
Monograph:
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Investigating the dispersal of microbes in a hospital setting following hand drying using either paper towels or a jet air dryer

Final report

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Summary

- All surfaces and the volunteers' clothing/boding showed significantly higher contamination with bacteriophage following use of a jet air dryer (JD) compared with paper towels (PT).
- Of the 8 surfaces investigated following hand contact post hand drying, only 5 showed detectable levels bacteriophage after PT use, whereas all surfaces showed contamination after JD use.
- Direct and indirect transfer of bacteriophage from the volunteer's apron - used as measure of clothing/body contamination - was observed only following hand drying using a JD.

Introduction

Nosocomial pathogens can persist on inanimate surfaces and be acquired at a high rate by hands after contact with environmental surfaces (1, 2). Prevalence of bacterial pathogens on hands and inanimate surfaces varies according to environment and hygiene (2, 3), with persistence of different bacteria ranging from a few minutes to several hours on hands (3, 4) and up to several months on surfaces (1, 5, 6).

The process of hand drying is an integral step of hand hygiene (7), and is essential in minimising the risk of pathogen spread (8-10), particularly in healthcare environments (7, 11). Residual moisture in hands has been associated with increased microorganism transfer from hand to surfaces; in one study, moisture was reduced by 91% after 8 seconds of drying using paper towels (PT), whereas 10 seconds of use of an air dryer achieved a decline of only 12% (8).

Public washrooms/toilets are serviced by a variety of hand-drying methods, e.g. jet air dryer (JD), warm air dryer, and PT. A previous study examined the risk of environmental bacterial contamination in hospital toilets, including by antibiotic resistant bacteria, associated with different hand-drying methods (12). Results showed less droplet/microbe dispersion following hand drying with paper towels than with a JD. As a result, higher contamination of the floor, dryer unit and dust in JD toilets was observed compared with those using PT, and also a greater range of bacteria types being recovered from the former.

These observations show the impact of the hand-drying method on the risk of contamination of the washroom/toilet environment. Importantly, these differences could also potentially affect the spread of pathogens beyond the toilets, especially noting that in hospitals these are used by staff, visitors and patients. Notably, also, patients can be particularly susceptible to infection. The relative risks of pathogen spread beyond the hospital toilet according to the hand drying method used have yet to be clarified.

Bacteriophages have been used successfully as surrogates (13, 14) to measure microbe survival on hands and surfaces, the duration of transmission risk (15, 16) and environmental contamination (17). In particular, bacteriophage PR772 was previously used to investigate surface contamination in a healthcare setting (17), as it is innocuous to humans and the environment (14, 18). This study aimed to investigate whether microorganisms that remain present on hands and/or contaminate the user during hand drying in the toilet can transfer

beyond the washroom to hospital and near patient surfaces, using a bacteriophage as an indicator of microbial contamination.

Material and methods

Preparation of bacteriophage using a continuous culture bioreactor

Bacteriophage PR772 (BAA-769-B1) and host strain *Escherichia coli* K12 (BAA-769) were obtained from the American Type Culture Collection (ATCC) and prepared following ATCC recommendations (19). Briefly, freeze dried *E. coli* was rehydrated in 30 ml of tryptic soy broth (TSB) and grown overnight at 37 °C. The overnight culture was diluted 30-fold using fresh TSB and incubated for 4 additional hours, prior to inoculate 20 µl of PR772 stock. Following overnight incubation at 37°C with shaking, bacteria were pelleted (3750 rpm, 30 min, 4°C), the virus-containing supernatants were filtered using 0.22 µm filters and bacteriophage filtrate was enumerated using a plaque assay method as previously described (17, 18). The 10^7 pfu/ml filtrate was stored at 4°C.

The high volume of PR772, required for hand immersion, was prepared using a single stage chemostat model. The system consisted in a sterile 1 l glass vessel, containing ports for inlet and outlet airflow, media, sampling and waste. The chemostat was kept at 37°C, aerobically, with continuous stirring. The bioreactor was started with 1 l of brain heart infusion (BHI) broth, and temperature and airflow were allowed to normalise for 1 h, before adding 2 ml of an overnight culture of the *E. coli* strain K12. Five hours post bacteria incubation, 1 mL of 10^7 pfu/ml PR772 was inoculated into the vessel. The model was continuously fed with BHI broth at 0.9 h^{-1} (54 ml/h), and allowed to run overnight. The content of the vessel was centrifuged at 3750 rpm for 30 minutes to remove bacterial debris. The supernatant was filtered twice using bottle top filtration units, and enumerated using a plaque assay (17, 18). Filtrate was diluted to 10^7 pfu/ml of PR772 and 200 mL aliquots were kept at 4°C until use.

