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New sequential-touch method to determine bacterial contact transfer rate from finger to surface

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bacterial contact transfer, culture in situ, exponential decay, fomite, sequential touch.

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Abstract

Aims: Infection can occur via surface touch. A new method was developed to more accurately evaluate the bacterial transfer rate from a finger to a surface.
Methods and Results: *Staphylococcus aureus* was used as the model bacteria to inoculate a thumb. Sequential touches were made between the contaminated thumb and a series of clean microscope slides. The bacteria on the glass surface were cultured in situ, and the bacterial transfer rate was evaluated by fitting the colony-forming units (CFUs) on glass surfaces with the exponential decay function. The resident microflora on thumb were also used to validate the new method.
Conclusions: The average transfer rate was 12·9% for *S. aureus* under the baseline condition. The CFUs counted on the glass surfaces were well fitted by the exponential decay function. A set of trials with more surfaces resulted in a more accurate evaluation. No statistically significant difference was found in the estimated transfer rate between the standard strain and the resident microflora.
Significance and Impact of the Study: The new method significantly increases the accuracy of evaluation of the microbial transfer rate from a finger to a surface while maintaining a low workload.

Introduction

Surface microbial transfer occurs between human hands and surfaces through both direct and indirect contact (Barker *et al.* 2001; Nicas and Jones 2009; Julian 2011; Lopez 2013). It can cause high-risk infection in public places (Thornley *et al.* 2011; Meadow *et al.* 2014; Zhang *et al.* 2016; Xiao *et al.* 2018) and in confined spaces (Rheinbaben *et al.* 2000; Rowlands *et al.* 2014). Micro-organisms transfer to and from a solid surface when the surface is touched (Kotwal and Cannon 2014; Malcolm *et al.* 2017; Tamrakar *et al.* 2017). Some researchers have studied the spread of disease via fomite surfaces by quantifying the transmission of bacteria and/or viruses between the hands and various surfaces (Boone and Gerba 2007; Julian *et al.* 2010; Larocque *et al.* 2016).

The proportion of the target bacteria that transfer during a touch event is quantified by the bacterial transfer rate: the ratio of the number of bacteria transferred to the recipient surface to the original number of bacteria within the contact area on the donor surface (Mbithi *et al.* 1992; Sattar *et al.* 2001; Dawson *et al.* 2007; Verhaelen *et al.* 2013). In previous studies of the bacterial transfer rate (Mackintosh and Hoffman 1984; Chen *et al.* 2001; Rusin *et al.* 2002; Harrison *et al.* 2003; Lopez *et al.* 2013; Bhoonderowa *et al.* 2014), either the hand or the surface was inoculated with the targeted bacteria; a single touch was then performed between the hand and the surface, followed by a series of procedures that included hand and surface sampling and bacterial incubation.

Large deviations are generally found in the evaluated bacterial transfer rate (Koenig *et al.* 2016; Miranda and Schaffner 2016). Several factors can influence bacterial transfer during a single touch, including the type of surface (Rusin *et al.* 2002), the environmental conditions (Lopez *et al.* 2013) and the contact pressure (Mbithi

et al. 1992). Some factors are difficult to control, including the surface wetness (Sharps *et al.* 2012) and the specific gesture used while touching (Sharps *et al.* 2012). Averaging across replicates of the bacterial transfer tests enables a more accurate evaluation of the transfer rate, but at the cost of a heavier workload.

The surface bacteria sampling process also introduces errors in the evaluation of the bacterial transfer rate (Rose *et al.* 2004). Various sampling methods have been applied to quantify surface bacteria, including swabbing (Rusin *et al.* 2002; Lopez *et al.* 2013), eluting with eluent (Lingaas and Fagernes 2009; Brar and Danyluk 2013) and rubbing in a medium (Bellissimo-Rodrigues *et al.* 2017). Each method is useful in specific appropriate situations, but they generally lack sufficient efficiency and reliability in collecting surface bacteria. For example one frequently used method, surface sampling by swabbing (Rusin *et al.* 2002; Lopez *et al.* 2013), can introduce significant errors in both absorbing and irrigating bacteria (Rose *et al.* 2004; Ahnrud *et al.* 2018).

This study presents a new method to accurately quantify bacterial transfer from a finger to a solid surface. Surface sampling was simplified by direct culturing of the bacteria in situ. Touches were performed on a number of surfaces. The transfer rate was determined by fitting the series of colony-forming units (CFUs) on the surfaces with an established function. The goodness of fit was analysed to validate the new method, and the thumb's resident microflora were also used directly as the targeted bacteria to investigate the transfer rate.

Materials and methods

Ethic approval

Permission was obtained from the Human Research Ethics Committee, The University of Hong Kong (Ethic Approval number: EA1603004). In this study, all procedures were carried out in accordance with the relevant guidelines and regulations, as introduced below.

Materials

Staphylococcus aureus obtained from ATCC 25923 (HKM, Guangdong, China), and the resident microflora on thumb were also used to investigate bacterial contact transfer. Biosafety level 1 was required for use of the model bacteria. Microscope slides (ISOLAB Laborgeräte GmbH) were used as the sample surface, and sterile plastic petri dishes (ISOLAB Laborgeräte GmbH) were used to hold the sterile slides. Lysogeny broth (LB) and 1·5% LB agar (Hangzhou BAISI Biotechnology Co, Ltd, Zhejiang, China) were used to culture and count the target bacteria on the touched glass surfaces.

