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Fungal contamination of nebuliser devices used by people with cystic fibrosis

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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: 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 two 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.

1 Conclusion: Nebuliser devices are frequently contaminated by moulds and
2 yeasts and emphasis should be placed on ensuring adequate nebuliser
3 hygiene.
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9 *Keywords*

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14 *Biofilm, fungi, nebuliser, device*
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19 **Introduction**

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21 Nebulised drugs including antibiotics and mucolytics, remain essential in the
22 management of cystic fibrosis (CF). Their introduction has been instrumental
23 in improving clinical stability and life expectancy. Nebulisation of antibiotics
24 has the added advantage of delivering very high drug concentrations directly
25 to the site of infection while minimising side effects such as nephrotoxicity and
26 ototoxicity.
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39 Multiple drug regimens are often required to attain optimal outcome. This
40 dependence on time consuming regimens increases treatment burden and
41 often impacts on adherence. The introduction of devices such as the e Flow®
42 and iNeb® have helped to reduce some of this burden by improved portability,
43 usability and speed of administration(1). Despite these changes adherence to
44 treatment and nebuliser hygiene can remain low(2, 3).
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56 Previous studies have demonstrated a relationship between nebuliser hygiene
57 and the risk of bacterial infections(4-7). Despite the high prevalence of fungal
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1 colonisation and infection in patients with CF, the role of nebuliser devices as
2 a potential source of primary inoculum and re-infection has not been
3
4 investigated. *Aspergillus fumigatus* remains the most common fungal species
5
6 to affect patients with CF and can be isolated in up to 58% of sputum
7
8 samples. The fungus results in a spectrum of conditions ranging from
9
10 hypersensitivity to frank infection(8). These include bronchopulmonary
11
12 aspergillosis (ABPA), aspergillus bronchitis, aspergilloma and in post
13
14 transplant recipients, invasive aspergillosis. Other fungi such as
15
16 *Scedosporium apiospermum* have also been implicated in pulmonary
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18 exacerbation.
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26 The aim of this study was to analyse the fungal flora of nebuliser devices from
27
28 people with CF and to compare contamination rates between the various
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30 devices.
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36 **Materials and Methods**

37 Subjects

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41 Adult attending the Leeds CF Unit at St James University Hospital were
42
43 prospectively recruited. All patients had classical features of cystic fibrosis in
44
45 conjunction with either two mutations or two abnormal sweat tests. Following
46
47 recruitment, subjects were requested to bring their nebuliser devices to their
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49 next outpatient appointment. No further information was provided and
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51 cleaning was not mentioned. The study was approved by the North Leeds
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2 Device sampling
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4 Samples were obtained from the equipment by wetting a sterile cotton swab
5 with sterile water and swabbing each drug chamber. INeb horns were
6 swabbed separately. Some individuals with INebs had multiple chambers.
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8 Samples as well as negative controls were sent to the Mycology Laboratory
9 at Leeds General Infirmary.
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19 Laboratory investigations
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21 Swabs were plated out on Sabouraud agar and on Scel+ agar to select for
22 *Scedosporium* sp(9). All plates were incubated at 27°C for up to two weeks.
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24 Any mould or yeast growing on the plate was identified immediately or stored
25 at –80°C prior to being recovered and identified at a later date. Yeasts were
26 initially identified by germ tube test. All germ tube negative isolates were
27 identified by MALDI-TOF examination using the Bruker Biotyper system(10) or
28 where this was not successful, the Biomerieux API32C yeast identification
29 method. Moulds were identified by microscopic examination, or if this was not
30 sufficient, by sequencing of the ITS1, 5.8S and ITS2 region and comparing
31 the sequence with the Genbank database by BLAST search(11). Sputa were
32 treated with an equal volume of 0.1% dithiothreitol and a 10ul loopful
33 inoculated onto 3 Sabourauds agar plates which were incubated for 7 days at
34 28, 35 and 45°C. All moulds are routinely examined though not always
35 identified to species level.
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58 Analysis
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1 The proportion of devices with any fungal contamination were analysed
2 according to device using significance tests and assuming a normal
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4 distribution. Device cultures were compared with the previous 6 months of
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6 sputum cultures
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10 11 **Results**