Surface sampling

Four healthy volunteers >18 years old took part in the study. Hand drying was performed in a toilet of the Leeds General Infirmary (LGI, UK) accessible through a main hospital entrance, used by hospital staff, visitors and patients. Volunteers had the option to wear nitrile gloves (StarLab, UK) or use their bare hands. Volunteers sanitized their hands/gloved hands with alcohol hand gel prior to immersion in ~200 ml of 10^7 PR772 filtrate. Hands were shaken thrice to remove excess liquid and dried using either PT or a JD (Airblade, Dyson, UK). As the study aims to represent the multiple individuals that use toilet facilities in hospital public areas, each volunteer was requested to dry the hands in a normal fashion. Each volunteer performed the assay twice, once drying their hands with PT and the other time after using the JD. Two volunteers did their first assay with PT, and the other two volunteers started with the JD. Sampling was spaced in a 5 week period.

Each volunteer wore an apron to enable measurement of body/clothing contamination during hand drying. Each volunteer's non-dominant hand (palm and finger tips) was sampled immediately after drying to measure (baseline levels of) hand contamination prior to environmental sampling. The volunteers then walked from the toilet on a pre-set route

that included different hospital public/clinical areas, and samples were collected from environmental surface sites following contact with either their dominant (still contaminated) hand, or apron. The surfaces sampled following hand contact included door handle (push and pull), stairs handrail, buttons (lift and ward access), ward phone, ward waiting room chairs, and stethoscope tubing. The volunteers touched the surfaces as they would for standard use. To investigate microbial transfer from clothing, the volunteers placed a stethoscope around their neck, as routinely practised by clinical staff, leaving the ends (chest piece and earpiece) in contact with the apron for ~7 min. Volunteers were also asked to cross their arms across their chest for 2 min, followed by resting them on the arms of a chair for 3 min. Each surface was swabbed with a 3M sponge-stick moistened with neutralising buffer (SLS, UK), and was disinfected with chlorine wipes pre- and post-sampling.

DNA extraction

Each sponge was processed on the same day of sampling by pipetting and transferring the buffer to sterile vials. DNA extraction was performed in duplicate from 1 ml of fluid, using the QIAamp UltraSens Virus kit (Qiagen, UK) as recommended by the manufacturer; with exception of the extended lysate centrifugation of 3200 x g for 10 min. DNA extraction of the PR772 stock used for hand immersion was also performed in duplicate. All DNA samples were quantified using a Nanodrop 2000c (Thermo Scientific, UK) and normalised to 5 ng/µl.

Real-time quantitative PCR (qPCR) assays

The genes P3 and P12 of bacteriophage PR772 were amplified using primer pairs previously validated for qPCR (Table 1). The PCR product was purified using the QIAquick PCR purification kit (Qiagen, UK), and dsDNA DNA was quantified using Qubit 2.0 fluorometer (Invitrogen, UK). DNA standard curves of viral DNA ranging from $5x10^9$ copies/µL to 500 copies/µL, in a 10-fold dilution series, were prepared as previously described (20). qPCR reactions containing final concentration of SYBR Green 1x Master Mix (Qiagen, UK), 0.6 µM primers (Table 1) and 25 ng of DNA template were prepared to a final volume of 20 µL. Reactions were analysed in a Rotor-gene Q (Qiagen, UK) using the following conditions: 95 ºC for 5 min, and 95 ºC for 10 s, 58 ºC for 15 s, 72 ºC for 20 s, repeated for 40 cycles. Standard curves were included on each qPCR plate in triplicate and used to convert threshold cycle values to copies per µL of template. Limit of detection was established at 500 copies. The same concentration of DNA template and DNA standard was used in each reaction. Each DNA extraction was run in duplicate for both genes. All samples from one surface (JD and PT) were analysed together on the same qPCR run.

Table 1. Primer sequences used for amplification of P3 and P12 gene of PR772 by standard and qPCR reactions.

