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A Sticking Point in Assessing Bacterial Contamination: Adhesive Characters of Bacterial Specializations, Swab Features, and Fomite Surface Properties Skew Colony Counts

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Abstract

Relative bacterial load as assessed by swabbing of surfaces is used to make critical decisions about safety in medical, food and athletic venues, with little consideration of bacterial attachment features (capsules, pili, flagella), swab type, or adhesive properties of fomites. To consider the impact of these parameters, a known quantity of bacteria with different adhesive specializations was applied to fomites of varying topography and surface energy and retrieved using multiple types of swabs. Swab type affected the total number of bacteria retrieved but had little effect on proportion of bacterial species collected (p = 0.455, by paired t-test). Mutant strains were observed for *E. coli* to determine contribution of surface features to fomite adhesion. Pili and flagella had greatest impact on retrieval from fomites with varied topography (ANOVA $F_{(44,4)}$ = 6.099; p = 6.0×10^{-4}), whereas surface chemistry and capsule chemistry had greatest impact on retrieval of species from fomites of different surface energies (ANOVA $F_{(20,3)}$ = 52.08, p= 1.24×10^{-9}). Adhesive properties of additional surface structures may need to be assessed and a more quantifiable study of fomite topography needs to be explored. Ultimately, a paradigm needs to be devised to make accurate comparisons of CFUs retrieved by swabbing surfaces for microbial contaminants.

Keywords: Bacterial Adhesion, Pili, Flagella, Swabbing, Fomites, CFUs.

INTRODUCTION

Swabbing of surfaces to determine bacterial load is an industry standard used by hospitals (Smith *et al*., 2013), clinics (Spratt *et al.,* 2014), nursing homes (Vesley *et al.,* 1987; Rouch and Marchese, 2017), organized sports (Davies *et al.,* 2017; Dyer *et al.,* 2018), forensic investigators (You *et al.,* 2019) and food processors (Garayoa *et al.* 2017, Keeratipibul, 2017) among others. The resultant colony counts are used to determine food and water safety (Jarvis *et al*., 2007), make medical diagnoses (Kwon *et al.,* 2012; Hsu *et al*., 2019), verify advertised over-the-counter dosages (Goldstein *et al*., 2013) and evaluate sterility of medical equipment (Chernecky and Waller, 2010; Maki *et al.,* 1977).

Adhesion of bacteria to cell surfaces is a critical step in pathogenesis (Hori and Matsumoto, 2010). Surface components which promote adhesion include: flagella, chemotactic proteins, fimbrial adhesins, lipopolysaccharides and capsules (Willis and Whitfield, 2013). Due to these adhesive features, the surface properties of fomites to which microbes adhere and the swabs used for their retrieval (and subsequent release into media), counts from standard swabbing techniques may not be accurate representations of the contaminating microbial community. Additionally, many bacteria produce surface biosurfactants which can interfere with adhesion (Giri *et al.,* 2019).

Role of protective external bacterial features in adhesion: capsules and glycocalyces

The capsular polysaccharides of many bacterial species, which prevent desiccation and promote virulence by avoiding degradation, appear to have variable effects on adhesion (Rubinchik *et al.,* 2014; Barato *et al.,* 2016; Xu *et al.,* 2016). Capsules serve in electrostatic evasion of phagocytes (Willis and Whitfield, 2013). In *E. coli*, alteration of capsular carbohydrates has been linked to reduced toxicity (Di *et al.,* 2017). Conversely, *Staphylococcus epidermidis* produces a negatively charged glycocalyx slime layer, which forms a hydrophobic biofilm as well as a fibrinogen binding protein, both of which have been implicated in adhesion to prosthetics (Nilsson, *et al.*, 1998). Once microbes form biofilms on medical device surfaces, they are extremely difficult to remove (Lin *et al*., 2017).

