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Thinking inside the box: nebulizer care, safe storage, and risk of infection in cystic fibrosis

Lauren Alexander[1,2], Jane Carson[1,2], John McCaughan[1,3], John Edmund Moore[1,4], Beverley Cherie Millar[1,4]

TO THE EDITOR:

Patients with cystic fibrosis (CF) are recommended to wash and disinfect their nebulizers on a regular basis, ideally after each use,[1] both to ensure that devices are maintained properly for optimal drug delivery and to minimize infection risks. In practice, approaches to nebulizer hygiene vary among pediatric[2,3] and adult patients, both in the home[2] and hospital environments.[4] Recently, Riquena et al.[5] demonstrated a contamination rate of 71.6% of the nebulizers used by CF patients who were chronically colonized with *Pseudomonas aeruginosa.* Nebulizers were contaminated with clinically significant organisms, including *Stenotrophomonas maltophilia* (11.9%), nonmucoid *P. aeruginosa* (4.8%), *Staphylococcus aureus* (4.8%), and *Burkholderia cepacia* complex (2.4%), as well as yeasts and filamentous fungi. Overall, such contamination was exacerbated by the use of tap water and outdoor drying of nebulizers, concurrent with poor nebulizer hygiene among patients.

Recently, CF centers in the United Kingdom highlighted a common practice to wash and store clean devices in sealed plastic boxes.[4] Given that there is no evidence in the published literature regarding microorganisms found in nebulizer storage boxes, we examined the microbiology of such boxes used during inpatient stays to help guide safe practice recommendations for the storage of nebulizers after cleaning/disinfection.

We collected 24 disposable plastic storage boxes (approximate dimensions: 152 mm in length × 98 mm in width × 68 mm in depth) used during inpatient stays from 15 pediatric patients and a new/unused control box. All microbiological analyses were performed blinded. Microbiology rinse cultures were performed aseptically on each box by adding 18 mL of 0.1% (w/v) peptone saline diluent (CM0733; Oxoid Ltd., Basingstoke, United Kingdom) into the box and agitating the diluent for 10 min. Resulting rinses were cultured aerobically on Columbia agar (CM0331; Oxoid Ltd.) supplemented with 5% (v/v) defibrinated horse blood (SR0050; Oxoid Ltd.) at 37°C/48 h, as well as in nonselective enrichment broth (Mueller-Hinton Broth; CM0405; Oxoid Ltd.) at 37°C/48 h and on Sabouraud dextrose agar with chloramphenicol (PO0161; Oxoid Ltd.) at 25°C/5 days, for the detection of yeasts/fungi. Resulting bacterial colonies were identified using matrix-assisted laser desorption/ionization, time-of-flight mass spectrometry, and fungal colonies were identified using internal transcribed spacer/PCR/DNA sequencing. Microbiological analysis of boxes was subsequently compared with contemporary sputum microbiology from respective patients.

Eighty percent of the patients had at least one of their storage boxes positive for bacteria (Table 1). Overall, 20 boxes (83%) were positive for bacteria; however the majority of these (65%) had a contamination rate of $< 10^3$ CFU/box, whereas 15% of positive boxes were contaminated between 10^3–10^4 CFU/box, with the remainder (20%) contaminated between 10^4–10^5 CFU/box. The most highly contaminated box harbored 5.4×10^4 CFU/box. Bacterial diversity demonstrated a predominately gram-positive flora, representing 15 genera and 22 species. *Micrococcus luteus* and *Dermacoccus nishinomiyaensis* were the most commonly isolated species, with coagulase-negative staphylococci and the viridans group (oral) streptococci having the greatest species diversity within their respective genera. Gram-negative bacteria were in the minority, representing 8.3% of bacterial species isolated, namely *Stenotrophomonas maltophilia* and *Neisseria flava/perflava/subflava.* Fungi were isolated from 4 (26.7%) of 15 boxes and included *Penicillium* sp., *Penicillium expansum*, *Cladosporium* sp. and *Candida albicans*.

With the exception of *Stenotrophomonas maltophilia*, none of the organisms identified are considered major pathogens of CF. None of the boxes grew organisms which were contemporary to the organisms found in patients' sputum (Table 1). Most of the organisms identified were of skin, mouth, or throat/oropharyngeal origin. In contrast to Riquena et al.,[5] a recent study in the USA[3] found contamination of nebulizers used by pediatric CF patients, the most frequently observed microbial contaminants being viridans streptococci, *Micrococcus* sp., coagulase-negative staphylococci, and *Candida albicans.* Our findings in relation to storage boxes largely concur with those of the US report[3] in terms of bacterial contamination. Our study demonstrated the presence of yeast and fungal contaminants, similar to the Brazilian report.[5] The occurrence of fungi may be due to inadequate drying of nebulizer parts prior to storage, which emphasizes the importance of thorough drying prior to storage.