Sterilization

The microscope slides and LB media were sterilized as previously described (Sattar *et al.* 2001). Briefly, the slides were carefully washed, and liquid LB and LB agar were prepared separately in conical flasks and sterilized over 20 min in an autoclave above 121°C. Using sterile forceps, the slides were then dried on an alcohol lamp and transferred to a petri dish after cooling. The LB agar in a conical flask was put into a 45°C water bath to keep it warm for use.

Handwashing

The skin of the hands is heavily colonized by bacteria, at a density of more than 10^4 CFU·per cm^2 (Boyce and Pittet 2002; Fierer *et al.* 2008). The researcher's hands were washed following the WHO guidelines for handwashing (page 14) (Boyce *et al.* 2009): hands were wetted and lathered with a non-antimicrobial liquid soap, scrubbed for 20–30 s, rinsed with clean running water and finally dried with an air dryer. The researcher's hands were also washed after the experiment, following the same procedure.

For the experiment using *S. aureus*, the hands were repeatedly washed following the same procedure. For the experiment in which the resident microflora on the thumb were used, a single session of handwashing was sufficient, and the quantity of remaining bacteria was found to be appropriate for observation of contact transfer.

Bacterial inoculation

For transfer of *S. aureus*, the external standard bacterial strain was inoculated on the thumb as the donor. A suspension of *S. aureus* at a concentration of 10^5–10^6 CFU·per ml obtained via overnight culture in liquid LB medium at 37°C was used for surface inoculation (Sattar *et al.* 2001; Lopez *et al.* 2013). The researcher's washed left thumb was inoculated with the *S. aureus* suspension with the following procedure. A 2-μl suspension of undiluted (or a 10-fold dilution) as the high (or low) concentration was dropped on the thumb and spread over an area of approximately 1 cm^2 with the pipette tip, resulting in an inoculated thumb with 200–2000 CFU as the inoculation of high concentration and 20–200 CFU as the inoculation of low concentration. The inoculated thumb was then allowed to visibly dry before the contact experiments began.

Surface touch

The surface touch experiments were conducted in a biosafety cabinet (Figure 1a). The touch experiment was performed by a single adult researcher. The petri dish with the slide was placed on an electronic scale. The contaminated thumb was pressed against the slide surface and held for 10 s after the load force reached 800 g (Pancic *et al.* 1980; Ansari *et al.* 1988; Mbithi *et al.* 1992; Rusin *et al.* 2002; Lopez *et al.* 2013; Stals *et al.* 2013). The touch was performed between the thumb and 30 slides in sequence as a set of sequential-touch trials. An untouched slide in a culture dish was also included in each set of trials as the blank control. An error of ±40 g for each single touch was acceptable. If this limit was exceeded, all samples in the sequential-touch trial were discarded. At this pressure, the contact area between the thumb and the glass surface was 3·6 cm^2; therefore, the pressure was approximately 0·2–0·3 kg cm^{-2}, which is consistent with values reported in previous studies (Chen *et al.* 2001; Rusin *et al.* 2002; Paulson 2005; Lingaas and Fagernes 2009; Julian *et al.* 2010; Stals *et al.* 2013; Greene *et al.* 2015).

Two groups of studies were conducted. First, two concentrations of *S. aureus* were used as the model bacteria for inoculation on the repeatedly washed thumb, and three sets of trials were performed with each concentration. Second, the thumb's resident microflora were directly applied for six sets of trials. The temperature in the laboratory was maintained at 22°C ± 1°C with a relative humidity of 65–75%.

Bacterial incubation and quantification

The bacteria on the slide were cultured in situ by directly covering with a layer of LB agar, in reference to the methods of contact plate (Scott and Bloomfield 1990; Gomez *et al.* 2012) and bacterial growth on surface (Yamada *et al.* 2010; Nazemi *et al.* 2018). The cultured CFUs on each slide were counted as the number of bacteria transferred during the surface touch. The touched slides were covered with 45°C liquid LB agar within 10 min after the touches, as shown in Fig. 1b. After the LB agar solidified, the petri dish was inverted and stored in an incubator at 37°C, and the CFUs were counted after 4 days of culture (Fig. 1c).

Calculation of bacterial contact transfer rate

Bacterial transfer in a single touch

During each touch between a bacteria-contaminated thumb and a clean surface, a proportion of the bacteria could transfer (Hori and Matsumoto 2010). The bacterial transfer rate was calculated with Equation 1.

$$\tau = \frac{\Delta C}{C} \quad (1)$$

where τ is the transfer rate in a single touch, ΔC the number of bacteria transferred during the touch and C the initial number of bacteria on the thumb before the touching experiment.