Primer	Sequence	Amplicon size (bp)	Reference
P12 Forward	5'-AATCCACCTTTGGCGACTTC-3'	108	(14)
P12 Reverse	5'-CCAGTACCTTTGGCAGAATCAG-3'		
P3 Forward	5'-CCCATTAAGTACGGCGATGTTATG-3'	102	(14)
P3 Reverse	5'-GGCAAGCGGAACCCAATAG-3'		

Data analysis

The changes in bacterial counts were calculated based on logarithms of 16S rRNA gene copy numbers to achieve normal distribution. SPSS version 23 was used for analysis of log transformed data. Statistical significance was assessed using a two-sided Wilcoxon Signed Rank test for related samples, i.e. to compare samples within the same method; and using a two-sided Mann-Whitney U test for independent samples, i.e. to compare samples between JD and PT methods. Both tests were assessed using a 95% confidence interval; $p \leq 0.05$ was considered statistically significant.

Ethical approval

The recruitment of volunteers following informed consent was approved by the School of Medicine Research Ethics Committee, University of Leeds (reference MREC 18-094). Authorisation to perform the study in LGI was approved by the Leeds Teaching Hospitals NHS Trust Research & Innovation Department.

Results

Hand and body contamination following hand drying

One female and three male volunteers took part in the study, of which two are right-handed and the other two are left handed. Paper towel drying was performed for an average of 12s, using 3 to 5 towels, whereas jet air drying was performed for an average of 10s. Each sample collection period lasted for 73 min on average. Three volunteers chose to wear gloves, whereas one volunteer preferred to apply the phage directly on their hands.

There was no significant difference ($p = 0.668$) in bacteriophage recovery between assays performed with and without gloves (Fig. 1a). Similarly, bacteriophage dispersion and recovery was not significantly affected by the use of gloves when hand drying was performed with either JD or PT (Fig. 1B).

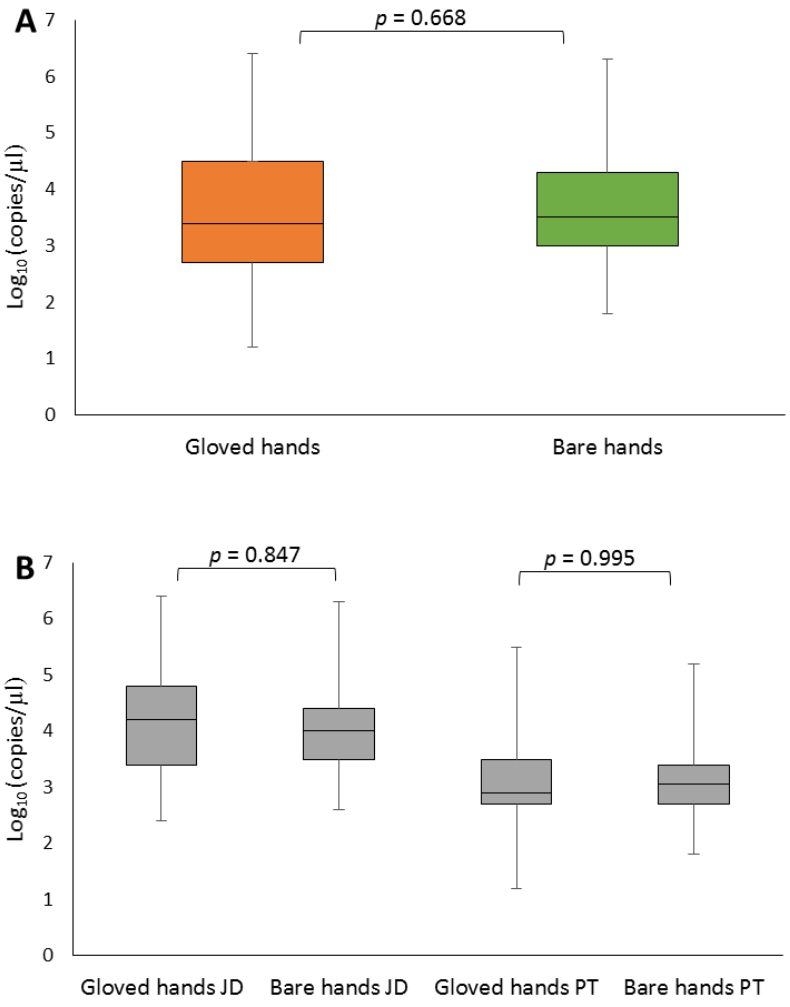

Fig. 1. Box plot in log_{10} (copies/µl) of gene P3 results from assays performed with and without gloves for a) all samples; b) each hand drying method used. Statistical analysis was performed using the Mann-Whitney U test.

