Since January 2020 Elsevier has created a COVID-19 resource centre with free information in English and Mandarin on the novel coronavirus COVID-19. The COVID-19 resource centre is hosted on Elsevier Connect, the company's public news and information website.

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Major Article

Microbial contamination of powered air purifying respirators (PAPR) used by healthcare staff during the COVID-19 pandemic: an in situ microbiological study

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Key Words:
Disinfection
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Background: Powered air purifying respirators (PAPR) are an option for healthcare workers requiring respiratory protection during the current COVID-19 pandemic; they are shared between multiple people. PAPR hoods are intended for multiple uses by a single user and may pose an infection risk between wearers.
Methods: Internal components of PAPR hoods and corrugated air supply hoses were swabbed for evidence of bacterial, fungal, common respiratory viruses and severe acute respiratory syndrome-coronavirus-2 (SARS-CoV-2) contamination.
Results: Twenty-five PAPR hoods were swabbed; 10 (40%) returned positive results. Bacterial growth was detected on six PAPR; five of the PAPR tested positive for fungal growth; all tested negative for SARS-CoV-2 and common respiratory viruses.
Conclusions: Bacteria and fungi can remain on internal components of PAPR hoods and air supply hoses despite following recommended disinfection procedures. PAPR hoods have the potential to act as fomites, cross-infecting wearers, and patients. Current guidelines for disinfecting PAPR hoods may not be effective for use in high risk healthcare environments.

BACKGROUND

In response to the coronavirus 2019 disease (COVID-19) pandemic, public health bodies have recommended that health care workers (HCWs) wear respirators as part of personal protective equipment (PPE) whenever there is risk of aerosol generating procedures (AGP), and at all times where patients with or suspected of having COVID-19 are treated.[1]

Powered air purifying respirator (PAPR) assemblies, also referred to as 'power hoods,' are an option available to HCWs requiring high level respiratory protection, those who fail a fit test for disposable respirators, or for HCWs that cannot tolerate prolonged use of standard respirators. PAPR are utilized during the highest risk aerosol generating procedures and have been shown to offer better conditions for the wearer when performing complex airway procedures compared to reusable respirators.[2,3] There is evidence to suggest that females and non-Caucasian people are less likely to pass fit tests for FFP3 respirators.[4-11] PAPR consist of powered fan units connected via corrugated hosing to hoods. Filtered air, under positive pressure is supplied to the wearer; this and the wearer's exhaled breath are expelled around the edges of the hood and through unshrouded expiratory valves; the hoods consist of a clear visor, stitched to fabric. PAPR and these hoods were originally developed to provide respiratory protection in industrial use to help combat pulmonary contamination by non-biological particulates. They have been adapted for use during the COVID-19 pandemic by upgrading the particulate filters, but the use of PAPR in clinical environments is not universally accepted.[12,13]

The fidelity of the hood relies on the assumption that high flow forced air prevents the wearer both from inhaling particles directly from the environment, and, importantly, from contaminating the

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internal surfaces of the air supply hosing. Such protection may be nullified if users follow guidance to don hoods before activating the fan unit or the filter air inlet becomes obstructed during use.[14] These problems are of particular significance in the current pandemic because individuals can be clinically asymptomatic but test positive for severe acute respiratory syndrome-coronavirus-2 (SARS-CoV-2),[15] putting other staff sharing the same hood at risk. Healthcare workers have expressed other infection concerns about PAPRs, including fomite dispersal during handling, aerosol deposition of respiratory and other secretions,[13] and lack of confidence in the decontamination process.

Current local guidelines from infection control services recommend the use of disinfection wipes for cleaning and disinfection, but do not address how the internal parts of the PAPR hoods and air supply hoses should be treated.[16] There has been minimal research published examining the efficacy of different decontamination methods, although cleaning alone and cleaning plus disinfection have both been shown to be effective in removing the influenza virus from the external components of PAPR;[13] we can find no studies that have examined the contamination or disinfection of internal components.

As PAPR hoods and air supply hoses are intended for multiple uses by a single user, the manufacturer has not determined how the internal surfaces of the PAPR components might be disinfected,[17] and so no evidence-based protocol exists for disinfecting the hoods before their re-use, when demand for high level respiratory protection outstrips supply. Evidence suggests that viral particles may persist on surfaces, in an infectious state, for prolonged periods.[18,19] In addition, fomite and aerosol transmission is suspected for SARS-CoV-2,[20,21] and therefore the probability exists that PAPRs may potentially contaminate sterile fields.[12] Given these concerns, we conducted a study to test our hypothesis that PAPR hoods and their air supply hoses could remain colonized with bacteria, fungi or detectable viral DNA/RNA following protocol-driven cleaning and disinfection.