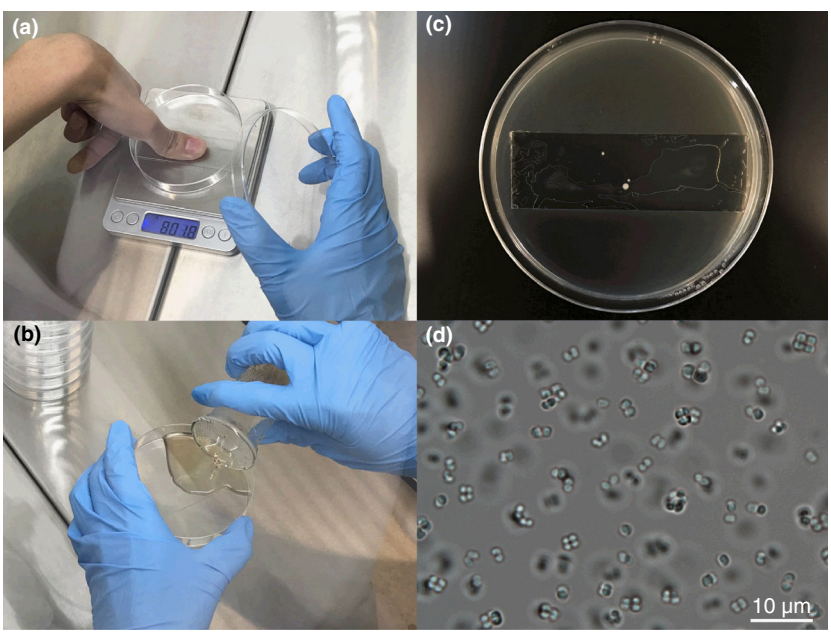

Figure 1 Test procedure for bacterial transfer from thumb to glass surface. (a) Inoculated thumb is pressed on a clean slide inside a sterile petri dish. Pressure is controlled by placing the slide and dish on an electronic scale. (b) Warm liquid LB agar is poured into the petri dish, covering the touched glass slide. (c) Bacterial colonies after 4 days of growth under LB agar. (d) Optical microscopic view of sample of resident microflora from thumb. [Colour figure can be viewed at wile yonlinelibrary.com]

Bacterial transfer in sequential touches

Before any differences were identified between bacterial individuals, we found it reasonable to assume that with each touch, each bacterial individual (or aggregation thereof) on the thumb had the same probability of being transferred to the glass surface. The expected transfer rate of all bacteria on the thumb was equal to the transfer probability of each bacterial individual. Because the physical parameters were kept constant during the surface touches, the bacterial transfer rate was considered to be constant during the sequential touches.

If a thumb was used to touch N slides in sequence, the number of bacteria transferred to each slide surface was set as ΔC_1, ΔC_2, …, ΔC_N. Equation 1 suggests that, after the first touch, the number of bacteria remaining on the thumb was $C-\Delta C_1 = C-\tau C = (1-\tau)C$. It could also be inferred that after the second touch, it became $C-\Delta C_1-\Delta C_2 = C-\tau C-\tau[(1-\tau)C] = (1-\tau)^2 C$. As such, after the N-th touch, it was $C-\Delta C_1-\Delta C_2-\ \dots\ -\Delta C_N = (1-\tau)^N C$. A general form is shown in Equation 2.

$$\sum_{i=1}^{N}\Delta C_i = \left[1-(1-\tau)^N\right]C \qquad (2)$$

where $\sum_{i=1}^{N}\Delta C_i$ is the total number of bacteria transferred to N surfaces during the sequential touches.

Equation 2 shows that as the number of touches (N) approaches infinity, the total number of transferred bacteria ($\sum_{i=1}^{N}\Delta C_i$) approaches the initial number of bacteria on the donor surface (C).

Bacterial transfer during the n-th touch

According to Equation 2, the number of transferred bacteria (ΔC_n, $1 \le n \le N$) during the nth touch can be derived as shown in Equation 3.

$$\Delta C_n = \sum_{i=1}^{n}\Delta C_i - \sum_{i=1}^{n-1}\Delta C_i = \left[\tau(1-\tau)^{n-1}\right]C \qquad (3)$$

A relationship was constructed to connect three parameters: the initial number of bacteria on the donor thumb (C), the bacterial transfer rate (τ) and the number of bacteria transferred during each touch in a set of trials (ΔC_1, ΔC_2, …, ΔC_N). The number of bacteria transferred with each touch (i.e. the left-hand side in Equation 3) exponentially decayed, with the same mechanism as in radioactivity (Martin 2006) and heat and mass transfer (Bergman *et al.* 2011).

Following the logic of previous studies, the transfer rate τ was directly obtained by ΔC and C, as in Equation 1. If the initial number of bacteria on the donor thumb (C) was unknown or difficult to measure, the sequential-touch method could be used. Following Equation 3, we set $N = 2$ and used the thumb to make identical touches on a pair of surfaces to obtain two sets of solutions for (i, ΔC_i), that is (1, ΔC_1) and (2, ΔC_2). τ and C could then be solved simultaneously. To increase the accuracy of evaluating τ, N could be set larger than 2. An array of ΔC (ΔC_1, ΔC_2, … , ΔC_N) was then obtained, and the values of τ could be evaluated by fitting the data with the formula introduced below.

