotes abscess formation. 2014. *Proc Natl Acad Sci*. Mar 11; 111(10).
6. **Nessar R**, et al. *Mycobacterium abscessus*: a new antibiotic nightmare. 2012. *J Antimicrob Chemother.* 67(4):810-8.
7. **Griffith DE**, et al. An official ATS/IDSA statement: diagnosis, treatment, and prevention of nontuberculous mycobacterial diseases. 2007. *Am J Respir Crit Care Med*. 175: 367–416.
8. **Cowan MM.** Plant products as antimicrobial agents. 1999. *Clin Microbiol Rev* 12:564-82.
9. **Savoia D.** Plant-derived antimicrobial compounds: alternatives to antibiotics. 2012. *Future Microbiol.* 7(8):979-90.
10. **Hemaiswarya S**, et al. Synergism between natural products and antibiotics against infectious diseases. 2008. *Phytomedicine* 15:639–52.
11. **CLSI**. Laboratory detection and identification of mycobacteria; proposed guideline. 2007. M48-P. C.L.S.I., Forbes, B.A.
12. **CLSI**. Susceptibility testing of mycobacteria, nocardiae, and other aerobic actinomycetes; approved standard, 2nd ed. 2011. M24–A2. C.L.S.I., Wayne, PA.
13. **Eloff JN**. A sensitive and quick microplate method to determine the minimal inhibitory concentration of plant extracts for bacteria. 1998. *Planta Med.* 64(8):711-3.
14. **Odds FC.** Synergy, antagonism, and what the checkerboard puts between them. 2003. *J Antimicrob Chemother*. 52(1):1.
15. **Mulyaningsih S**, et al. Synergistic properties of the terpenoids aromadendrene and 1,8-cineole from the essential oil of *Eucalyptus globulus* against antibiotic-susceptible and antibiotic-resistant pathogens. 2010. *Phytomedicine* 17, 1061–66.
16. **Tyagi P**, et al. Bactericidal activity of curcumin I is associated with damaging of bacterial membrane. 2015. *PLoS One.* 10(3): e0121313.
17. **Baldwin PR**, et al. Monocarbonyl analogs of curcumin inhibit growth of antibiotic sensitive and resistant strains of *M. tuberculosis*. 2015. *Eur J Med Chem.* 6;92:693-9.