Therefore, what is the significance of the storage boxes being largely contaminated with oral and environmental organisms? Although the organisms detected are not believed to be clinically significant, such organisms

1. Northern Ireland Working Group on Nebuliser Care and Hygiene in Cystic Fibrosis, Belfast, Northern Ireland.
2. Northern Ireland Paediatric CF Centre, Department of Physiotherapy, Royal Belfast Hospital for Sick Children, Belfast, Northern Ireland.
3. Department of Medical Microbiology, Royal Group of Hospitals, Belfast, Northern Ireland.
4. Northern Ireland Public Health Laboratory, Department of Bacteriology, Belfast City Hospital, Lisburn Road, Belfast, Northern Ireland.

Table 1. Comparison between microbial contaminants found in plastic storage boxes used to store nebulizers and current sputum microbiology in patients with cystic fibrosis.

Patient	Box	Sputum
1	*Staphylococcus epidermidis*	*Pseudomonas aeruginosa*
2	*Micrococcus luteus*, *Dietzia cinnamea*	*MRSA*
3	*Staphylococcus capitis*, *Dermacoccus nishinomiyaensis*, *Kocuria rhizophila*, *Corynebacterium afermentans*, *Paenibacillus macerans*, *Bacillus licheniformis*	*Pseudomonas aeruginosa*
4	*Micrococcus luteus*, *Gemella haemolysans*, *Streptococcus sanguinis*, *Rothia aeria*, *Rothia dentocariosa*, *Dermacoccus nishinomiyaensis*, *Bacillus licheniformis*, *Kocuria rhizophila*, *Streptococcus parasanguinis*, *Rothia mucilaginosa*	*Stenotrophomonas maltophilia*
5	*Streptococcus parasanguinis*, *S. mitis*, *S. oralis*, *Streptococcus* sp., *Neisseria flava*, *N. perflava*, *N. subflava*	Yeasts
6	*Staphylococcus warneri*, *Stenotrophomonas maltophilia*, *Microbacterium paraoxydans*, *Bacillus licheniformis*, *Penicillium expansum*, *Penicillium* spp., *Candida albicans*	*Pseudomonas aeruginosa*
7	No growth	*MRSA*
8	*Micrococcus luteus*	*Long-standing* ABPA
9	*Streptococcus mitis*, *S. oralis*, *S. sanguinis*, *S. parasanguinis*	*Pseudomonas aeruginosa*
10	*Micrococcus luteus*, *Bacillus* sp., unidentified fungus	*Staphylococcus aureus*
11	*Micrococcus luteus*	*Staphylococcus aureus*
12	*Staphylococcus saprophyticus*, *Dermacoccus nishinomiyaensis*,*Cladosporium* spp.	*Staphylococcus aureus*, *Haemophilus influenzae*, *Stenotrophomonas maltophilia*
13	*Brevibacillus* sp., *Dermacoccus nishinomiyaensis*, unidentified fungus	*Pseudomonas aeruginosa*
14	No growth	*Pseudomonas aeruginosa*, *Staphylococcus aureus*, ABPA
15	No growth	*Staphylococcus aureus*, *Haemophilus influenzae*
Control	*Staphylococcus epidermidis*	New control box

MRSA: methicillin-resistant *Staphylococcus aureus*; and ABPA: allergic bronchopulmonary aspergillosis.

may harbor antibiotic resistance gene determinants and, if nebulized, could provide a reservoir for such determinants to be horizontally transferred to established CF pathogens in the lung, thereby potentially increasing the antimicrobial resistance burden. Studies are therefore required to elucidate the potential for such horizontal gene transfer events from nonpathogenic to pathogenic organisms.

The efficiency of nebulizer cleaning and disinfection will directly affect the hygienic status of boxes, used subsequently for the storage of nebulizers. Therefore, in alignment with current evidence, patients should wash and disinfect their nebulizers after each use with steam disinfection in a baby bottle disinfector and leave their nebulizers in such disinfector units until next required.[6] Where storage in the steam disinfector is not practical, then, after disinfection, nebulizers should be air dried fully and stored on absorbent tissue in dedicated clean storage boxes, separate from those used to wash nebulizers.

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