Method for evaluating τ and C

Combined with Equation 2, the parameter C was removed from Equation 3 to reconstruct the relationship between the number of a touch (n) and the number of bacteria transferred with the nth touch (ΔC_n) during the sequential touches, namely $\Delta C_n = f_\tau(n)$, as an exponential decay function with only one coefficient, τ, as shown in Equation 4. The optimal solution for the coefficient, the transfer rate (τ), was then determined with the least-squares method. As such, the initial number of bacteria on the donor thumb (C) could be calculated using Equation 2.

$$\Delta C_n = \left[\tau(1-\tau)^{n-1}\right]\cdot\left[\frac{\sum_{i=1}^{N}\Delta C_i}{1-(1-\tau)^N}\right] = \frac{\tau(1-\tau)^{n-1}}{1-(1-\tau)^N}\left(\Delta C_1+\Delta C_2+\cdots+\Delta C_N\right) \qquad (4)$$

Equation 4 suggests that the transfer rate (τ) for bacterial transfer from a thumb to N surfaces could be calculated indirectly by fitting the series of CFUs on the N surfaces, namely ΔC_n ($1 \le n \le N$).

Statistical analyses

Origin 8·6 was used for data analysis and for generation of figures. Student's independent-samples t-test was performed to determine whether a statistically significant difference was present in the transfer rate between the different concentrations of *S. aureus* and between the use of *S. aureus* and the resident microflora.

Results

Transfer of *S. aureus* from thumb to glass slide

Two concentrations of *S. aureus* were used for transfer from a thumb to glass surfaces, as shown in Fig. 2a–f. (The data are listed in Table S1). In each trial set, the number of CFUs counted on the touched glass slides showed a decreasing trend as the sequence of contact progressed. Via data fitting, transfer rates of 11·4, 12·8 and 21·0% were obtained with the lower *S. aureus* concentration and rates of 11·7, 12·1 and 8·59% were obtained with the higher *S. aureus* concentration. No statistically significant difference

was observed in the transfer rate between the two concentrations of *S. aureus* ($P = 0{\cdot}252$). The average transfer rate for the six sets of trials was 12·9%, with a small standard deviation (SD; 3·84%). The adjusted R^2-square (adj. R^2) values in the six sets of trials ranged from 0·371 to 0·809 (mean, 0·563), which indicates a good data fit for evaluation of the transfer rate.

Different numbers of touches and CFUs

The reliability of the new method was analysed in terms of the number of touches used during a set of trials and the number of CFUs counted on the series of touched surfaces. The SD of the evaluated transfer rate and the adj. R^2 were used as parameters to reflect the goodness of data fitting to allow comparisons between different numbers of touches and different numbers of CFUs (Fig. 3).

The different amounts of data captured from the six arrays of 30 CFU values in Fig. 2 were used for fitting. The use of more CFU values for data fitting, representing more touches, resulted in smaller deviations in the evaluated transfer rate (Fig. 3a) and larger adj. R^2 values in data fitting (Fig. 3b). The deviations in the evaluated transfer rate decreased as the number of sequential touches increased. An obvious decrease in the deviation and an increase in the adj. R^2 values were observed as the number of touches increased from 20 to 30.

The total number of CFUs on the series of touched slides showed little influence on the transfer rate evaluation, but significant deviations were observed in the evaluated transfer rate (Fig. 3c) and unreliable data fitting were observed in the evaluation (Fig. 3d) if fewer than five CFUs were counted for the whole series of touched slides.

Transfer of resident microflora

The thumb's resident microflora were directly applied as the targeted bacteria for contact transfer (Fig. 4). Via data