qPCR results for environment/surface recovery of bacteriophage PR772 were similar for P3 and P12 genes, and so only the P3 gene results are discussed below (Table 2, and Fig.2-4). Results for the P12 gene are shown as supplementary data in Annex I (Table S1, and Fig. S1-S3). Furthermore, qPCR assays for gene P3 have a reported higher efficiency compared to gene P12 (98% vs 92%) (14).

Both JD and PT methods significantly (p<0.05) reduced bacteriophage contamination of the hands by 2 and 3 log_{10} copies/µl, respectively (Table 2 and Fig. 2). Apron (simulated trunk/clothing) contamination by bacteriophage during hand drying was significantly higher (p<0.05) after JD use. The levels of PR772 detected on the volunteers' hands at the end of the experiments suggested gross persistence of bacteriophage contamination throughout the sampling period (Fig. 2).

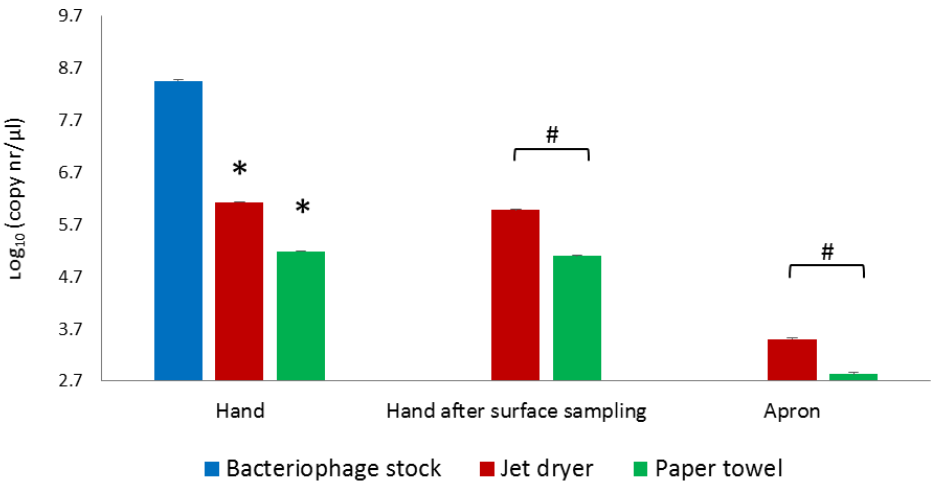

Fig. 2. qPCR results for detection of the gene P3 of bacteriophage PR772 from surfaces exposed to bacteriophage. * $p<0.05$ on the Wilcoxon Signed Rank; # $p<0.05$, Mann-Whitney U test.

Table 2. Mean values of the gene P3 of bacteriophage PR772 recovered from each environment/surface tested. Results are shown in copies/µl and as logarithms.

Sample	**P3 (copies/µl)**		**P3 (log_{10} copies/µl)**	
Bacteriophage stock	3.39×10^8		8.5	
	JD	**PT**	**JD**	**PT**
Hand (start of the experiment)	1.5×10^6	1.8×10^5	6.1	5.2
Door (pull)	4.7×10^4	6.1×10^2	4.5	2.6
Stairs handrail	3.6×10^4	3.5×10^3	4.5	3.4
Door (push)	6.3×10^4	3.4×10^3	4.5	3.3
Lift button	1.7×10^4	4.1×10^3	3.7	3.0
Chair	6.7×10^4	2.7×10^3	4.7	3.3
Phone receptor	6.7×10^3	6.1×10^2	3.7	2.6
Ward access button	2.2×10^3	3.5×10^2	3.1	2.4
Stethoscope tubing	1.1×10^4	7.6×10^2	3.8	2.8
Stethoscope (chest piece & headset)	3.0×10^3	6.92×10^2	3.4	2.7
Apron	6.1×10^3	1.2×10^3	3.5	2.8
Arm chair	1.1×10^3	5.2×10^2	2.9	2.6
Hand (end of the experiment)	1.1×10^6	1.4×10^5	6.0	5.1