METHOD

Study design and setting

After approval from the Brighton and Sussex University Hospitals NHS Trust, UK, Executive Board, we conducted an observational, laboratory study to test the PAPR hoods in use in our institution. Over a 48 hour period in June 2020, 25 PAPR hoods and their attached corrugated air supply hoses, approximately 25% of our institution's circulating stock, were tested for evidence of bacterial, fungal, respiratory viral and SARS-CoV-2 colonization, generating a total of 100 swabs for analysis.

Hoods

We examined the 3M Scott Safety FH1 and FH2 PAPR headtops, (3M Scott, Skelmersdale, UK) that are used at our institution. The FH1 headtop is a half hood that provides a loose neoprene seal around the face; the FH2 headtop has a shoulder cape that provides neck and shoulder protection (Fig 1). Hood assemblies are cleaned with disinfectant wipes after use (benzalkonium chloride 0.54 g, didecyldimonium chloride 0.54 g, and phenoxyethanol 0.6 g per 100 g; GAMA Healthcare, Watford, UK).

Swabbing

Previously used but cleaned and disinfected PAPR were tested in the clinical environments in which they were stored and donned. The hood-holder manipulated the mask to allow the swab-taker access to the target areas to take samples (Fig 2). Each PAPR was swabbed for bacteria, fungi, respiratory viruses (extended respiratory panel) and SARS-CoV-2. Bacterial and fungal samples were taken with Transwab Amies Charcoal swabs (MW171, Medical Wire & Equipment, UK), and viral samples taken with Sigma Virocult swabs (MW951S, Medical Wire & Equipment, UK). Swabs were moistened with sterile 0.9% sodium chloride solution.

Each PAPR hood had 4 swabs: 2 charcoal and 2 viral, one of each from 2 predefined areas: first, inside of the clear plastic visor including the expiratory valve, and second, inside the corrugated air supply hose attached to the hood. The visor and expiratory valve were divided in to two halves, one for the charcoal swab, and one for the viral swab. The expiratory valve was swabbed first with care taken to apply the swabs uniformly over the surface and then over the respective side of the visor in a linear, up-down, fashion with care taken to access any corners and cover the full area of the visor. The air supply hoses were also divided in two halves, one for each type of swab. The swab was passed from the rim down to approximately 10 cm, the length of the swabs, with care taken to swab within the ridges of the hose corrugations.

Investigators and PPE

All samples were taken by consultant anaesthetists experienced in the wearing of FFP3 respirators and PPE, and conducting procedures with aseptic non-touch technique (ANTT). Investigators wore PPE to

Fig. 1. 3M Scott FH1 and FH2 *Headtops* and PAPR arrangement.

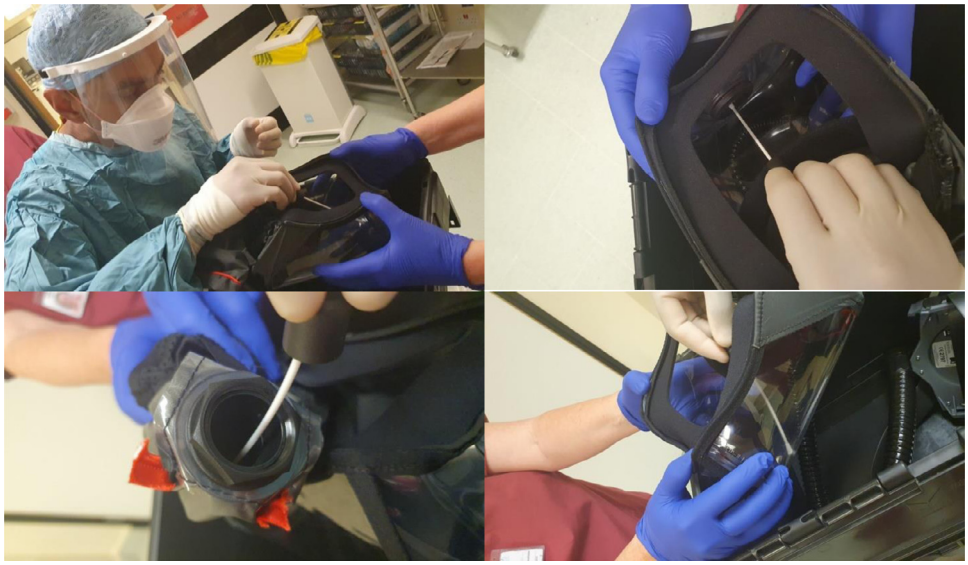

Fig. 2. Swabbing PAPR hoods and fan tubing.