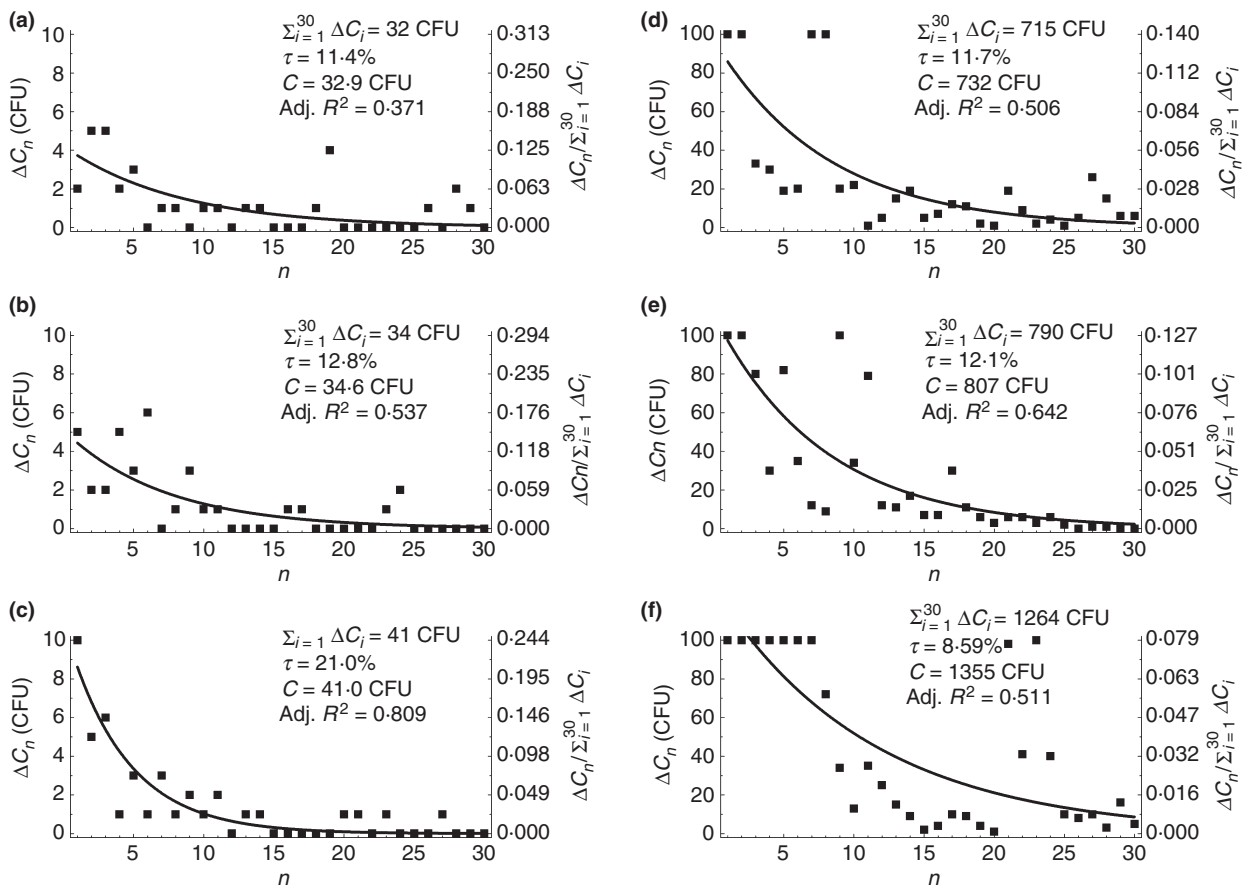

Figure 2 Transfer of *S. aureus* during sequential touches between a contaminated thumb and 30 clean slides. Six sets of trials were performed with inoculated *S. aureus* at low (a-c) and high (d-f) concentrations. For each trial, the CFUs counted on each slide are shown as square points, and the 30 CFU values fitted with Equation 4 are shown as fitting curves. The evaluated transfer rate, the evaluated initial number of bacteria on the donor thumb and the adj. R^2 value based on data fitting are listed for each set of trials. (CFUs counted on each touched slide are listed in Table S1.)