Surface contamination following hand contact

Several environmental samples were collected from hospital public and ward areas, following contact with bacteriophage contaminated hands dried with either JD or PT. All

surfaces (n=8) investigated following JD use (100%) showed bacteriophage contamination above the limit of detection, whereas this occurred for only 5 surfaces (62.5%) when PT were used (Fig. 3). Average surface contamination following hand contact was >10-fold higher post JD versus PT use (4.1 $\log_{10}$ copies/µl and 2.9 $\log_{10}$ copies/µl, respectively). For all samples, there was a significantly (p<0.05) higher level of surface contamination following hand drying with the JD versus PT. Samples obtained from smaller surface areas, e.g. buttons, showed lower bacteriophage contamination (3.1 and 3.7 $\log_{10}$ copies/µl for JD; 2.4 and 3.0 $\log_{10}$ copies/µl for PT). Interestingly, simulated use of a hospital phone for 10 s resulted in detectable contamination only following JD use. Similar results were observed on the samples collected from the pull door handle.

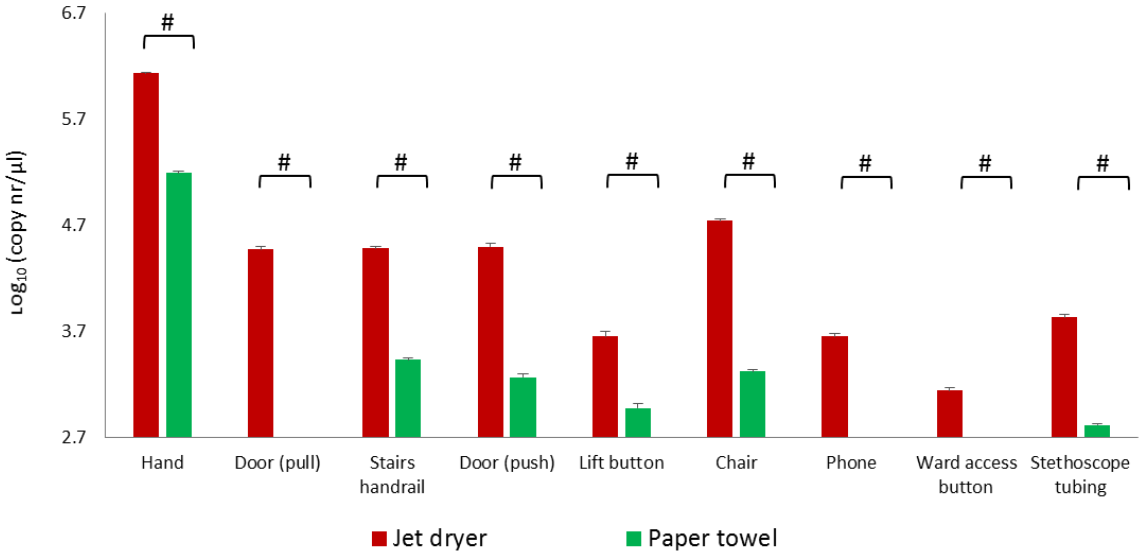

Fig. 3. qPCR results for detection of gene P3 of the bacteriophage PR772 from environmental samples following contact with contaminated hand. # p<0.05, Mann-Whitney U test.

Bacteriophage transfer from apron/clothing

Phage dispersal to the apron was 3.5 and 2.8 $\log_{10}$ copies/µl after use of JD and PT, respectively (Table 2). However, only surfaces samples following JD use had detectable transfer of bacteriophage from aprons, with an average value of 3.2 $\log_{10}$ copies/µl, likely reflecting the higher levels of apron phage contamination after this hand drying method. The stethoscope surfaces in contact with the apron showed a significantly (p<0.05) higher PR772 contamination after JD compared with PT use (Fig. 4). Bacteriophage contamination of the chair arm was detected only following JD use. A non-significant trend for increased chair arm contamination was observed for the P3 gene following JD versus PT use (p<0.076); a significantly higher level of contamination associated with JD use was observed for the P12 gene (Fig. S3). Chair arm samples were collected following indirect contact with the apron, i.e. after the volunteers' crossed arms contacted the apron and then touched the chair

arms. These observations suggest that both direct and indirect microbial transference from body/clothing is possible following user contamination during hand drying.

