Journal of Cystic Fibrosis 15 (2016) 74–77

Journal of Cystic Fibrosis

www.elsevier.com/locate/jcf

Original Article

Fungal contamination of nebuliser devices used by people with cystic fibrosis

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Received 15 April 2015; revised 4 June 2015; accepted 10 June 2015
Available online 20 June 2015

Abstract

Background: Poor nebuliser hygiene can result in bacterial contamination and risk of infections. The aim of this study was to assess the prevalence of fungal contamination of nebulisers used by adults with cystic fibrosis.
Methods: A total of 170 nebulisers from 149 subjects were screened by wetting a sterile cotton swab with sterile water and swabbing each drug chamber. The swab was then plated out on Sabouraud and on Scel + agar and incubated at 27 °C for up to 2 weeks.
Results: Fungal cultures were positive in 86 (57.7%) patient's devices. In 28/149 (18.8%), 39/149 (26.2%), 47/149 (31.5%) and 20/149 (13.4%) of subjects *Aspergillus* species, yeasts, moulds and both yeasts and moulds were isolated respectively. There was no difference in contamination rates between different devices.
Conclusion: Nebuliser devices are frequently contaminated by moulds and yeasts and emphasis should be placed on ensuring adequate nebuliser hygiene.

Keywords: Biofilm; Fungi; Nebuliser; Device

1. Introduction

Nebulised drugs including antibiotics and mucolytics, remain essential in the management of cystic fibrosis (CF). Their introduction has been instrumental in improving clinical stability and life expectancy. Nebulisation of antibiotics has the added advantage of delivering very high drug concentrations directly to the site of infection while minimising side effects such as nephrotoxicity and ototoxicity.

Multiple drug regimens are often required to attain optimal outcome. This dependence on time consuming regimens increases treatment burden and often impacts on adherence. The introduction of devices such as the e Flow® and iNeb® has helped to reduce some of this burden by improved portability, usability and speed of administration [1]. Despite these changes adherence to treatment and nebuliser hygiene can remain low [2,3].

Previous studies have demonstrated a relationship between nebuliser hygiene and the risk of bacterial infections [4–7]. Despite the high prevalence of fungal colonisation and infection in patients with CF, the role of nebuliser devices as a potential source of primary inoculum and re-infection has not been investigated. *Aspergillus fumigatus* remains the most common fungal species to affect patients with CF and can be isolated in up to 58% of sputum samples. The fungus results in a spectrum of conditions ranging from hypersensitivity to frank infection [8]. These include bronchopulmonary aspergillosis (ABPA), aspergillus bronchitis, aspergilloma and in post transplant recipients, invasive aspergillosis. Other fungi such as *Scedosporium apiospermum* have also been implicated in pulmonary exacerbation.

The aim of this study was to analyse the fungal flora of nebuliser devices from people with CF and to compare contamination rates between the various devices.

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http://dx.doi.org/10.1016/j.jcf.2015.06.004

2. Materials and methods

2.1. Subjects

Adults attending the Leeds CF Unit at St James University Hospital were prospectively recruited. All patients had classical features of cystic fibrosis in conjunction with either two mutations or two abnormal sweat tests. Following recruitment, subjects were requested to bring their nebuliser devices to their next outpatient appointment. No further information was provided and cleaning was not mentioned. The study was approved by the North Leeds Ethics Committee (11/YH/0296).

2.2. Device sampling

Samples were obtained from the equipment by wetting a sterile cotton swab with sterile water and swabbing each drug chamber. iNeb horns were swabbed separately. Some individuals with iNebs had multiple chambers. Samples as well as negative controls were sent to the Mycology Laboratory at Leeds General Infirmary.

2.3. Laboratory investigations

Swabs were plated out on Sabouraud agar and on Scel + agar to select for *Scedosporium* sp. [9]. All plates were incubated at 27 °C for up to 2 weeks. Any mould or yeast growing on the plate was identified immediately or stored at −80 °C prior to being recovered and identified at a later date. Yeasts were initially identified by germ tube test. All germ tube negative isolates were identified by MALDI-TOF examination using the Bruker Biotyper system [10] or where this was not successful, the Biomerieux API32C yeast identification method. Moulds were identified by microscopic examination, or if this was not sufficient, by sequencing of the ITS1, 5.8S and ITS2 region and comparing the sequence with the Genbank database by BLAST search [11]. Sputa were treated with an equal volume of 0.1% dithiothreitol and a 10 μl loopful inoculated onto 3 Sabourauds agar plates which were incubated for 7 days at 28, 35 and 45 °C. All moulds are routinely examined though not always identified to species level.

2.4. Analysis

The proportion of devices with any fungal contamination was analysed according to device using significance tests and assuming a normal distribution. Device cultures were compared with the previous 6 months of sputum cultures.

3. Results

A total of 170 nebulisers, some with multiple chambers were sampled, representing a mean of 1.87 and median of 2 (range 1–4) devices from 149 subjects.

Overall, 86/149 (57.7%) of subjects had positive fungal cultures from at least one of their devices, with 39/149 (26.2%) being yeasts, 47/149 (31.5%) moulds and 20/149 (13.4%) a combination of yeasts and moulds.

There was no significant difference in the contamination rates of iNeb and Eflow devices ($p > 0.05$) (Table 1). While the contamination rate of Ventstreams appeared lower, numbers of devices used were small. Similar rates of fungal contamination were seen for iNeb chambers and horns. There was no correlation between medication administered and rate of contamination (Table 2).

Aspergillus fumigatus was the most frequent mould isolated (Table 3) followed by *Penicillium* sp. *P. commune* was the most common identifiable species from this genus. A number of *Lecanicillium* sp. isolates were also identified. *Exophiala* sp. were isolated from five devices. One was identified as *E. oliosperma,* one as *E. jeanselmei* and three as *Exophiala* sp. None were *E. dermatiditis*. Thus a total of 4/149 (2.7%) of all

Table 1
Fungal contamination rates for the iNeb, Eflow, Sidestream and Venstream nebulisers.

Device	No.	No. positive	% Positive
iNeb			
Horn	94	31	33.0
Drug chamber	199	71	35.7
Total	293	102	34.8
Eflow	48	21	43.7
Sidestream	22	9	40.9
Ventstream	6	1	16.7
Total nebulisers	170	62	36.5

Table 2
Positive fungal isolates identified from different devices according medication type.

Medication	Device	No.	No. positive	% Positive
7% NaCl	iNeb	24	11	45.8
	Eflow	5	2	40
	Sidestream	2	0	0
	Ventstream	1	0	0
7% NaCl and ventolin	Ventstream	1	1	100
Promixin	iNeb	57	19	33.3
DNase	iNeb	81	33	40.74
	Eflow	24	13	54.17
	Sidestream	14	7	50.00
Tobramycin	iNeb	25	5	20.00
	Eflow	10	5	50.00
	Pari Ic	2	1	50.00
	Ventstream	1	0	0.00
Aztreonam for inhalation solution	Eflow	3	0	0
Ventolin	iNeb	8	1	12.50
	Eflow	6	1	16.67
	Sidestream	4	0	0.00
	Ventstream	1	0	0.00
Ventolin and atrovert	Venstream	1	0	0
	Sidestream	1	1	100
Bricanyl	Sidestream	1	1	100
Combivent	iNeb	1	0	0
Atrovent	iNeb	1	1	100
Taurolidine	Ventstream	1	0	0