Interaction of South Asian Spices with Conventional Antibiotics: Implications for Antimicrobial Resistance for Mycobacterium abscessus and Cystic Fibrosis

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Abstract

Background: Antimicrobial resistance (AMR) has rendered certain species of Mycobacterium difficult to treat clinically, particularly, the nontuberculous Mycobacterium, Mycobacterium abscessus. This bacterium is emerging in specific disease populations, including amongst cystic fibrosis (CF) patients, where AMR represent a true treatment dilemma. Therefore, any innovation with traditional antimicrobial compounds in spices, which increases the potency of existing conventional antibiotics should be investigated. Methods: M. abscessus isolates (n=9 multidrug-resistant clinical isolates from CF patients + 1 Reference Strain) were examined for their direct susceptibility to 27 spices, as well as the interactive effect of this spice combination to their susceptibility to amikacin and linezolid antibiotic, with standard disk diffusion assay. Results: Five isolates of M. abscessus (5/10; 50%) failed to grow on the spice enriched medium, which included four clinical isolates and the National Culture Type Collection (NCTC) Reference Strain. Of the remaining five isolates which grew on the spice medium, no cultural phenotypic differences were observed, compared to unsupplemented controls. In the case of both amikacin and linezolid, the zone of inhibition increased with the inclusion of the spices. Initially, all isolates of M. abscessus were fully resistant to linezolid (mean zone of inhibition = 0 mm), and growth was to the edge of the antibiotic disk, whereas when in the presence of spices, large zones of inhibition were observed (mean zone of inhibition = 33.3 mm). With amikacin, the mean zone of inhibition increased from 23.2 mm to 32.0 mm, in the presence of spices. Conclusion: These data suggest that the spices were interacting synergistically with the antibiotics, thus making the antibiotic more potent against the bacteria tested. This study is significant as it demonstrates a positive interaction between spices and the conventional antimycobacterial antibiotics, amikacin, and linezolid. Given the burden of AMR to M. abscessus, particularly in a patient with chronic disease such as CF, any food-related innovation that can help maximize the potency of existing antimycobacterial antibiotics is to be encouraged and developed. The specific mechanism as to how spices increase the potency of such antibiotics with M. abscessus needs to be elucidated, as well as novel food (spice) delivery modalities developed, including novel medicinal foodstuffs or functional foods, that can harness this beneficial effect in vivo to medicine and society.

Keywords: Antimicrobial resistance, cystic fibrosis, infection, Mycobacterium abscessus, nontuberculous Mycobacteria

Introduction

Historically, spices have been used around the world to flavor, create aroma, color, and preserve foodstuffs. The antimicrobial properties of spices have been well documented in the published scientific/medical literature, as recently reviewed in the seminal review by Liu et al.¹¹

The long-term use of several classes of antibiotic agents, including aminoglycosides, beta-lactams, fluoroquinolones, macrolides, and polymyxin, for the prophylaxis, maintenance, and treatment of bacterial respiratory pathogens causing chronic chest infections in patients with cystic fibrosis (CF) has important consequences for the persistence of respiratory pathogens,

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including the emerging nontuberculous Mycobacteria (NTM), in particular, *Mycobacterium abscessus*. *M. abscessus* infection is CF has gradually emerged from early reports of its prevalence of 1.3% in 1984\cite{2} to 32.7% in adult CF patients in the US,\cite{3} although prevalence rates from the US CF Foundation Registry are 12%.\cite{4} This infection is associated with chronic persistence of the organism in the lower airways, once colonization has occurred, leading to increased morbidity and mortality in some patients.\cite{5} Furthermore, the presence of this organism in patients’ sputum is usually a contraindication for lung transplantation. Hence, it is important to be have efficacious antibiotics available to attempt to eradicate the organism on the first isolation from sputum, as well as to chronically manage the organism, particularly with acute pulmonary exacerbation. One worrying microbiological characteristic to emerge from studies to date is the relative antibiotic resistance of this organism, where there is usually a high degree of antibiotic resistance to several classes of antibiotics.\cite{6}

While there are several reports on the antibacterial activity of spices on other species of *Mycobacterium*, particularly *M. tuberculosis*\cite{7,8}, there have been no reports on the activity of spices on *M. abscessus*. Therefore, it was the aim of the current study to explore potential antimycobacterial activity of South Asian spices, by examining: (i) a direct antibacterial effect of spices on growth and proliferation of *M. abscessus* isolates and (ii) to investigate if the presence of a wide variety of spices alter antimicrobial activity of two commonly used antibiotics (amikacin and linezolid), in the treatment of *M. abscessus* pulmonary infection.

**Methods**

**Spices employed in this study**

Twenty-seven spices were examined in this study, as detailed in Table 1. All spices were purchased from retail markets in the UAE, namely at Al Ain and Dubai, between July 2016 and July 2017. Spices were individually collected in sealed plastic food bags and were transported to the laboratory for microbiological examination at ambient temperature.