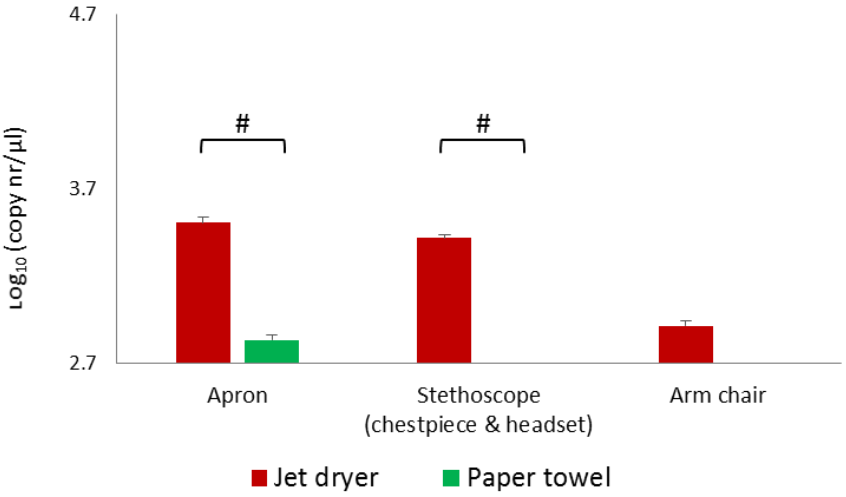

Fig. 4. qPCR results for detection of gene P3 of the bacteriophage PR772 from environmental samples obtained after contact with contaminated apron. # $p<0.05$, Mann-Whitney U test.

Discussion

Transmission of pathogens can occur via contaminated hands of patients or hospital workers (2, 3, 21-23). This is concerning given the prevalence of bacteria, including multidrug resistant pathogens, in healthcare settings (2, 22, 24, 25). Hand hygiene is therefore a key intervention for the prevention of healthcare-associated infections (2, 7, 24, 26). Hand drying is an integral part of this intervention, reducing the potential for transmission of infectious agents (7, 11), as bacteria transfer is more likely to occur from wet skin to other surfaces (8).

Choice of an appropriate hand drying method in hospital facilities is critical, as public areas are shared by staff, patients and visitors, creating a potential niche for transfer/acquisition of pathogens to/from surfaces including those in clinical areas (27). Previous studies compared bacterial dispersion (10, 28), user contamination (10), and environmental contamination in hospital toilets following JD or PT use (12). Overall, a higher rate and extent of dispersal of microbes to surfaces in the toilet/washroom and onto the user him/herself have been observed following JD compared with PT use.

In the present study, we investigated whether residual microbial contamination of hands and body following hand drying in toilets facilitates microbe dispersal beyond the washroom into hospital public and clinical areas. We used a chlorine sensitive bacteriophage (PR772) as surrogate for bacterial potential pathogens (14, 18), using qPCR to quantify the extent of contamination of different environmental surfaces, which could be expected to be touched after exiting the toilet/washroom. When enumerating bacteriophage levels, qPCR assays generally yield faster and more precise results than plaque based tests (29).

The recommended hand washing practices for healthcare workers (7, 11) are often not followed, with reported rates of staff adherence to hand hygiene guidelines under 6% (30, 31), and an average of 40% (7, 11), across multiple institutions. Furthermore, observational studies of hand washing in community toilets, have reported washing times between 5 and 9 seconds (32-34), far inferior to the 20 seconds recommended by the Centers for Disease Control and Prevention and the World Health Organisation (7, 11). Hand drying is therefore important to reduce the contamination remaining in hands following inadequate hand washing.

In this study, drying of hands that were still contaminated (as can frequently occur after washing) significantly reduced the microbial burden, with a significantly greater effect seen following PT compared with JD use. Other studies have reported PT to be more effective at removing water (8) and bacteria (28, 35, 36) from hands/washed hands compared with air drying methods. This is possibly related to the friction used during PT drying, which improves bacterial removal (35, 36). However, in all our assays bacteriophage was still present on the volunteers' hands when leaving the toilet. The level of contamination we measured at that stage was consistent with the 4 to 6 $\log_{10}$ colony forming units of bacteria that have been reported in the hands of healthcare workers following regular wash with plain or antimicrobial soap (3, 11, 37).

