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Bacterial transfer to fingertips during sequential surface contacts with and without gloves

Marco-Felipe King[1] 🆔 | **Martín López-García**[2] 🆔 | **Kalanne P. Atedoghu**[1] | **Nan Zhang**[3] | **Amanda M. Wilson**[4] 🆔 | **Martijn Weterings**[5] | **Waseem Hiwar**[1] 🆔 | **Stephanie J. Dancer**[6,7] 🆔 | **Catherine J. Noakes**[1] 🆔 | **Louise A. Fletcher**[1] 🆔

[1]School of Civil Engineering, University of Leeds, Leeds, UK

[2]School of Mathematics, University of Leeds, Leeds, UK

[3]Department of Mechanical Engineering, The University of Hong Kong, Hong Kong, China, SAR

[4]Department of Community, Environment and Policy, Mel and Enid Zuckerman College of Public Health, University of Arizona, Tucson, Arizona

[5]Institute of Life Technologies, University of Applied Sciences and Arts Western, Sion, Valais-Wallis, Switzerland

[6]School of Applied Sciences, Edinburgh Napier University, Edinburgh, UK

[7]Department of Microbiology, Hairmyres Hospital, NHS Lanarkshire, Glasgow, UK

Correspondence
Marco-Felipe King, School of Civil Engineering, University of Leeds, Woodhouse Lane, Leeds LS29JT, UK.
Email: m.f.king@leeds.ac.uk

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Abstract

Bacterial transmission from contaminated surfaces via hand contact plays a critical role in disease spread. However, the fomite-to-finger transfer efficiency of microorganisms during multiple sequential surface contacts with and without gloves has not been formerly investigated. We measured the quantity of *Escherichia coli* on fingertips of participants after 1-8 sequential contacts with inoculated plastic coupons with and without nitrile gloves. A Bayesian approach was used to develop a mechanistic model of pathogen accretion to examine finger loading as a function of the difference between *E coli* on surfaces and fingers. We used the model to determine the coefficient of transfer efficiency (λ), and influence of swabbing efficiency and finger area. Results showed that λ for bare skin was higher (49%, 95% CI = 32%-72%) than for gloved hands (30%, CI = 17%-49%). Microbial load tended toward a dynamic equilibrium after four and six contacts for gloved hands and bare skin, respectively. Individual differences between volunteers' hands had a negligible effect compared with use of gloves ($P < .01$). Gloves reduced loading by 4.7% (CI = −12%-21%) over bare skin contacts, while 20% of participants accrued more microorganisms on gloved hands. This was due to poor fitting, which created a larger finger surface area than bare hands.

KEYWORDS

bacterial transmission, Bayesian model, *Escherichia coli*, hospital gloves, hospital surface contact, transfer efficiency

1 | INTRODUCTION

The transmission of infection has significant economic and societal implications, but quantifying or establishing how pathogens are transmitted is often challenging due to complex interactions between air and surfaces, variability in behaviors, and difficulty detecting viable pathogens in indoor environments.[1-3] The transmission of multidrug-resistant Gram-negative bacilli is a significant problem in UK and US hospitals, and from May 2018 to 2019, there were 24 879 *Escherichia coli* (*E coli*) bacteremia hospital-onset

cases.[4,5] With an average cost of £5239 ($6600 USD) in hospital care per case,[6] the prevention of hospital-acquired infections (HAIs) is a major priority for both the UK National Health Service and health organizations worldwide. While the transmission routes for these pathogens are still poorly understood,[7] it is thought that 20% of HAIs are spread through direct or indirect hand-to-mucosa contact.[8] Hands of healthcare workers provide a dynamic vector for the transfer of microbes from contaminated hospital surfaces to susceptible patients.[9,10] Although there is strong emphasis on hand hygiene, the role of indirect contact, surface, or fomite transmission through contaminated surfaces may be underestimated.[11] Since there is also a relationship between contact and airborne transmission, pathogens are known to deposit onto surfaces from the air and may then survive for many hours.[12-15]

The transfer efficiency (λ) between microorganisms on surfaces and hands is an important parameter for understanding infection transmission risk.[16,17] It is used in contact transmission models to predict contamination of hands and hence model the exposure of patients and healthcare workers to infectious pathogens. Values for λ are therefore derived from experimental studies and are known to vary with microorganism, surface material, use of gloves, and type of contact. In terms of relevant hospital-acquired pathogens, the transfer efficiency of Gram-negative bacteria such as *E coli* and *Acinetobacter baumannii* from dry non-porous fomites to fingertips has been reported to lie between 0.1% and 76%[18] under dry air conditions: 20%-40% relative humidity. Since experimental variability is so large, it is hard to say whether higher environmental humidity increases this transfer rate. However, for 40%-65% relative humidity, experiments have reported values between 0.3% and 100%

Practical Implications

- Transfer efficiency is an important parameter in contact infection transmission models.
- Approximate Bayesian computation is a flexible method for fitting a model to data.
- Swabbing efficiency was found to be highly significant in this experimental study, and needs to quantified in future experiments.
- Finger surface area is increased by gloves that do not fit well, and this encourages the acquisition of microorganisms.
- Gloves should be the correct size and fingers should fit snugly.
- Healthcare staff should be reminded about the need to change gloves between specific duties in accordance with local protocol.

for surfaces such as glass, stainless steel, ceramic, and granite with an average λ of 52% (σ = 19%) (see Table 1).[18,19] The use of nitrile gloves has been shown to reduce *Staphylococcus aureus* transfer to 38% (σ = 18%),[20] while no significant difference in transfer between Gram-positive and Gram-negative bacteria has been found without the use of gloves.[19] When adding an intermediary plastic or metal surface, bacteria are transferred between gloved hands with efficiencies ranging from 0.1% to 75% (mean = 37%, σ = 32%)[20]; applying a twisting shear stress seems also to increase transfer between

TABLE 1 Representative transfer efficiencies for fomite to hand (λ) in recent literature with similar experimental conditions (environmental relative humidity 40%-65%)

Organism	Surface	Gloves	Mean % (min-max)	Std. Dev. σ	References
E coli	Acrylic	None	53 (30-98)	28	Lopez et al[19]
	Glass	None	79 (38-100)	27	
	Ceramic tile	None	61 (4-100)	45	
	Laminate	None	27 (2-77)	30	
	Stainless steel	None	54 (24-99)	24	
	Granite	None	37 (0.3-100)	39	
	Stainless steel	None	85	5	Arinder et al[46]
	Plastic	None	12	9	Bartz et al[47]
M luteus	Stainless steel	None	40	–	Rusin et al[23]
	Plastic	None	42	–	
S aureus	Acrylic	None	47 (24-67)	18	Lopez et al[19]
	Glass	None	46 (26-66)	16	
	Ceramic tile	None	55 (28-78)	19	
	Laminate	None	62 (31-90)	25	
	Stainless steel	None	48 (17-86)	25	
	Stainless steel	Nitrile	51	16	Koenig et al[20]
	Plastic	Nitrile	25	10	

Note: Values are given as percentages.

surfaces by a factor of three.[21] After a third contact, transfer efficiency substantially reduces to 1.25% (σ = 0.9%)[21] and transfers to and from porous surfaces, including fabrics to hands, are often slightly lower (mean = 0.5%).[19,22] Although these percentages may seem small, putting them into a clinical context such differences could significantly alter patient outcome.