12 A total of 170 nebulisers, some with multiple chambers were sampled,
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14 representing a mean of 1.87 and median of 2 (range 1-4) devices from 149
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16 subjects.
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24 Overall, 86/149 (57.7%) of subjects had positive fungal cultures from at least
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26 one of their devices, with 39/149 (26.2%) being yeasts, 47/149 (31.5%)
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28 moulds and 20/149 (13.4%) a combination of yeasts and moulds.
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34 There was no significant difference in the contamination rates of iNeb and
35
36 Eflow devices ($p > 0.05$) (Table 1). While the contamination rate of
37
38 Ventstreams appeared lower, numbers of devices used were small. Similar
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40 rates of fungal contamination were seen for iNeb chambers and horns. There
41
42 was no correlation between medication administered and rate of
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44 contamination (Table 2).
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51 *Aspergillus fumigatus* was the most frequent mould isolated (Table 3)
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53 followed by *Penicillium* sp. *P. commune* was the most common identifiable
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55 species from this genus. A number of *Lecanicillium* sp. isolates were also
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57 identified. *Exophiala* sp, were isolated from, five devices. One was identified
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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 study subjects had one or more devices contaminated with *Exophiala* sp.

The most frequent yeast to contaminate devices was *Candida guilliermondii* followed by *C. parapsilosis*. Contamination with environmental basidiomycetous yeasts in the genera *Rhodotorula* and *Cryptococcus* (but not *Cr. neoformans*) was also common. The commonest cause of oral colonisation and infection, *C. albicans* was isolated on a single occasion.

When the results of culture from devices was compared with sputum culture from the previous six months, no obvious correlation was seen between positive culture from devices and previous sputum specimens ($p > 0.05$). Most people with CF who were sputum culture positive for *A. fumigatus* (46/50) did not have devices contaminated with this fungus. Of the six study subjects with *Exophiala* sp. or black yeasts isolated from devices, none had *Exophiala* sp. isolated from sputum culture in the previous six months. Of seven study subjects who had black yeasts isolated from sputum culture in the previous 6 months (provisionally identified as *Exophiala* sp.), none had *Exophiala* isolated from devices. No *Scedosporium* sp. were isolated from any device. All controls samples were negative.

Discussion

1 Although there is an increasing body of evidence supporting a relationship
2 between nebuliser hygiene and the risk of bacterial infections(4, 5), there is a
3 paucity of data on fungal contamination. A recent one day study by Jadhay et
4 demonstrated a high prevalence of fungal colonization of oxygen humidifier
5 and Hudson's nebuliser chambers in ICUs, wards, the casualty and OPD(6).
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7 Significantly, 15% of devices remained colonised despite disinfection with
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The present study has shown a high prevalence of contaminated nebuliser devices used by patients with CF. Over 57% of subjects had positive fungal cultures in at least one device including yeasts, moulds or a combination of both. There appears to be no significant difference between the rates of contamination between the various devices. We were surprised by the similarity in the contamination rate of conventional nebulisers and those with mesh technology. We had expected to find an increase rate of contamination in the conventional devices and had not expected to isolate fungus from the horn of the ineb. While contamination of Ventstreams appeared low, numbers were too small for the results to be meaningful.

We routinely instruct patients on how to follow the manufacturer's guidelines for cleaning the various devices. It is likely that such protocols are only partially adhered to and that following initial contamination, further cleaning becomes less effective due to the presence of polymicrobial biofilms.