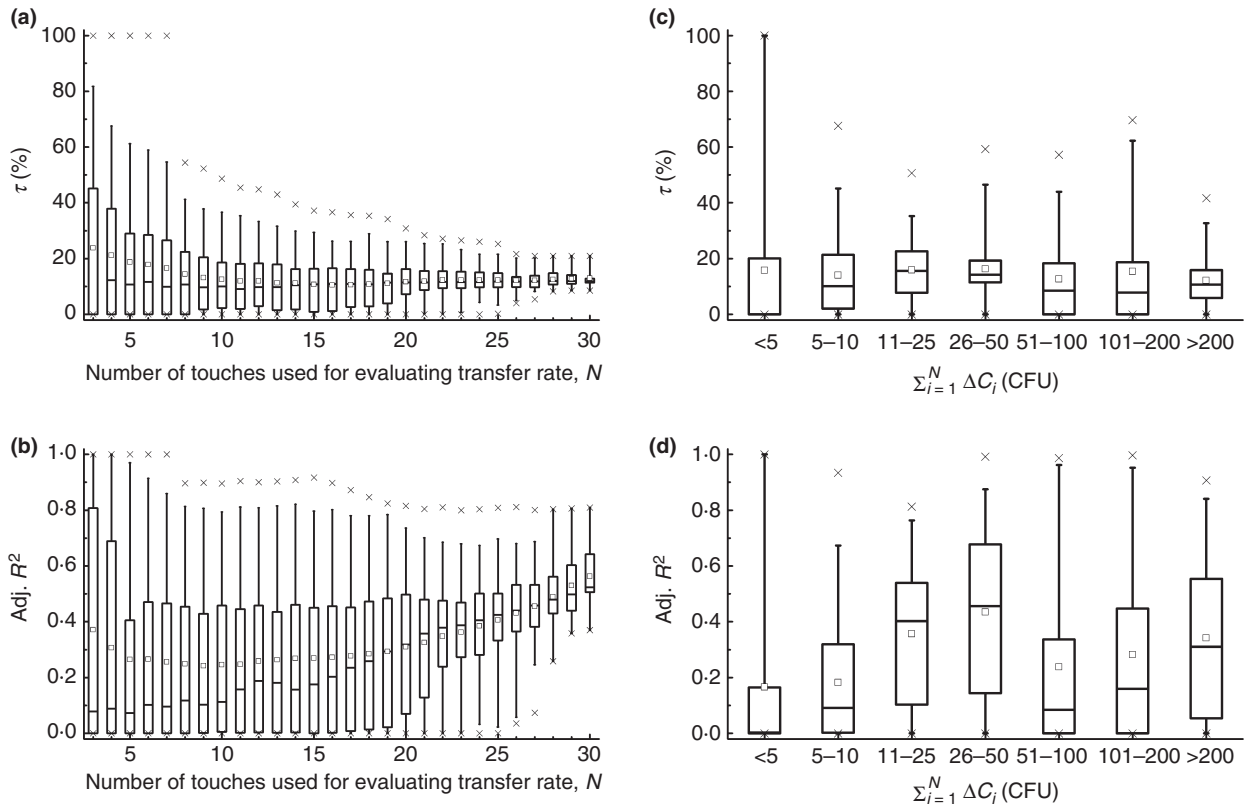

Figure 3 Evaluation of the goodness of data fitting in terms of the number of touches and the total number of CFUs counted in a set of trials. Two parameters—the transfer rate and the adj. R^2 value—varied with the number of touches (a and b) and the total number of CFUs (c and d), and are presented to evaluate the data fitting. Squares, horizontal lines, boxes, whiskers and cross labels in the box charts represent the mean, median, 25% to 75% range, 5% to 95% range and 1% to 99% range respectively.

fitting, the average transfer rate from six replicates was 12·8% (SD, 3·84%). No statistically significant difference in the transfer rate was observed between the trials with inoculated *S. aureus* and those with the thumb's resident microflora ($P = 0·968$), but the adj. R^2 values for fitting the CFUs for skin bacterial transfer (mean, 0·432) were not as good as those with the model bacteria *S. aureus* (mean, 0·563).

Discussion

The aim of this study was to propose and evaluate a simple method to accurately calculate the microbial transfer rate during a surface touch. First, the method of surface sampling was simplified, as the bacteria on the touched surface were cultured in situ (Fig. 1b,c), which required less sampling intervention and thus resulted in reduced variability. Then, based on the new sampling method, we used 'sequential touches' to evaluate the bacterial transfer rate from a finger to a surface while maintaining a low labour intensity. In the sequential touch, one thumb (as the bacteria donor) was used to touch 30 pieces of clean glass surfaces to obtain 30 values of CFUs. The new method did not require the initial number of bacteria on the thumb to be evaluated; instead, an optimal value for the transfer rate was obtained by fitting the 30 CFUs with the established function (Equation 4).

The exponential-decay function (Equation 4) assumes that each bacterial individual has the same probability of transferring during the sequential touches. If we averaged the replicates of the trials in Figs 2 and 4, the adj. R^2 value of around 0·9 (Fig. S1) indicated a good fit by Equation 4, which confirmed the assumption of exponential decay as the premise of the new method. (Further explanation is given in the Supporting information). The significant fluctuations of the data points around the fitting lines in Figs 2 and 4 are considered to indicate the intrinsic randomness of the transfer event; thus, they do not serve as a reason to reject the trend of exponential decay.

The parameters applied in the new method were analysed. The small SD among the trials was a result of the sequential touches with a large number of glass slides and the significant number of CFUs counted on the slides.