A simple glance at Table 1 might suggest that these data are normally distributed, but reporting of mean and standard deviations does not reveal a full picture of the experimental data set. Data sets represented solely by a mean and standard deviation are suggestive of normally distributed data; the criteria for which is that variance is finite with negative and positive values possible. However, when taking the range into account, which is strictly positive, we can see that the assumption of normality may not be the best representation of these data. As a result, the median and 95% confidence interval plus visual representation in the form of histograms or similar would be strongly advised going forward.

Although previous studies have investigated the transfer efficiency of various microorganisms during a single contact from fomite to finger[18,19,23] and finger to fomite,[23-25] only a small number of studies have considered more than one surface contact.[21,26] Repeated contact with a surface covered in fluorescent powder shows that skin became saturated after six contacts,[27] while a separate study using fluorescent particles found an equilibrium after five contacts.[21] Laboratory studies of bacterial finger loading from sequential surface contacts have not previously been undertaken; hence, it is not known whether this saturation happens for surfaces contaminated with bacteria. We hypothesize that a dynamic equilibrium may exist during bacterial transfer between fingers and surfaces with similar levels of contamination. This hypothesis is based on the difference between contamination levels on the surface and the skin (ie, the gradient) dictating the transfer of microorganisms in both directions.[26]

Escherichia coli, which has traditionally been used as a fecal indicator, has also been used as a model Gram-negative pathogen in previous transfer studies since it is safer for direct hand-to-surface contact in transfer studies than pathogens of interest and is still considered representative of several multidrug-resistant organisms such as Klebsiella spp., *Pseudomonas aeruginosa*, and Shigella spp.[19,23] Specific transmission parameters, such as transfer efficiencies from fomite to finger, are needed to measure the risk of pathogen transmission; this will enable the use of quantitative microbial risk assessment in diverse indoor environments, for example, hospitals, offices, and transportation.

The aim of this study is to quantify the transfer of *E coli* between plastic surfaces and fingers through sequential contacts and to determine how the wearing of nitrile gloves affects this transfer. An experimental study is carried out to measure contamination of fingers of volunteers following sequential contact with between 1 and 8 surfaces. Significant novelty is through fitting the data to a model using approximate Bayesian computation to assess experimental variability and to estimate values for the transfer efficiency for gloved and un-gloved hands.

2 | METHODOLOGY

In summary, participants were asked to touch a sequence of up to eight *E coli* inoculated plastic coupons using each of their fingers in turn (thumbs were controls) (see Figure 1). All participants took part in the investigation with and without nitrile gloves. Fingertips were sampled and swabs plated onto selective growth media for quantitative evaluation. A detailed method is laid out in what follows.

2.1 | Preparation of volunteer hands (n = 35)

Thirty-five volunteers participated in the study over a 7-week period: 21 males and 14 females aged 21-45 years from the University of Leeds. Their participation and experimental protocol were approved by the University of Leeds Research Ethics Committee (Reference: MEEC 17-021). Participants washed their hands with warm water and liquid soap for 30 seconds and then rinsed their hands with warm running tap water. Hands were dried using paper towels until visibly dry. Fingertip temperature and pH were recorded using a decontaminated thermometer (Boots) and litmus paper (Fisher Scientific). A control sample swab from both thumbs of the participant was taken before experiments commenced. During the gloved experiments, the participant donned single-use Bodyguards Finite P Indigo AF Nitrile Powder Free Examination Gloves MFNP100 (Polyco Healthline) while under observation by the researchers.

2.2 | Preparation of inoculum

A laboratory strain of *E coli* was prepared at the beginning of the experiment phase using a loopful of bacteria transferred to 100 mL of nutrient broth (Oxoid Ltd). This was incubated at 37°C for 18 hours with a 10-mL sample centrifuged for 30 minutes. The pellet was resuspended in 10 mL of 98% Ringer's solution (Oxoid Ltd) and 2% Tween-80 (Oxoid Ltd).[18] Serial dilution followed by culture on
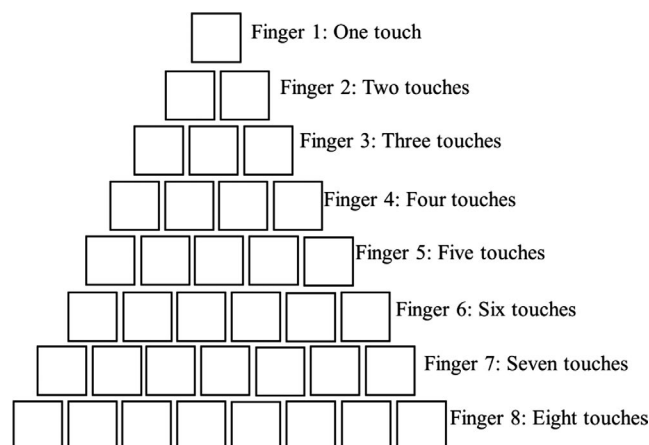

FIGURE 1 Schematic diagram showing the sequence for touching inoculated surfaces with fingertips

TABLE 2 Simulation parameter list

Parameter name	Symbol	Prior distribution	References
Swabbing efficiency (%)	s_{eff}	$N_{[0,50]}(22, 4)$	Moore et al[29]
Area of finger (cm^2)	A_f	$N_{[1,3]}(1.71, 0.34)$	Measured
Inoculum (CFU/mL)	$C^{(s)}$	$U(5 \times 10^7, 5 \times 10^9)$	Measured
Survival	d	$N_{[1 \times 10^{-3}, 1.5 \times 10^{-1}]}(8.6 \times 10^{-3}, 9.9 \times 10^{-3})$	Measured
Transfer efficiency (%)	λ_g	$N_{[0,100]}(27, 30)$	López et al[19]

Note: Key: $g \in \{G = Gloved, U = Un\text{-}gloved\}$. $N_{[a,b]}(\mu, \sigma)$ represents a normal distribution with mean μ and standard deviation σ, truncated between a and b.

tryptone bile X-glucuronide (TBX) (Oxoid Ltd) was used to approximate the concentration (~1.12 × 10^9 CFU/mL).