1 There was no apparent correlation between fungal contamination of nebuliser
2 devices and previous sputum mycology. Whilst these results do not exclude
3 the role of the nebuliser device as a potential reservoir for pathogenic fungi
4 much more in depth longitudinal studies are needed.
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11 *Aspergillus fumigatus* was the most frequent mould isolated followed by
12 *Penicillium* sp with yeasts being found on many devices. While yeasts are
13 regularly isolated from sputum specimens of people with CF, yeasts are not
14 generally thought to represent a major clinical problem(12). It was notable that
15 the distribution of yeasts isolated from nebuliser devices did not correlate with
16 yeasts that normally colonise or infect the oral cavity. For example *Candida*
17 *albicans* is a common isolate from CF sputum and assumed to represent oral
18 flora but only one device was contaminated with this species. The yeasts
19 contaminating the nebuliser devices were often those associated with the
20 formation of biofilms such as *C. parapsilosis*. Specimens also included
21 environmental yeasts such as *Rhodotorula* sp, which are infrequently isolated
22 from clinical specimens due to their inability to grow well at 37C.
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43 In clinical practice we have recognised significant differences in patient
44 adherence to cleaning regimens with some nebuliser and compressors being
45 returned in a poor state of cleanliness. Education and adherence with the
46 manufacturers cleaning regimens can reduce contamination, highlighting the
47 importance of regular cleaning and disinfection(13). Further research is
48 needed to assess the relative effectiveness of different cleaning methods.
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58 There is presently no data on the rate of fungal contamination of the new dry
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1 powder antibiotic inhalers although the risk of contamination is likely to be
2 significantly lower in these disposable devices.
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7 In conclusion, fungal contamination of nebuliser devices used by patients with
8 CF appears common and is not device-specific More emphasis needs to be
9 placed on improving nebuliser hygiene through education and appropriate
10 cleaning regimens.
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22 **Acknowledgements**

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26 Novartis
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Table 1

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

Table 2

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 lc	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

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

Table 3

	Previously Reported from CF sputum	Previously Reported from non-CF sputum	Total no. of devices	% of devices	Total no. study participants with at least one contaminated device	% of study participants
<i>Aspergillus fumigatus</i>	+	+	24	9	20	13
<i>Aspergillus niger</i>	+	+	3	1	3	2
<i>Aspergillus versicolor</i>	+	+	1	<1	1	<1
<i>Cladosporium sphaerospermum</i>	+	+	1	<1	1	<1
<i>Exophiala oligosperumum</i>			1	<1	1	<1
<i>Exophiala sp.</i>	+	+	3	1	2	1
<i>Exophiala jeanselmei</i>			2	<1	1	<1
<i>Lecanicillium lecanii</i>			5	2	3	2
<i>Lecanicillium sp.</i>			2	<1	2	1
<i>Microsporum fulvum</i>			1	<1	1	<1
<i>Myxotrichum sp.</i>			1	<1	1	<1
<i>Penicillium commune</i>			4	<1	4	3
<i>Penicillium glabrum</i>			1	<1	1	<1
<i>Penicillium griseofulvin</i>			1	<1	1	<1
<i>Penicillium coryphilum</i>			1	<1	1	<1
<i>Penicillium digitatum</i>		+	2	<1	2	1
<i>Penicillium sp.</i>	+	+	10	4	5	3
<i>Rhizopus oryzae</i>			1	<1	1	<1
<i>Scopulariopsis chartarum</i>			1	<1	1	<1
<i>Ulocladium chartarum</i>			2	<1	1	<1
<i>Aureobasidium pullulans</i>		+	2	<1	1	<1
<i>Candida albicans</i>	+	+	1	<1	1	<1
<i>Candida guilliermondii</i>		+	36	13	22	15

<i>Candida holmii</i>			1	<1	1	<1
<i>Candida krusei</i>		+	2	<1	1	<1
<i>Candida lipolytica</i>			1	<1	1	<1
<i>Candida parapsilosis</i>	+	+	29	11	20	13
<i>Candida pelliculosa</i>			3	1	2	1
<i>Candida sake</i>			1	<1	1	<1
<i>Candida sp.</i>	+	+	1	<1	1	<1
<i>Candida zeylanoides</i>		+	1	<1	1	<1
<i>Cryptococcus sp.</i>		+	1	<1	1	<1
<i>Cryptococcus albidus</i>			4	1	3	2
<i>Cryptococcus unigutatus</i>			10	4	5	3
<i>Cryptococcus carnescens</i>			1	<1	1	<1
<i>Rhodotorula glutinis</i>	+		24	9	17	11
<i>Rhodotorula mucilaginosa</i>	+		21	8	17	11
<i>Rhodotorula minuta</i>			5	2	4	3
<i>Rhodotorula sp.</i>		+	1	<1	1	<1
<i>Sporobolomyces roseus</i>			1	<1	1	<1
<i>Trichosporon asahii</i>		+	1	<1	1	<1

Table 3:

+Previous reports of fungal isolates from CF and non CF sputum respectively. Number of devices with positive fungal isolates and number of study participant with at least one infected device.