protect themselves from potentially colonized PAPR and to prevent inadvertent contamination of the equipment by the investigators. All investigators wore FFP3 respirators without an expiratory valve. The 'hood-holder' wore fresh latex free sterile gloves, and eye protection; the 'swab-taker' wore freshlatex free sterile gloves, fresh surgical gowns, and eye protection; the investigator responsible for sample labeling and bagging wore non-sterile latex free gloves, and eye protection. Swabbing was carefully planned and conducted so that each swab consistently sampled the same area of respective hoods and air supply hoses, minimizing overlap and the potential to remove organisms.

Traceability and labeling

The locations of PAPR across the institution were identified. Hoods were marked with a unique code that corresponded with the code for their respective swabs and allowed for the location of any PAPR to be identified. For example, 'RSCH_ENT_1' refers to a PAPR that is kept in the Ear, Nose and Throat (ENT) outpatient department at the Royal Sussex County Hospital. Where the PAPR already had a number designation in a clinical area, this was reflected in the assigned code.

Laboratory testing and reporting

Bacterial testing

Transwab Amies Charcoal swabs were cultured onto Blood (Oxoid Colombia Agar with horse blood, Code: PB0122, distributed by ThermoFisher, UK) and Chocolate (Oxoid Colombia Agar with Chocolated Horse Blood, Code: PB0124, ThermoFisher, UK) agar plates and incubated in carbon dioxide (CO_2) for 24 hours. Any growth was identified using the MALDI-TOF system (Bruker, USA).

SARS-CoV-2 (COVID-19) testing

Testing for SARS-CoV-2 was performed by real-time RT-PCR method. Four hundred micro litre of each of the Sigma Virocult swabs with added 5 μL internal control were extracted using the Kingfisher Flex system (ThermoFisher, UK) with elution volume of 50 μL. Ten micro liter of extracted sample material was then added to 15 μL PCR master mix of the CE-marked Bosphore Novel Coronavirus (2019-nCoV) Detection v2 Kit (manufactured by Anatolia Geneworks, Turkey and supplied by Launch Diagnostics, UK). This is then amplified on the Applied Biosystems 7500 machine (Applied Biosystems, USA) using the thermal protocol for Bosphore Novel Coronavirus (2019-nCoV) Detection Kit v2 as per manufacturer recommendation. The targets for the test include (orf1ab & Egene). The manufacturer reported 100% specificity and 95% sensitivity (with analytical detection limit of 25 copies/rxn).

Respiratory panel testing

Testing for the respiratory panel was performed by real-time RT-PCR method. Four hundred micro liter of each of the Sigma Virocult swabs was extracted using the Nuclisens EasyMag system (Biomerieux, France) with elution volume of 100 μL. The respiratory panel multiplex RT-PCR detection kit (CE-marked Bosphore Respiratory pathogen panel v6, manufactured by Anatolia Geneworks, Turkey and supplied by Launch Diagnostics, UK) 7 mini-multiplexes with each of the mini-multiplexes has their own integrated internal control. This multiplex RT-PCR panel can detect 19 targets including: influenza A (pandemic H1N1 or non-pandemic H1N1), influenza B, parainfluenza 1-4, RSV, Enterovirus, Parechovirus, Rhinovirus, seasonal Coronaviruses (NL63, 229E, OC43, & HKU), Human metapneumovirus, Human bocavirus, Adenovirus, and Mycoplasma pneumoniae. Ten micro liter of extracted sample material was then added to 15 μL PCR master mix of each of the mini-multiplexes. These were then amplified on the Applied Biosystems 7500 Fast machine (Applied Biosystems, USA) using the thermal protocol for Bosphore Respiratory pathogens panel detection kit v6 as per manufacturer recommendation. The manufacturer reported 100% specificity and 95% sensitivity.