2.3 | Preparation of surface coupons

High-pressure laminate plastic (ELS Panels) was marked into 3 cm × 3 cm squares using an indelible marker. Since autoclaving damaged the surface properties, this was sterilized by submerging in 70% isopropyl alcohol, allowed to dry overnight in a fume cupboard, and screened for live organic bioburden using an adenosine triphosphate swab (Hygiena Int.) as a qualitative assurance. A 100-μL aliquot of culture solution was pipetted onto the marked coupons, spread out using a sterile spreader, and allowed to air-dry at 21°C ± 1°C and 48% ± 2% relative humidity for 60 minutes.

2.4 | Fingertip contacts and sampling

Coupons were flat on a laboratory bench and could not be moved by participants. Participants touched the inoculated coupons with one fingertip at a time at an interval of 1 second following the pattern in Figure 1. The first fingertip was used to touch one inoculated square surface, the second fingertip to touch two inoculated square surfaces, and so on, until the eighth fingertip touched eight inoculated square surfaces. Fingers were used in a pre-defined randomized manner (excluding thumbs) such that if, for example, the index finger of the right hand was used as finger 1 in one set of experiments, it might have been used as finger 5 in another volunteer's experiment set. Participants were trained to apply 50 g (±5 g) pressure for 1 second during each surface contact using a top-balance (sensitivity 0.002 g), which relates to a "light-touch".[28] Contact with the surface was standardized to a time of 1 second.

Immediately after a fingertip was used to touch the required number of inoculated coupons, the fingertip was sampled with a sweeping and rotating motion using a sterile cotton swab moistened with sterile Ringer's solution + Tween-80 solution. This was done to remove *E coli* transferred to the fingertip during surface contacts.[29] All samples were transferred to 10 mL Ringer's solution + Tween-80, shaken for 30 minutes at 36.6°C before being serially diluted (1 mL in 10 mL), and then, 0.1 mL was spread onto Petri dishes containing TBX agar. Plates were incubated for

24 hours at 37°C, and visible colonies were counted. The experiment was crossed as all participants performed un-gloved conditions followed by gloved conditions to avoid cross-contamination (so not counterbalanced); however, whichever finger they used to touch a surface was randomized à priori but maintained for both conditions.

2.5 | Statistical analysis

Statistical analysis of the experimental data was carried out using R (R project version 3.3.2) to investigate the effect of gloves, number of sequential contacts, and the participants' implicit differences in pressure and contact surface area on CFU loading. À priori sample size was estimated conservatively at 35 based on a medium effect size of 0.71.[18,23] Welch's two-tailed *t* test was used to assess statistical differences between groups as a preliminary measure.

A linear mixed-effects model[30] with $\log_{10}(CFU)$ as a dependent variable was applied using the *lme4 R* package (version 1.1-18-1)[31] to investigate the joint effect of gloves, contact number (fixed effect), and individual variability between participants (random slopes). Mixed-effects models account for shared variance within participants while modeling between-participant differences (see Appendix S1 for mathematical details).

Analysis of deviance using the Wald chi-square test was used to extract *P*-values for the model. Significant *P*-values were reported as $P < .05, .01, .005, .001, .0005,$ or $.0001$ or, if nonsignificant, as $P > .05$.

2.6 | Modeling of bacterial counts on fingers

Current microbial loading on fingers (C_n) after contact n can be described using a recurrent relation that is dependent on previous loading (C_{n-1}), surface loading ($C^{(s)}$), and a transfer efficiency parameter specific to that surface type and microorganism (λ).[19,23] Participant finger area was measured with a ruler: mean of 1.71 cm^2 and standard deviation 0.34 cm^2. But the exact contact area (A_f) is not known and hence must be estimated. Exact inoculate numbers on the surface ($C^{(s)}$) after die-off % (d) are not known ($C^{(s)} \cdot d$) either and so are estimated (see Table 2). A modified version of Julian

et al's equation[32] is given in Equation 1 to account for finger area and die-off. A subscript g was introduced to denominate whether the hands were gloved or un-gloved, creating two forms of the equation ($g \in \{G = Gloved, U = Un - gloved\}$).

$$c_{g,n} = c_{n-1} + \lambda_g \left[(C^{(s)} \cdot d) \cdot A_f - c_{g,n-1} \right] \qquad (1)$$

In this equation, transfer efficiency (λ) represents the ratio of the number of bacteria recovered from the finger after contact with the inoculated surface divided by the number of bacteria present on the surface during the contact. Transfer efficiency is notoriously complex to measure, however, and often thought to be underestimated due to variability in initial inoculum, bacterial inactivation rates, and swabbing efficiencies during the experiment.[23,33] The approximate Bayesian computation (ABC) method[34] was used to assess variability of measured and estimated variables. This method compares predictions of Equation 1 using a random sample of variable values against experimental data and ranks the closeness of the prediction through the calculation of the Euclidian distance (see Appendix S2). The vector of variables that produce the closest prediction is then chosen as "best." However, multiple combinations may be "best" and so this method allows us to evaluate these variabilities by selecting the variable combinations that yield the lowest 1% of Euclidian distances. A description of the algorithm is given in Appendix S2. The code was written in MATLAB (version 2017a) to fit Equation (1) to the experimental data while calibrating transfer efficiency that optimally represents the mechanism of sequential contacts. It also included the effect of variability in initial surface inoculum concentration ($C^{(s)}$), inactivation of bacteria during drying (d), and sampling efficiency using cotton swabs (S_{eff}).[18] Since swabs are known to underestimate the CFU count because bacteria are retained in the fibers,[35] we took this into consideration when comparing with the experimental data by artificially multiplying by a swabbing efficiency chosen from Table 2 for each case: $C_{g,n} \cdot S_{eff}$. We assumed that all other parameters were the same between cases, regardless of whether the participant wore gloves (eg, the surface inoculum did not vary just because the participant wore gloves, although we will later see that finger area might).

The CFU concentration in the initial inoculum on the surface ($C^{(s)}$) was represented by a uniform distribution between 5×10^7 CFU and 5×10^9 CFU per mL.[18] Survival of bacteria over the 60-minutes drying time ($d = 1 - die off$) was represented by a normal distribution with an arithmetic mean 99.14% and standard deviation of 0.99%,[36] which evaluated through separate laboratory experiments and were in keeping with current literature. It is assumed that bacteria were evenly distributed over the inoculated coupon surface area. All variable distributions were appropriately truncated to avoid negative values (see Table 2). An initial guess or prior guess of transfer efficiency is taken from the work by Lopez et al.[19] The parameters used in the ABC method are summarized in Table 2, and the ABC algorithm is described in Appendix S2.