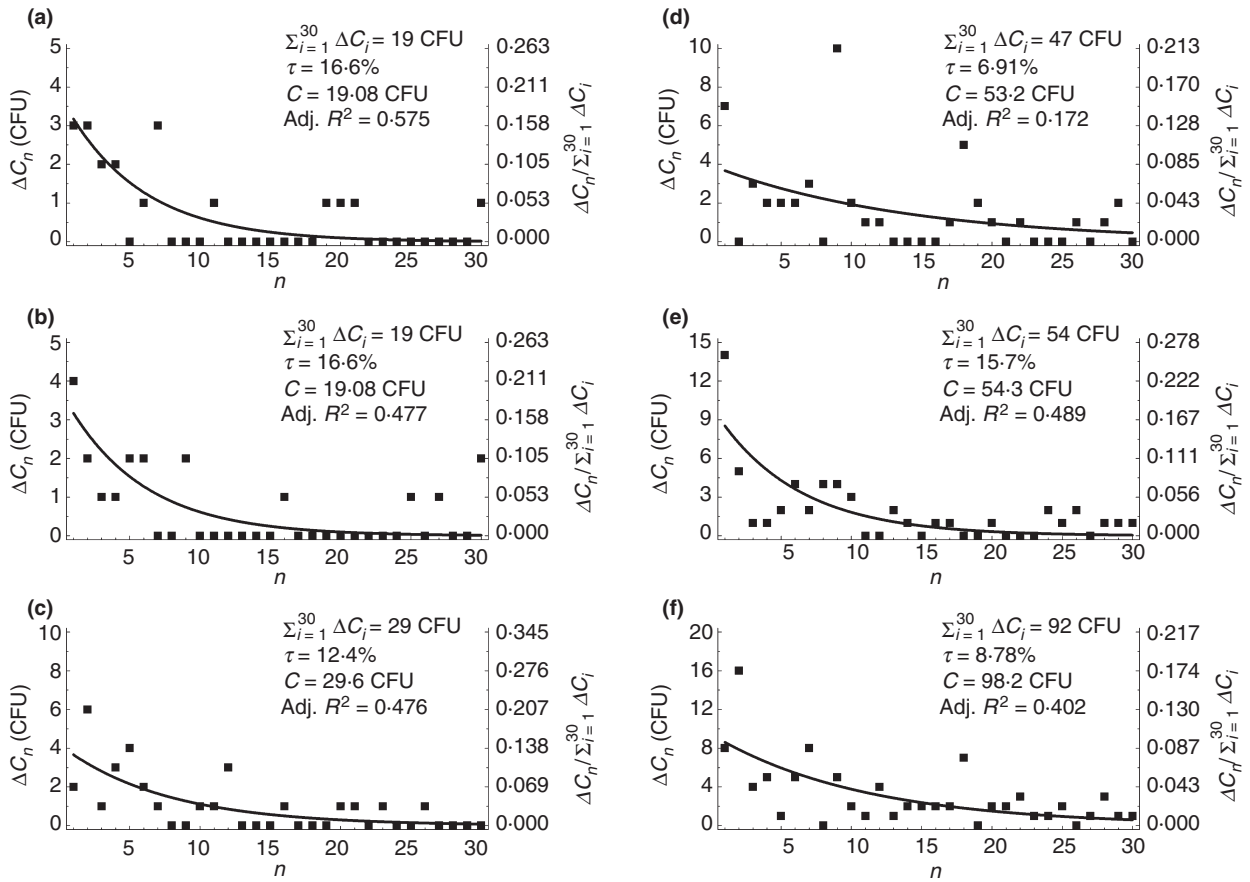

Figure 4 Transfer of resident microflora on the thumb during sequential touches between a contaminated thumb and 30 clean slides. Six sets of trials (a-f) were performed. For each trial, the CFUs counted on each slide are shown as square points, and the 30 CFU values fitted with Equation 4 are shown as fitting curves. The evaluated transfer rate, the evaluated initial number of bacteria on the donor thumb and the adj. R^2 value based on data fitting are listed for each set of trials. (CFUs counted on each touched slide are listed in Table S1.)

The use of a larger number of touches with the sequential-touch method improved the accuracy and reliability of evaluation, as shown in Fig. 3a,b, but it also increased the labour intensity. Thirty touches is considered appropriate for the sequential-touch method; it enables evaluation of the transfer rate while maintaining an efficient workload. Because plate counting used tens of plates to test the sampled bacteria in 10-fold dilution (Lopez *et al.* 2013; Stals *et al.* 2013), this new method with 30 touches reduced the workload while avoiding unnecessary sampling intervention and maintaining the accuracy of the results.

The concentration of *S. aureus* inoculated on a thumb should not be too high or too low. Inoculation with an excessive concentration of bacteria may not be a serious problem, but it may result in difficulty in counting the CFUs on the first few touched glass slides. In practice, a sufficient number of sequential touches can reduce the problem caused by an excessive bacterial concentration. In contrast, the series of CFU values is meaningless and inappropriate for data fitting if the inoculated bacterial concentration is too low, as shown in Fig. 3c,d. Thus, an appropriate number of bacteria on the thumb should be ensured before any touching experiments are performed.

Although the CFU values in Figs 2 and 4 fluctuate significantly, the average adj. R^2 value of around 0·5 indicates an acceptable level of data fitting. A relatively low SD among the sets of trials (as shown in Figs 2 and 4) indicates accurate evaluation by the new method. The average relative SD (i.e. the ratio of the SD to the average transfer rate) in this study was 29·9%, which is significantly smaller than those in the literature, in which the average relative SDs exceed 40% (Julian *et al.* 2010; Sharps *et al.* 2012; Brar and Danyluk 2013; Lopez *et al.* 2013; Stals *et al.* 2013; Tuladhar *et al.* 2013; Greene *et al.* 2015) and many even exceed 70% (Julian *et al.* 2010; Lopez *et al.* 2013; Stals *et al.* 2013; Tuladhar *et al.* 2013; Greene *et al.* 2015). For the new method, however, the

goodness of evaluating the initial number of bacteria on the thumb was not validated.

However, verification of the evaluated transfer rate against previous findings is difficult because the experimental conditions varied in the previous studies. Mackintosh and Hoffman (1984) found that the transfer rate from finger to fabric varied significantly according to the type of bacteria. Touch behaviour was well controlled by Lopez *et al.* (2013), but large errors remained in their evaluation of the microbial transfer rate. Unfortunately, we did not find any studies in which the bacterial transfer rate from a naked finger (without a glove) to a nonporous surface was evaluated.

The application of sequential touches was a result of the simplified method of counting surface bacteria. Using the traditional single-touch method, the bacteria on both the thumb and the surface must be sampled. Swabbing of the sampled surface followed by plate counting is the most widely used method for determining transfer rates (Mackintosh and Hoffman 1984; Chen *et al.* 2001; Rusin *et al.* 2002; Harrison *et al.* 2003; Lopez *et al.* 2013; Bhoonderowa *et al.* 2014). However, the efficiency of surface sampling by swab has been shown to be low and to vary across surfaces (Rose *et al.* 2004). In addition, the processes, such as irrigating bacteria from the swab, sample dilution and spread plates, were often poorly controlled and resulted in significant errors.