Fungal testing

Transwab Amies Charcoal swabs were cultured onto Sabouraud dextrose agar plates (Oxoid Sabouraud dextrose agar with Chloramphenicol, Code: PO0161, ThermoFisher, UK) and incubated in air at 35°C for 5 days. Any growth of fungi was then identified macroscopically and/or microscopically as appropriate.

RESULTS

Of the 25 PAPR hood and air supply hose assemblies swabbed, ten (40%) returned a positive result (Table 1).

Table 1
Positive swab results

Hood	Model	Part	Bacteria	Viral panel	SARS-CoV-2	Fungal	Storage
4	FH1	Visor	Bacillus simplex	x	x	x	Open ward
12	FH1	Visor	x	x	x	NSEM	Drying hook
13	FH1	Visor	x	x	x	NSEM	Open box
15	FH1	Visor	x	x	x	NSEM	Open box
16	FH1	Hose	Kocuria rhizophilia	x	x	x	Sealed box
19	FH1	Visor	x	x	x	NSEM	Sealed box
20	FH1	Visor	Bacillus weihenstephensis	x	x	x	Open box
21	FH2	Hose	Micrococcus luteus	x	x	x	Open box
23	FH1	Visor	Staphylococcus epidermidis	x	x	NSEM	Open box
25	FH2	Visor	Micrococcus luteus	x	x	x	Open box
25	FH2	Hose	Micrococcus luteus	x	x	x	Open box

FH1/FH2, model of 3M Scott PAPR Headtop; NSEM, nonsporulating environmental mould; x, negative swab.

Bacterial testing

There was bacterial growth detected on 6 PAPR hoods. Bacteria were detected on the visor only of 3 hoods, corrugated air supply hose only of 2 PAPR assemblies. Bacteria were detected on both the visor and corrugated air supply hose of 1 hood. The bacteria detected were Bacillus simplex, Kocuria rhizophilia, Bacillus weihenstephensis, Micrococcus luteus, and Staphylococcus epidermidis.

Fungal testing

Five of the PAPR hoods were positive for fungal growth; all grew non-sporulating environmental mould (NSEM) which could not be identified.

SARS-CoV-2 (COVID-19) and respiratory panel testing

All PAPR hood and hose assemblies sampled tested negative for SARS-CoV-2 (COVID-19) and respiratory viral panel testing.

DISCUSSION

To our knowledge, this is one of the first studies conducted during the COVID-19 pandemic to report contamination of internal components of PAPR. Despite cleaning and disinfection according to recommended protocols in the UK during the 2020 COVID-19 pandemic, bacteria and fungi remained at detectable levels on internal surfaces of the hoods and corrugated air supply hoses.

Staphylococcus epidermidis, Kocuria rhizophilia, and Micrococcus luteus are Gram positive cocci. Staphylococcus epidermidis is a skin commensal that can cause infections of intravenous lines and prosthetic materials, including artificial heart valves and orthopedic joints. Kocuria rhizophiliais found in the oropharynx, oral mucosa, and skin, but does not usually cause human infections except rarely in severely immunocompromized patients. Micrococcus luteus is part of the normal bacterial flora of human skin and is not generally pathogenic. Bacillus simplex and Bacillus weihenstephensis are gram positive bacilli that can colonize skin and are found in soil; they are unlikely to be pathogens in humans, although Bacillus simplex has been implicated in food borne disease and as a potential cause of brain abscess.[22]

The clinical impact of NSEMs is difficult to qualify; they do not sporulate, and so are difficult to differentiate. They are usually not pathogenic except occasionally in immunocompromized patients. Notably, however, NSEM survived optimal care in one hood (controlled storage, double cleaning and disinfection, tracked use, user log, cleaning diary), even though it had not been worn for 6 days.

The risk to PAPR users, at our institution, of pathogenic cross infection during the study period seems minimal. However, we think that it is reasonable to infer from our results that more pathogenic organisms could survive cleaning and disinfection procedures, and risk cross-infecting subsequent users. Recent studies have demonstrated the ability of SARS-COV-2 to persist for prolonged periods of time in an infectious state on a variety of surfaces[19]; it is likely none of previous wearers of these hood systems was infected with SARS-CoV-2, but this is may be of greater concern as more virulent mutant strains of SARS-CoV-2 arise.