3 | EXPERIMENTAL RESULTS

3.1 | Finger loadings

Figure 2 shows the comparison of $\log_{10}$CFU values with and without gloves for all participants. Each participant's un-gloved and gloved results are plotted together to show the distribution of loading among all participants (labeled 1-35). Overall, a mean decrease of 0.19 $\log_{10}$ CFU (equivalent to a mean of 4.7%, confidence interval = −12% to 21%) is found for gloved contacts compared with the un-gloved touch, which is statistically significant ($P < .001$). However, it must be noted that 8 out of the 35 participants did accrue higher CFU counts with gloves than without after 1 contact, while 17% showed no detectable difference.

3.2 | Effect of gloves

A comparison plot of *E coli* loading on fingertips for both gloved and un-gloved hands after sequential contacts is shown in Figure 3. Pairwise comparison of gloved and un-gloved loadings reveals a statistically significantly difference for all contact counts ($P < .005$). Following one contact, 44% (CI = 42%-47%) more accretion occurs on bare skin than on the gloved finger (Welch's $t = 2.4$, $P < .05$). This difference increases to a maximum of 50% (CI = 33%-66%) at five contacts. On average, gloves reduce CFU loading by 4.7% (CI = −12% to 21%). The linear mixed-effects model allows a more complex analysis taking into account the non-aggregated data set. It confirms that gloves had a significant effect on lowering CFU loadings: 4.1 $\log_{10}$ CFU (95% CI = 4.0-4.2 $\log_{10}$ CFU) vs 4.4 $\log_{10}$ CFU (CI = 4.3-4.4 $\log_{10}$ CFU) for un-gloved contacts, with Welch's t test supporting this conclusion $t = 4.61$, $P < .0001$. The effect of number of contacts on loading is comparatively low, contributing on average 0.05 $\log_{10}$ per extra contact.

3.3 | Effect of individual participant variability

Examining data from individual participants, correlation between gloved and un-gloved burdens is significant ($P < .01$), meaning that if a participant acquired a large microbial burden when gloved, then they were also likely to exhibit a high CFU burden when un-gloved. However, the individual random effect of the participant is modest in comparison with the spread within the gloved and un-gloved experiments. Variance contributed by the participant when gloved was twice as high (0.08 $\log_{10}$ CFU) than when un-gloved (0.04 $\log_{10}$ CFU), whereas variance between participants during different contacts counts was comparatively small (0.0007 $\log_{10}$ CFU). After hand washing, participants' hand pH values ranged from 5.0 to 7.0 while hand temperatures ranged from 29 to 39°C, median = 33°C. No statistically significant effect on transfer efficiency was found relating to hand pH ($X_1^2 = 0.75$, $P > .05$) nor hand temperature ($X_1^2 = 0.42$, $P > .05$). Overall, no significant interaction ($t = 0.68$, $P > .05$) was
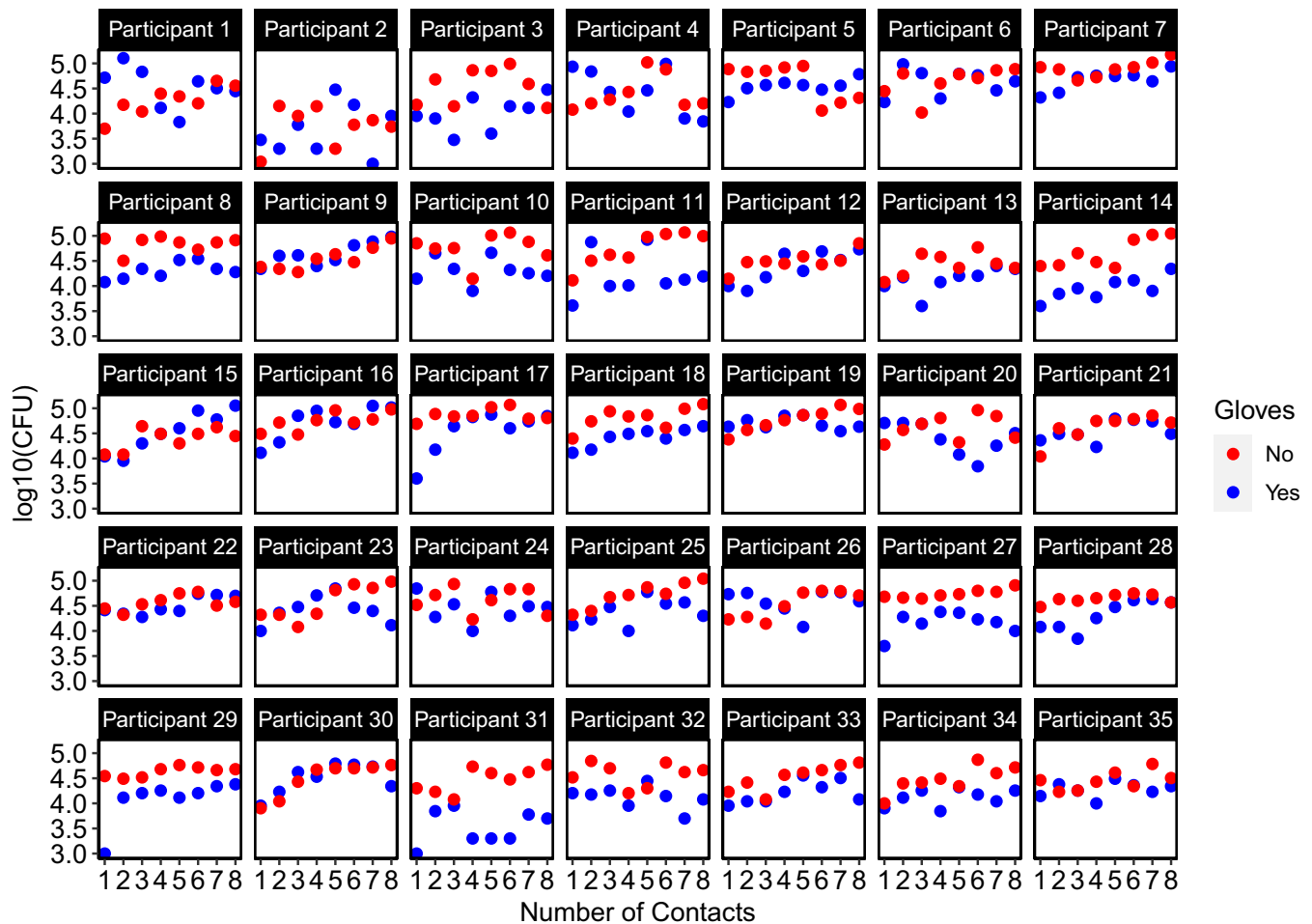
FIGURE 2 Plots of $\log_{10}$CFU for gloved and un-gloved experiments separated by participant
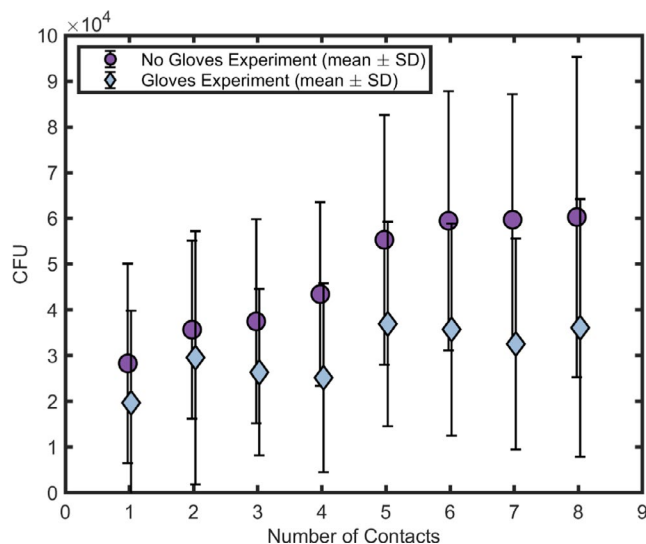

