TO THE EDITOR:

Patients with cystic fibrosis (CF) are recommended to wash and disinfect their nebulizers on a regular basis, ideally after each use, 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.(3) 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 (approximately 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 blindfolded. 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; Hinton Broth; CM0405; Oxoid Ltd.) at 25°C/5 days, for the detection of yeasts and filamentous 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 × 10^5 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 flavaperflava/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., 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. 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...
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. 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.

### AUTHOR CONTRIBUTIONS

All authors contributed to the design, execution, analysis, and writing of this letter.

### FUNDING SOURCES

All authors are members of the Northern Ireland Working Group on Nebuliser Care and Hygiene in Cystic Fibrosis, and this group has received an unrestricted medical educational grant from Vertex Pharmaceuticals Incorporated (Grant no. ME-2015-104608). Vertex Pharmaceuticals Incorporated was not involved in the study design, in the collection, analysis and interpretation of data, in the writing of the report, or in the decision to submit the article for publication.

### REFERENCES

1. Saiman L, Siegel JD, LiPuma JJ, Brown RF, Bryson EA, Chambers MJ, et al. Infection prevention and control guideline for cystic fibrosis: 2013 update. Infect Control Hosp Epidemiol. 2014;35 Suppl 1:S1-567. https://doi.org/10.1086/676862
2. MacFarlane M, Carson L, Crossan A, Bell J, Moore JE, Millar BC. Nebuliser cleaning and disinfection practice in the home among patients with cystic fibrosis. J Infect Prev. 2019. http://dx.doi.org/10.1177/175717419855603 [Epub ahead of print]
3. Murray TS, O’Rourke TK Jr, Feinn R, Drapeau G, Collins MS. Nebulizer cleaning and disinfection practices in families with cystic fibrosis: The relationship between attitudes, practice and microbe colonization. J Cyst Fibros. 2019. pii: S1569-1993(19)30112-2.
4. Bell J, Moore JE, Millar BC. Cleaning of inpatient nebulizer devices in cystic fibrosis patients: the urgent need for universal guidelines. J Hosp Infect. 2018;100(3):e64-e66. https://doi.org/10.1016/j.jhin.2018.06.025

5. Riquena B, Monte LFV, Lopes AJ, Silva-Filho LVRFD, Damaceno N, Aquino EDS, et al. Microbiological contamination of nebulizers used by cystic fibrosis patients: an underestimated problem. J Bras Pneumol. 2019;45(3):e20170351. https://doi.org/10.1590/1806-3713/e20170351

6. Hohenwarter K, Prammer W, Aichinger W, Reychler G. An evaluation of different steam disinfection protocols for cystic fibrosis nebulizers. J Cyst Fibros. 2016;15(1):79-84. https://doi.org/10.1016/j.jcf.2015.07.005