PAPRs are available to clinicians who conduct high risk (aerosol generating) procedures,[2,3] and those who fail fit-testing for disposable FFP3 respirators or reusable half-face respirators. Contamination of hood assemblies may be more likely if the hood is donned before the fan unit is activated (as advised by Health Protection Scotland),[14] and if the inlet to the air filter is blocked (allowing recirculation of exhaled breath inside the hood and air supply hose). People with prominent noses are more likely to pass respirator fit tests,[23-26] Caucasian males being the gender and racial phenotypes of the population trialed during PPE design.[4-10] Our results suggest that obligate PAPR wearing by non-Caucasians and women may place users at even further risk of pathogenic cross-contamination, and, potentially, COVID-19-related morbidity and mortality.[27,28] There remains a dearth of comparative studies involving females and non-Caucasians, but there is a growing body of anecdotal and empirical evidence supporting suggestions of structural sex and racial bias in the provision of respiratory PPE internationally.[4,5,7-11] During this pandemic, confidence in PPE has been reduced by concerns over personal safety,[29] conflicting, frequently changing, advice,[30] difficulties with availability,[31] and UK public health PPE guidance is not in line with other international standards.[32] The PAPR systems used at our institution are not designed for medical use and have an expiratory valve that vents unfiltered exhaust gas (a mix of exhaled and fresh air) potentially exposing patients and other staff to cross infection from contaminated hoods.

PAPR hoods and air supply hoses are designed for multiple uses by a single user and the cleaning and disinfection of internal components is difficult rand not considered by the manufacturer. Fabric and foam components cannot be immersed in disinfecting fluid, washed mechanically or sterilized with ethylene dioxide, radiation, steam, or vaporized hydrogen peroxide (VHP)[17]; disinfectant wipes commonly used in the UK may cause functional degradation.[16]

Our study has several limitations. The charcoal and viral swabs are intended for mucosal and not surface sampling. We dampened the swabs to recreate mucosal moisture, but may have reduced the chances of true positive tests by doing this. It may be that the swabbing techniques we used have low detection rates, requiring a larger sample size to detect viral contamination. Although we isolated several microbial species, we are unable to correlate our findings with cases of hospital acquired infection. The hoods were tested in the order their results are presented and the distribution of organisms would

suggest that investigators did not cross-contaminate hoods whilst conducting this study.

We noted a wide variation in the storage of cleaned hoods, including examples both of poor practice (left lying open on a trolley next to a ward bay accommodating patients with COVID-19, uncontrolled storage in open boxes, and variations in drying techniques), and of good practice (controlled storage, after double cleaning and disinfection, with tracked locations of use, a strict log of previous wearers, and cleaning diaries, always stored in the same box). It is possible that the microorganisms cultured were introduced from the storage environment – we did not swab the external components of the PAPR nor did we swab boxes, if used to store cleaned PAPR. However, it is unlikely that the storage environment would contaminate the inside of the air supply hoses. The clinical activities of those wearing the PAPR are unknown. Only the hoods used by ENT staff had any element of tracking and then only details of the wearer were recorded, not the patients seen or cared for, or procedures conducted whilst wearing the PAPR.

All hoods except one had been worn within 24 hours of testing but without adequate time logs, we were unable to ascertain how long; a longer time length of time since the last use may reduce the chance of successfully culturing bacteria and after 72 hours it is difficult to retrieve viral materials from hard surfaces. One hood assembly was last worn 6 days before swabbing (one of several, tracked, PAPR), and this grew a NSEM.

We examined 25 hoods and air supply hose assemblies, approximately 25% of our institution's circulating stock, and consider that this represented an adequate sample to test our hypothesis. PAPRs are an important component in the range of respiratory PPE available to healthcare staff in pandemics.[2,3,33,34] It is our opinion that, given the constraints outlined by both the manufacturer and the distributor, the hoods tested cannot be adequately disinfected for use in high risk healthcare environments and the systems may support fomite transmission; in the short-term this may be overcome by issuing HCWs with personal PAPR hoods and air supply hoses.

Urgent investment and research is required to develop more effective respirators that fit better, equipment designed for clinical environments that can be adequately disinfected, and comparative studies to identify optimal equipment, cleaning, disinfection, and storage. This work cannot succeed in isolation and industry, the manufacturers of respirators, must be engaged to collaborate with clinicians to develop PAPR systems that are fit for purpose in high risk healthcare settings.

CONCLUSIONS

This study demonstrates that disinfection guidelines in the UK during the COVID-19 pandemic may not be effective in disinfecting PAPR hood and air supply assemblies. Organisms that survive the disinfection process can persist for more than 5 days.

ETHICAL APPROVAL

Not required.

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