FIGURE 3 Mean of measured *E coli* retrieved from fingertips after each sequential surface contact. Error bars show standard deviations. N = 35 participants

found between the use of gloves and the number of times surfaces were touched, meaning that the effect of wearing gloves does not appear to have a multiplicative protective effect on CFU loadings as

contact count increases. Variance in loadings between participants, for which the model does not account, was found to be 0.06 $\log_{10}$ CFU. As a result, the effect of gloves dominated other factors, but a small but still significant portion of the results may be dictated by unobserved or latent variables.

3.4 | Effect of sequential contacts

Oldham's method[37] was used to calculate the correlation between fingertip CFU counts from the first contact (baseline) vs CFU counts after n contacts. This suggests that the amount of *E coli* accrued during the first contact has a statistically significant effect on loadings up to, and including, contact number 4 ($P < .001$). Figure 4 shows boxplots of percentage differences of CFU between subsequent contacts ($(c_{g,n} - c_{g,n-1})/c_{g,n-1} \times 100$), where n represents the contact number and g is either gloved or un-gloved. The median magnitude loading of *E coli* on fingers decreases steadily for gloved fingers from contacts 1 to 4 but does not do so monotonically for un-gloved hands. By contact 4 for gloved fingers, there is on average 19% less *E coli* on fingers than during contact 3. This trend continues apart from contact 4 to 5, where a reversal can be seen, suggesting that bacteria are readily deposited back to the surface from gloved fingers
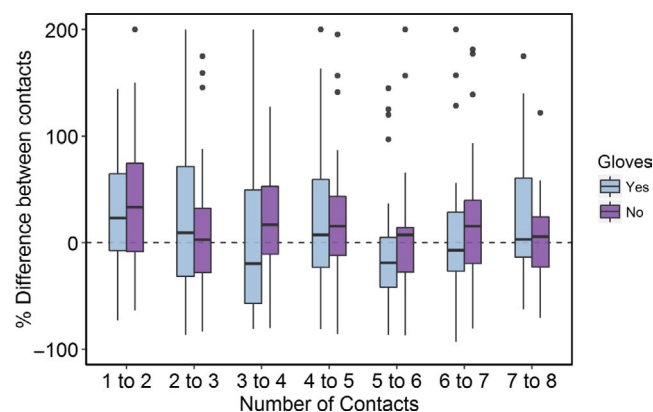
FIGURE 4 Percentage loading difference between subsequent surface contacts. Whiskers represent 1.5 × 25th and 75th percentiles, respectively. N = 35 participants
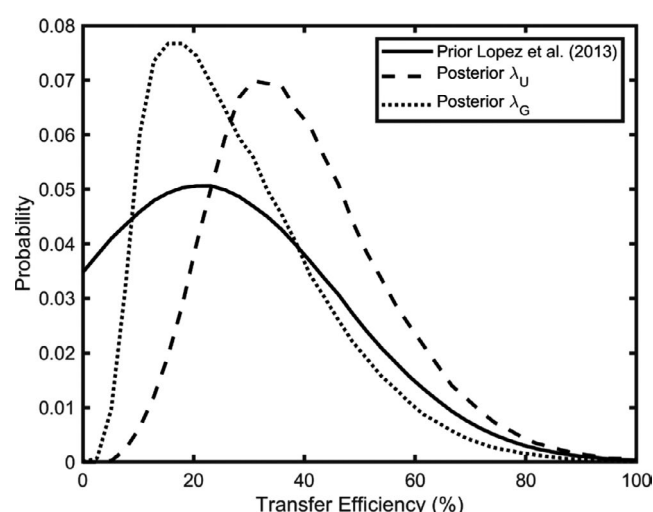

FIGURE 5 Histograms of the prior distribution of λ from Lopez et al[19] and the estimated posterior distributions of λ_G and λ_U by ABC

and a dynamic equilibrium may then exist. There is a statistically significant jump from 4 to 5 contacts in the gloved case (*t*-value = 2.31, *P* < .05) that is similar to the initial % increase but which does not exist in the un-gloved cases until contact 6 (*t*-value = 0.36, *P* > .05). Significant levels of stochasticity or loading variance increase after the first contact as can be seen by the whiskers in Figure 4, which on average are larger for un-gloved hands by 16%.

3.5 | ABC modeling results

3.5.1 | Predicting transfer efficiency (%) using the ABC method

The ABC algorithm was used to compute transfer efficiency using Equation 1 instead of the linear mixed-effects model, which does not account for vagaries of pathogen transfer mechanisms. Density histograms of predicted transfer efficiencies (λ_G and λ_U), which most closely represent the experimental CFU for all

contacts, are plotted in Figure 5. These are used to estimate mean, standard deviation, and 95% CI for gloved and un-gloved contacts and are presented in Table 3. The prior experimental distribution of transfer efficiencies collated from Lopez et al's[19] experimental data used as an initial guess or prior distribution is laid under the posterior predictions.[16] We note that the gloved transfer efficiency tends to be lower than that reported in Table 2 and that the un-gloved version is higher.