Some studies modified the sampling method using direct elution of bacteria from the skin or other surfaces. Waterman *et al.* (2006) used the glove juice method to sample bacteria from the hands; the sampling solution was poured into the worn glove from the wrist and sampled after a period of rubbing and irrigating. Conover and Gibson (2016) used the glove juice method to evaluate the efficacy of soaps. Lingaas and Fagernes (2009) put the whole hand into a sterile bag instead of a glove for hand sampling and put the finger into a self-made finger stall for finger sampling. The European Norm 1500, as a standard method to evaluate the efficacy of hand hygiene, has been used to quantify hand bacteria (Kampf and Ostermeyer 2002; Rotter 2004; Wilkinson *et al.* 2013). Bellissimo-Rodrigues *et al.* (2017) followed the European Norm 1500 standard to sample *Escherichia coli* on fingers by directly rubbing the fingers in a petri dish of tryptic soy broth. Other methods have also been attempted. Brar and Danyluk (2013) put contaminated tomatoes and a removed glove into sampling bags with eluent and irrigated the bacteria into the eluent by shaking the bag, and Sattar *et al.* (2001) and Hübner *et al.* (2011) eluted incubated bacteria by pressing a finger or fabric onto the mouth of the vial containing the eluent; however, these modified methods were more labour-intensive and required more manual operation (Lopez *et al.* 2013), although they did improve the efficiency of surface bacterial sampling. Therefore, each method has a specific area of use, but none has been widely accepted as a replacement for swabbing to quantify the microbes on a surface (Lopez *et al.* 2013).

In the method proposed in this study, in situ culture of bacteria allowed us to avoid the common problems of surface sampling. Each sterilized glass slide used as a bacteria recipient was packed in a petri dish, as shown in Fig. 1a. Immediately after being touched by a contaminated thumb, the touched glass slides were covered directly with LB agar (Fig. 1b). It was easy to count the CFUs growing between the agar and glass surface (Fig. 1c), and the small size of the colonies in these anoxic environments (Fig. 1c) reduced the likelihood of the colonies mixing during culture.

The new method is analogous to the contact plate method (Scott and Bloomfield 1990; Gomez *et al.* 2012), but without the step of plate uncovering, which avoids a loss of sampling efficiency. Contact plate as a kind of surface sampling method involves additional bacterial transfer events—both the thumb and the surface involved in a touch event were sampled by, respectively, touching a plate agar (Chowdhury *et al.* 2018). The two additional transfer events, which may have two different transfer rates, could affect the evaluation of the transfer rate between the touched finger and the surface.

The new method was also applied to evaluate the transfer rate of the resident dermal microflora on the thumb. The average transfer rate of the resident microflora shown in Fig. 4 was similar to that of *S. aureus* shown in Fig. 2. No significant difference was seen in the transfer rate between the resident microflora and *S. aureus* as one kind of transient microflora on the thumb. One possible explanation is that the bacteria applied in the trials may have a similar property of adhering to skin and surfaces; however, it is also possible that the transfer rate is independent of the type of bacteria. It is known that bacteria usually exist collectively and have a layer of adhesive coating outside their cells (Busscher and Weerkamp 1987; Garrett *et al.* 2008; Hori and Matsumoto 2010). In this case, the differences in the transfer rates among the types of microbes in previous studies (Mackintosh and Hoffman 1984; Scott and Bloomfield 1990; Rusin *et al.* 2002; Lopez *et al.* 2013) may have resulted from errors in the trials, but this hypothesis should be further verified in a subsequent study.

The subject's were repeatedly washed before inoculation with *S. aureus*. To examine the effectiveness of this repeated handwashing regimen, we carried out the 30-time sequential-touch trials using the two-round washed hand (with an interval of less than 30 min) as a pretest on the efficiency of the handwashing. As a result of this

regimen, for each of all three replicates, no more than one colony grew on the 30 total slides. When testing for the resident microflora, repeated handwashing can result in an insufficient number of CFUs on the recipient surface, which can affect statistical evaluation of the transfer rate. To maintain the resident microflora at a sufficient number of CFUs to observe and enumerate the bacteria, the researcher was only allowed to perform one set of trials per day.

The proposed method was used to evaluate the number of bacteria on a donor surface by testing only the recipient surfaces. The new method could be applicable in situations in which direct testing of bacteria on the donor surface is difficult, such as those involving skin, porous surfaces and food. In some situations, the bacterial quantities on both the donor and recipient surfaces are difficult to evaluate via in situ culturing, such as finger-to-finger (Rheinbaben *et al.* 2000; Greene *et al.* 2015) or finger-to-clothing (Mackintosh and Hoffman 1984; Scott and Bloomfield 1990) contact. In such scenarios, both surfaces can be considered donors to touch a series of prepared clean surfaces as the method above, and the initial number of bacteria on each of the two target surfaces and the transfer rate in their touch can be then estimated.

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Conflict of Interest

No conflict of interest declared.

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Table S1. CFUs counted on each touched slide in each series of sequential touches (ΔC_n).

Table S2. Proportion of CFUs on each touched slide to all CFUs in the corresponding sequential touches ($\Delta C_n/\Sigma 30\ i = 1 \Delta C_i$).

Figure S1. Proportion of CFUs on each touched slide, averaged from (a) six sets of trials using *Staphylococcus aureus*; (b) six sets of trials using resident microflora; and (c) all 12 sets of trials. The data in each plot of Fig. S1 are listed in Table S2 and were fitted to a curve with Equation 4 from the main text.