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The antimicrobial susceptibility of non-tuberculous mycobacteria

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Summary *Objectives:* Pulmonary non-tuberculous mycobacterial infection (NTM) is a challenging and increasingly prevalent infection. Antimicrobial resistance is common and may be associated with poor outcomes. This retrospective study aimed to report longitudinal trends in mycobacterial isolation and NTM drug susceptibility.
Methods: Mycobacterial culture and drug sensitivity testing results were obtained over a 13 year period. Drug sensitivity testing was performed by broth macrodilution for slow-growing mycobacteria and disc diffusion for rapidly growing mycobacteria.
Results: Culture results were obtained from 109,311 samples (31,758 subjects) of which 5960 samples (1209 subjects) isolated NTM over 13 years. Drug susceptibility results were obtained for 2637 NTM isolates (898 subjects). NTM isolation increased over time, driven by the *Mycobacterium avium* complex and *Mycobacterium abscessus*. Amongst most species resistance to the key agents clarithromycin and amikacin was rare. The highest rate of resistance was found in *M. abscessus* and *Mycobacterium simiae*. Most *M. abscessus* isolates were sensitive to macrolides, aminoglycosides and tigecycline; *M. simiae* isolates were only consistently sensitive to clofazimine, amikacin and cycloserine.
Conclusions: NTM isolation is increasingly common in our centre. Reassuringly, resistance to clarithromycin and amikacin is rare in most species. Tigecycline, cycloserine and clofazimine may be useful in the treatment of the most resistant species, *M. abscessus* and *M. simiae*.

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Introduction

Non-tuberculous mycobacteria (NTM) are ubiquitous organisms found in the environment throughout the world.[1] They may cause infection in humans at any bodily site, with pulmonary disease being the most common. Affected individuals often have an underlying respiratory disease, such as chronic obstructive pulmonary disease or bronchiectasis, but infection may also develop in seemingly healthy persons.[2] The prevalence of pulmonary NTM isolation has been shown to be increasing in many countries including the UK,[3–5] however NTM are frequent contaminants and isolation does not necessarily equate to disease. Treatment may be prolonged and cure is not always possible. Resistance to antimycobacterial drugs is common, however the correlation between *in vitro* sensitivity and *in vivo* treatment outcomes for some drugs and NTM species has been observed to be poor[6,7] and whilst their role in guiding treatment remains under debate[6,8] resistance to certain agents such as macrolides and the *Mycobacterium avium* complex (MAC) is associated with a poor outcome.[9–12] The aims of this study were to describe the longitudinal trends in NTM isolation in a UK tertiary referral centre and to report the patterns of drug sensitivity testing (DST) for key species.

Materials and methods

Data collection

Mycobacterial culture and drug sensitivity testing (DST) results were obtained from the electronic results system from the Microbiology department of the Royal Brompton and Harefield NHS Foundation Trust, London, UK (RBHT). Data was obtained for all samples received between January 2000 and June 2014. DST results were excluded for samples received from April to May 2011 and September and December 2012 as during these periods the laboratory was closed for refurbishment and samples were tested externally. For the calculation of NTM prevalence, activity data for RBHT (excluding the departments of cardiology and cardiac surgery which are unlikely to have contributed any specimens) was available from 2010 onwards from our administrative database.

Mycobacterial culture and identification

Clinical samples were prepared according to a standard protocol and inoculated into BACTEC 960 mycobacterial growth indicator tubes (MGIT) and Lowenstein-Jensen slopes for mycobacterial culture. Liquid cultures were kept for 42 days and solid media for 8–12 weeks. Mycobacteria were identified using biochemical and phenotypic tests and Accuprobe (Gen-Probe, San Diego, CA, USA) DNA probes. For species not identified by Accuprobe, from 2004 the GenoType Mycobacterium line probe assay was used and from April 2005 the GenoType Mycobacterium CM/AS assays were used (Hain Lifescience, Nehren, Germany). Species which could not be identified by either method were sent for external identification at the Public Health England National Mycobacterium Reference Laboratory.

Drug sensitivity testing

For slow-growing mycobacteria (SGM) DST was performed using the broth macrodilution method. Until March 2011 the BACTEC 460 system was used with 12 antibiotics at a single concentration: streptomycin (2 μg/ml), isoniazid (2 μg/ml), rifampicin (2 μg/ml), ethambutol (10 μg/ml until November 2006, 5 μg/ml thereafter), capreomycin (10 μg/ml), cycloserine (80 μg/ml), ethionamide (10 μg/ml), rifabutin (2 μg/ml), ciprofloxacin (2 μg/ml), amikacin (25 μg/ml), clofazimine (0.8 μg/ml) and clarithromycin (2 μg/ml). From June 2011 onwards the BACTEC MGIT 960 and BD EpiCenter™ TB eXiST system (Becton Dickinson) was used. For MAC the drugs tested were: amikacin (2.0 μg/ml, 4.0 μg/ml and 8.0 μg/ml), clarithromycin (16 μg/ml, 32 μg/ml and 64 μg/ml), ethambutol (2.0 μg/ml, 4.0 μg/ml and 8.0 μg/ml), rifampicin (0.5 μg/ml, 2.0 μg/ml and 8.0 μg/ml). Rifampicin resistant isolates were also tested against rifabutin (0.12 μg/ml, 0.5 μg/ml and 2.0 μg/ml) and clarithromycin resistant isolates against ciprofloxacin (1.0 μg/ml, 2.0 μg/ml and 4.0 μg/ml). Other SGM were tested against amikacin (32 μg/ml), clarithromycin (16 μg/ml), ethambutol (5.0 μg/ml) and rifampicin (1.0 μg/ml). Rifampicin resistant isolates were also tested against rifabutin (2.0 μg/ml) and clarithromycin resistant isolates against ciprofloxacin (2.0 μg/ml).

For rapidly growing mycobacteria (RGM) sensitivity was tested using the disc diffusion method (based on the British Society for Antimicrobial Chemotherapy (BSAC) disc susceptibility testing method) against the following antibiotics: amikacin 30 μg/ml, azithromycin 15 μg/ml, cefoxitin 30 μg/ml, ciprofloxacin 1 and 5 μg/ml, clarithromycin 15 μg/ml, meropenem 10 μg/ml, tigecycline 15 μg/ml, moxifloxacin 1 μg/ml, fusidic acid 10 μg/ml, kanamycin 25 μg/ml, vancomycin 25 μg/ml, gentamicin 10 μg/ml and linezolid 10 μg/ml.

Data analysis

Raw data were pre-processed using Microsoft Excel 2001 for Mac (Microsoft, Redmond, WA, USA) and then imported into *R* version 3.0.4[13] for further analysis. Plots were created using the *ggplot2* package.[14]

Results

Data were obtained for 109,311 samples from 31,758 subjects. The number of samples received for culture steadily increased over time, from 4722 in 2000 to 9938 in 2013, the most recent year for which there was complete data. Whilst the number of samples culture positive for *Mycobacterium tuberculosis* (MTB) changed little over time, there was an increase in the number of NTM isolates from 137 in 2000 (2.9% of all samples, 73% of all positive cultures) to 759 in 2013 (7.6% of all samples, 92% of all positive cultures). Similarly the number of individual subjects providing samples for mycobacterial culture (Fig. 1) increased from 1753 in 2000 to 4561 in 2013 and whilst the number of individuals culture positive for MTB fell, there was a five-fold increase in the number of individuals culturing NTM, from 62 in 2000 to 301 in 2013.

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