Table 3 shows the mean, standard deviation, and 95% confidence intervals of transfer efficiencies as calculated using the ABC method. A statistically significant difference exists between both estimates (*P* < .0001).

The results of the ABC algorithm lead to the posterior bivariate diagrams of Figure 6 that shows the relationship between independent variables $CFU^{(s)}, A_f, d, S_{eff}$ against transfer efficiency (λ_U). Red dotted lines indicate prior means for the independent variables from either experimental data or literature. The ABC algorithm is able to learn about each parameter individually, and little correlation between parameters is visible. We can also see that the prior mean values are not far away from the posterior predictions, but in all cases, a distribution of other "good" or valid combinations is also given.

Finger loading predictions were then plotted using the newly calibrated parameter set ($\lambda_g, c^{(s)}, A_f, d, S_{eff}$) for 8 surface contacts using the model in Equation 1 (see Figure 7). Standard deviations were also included of the 1% "best" predictions to highlight the effect of parameter combinations. All predictions for both cases pass through the error bars of the experimental data, highlighting that the model can represent both mean and variability of the experiment. All un-gloved predictions are within one standard deviation of the experimental mean, whereas the gloved predictions are captured within the 95% confidence interval range. Quantitatively, we note that this represents an average of 11% relative error (min 4%, max 23%) between prediction for the un-gloved cases and a 28% error for the gloved case (min 14%, max 50%).

4 | DISCUSSION

Between participants, variation appears to be modest in comparison with the effect of a glove barrier, meaning that contact surfaces area, roughness, skin temperature, pH (in un-gloved tests), and contact pressure are less relevant during glove use. A possible explanation for higher transfer rates with un-gloved hands may be the

TABLE 3 Transfer efficiency (%) statistics for un-gloved and gloved contacts

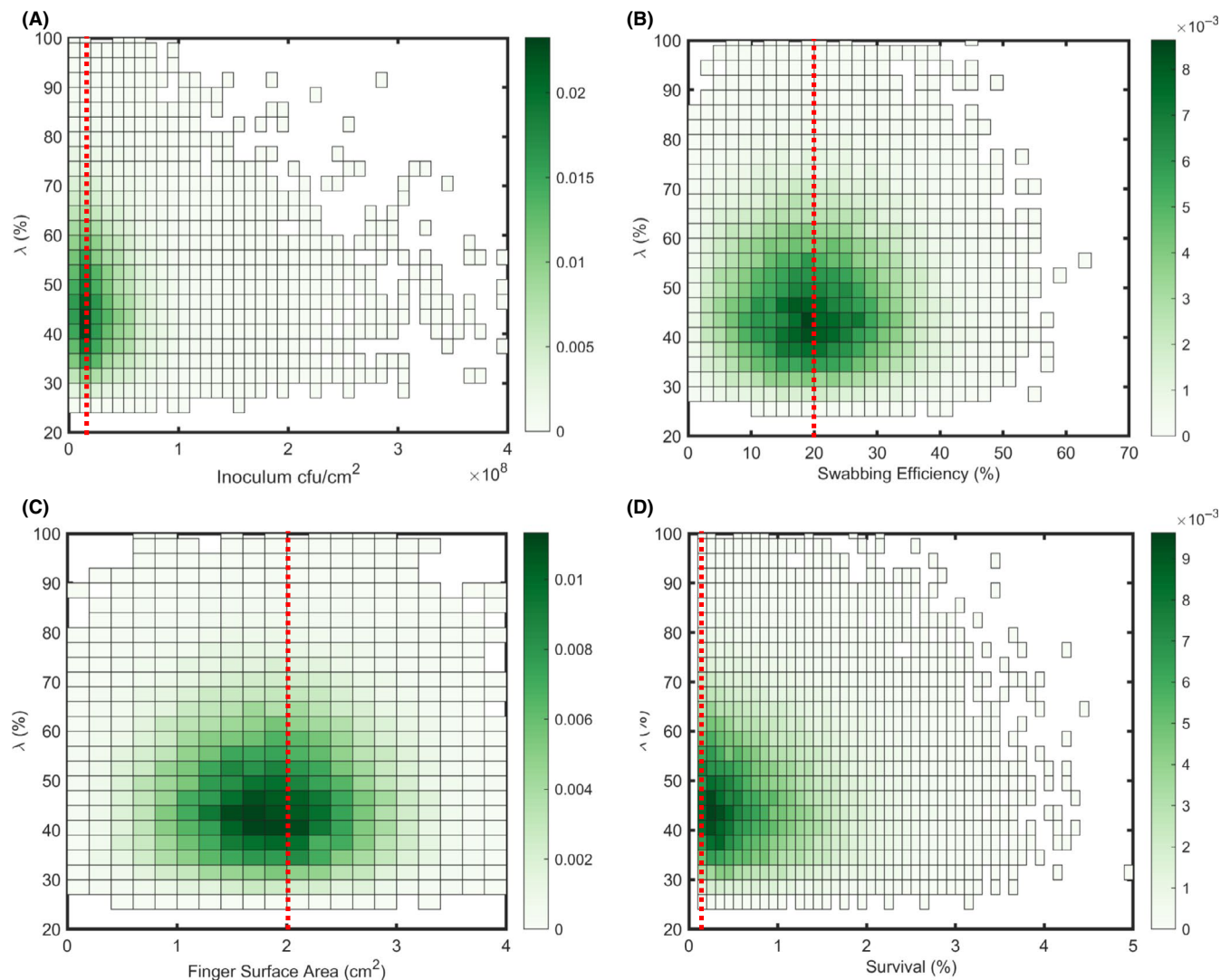|	Un-gloved λ_U (%)	Gloved λ_G (%)
Mean	49	30
St. dev.	12	10
95% confidence interval	32-72	17-49

FIGURE 6 Bivariate histograms of independent variables on transfer efficiency prediction for un-gloved hands. Top left - Inoculum vs \lambda, Top-right - Swabbing efficiency vs \lambda, Bottom left - Finger surface area vs \lambda and Bottom right- Survival vs \lambda. Red lines indicate prior means

hydrophilic properties of *E coli*, where the organism may be attracted to the moisture on hands.[38] While it is uncertain if un-gloved hands show higher loadings because microorganisms are trapped in the skin crevices, creating a higher surface area compared with gloved hands, the skin surface may have reached an intrinsic maximum carrying capacity and cannot physically accrue any more microorganisms.[39] We also note from our own unpublished pressure data from a separate experiment that participants tend to apply less pressure when not wearing gloves, making these findings potentially "best-case" scenarios.