Fungal contamination of nebuliser devices used by people with cystic fibrosis

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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: 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 two 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

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® have 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.

Materials and Methods

Subjects

Adult 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).

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.

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 two 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 10ul 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.**

Analysis

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

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 study subjects had one or more devices contaminated with *Exophiala* sp.

The most frequent yeast to contaminate devices was *Candida guilliermondii* followed by *C. parapsilosis*. Contamination with environmental basidiomycetous yeasts in the genera *Rhodotorula* and *Cryptococcus* (but not *Cr. neoformans*) was also common. The commonest cause of oral colonisation and infection, *C. albicans* was isolated on a single occasion.

When the results of culture from devices was compared with sputum culture from the previous six months, no obvious correlation was seen between positive culture from devices and previous sputum specimens ($p > 0.05$). Most people with CF who were sputum culture positive for *A. fumigatus* (46/50) did not have devices contaminated with this fungus. Of the six study subjects with *Exophiala* sp. or black yeasts isolated from devices, none had *Exophiala* sp. isolated from sputum culture in the previous six months. Of seven study subjects who had black yeasts isolated from sputum culture in the previous 6 months (provisionally identified as *Exophiala* sp.), none had *Exophiala* isolated from devices. No *Scedosporium* sp. were isolated from any device.

All controls samples were negative.

Discussion

Although there is an increasing body of evidence supporting a relationship between nebuliser hygiene and the risk of bacterial infections(4, 5), there is a paucity of data on fungal contamination. A recent one day study by Jadhay et al demonstrated a high prevalence of fungal colonization of oxygen humidifier and Hudson's nebuliser chambers in ICUs, wards, the casualty and OPD(6). Significantly, 15% of devices remained colonised despite disinfection with 70% alcohol.

The present study has shown a high prevalence of contaminated nebuliser devices used by patients with CF. Over 57% of subjects had positive fungal cultures in at least one device including yeasts, moulds or a combination of both. There appears to be no significant difference between the rates of contamination between the various devices. **We were surprised by the similarity in the contamination rate of conventional nebulisers and those with mesh technology. We had expected to find an increase rate of contamination in the conventional devices and had not expected to isolate fungus from the horn of the ineb.** While contamination of Ventstreams appeared low, numbers were too small for the results to be meaningful.

We routinely instruct patients on how to follow the manufacturer's guidelines for cleaning the various devices. It is likely that such protocols are only partially adhered to and that following initial contamination, further cleaning becomes less effective due to the presence of polymicrobial biofilms.

There was no apparent correlation between fungal contamination of nebuliser devices and previous sputum mycology. Whilst these results do not exclude the role of the nebuliser device as a potential reservoir for pathogenic fungi much more in depth longitudinal studies are needed.

Aspergillus fumigatus was the most frequent mould isolated followed by *Penicillium* sp with yeasts being found on many devices. While yeasts are regularly isolated from sputum specimens of people with CF, yeasts are not generally thought to represent a major clinical problem(12). It was notable that the distribution of yeasts isolated from nebuliser devices did not correlate with yeasts that normally colonise or infect the oral cavity. For example *Candida albicans* is a common isolate from CF sputum and assumed to represent oral flora but only one device was contaminated with this species. The yeasts contaminating the nebuliser devices were often those associated with the formation of biofilms such as *C. parapsilosis*. Specimens also included environmental yeasts such as *Rhodotorula* sp, which are infrequently isolated from clinical specimens due to their inability to grow well at 37C.

In clinical practice we have recognised significant differences in patient adherence to cleaning regimens with some nebuliser and compressors being returned in a poor state of cleanliness. Education and adherence with the manufacturers cleaning regimens can reduce contamination, highlighting the importance of regular cleaning and disinfection(13). Further research is needed to assess the relative effectiveness of different cleaning methods. There is presently no data on the rate of fungal contamination of the new dry

powder antibiotic inhalers although the risk of contamination is likely to be significantly lower in these disposable devices.

In conclusion, fungal contamination of nebuliser devices used by patients with CF appears common and is not device-specific. More emphasis needs to be placed on improving nebuliser hygiene through education and appropriate cleaning regimens.

Acknowledgements

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