**Microbiological investigations**

*M. abscessus* isolates (*n = 10*) were obtained from the HSC Microbiology Culture Repository, MicroARK (www.microark.com), housed at the Northern Ireland Public Health Laboratory, at Belfast City Hospital. These isolates consisted of nine clinical isolates obtained from patients with CF and one Reference Strain (*Mycobacterium abscessus* National Culture Type Collection [NCTC] 13031) obtained from the Public Health England (Colindale, London, UK). All isolates had been historically stored on slopes of Lowenstein-Jensen medium in glass universal containers at ambient temperature. All isolates were recovered and passaged twice on Columbia agar base (Oxoid CM0331; Oxoid Ltd., Basingstoke, UK) supplemented with 5% (v/v) defibrinated horse blood, which was incubated at 37°C for 5 days, before employment in the current study. A fresh culture of each isolate was prepared as described above and was harvested into 0.1% (w/v) peptone saline (CM0733) to yield a 0.5McFarland inoculation standard, for subsequent experiments.

**Development of Spice Agar Medium**

Spices (*n = 27*) were employed in this experiment, as detailed in Table 1. Approximately 0.2 g of each spice was weighed and added to 30 ml absolute ethanol (Sigma Aldrich Ltd., UK) and allowed to infuse overnight at room temperature. Following this, the absolute ethanol containing the combined spices was added to 1 L of Mueller Hinton agar (MHA) (CM0337) and was sterilized by autoclaving, prior to the pouring of MHA plates containing *circa* 0.02% (w/v) of each spice, to produce Spice Agar Medium (SAM). Unsupplemented MHA plates were also prepared as controls.

**Growth of Mycobacterium abscessus isolates on Spice Agar Medium**

*M. abscessus* isolates, as detailed above, were inoculated on SAM plates, as well as onto unsupplemented MHA plates (control) and were incubated as detailed above. The following culture parameters were examined as follows: (i) bacterial colony growth and proliferation, (ii) inhibitory effect of SAM medium, (iii) pigment production, and (iv) smooth to rough colonial conversion.

**Interference with antibiotic susceptibility**

An experiment was designed to investigate the effect of spices on antibiotic susceptibility on *M. abscessus* isolates, as detailed

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**Table 1: Spices collected and employed in this study**

| Spices                  |
|------------------------|
| BBQ: Barbecue          |
| Baharath powder         |
| BBQ spices              |
| Biryani powder          |
| Cardamom powder         |
| Chili powder            |
| Cinnamon powder         |
| Coriander powder        |
| Cumin powder gyma       |
| Fish masala powder      |
| Garam masala powder     |
| Garlic powder           |
| Ginger powder           |
| Harr spices             |
| Kabsch spices           |
| Kashmiri chili powder   |
| Lemon powder            |
| Madras curry powder     |
| Mandi spices            |
| Meat masala             |
| Nutmeg powder           |
| Paprika powder          |
| Pepper powder black     |
| Kofa spices             |
| Tandoori spices         |
| Tikka spices            |
| White pepper powder     |
| Zaata (lebanon)         |

BBQ: Barbecue
The most significant finding of the current study was the interaction between spices and antibiotic susceptibility with M. abscessus. Antimicrobial resistance (AMR) has become a global public health problem which now attracts the attention of major political bodies, including the G7 nations. AMR is particularly problematic in several countries in South Asia, including India, Pakistan, Bangladesh, and Sri Lanka, countries which are major spice producers and consumers/users. The current in vitro study showed that in the presence of a combination of 27 spices at low concentration (circa. 0.02% [w/v]; 200 ppm), antibiotic susceptibility increased with two major antibiotics, commonly used to treat NTM infection in patients with CF, namely amikacin (aminoglycoside) and linezolid (oxazolidone). Our studies demonstrated that the spices did not directly inhibit the growth of all the M. abscessus, but were able to interact synergistically with the two antibiotics tested. At this stage, we do not have any evidence to indicate that this also occurs in the in vivo scenario in humans, but given the escalating problems associated with global AMR; this finding is of interest and warrants further investigation.

The consequences of such synergistic behaviour with the antibiotics are several folds, including (i) the lowering of minimum inhibitory concentrations of established pathogens against conventional antibiotics, effecting slowing down or indeed reversing the effects of antibiotic resistance development and (ii) reducing the need for employment of expensive new antibiotics and supporting the proper use of existing generic antibiotics, which are both widely available and cheap.

**Conclusions**

This study is significant as it demonstrates a positive interaction between spices and the conventional antimycobacterial antibiotics, amikacin, and linezolid. Given the burden of AMR to M. abscessus, particularly in a patient with chronic disease such as CF, any food-related innovation that can help maximize the potency of existing antimycobacterial antibiotics is to be encouraged and developed. The specific mechanism as to how spices increase the potency of such antibiotics needs to be elucidated, as well as novel food (spice) delivery modalities, developed, including novel medicinal foodstuffs or functional foods, that can harness this beneficial effect in vivo to medicine and society.

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Nil.

**Conflicts of interest**

There are no conflicts of interest.

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