Nevertheless, gloves generally afford a consistency in loadings, with a dynamic equilibrium appearing at four contacts. A similar trend was seen for un-gloved hands, although equilibrium was seen after six contacts. This value may shift depending on surface type or porosity and the difference in CFU values between finger and surface.[27] The highest stochasticity is observed from the first contact, as shown in Figure 4, leading to the conclusion that contact with a

single contaminated object such as a door handle may have greater variability than repeated contacts, say with a soiled computer keyboard. This reinforces that gloves should be removed after patient care and hands thoroughly washed. While gloves often became less contaminated, they facilitate transfer from fingers to surfaces more readily than un-gloved hands. Although an average of 5% difference between CFU glove loadings and un-gloved fingers may appear small, this could be clinically significant when dealing with pathogens with tiny infectious doses.

It is important to recognize that this laboratory study measured transfer under idealized conditions that possibly differ from real surface contacts in a hospital or other indoor environments including pressure, sheer force, or other ways people manipulate objects. The current analysis does not include quantification of asymmetric (bidirectional) transfer from fomite to fingertip and vice versa as sampling is destructive; however, it has been found previously that transfer from skin to fomite is often substantially smaller in
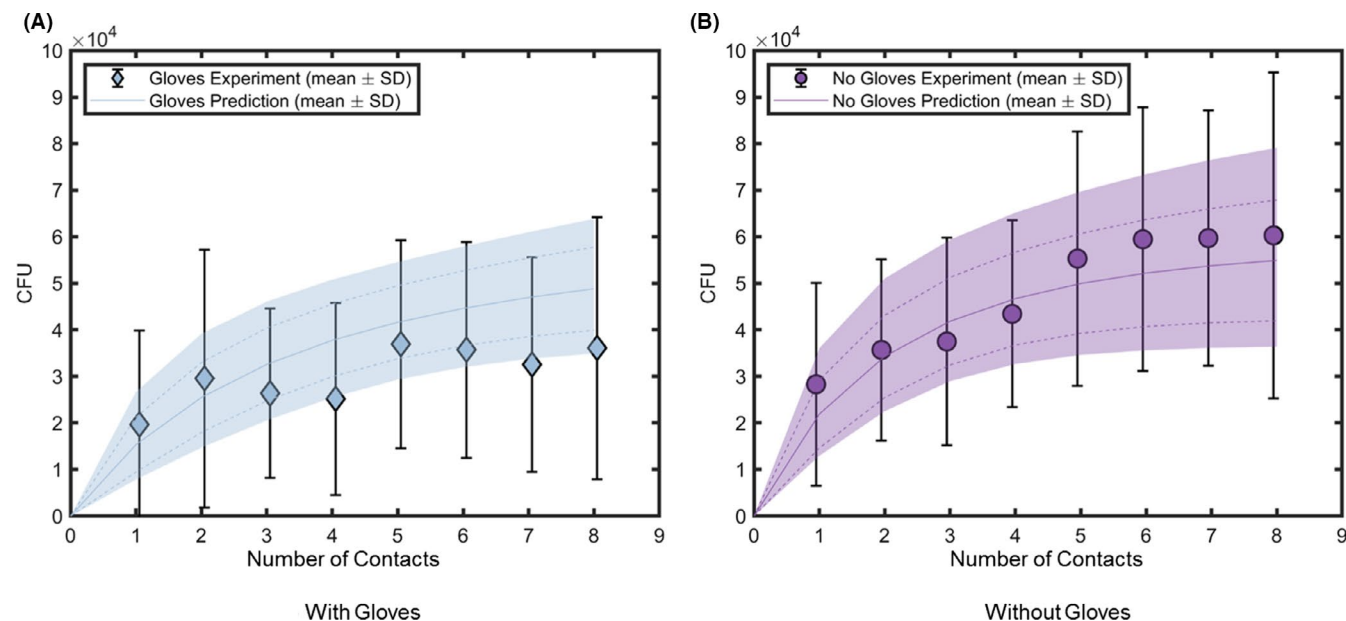
FIGURE 7 Predicted bacterial finger loadings using Equation 1 within the ABC algorithm with experimental data for (A) gloves and (B) without gloves. Shaded area represents 95% confidence intervals. N = 35 participants

magnitude, although not negligible.[19,23,33] Transfer efficiency is likely to also be related to inoculum quantity or more specifically to the difference in CFU counts between fomite and finger.[25] Percentage transfer of microorganisms appeared to decrease linearly as inoculum concentration increased, while the concentration gradient between surface and finger dictates the transfer efficiency.[40] Although Oldham's method shows that the effect disappears after multiple contacts, this hypothesis is still visible in Figure 4. The study utilizes an inoculum concentration range of ~5×10^7 to 5×10^9/mL, which could be representative of a sample of human fluid waste,[40] but is likely to be higher than that found on most surfaces in healthcare environments. Although variability is quantified through the ABC simulation process, latent variables may remain hard to quantify or unmeasured. Laboratory temperature and humidity conditions are quantitatively similar to experiments by Lopez et al[19] who found similar transfer efficiencies, but may not fully reflect surfaces in hospital patient rooms due to the lack of biofilm or other microorganism communities. Greene et al[18] systematically found lower transfer efficiencies (24% for un-gloved hands) suggesting either that surfaces were completely dry, that swabbing efficiency was overestimated, that bacterial inactivation was underestimated, or that initial inoculum was actually lower than anticipated. As a result, the ABC method allows all of these variables to be considered simultaneously and a posterior distribution to be given for transfer efficiency. Surfaces, in our experiment, were also deliberately inoculated to have a relatively uniform concentration on each surface, but some coagulation of fluid was still noticeable in the center of the coupon after an hour. Since similar conditions were used by Lopez et al,[19] it stands to reason that this would have happened in that study, also explaining their similar transfer efficiency rates.