We found that bacteriophage dispersal across hospital surfaces was more frequently detected when hands were dried using the JD; all of the surfaces investigated were contaminated with bacteriophage, compared with 62.5% of surfaces following PT use. This suggests a higher potential for microbial spread through the hospital following JD use, which is concerning as objects and surfaces can serve as reservoirs for microorganisms that can be acquired via hand contact (1, 38, 39). We found bacteriophage contamination following JD in areas of frequent contact, such as door handles or waiting room chairs. In addition, the contamination of items that are in close contact with healthcare professionals and patients, such as phones or stethoscopes, is particularly concerning.

As before (10), we observed user body contamination after both hand drying methods, which was significantly greater following JD use. Importantly, bacteriophage contamination of users that was observed after JD use was directly and indirectly transferred onto surfaces; conversely, microbe transfer from the subject's apron/trunk/arms to environmental surfaces was not seen following PT hand drying. These observations likely reflect the increased risk of subject contamination during hand drying by a JD due to splattering (droplet/particle dispersal) (9).

Conclusion

Microbial contamination of the user's hands/trunk remaining or occurring during hand drying in the washroom/toilet, can result in microbe dissemination to multiple surfaces in the hospital environment via hand and clothing/skin contact. This phenomenon is significantly more likely to occur after hand drying with a JD as opposed to PT use. A fundamental principle of infection prevention practice is to minimise the potential for microbe dispersal. Thus, our findings question the use of hand drying by a JD in a hospital setting.

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Appendix I

Table S1. Mean values of the gene P12 of bacteriophage PR772 recovered from each environment/surface tested. Results are shown in copies/µl and as logarithms.

Sample	P12 (copies/µl)		P12 (log_{10} copies/µl)	
	JD	PT	JD	PT
Bacteriophage stock	1.31×10^9		9.0	
	JD	**PT**	**JD**	**PT**
Hand (start of the experiment)	8.2×10^5	1.1×10^5	5.8	4.9
Door (pull)	2.7×10^4	3.9×10^2	4.1	2.2
Stairs handrail	1.8×10^4	1.9×10^3	4.1	3.0
Door (push)	5.4×10^4	4.1×10^3	4.3	3.1
Lift button	1.3×10^4	4.2×10^3	3.6	2.8
Chair	4.4×10^4	3.2×10^3	4.5	3.3
Phone	5.9×10^3	2.7×10^2	3.6	2.3
Ward access button	2.4×10^3	2.8×10^2	3.1	2.4
Stethoscope tubing	3.3×10^3	5.0×10^2	3.3	2.4
Stethoscope (chest piece & headset)	4.3×10^3	6.0×10^2	3.1	2.6
Apron	7.7×10^3	3.1×10^3	3.7	3.3
Arm chair	3.6×10^3	2.6×10^3	3.4	2.5
Hand (end of the experiment)	5.6×10^5	4.4×10^4	5.7	4.6

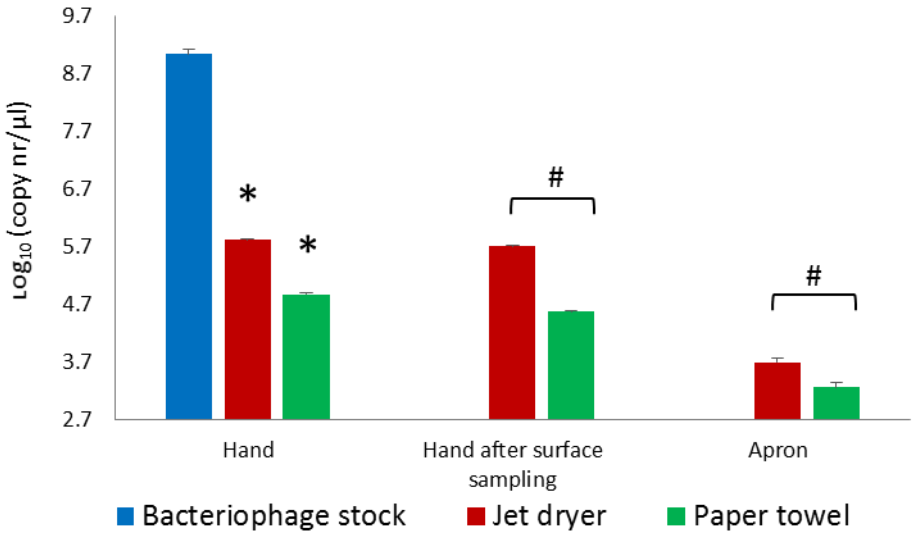

Fig. S1. qPCR results for detection of the gene P12 of bacteriophage PR772 from surfaces exposed to bacteriophage. * $p<0.05$ on the Wilcoxon Signed Rank; # $p<0.05$, Mann-Whitney U test.

