Microbial contamination of liposomal amphotericin B nebuliser devices in lung transplant patients☆

Contaminación microbiana de dispositivos de nebulización de anfotericina B liposomal en pacientes trasplantados pulmonares

Microbiological Procedure number 61 of the *Sociedad Española de Enfermedades Infecciosas y Microbiología Clínica* (SEIMC) [Spanish Society of Infectious Diseases and Clinical Microbiology] entitled "Microbiological methods for monitoring the cleaning, disinfection and sterilisation of medical devices" focuses on reusable medical devices qualified as semi-critical or medium risk; those that come into contact with the mucous membranes without penetrating sterile tissues and which require at least high-level disinfection. With this in mind, we would like to present a study about microbial contamination of liposomal amphotericin B nebulising equipment in lung transplant patients.

Nebulisers are devices for administering a drug through the airways by aerosol delivery. In the context of lung transplantation, home-administered prophylaxis with nebulised amphotericin B is effectively used for the prevention of infection by *Aspergillus* spp.[1] These devices are a potential source of infection in the event of microbial contamination.[2] Very few studies have assessed the incidence of microbial contamination in nebulisers and the clinical repercussions,[3] particularly in lung transplantation.[4] Moreover, there is a lack of consensus on the standardisation of cleaning and disinfection procedures and microbiological monitoring of these devices.[5] The aim of this study is to estimate the prevalence of microbial contamination in amphotericin B nebulising systems used by lung transplant patients, and to assess the impact on the patient's microbiology.

When the prophylaxis was starting, all patients were instructed on how to adequately clean and disinfect the nebuliser (washing with a brush, soap and water and immersion in 1% sodium hypochlorite solution). After the transplant, the patients had visits every month for the first year, every two months in the second year and then every three to four months. Sputum cultures were taken at these visits if the patient expectorated and fibreoptic bronchoscopy was also occasionally performed.

In 2012–2014, the nebulisation equipment of a cohort of lung transplant patients was sampled. We studied 75 cultures from 71 patients; 42 males, with a mean age of 49 (range: 5–64 years), and 29 females, with a mean age of 51 (22–66 years). Samples were taken from the pipette, the reservoir and tubing of the device. The median time between transplantation and collection of the sample was 666 days (range: 68–1470 days). The results of the cultures of respiratory samples collected in the three months before and after the sampling of the nebuliser were also reviewed.

Microbial contamination was detected in 53.3% of the nebulisers (Table 1). The contamination rate was significantly higher in the pipette and the reservoir (50.7%) than in the device tubing (11.5%) ($p < 0.005$). *Pseudomonas aeruginosa* was not isolated in any of the nebulisers.

Despite the microbial findings, the microorganism isolated in the nebuliser only corresponded with the isolate in respiratory samples in one of the 71 patients studied (1.4%). In this case, Proteus mirabilis was isolated in two sputum samples 14 and 24 days respectively after being found in the nebuliser.

Table 1
Microorganisms isolated in the nebulisers.

Nebulising devices studied (75)	Number	Percentage (95% CI)
Negative	35	46.7% (35.1–58.6%)
Positive[a]	40	53.3% (41.4–64.9%)
Non-fermenting Gram-negative bacillus	33	50.8%
Pseudomonas spp. (not *P. aeruginosa*)	19	–
Acinetobacter baumannii	2	–
Acinetobacter spp.	4	–
S. maltophilia	2	–
Other[b]	6	–
Enterobacteria	14	21.5%
Enterobacter spp.	7	–
K. pneumoniae	1	–
P. mirabilis	1	–
S. marcescens	1	–
Other[c]	4	–
Gram-positive flora	11	16.9%
Plasma-coagulase negative	4	–
Staphylococcus	1	–
Streptococcus spp.	2	–
S. aureus Bacillus spp.	4	–
Filamentous fungus (Penicillium)	2	3.1%
Yeast	4	6.2%
Candida spp.	3	–
Rhodotorula	1	–
Atypical mycobacteria (M. mucogenicum)	1	1.5%
Total microorganisms isolated	65	–

[a] On 16 devices the culture was polymicrobial.
[b] *Roseomonas* spp., *Shewanella* spp., *Sphingomonas* spp., *Ochrobactrum* spp.
[c] *Aeromonas* spp., *Pantoea* spp.

There have been few studies similar to the one we present here, where we estimate the prevalence of microbial contamination in amphotericin B nebulising systems used by lung transplant patients. In a 2005 study[4] patient adherence to the cleaning and disinfection protocol was poor, at only 39%. After those findings, patient education on care of the nebulising equipment was intensified.

The aforementioned Microbiological Procedure highlights the magnitude of the problem of nosocomial infection associated with semi-critical devices and the insufficient surveillance to which they are subjected. The procedure also strongly recommends periodic microbiological cultures as part of the quality control of the cleaning and disinfection process. However, this document does not review home-administered nebulised therapy devices in particular clinical contexts, such as cystic fibrosis or lung transplantation, which also require the same considerations.