The data enabled evaluation of the statistical properties of CFU loading on fingertips and transfer efficiency for a multiple sequential

contacts, and statistical[18,19,23] or risk analysis models[32,33,41] often consider CFU transfer from surface to fingertips to be normally distributed simply because data or distribution parameters are not readily available. We note that the current data show that CFU burdens on fingers after surface contacts are not normally distributed between groups nor within groups (Shapiro-Wilks test $P < .001$), nor are their variances equal between groups (heteroscedasticity).[18,23,42] Instead, they appear more like a lognormal distribution (based on a Box-Cox transformation with $\gamma = 0.8$). As a result, analysis of variance (ANOVA) or paired t tests without adjustment for unequal variances can produce invalid results leading to inaccurate recommendations and therefore should be used with caution. At first glance, it may be tempting to apply a repeated-measures ANOVA model to aggregated data. However, this aggregation confounds the random slope variance with residual error and reduces the error degrees of freedom. Although conditional independence is then met, the aggregation process precludes simultaneous generalization over participants and predictors[22] and so should equally be avoided. The assumption of conditional normality may also artificially inflate mean transfer efficiencies and reduce the likelihood of extreme transfer rates being observed.[16,17] Histograms are recommended to accompany experimental data which can allow the reader greater confidence in interpreting results. Additionally, the effect of swabbing method efficiency cannot be ruled out despite the reported normal distribution characteristics in the general literature.[29] In future, further granularity could be sought through the consideration of swabbing efficiency differences between surface or material types, as surface type has been shown to affect swabbing efficiency.[43] This would require experimental trials to investigate swabbing efficiency differences between gloved and un-gloved hands. Finally, even 35 participants produce a modest effect size, meaning that conservative power calculations should be conducted before experiments given the lack of normality.

CFU loadings on gloved hands were significantly lower on average for all contacts regardless of adjustment for the initial quantities accrued. This suggests that differences between gloved and un-gloved hands could have important implications. During an observational study conducted at a Welsh hospital,[44] healthcare workers performed four (σ = 3.4) surface contacts on average more with gloves than without (σ = 6.7). Additionally, direct patient contacts in the same study were 20% higher with gloves than without. It is not surprising then that one might expect gloves to afford consistently lower infection transmission risk. We note that 23% of participants actually accrued more pathogens on their gloved fingers than without in this current study. From visual recordings by the experimentalist, this was due to their hands being between glove sizes, that is, wearing gloves that were too large for their hands. While gloves are already well recognized as an important barrier for preventing pathogen transmission from healthcare workers hands to patients, this also has an implication for catering and food industries. The results suggest better fitting gloves are likely to become less contaminated during use if the correct size is worn.

5 | CONCLUSION

This study presents an investigation into the loading of viable *E coli* on fingers following sequential contacts with a contaminated plastic surface with gloved and un-gloved hands. Transfer efficiency with a smooth plastic surface for both gloved and un-gloved hands was higher than current literature suggests. By quantifying CFU on fingertips following 1-8 sequential contacts with inoculated surfaces, the following conclusions were drawn:

1. Gloves show lower burden only 80% of the time, but on average, burden on gloves is 5% lower than un-gloved hands. The individual effect on CFU loading from variation in participant variability is modest in comparison with the use of gloves. Therefore, choosing the correct glove size to avoid excess fabric accruing pathogens is critical in ensuring lower risk.

2. CFU loading reaches equilibrium on both gloved and un-gloved hands. The mean CFU burden on gloves peaks at five contacts compared with six for un-gloved hands before stabilizing through either equal transfer (up and down) or failed transfer from surface to finger. While in this study we demonstrate that less drastic changes in concentration occur after only 4 contacts, every contact with a surface could be the first exposure to a pathogen, making hand hygiene and the use of gloves important continued practices. Gloves showed decreasing loads on fingers during sequential contacts suggestive of transfer from finger to surface and should not be used for multiple patient care or cleaning episodes.

3. The ABC method provides a novel, accessible, and flexible method for parameter estimation. Transfer efficiency that best represents this experimental data set, estimated using the ABC model, was higher with bare skin (49%, 95% confidence interval

CI = 32%-72%) than gloved hands (30%, CI = 17%-49%), highlighting high variability arising from latent variables.

4. Repeated-measures ANOVA should be avoided for computing the crossed random effects of individual participants, and a linear mixed-effects model should be used instead.

5. Parameter estimation has a significant effect on simulated transfer efficiency. CFU loadings from surface to fingertip transfer are not a Gaussian distribution, and hence, care should be taken in using data from surface contact studies in infection risk assessments. An incorrect assumption about the distribution of the data may lead to inappropriate recommendations or interpretations especially after modeling multiple surface contacts where artificial inflation of the central tendency might occur.

The study adds significant weight to the methodological framework for data analysis in the indirect infection transmission arena by providing an approach to determine the transfer efficiency and uncertainty of parameters that affect it. It is conceivable that the future of this modeling will be used to investigate the effect of other environmental parameters such as hospital room layout[16] to predict the effects that perturbations of models can have in global pathogen accretion dynamics.

From a clinical perspective, the findings suggest that the use of nitrile gloves may afford more consistent bacterial loading characteristics when in contact with plastic laminate surfaces, which provide both lower and more predictable infection transmission risk. This upholds UK national recommendations for glove use during patient care, especially when fluids are involved[45]; however, the fit of the gloves was shown to have an important influence on the potential for bacterial contamination especially when in 23% of the cases, a higher loading was found. Glove fit is therefore of crucial importance.

CONFLICT OF INTEREST
None to declare.

AUTHOR CONTRIBUTION
Marco-Felipe King: Conceptualization (lead); Data curation (supporting); Formal analysis (lead); Funding acquisition (equal); Investigation (equal); Methodology (equal); Project administration (equal); Resources (equal); Software (equal); Supervision (lead); Writing-original draft (lead); Writing-review & editing (lead). **Martín**

López-García: Data curation (equal); Formal analysis (equal); Funding acquisition (supporting); Investigation (equal); Resources (equal); Software (equal); Writing-review & editing (equal). **Kalanne Princess Atedoghu:** Data curation (lead); Methodology (equal); Writing-review & editing (supporting). **Amanda Marie Wilson:** Writing-review & editing (equal). **Nan Zhang:** Investigation (equal); Writing-review & editing (equal). **Martijn Weterings:** Formal analysis (supporting); Methodology (supporting); Writing-review & editing (supporting). **Waseem Hiwar:** Data curation (supporting); Writing-review & editing (supporting). **Stephanie Dancer:** Funding acquisition (equal); Writing-original draft (equal); Writing-review & editing (equal). **Catherine Noakes:** Funding acquisition (lead); Writing-original draft (equal); Writing-review & editing (equal). **Louise Fletcher:** Conceptualization (equal); Funding acquisition (equal); Writing-review & editing (supporting).

ORCID

Marco-Felipe King https://orcid.org/0000-0001-7010-476X
Martín López-García https://orcid.org/0000-0003-3833-8595
Amanda M. Wilson https://orcid.org/0000-0003-3259-8169
Waseem Hiwar https://orcid.org/0000-0003-3742-3456
Stephanie J. Dancer https://orcid.org/0000-0002-8777-2614
Catherine J. Noakes https://orcid.org/0000-0003-3084-7467
Louise A. Fletcher https://orcid.org/0000-0001-7755-8561

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.