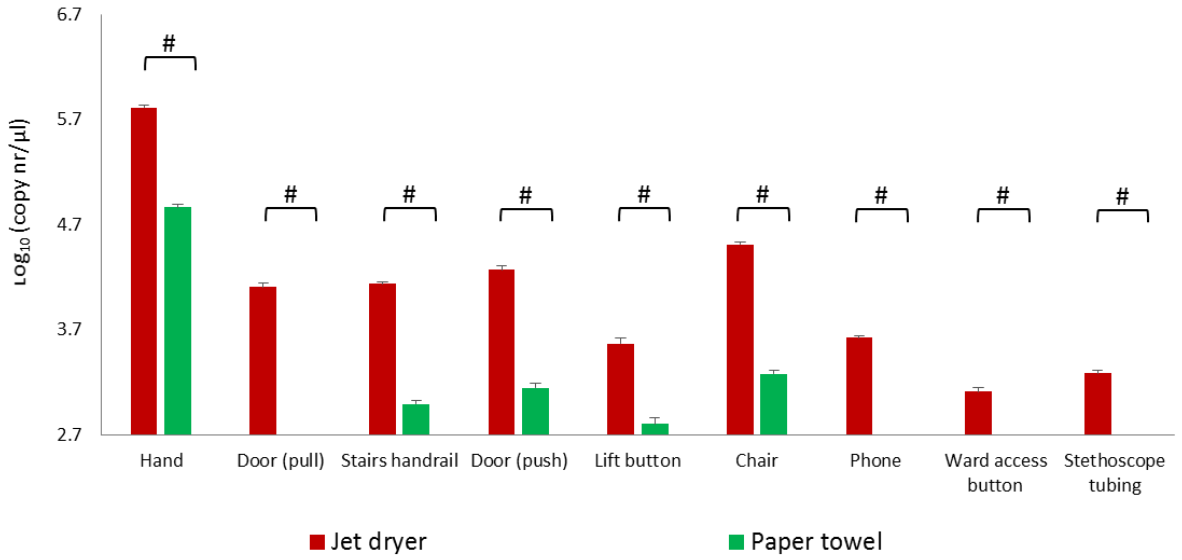

Fig. S2. qPCR results for detection of gene P12 of the bacteriophage PR772 from environmental samples following contact with contaminated hand. # p<0.05, Mann-Whitney U test

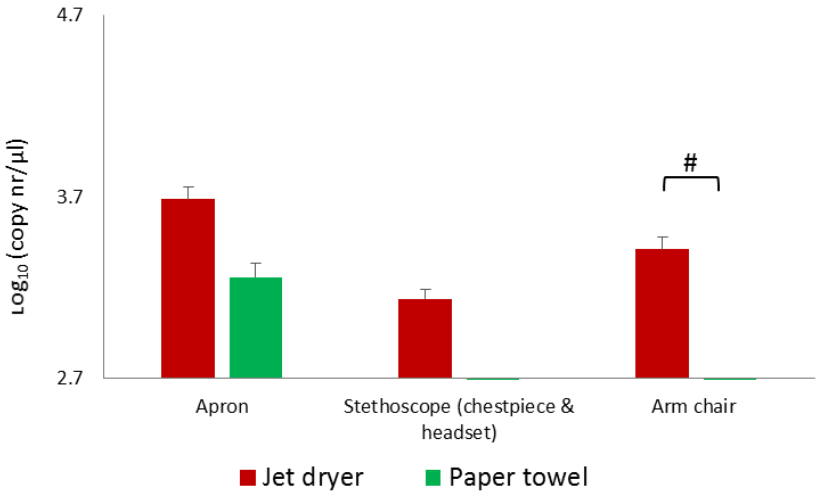

Fig. S3. qPCR results for detection of gene P12 of the bacteriophage PR772 from environmental samples obtained after contact with contaminated apron. # *p*<0.05, Mann-Whitney U test.