Despite the limitations of our study, we believe that this work demonstrates the need to standardise appropriate mechanisms for the disinfection of nebuliser devices. We also consider it necessary to protocolise stringent microbiological monitoring of these devices to assess the process; especially in lung transplant and other immunologically compromised patients.

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Discrepancy in the genotypic versus phenotypic testing for resistance to rifampicin in *Mycobacterium tuberculosis*. A case report☆

Discrepancia en la resistencia genotípica versus fenotípica a rifampicina en Mycobacterium tuberculosis. *A propósito de un caso*

Tuberculosis (TB) continues to be a serious worldwide public health problem today. According to the latest global data provided by the World Health Organisation (WHO), in 2016 there were approximately 10.4 million new cases and 1.7 million deaths worldwide from this infectious respiratory disease. Since the discovery of the first drugs, resistant strains began to appear, making it necessary to treat the disease with a combination of tuberculostatic drugs. Strains are considered to be multi-drug resistant (MDR) when resistance is detected to at least isoniazid (H) and rifampicin (R). When such resistance is not identified prior to the start of treatment, patients infected with mono-resistant strains have a high risk of developing additional resistance if they receive standard therapy.[1]

Nucleic acid amplification tests enable rapid identification of the *M. tuberculosis* complex (MTBc) in clinical samples. Xpert® MTB/RIF (Cepheid®) is a system capable of detecting MTBc and resistance to R within 2 h, with a sensitivity of 98% in samples with positive microscopy and 67% with negative microscopy, and with a specificity in both cases of 99%.[2] In 2011, the WHO recommended it as a first diagnostic step in countries with high rates of MDR-TB.[3]

We present the case of a patient diagnosed with pulmonary TB resistant to R according to Xpert® MTB/RIF (Cepheid®), but sensitive by the BD BACTEC™ MGIT™ 960 SIRE phenotypic method.

This was a 50-year-old male with a history of injecting drug use, active smoker, chronic alcoholism, and chronic hepatitis C.

He reported having had a cough for eight months, which was initially dry, but with purulent sputum in the previous few days, although no haemoptysis. He had no pyrexia, constitutional syndrome or apparent contact with individuals with respiratory disease. Chest X-ray showed right apical infiltrate (Fig. 1) and sputum smear microscopy was positive.

Molecular detection of MTBc performed by Xpert® MTB/RIF (Cepheid®) was positive and resistance to R was identified. Culture in BD BACTEC™ MGIT™ 960 liquid medium detected growth after 19 days of incubation. The antibiogram performed by BD BACTEC™ MGIT™ 960 SIRE revealed sensitivity to H, R, streptomycin (S) and ethambutol (E). In view of the discrepancy, the strain was sent to the Mycobacteria Genetics Group at the University of Zaragoza for sequencing of the *rpoB* gene resistance determining region; the L511P mutation was detected, which explained the difference.

As the patient was initially diagnosed with pulmonary TB resistant to R, it was decided to start treatment with the following regimen: two months of quadruple treatment (H + Z + E + levofloxacin) and completing up to 12 months with the first three. Although it was later reported that the mutation that conferred genotypic, but not phenotypic, resistance to R was L511P, the same treatment pattern was maintained, with good progress and negative repeat culture after two months of treatment.

The treatment of TB consists of two phases: intensive and continuation. The aim of the continuation phase is to prevent relapses after treatment is completed. In this phase, R plays a key role, as it is the most effective drug because of its sterilising effect capable of eliminating the persistent bacilli responsible for recurrences. Isolated resistance to R therefore determines the prognosis of MDR-TB.[4]

According to both the Mensa and Sanford antimicrobial therapy guidelines from 2016, in cases of resistance to R, treatment with H, Z and E is recommended for 12 months. In the first two months, levofloxacin or S can be added in patients with

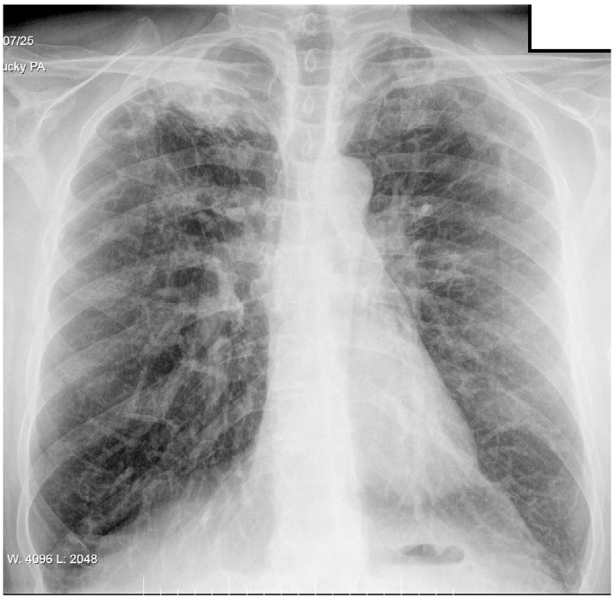

Fig. 1. Anterior/posterior chest X-ray on admission: right apical infiltrate.

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