Role of external cell wall bacterial structures: pili, flagella, and o-oligosaccharides

Pili come in a variety of shapes, locations, and functions; and multiple types can be found on the same cell. In *E. coli*, Type 1 pili (Kolenda *et al.,* 2019) and P pili are well documented adhesion structures (Hori and Matsumoto, 2010) associated with uropathogenicity (Mulvey *et al.,* 1998). The CFA-1 and CS pili are associated with enterotoxigenicity (Epler Barbercheck *et al.,* 2018). Pili associated helical adhesion proteins and pilins have been linked to increased adhesion to host cell surfaces enhancing virulence (Epler Barbercheck *et al*., 2018). Flagella also promote adherence by acting as adhesins, and increase virulence due to motility associated pathogen spread (Duan *et al.*, 2012). Mutant flagella or those inhibited by antiflagellin antibodies have significantly decreased adhesive properties (Giron *et al.,* 2002; *Roy et al*., 2009). The negative charge associated with the o-oligosaccharide of the gram negative outer membrane electrostatically repels phagocytes, increasing bacterial virulence and affecting their ability to bind to different fomites (Anderson and Young, 2016). Because wild type *E. coli* makes use of all of these structures as virulence factors, mutant knockout strains missing specific external components make an ideal study model.

Fomite features influencing microbial adhesion

Fomite adhesive properties, including surface energy and topography, may also alter adherence. It has been shown that the intrinsic surface properties of different types of plastics affect both long and short term bacterial adhesion to their surfaces (Cai *et al*., 2019). Variations in roughness on a nanoscale and surface chemical heterogeneity further confound assessment of bacterial adhesion onto fomites (Bradford *et al.,* 2018).

All surfaces have an inherent charge which creates weak charged interactions with other surfaces with which they come into contact. The smaller the structures (such as bacteria) and the more densely packed, the greater the number of small interactions that occur. These weak dipole-to-dipole like interactions constitute a van der Waal's bond, and increase adherence. This is a major component of Derjaguin-Landau-Verwey-Overbeek (DLVO) theory, which relies upon weakly

charged molecular interactions to explain the adhesive properties of cells (Hori and Matsumoto, 2010, Cai *et al.,* 2019).

Interaction of swabs and fomites

Both the swab composition and the pattern of swabbing can impact the debonding of a bacterium from a surface and its subsequent attachment to a swab (Keeratipibu *et al.,* 2017; You *et al.,* 2019). This transfer is less likely if a swab has lower surface energy than the fomite surface or is being moved in the same direction as the microbe-surface bond. Although rougher topography increases the total surface area of a fomite, it may offer fewer available bonding sites if microbes are unable to access the crevices. This should result in easier bacterial transfer during swabbing. Conversely, if bacteria are sequestered in the crevices of a rough surface, they may be unreachable by swabbing, reducing retrievability. On skin, tape-stripping has been suggested as a comparable or even preferable method for bacterial retrieval to address some of these issues (Ogai *et al.,* 2018).

Swab features influencing microbial adhesion

Polyester swabs are hydrophobic but may develop a negative static charge when rubbed on a surface during sampling (Maruf, 2019) which could result in electrostatic repulsion of negatively charged bacteria. Flocked nylon swabs have a brush-like texture which the manufacturer claims will better extricate surface microbes and a strand arrangement designed to use capillary action to maximize uptake (Becton Dickinson, 2019; Copan, 2019). While cotton swabs are considered neutral in generating a static charge, the fibers themselves have a net negative charge which could reduce microbial attachment (Cotton Fiber, 2012), increasing retrieval. You *et al.* (2019) found cotton swabs to be most effective in retrieving DNA samples of *E. coli* and *S. aureus* from hard surfaces. They also determined that wetting the swabs affected DNA retrieval; and called for a standardized sampling method.

Moisture regain values, which are a measure of absorptiveness, likely influence both bacterial adhesion to and release from swabs; and vary greatly between swab materials (polyester=0.4%, cotton=8.5%, nylon=4.0%) (Polyester, 2012). Additionally, variations in the swab surface area and sampling technique may alter results and reduce the comparability of studies.

This investigation elucidated the impact of the following factors on accurately determining the composition of microbial communities sampled by surface swabbing: 1) the role of fomite surface properties, 2) the efficacy of swabs in bacterial retrieval and release to media (transfer from fomite to fomite) and 3) the role of external bacterial features in microbial adherence to a surface and subsequent resistance to removal by swabbing.

MATERIALS AND METHODS

Handling of bacteria

See Table 1 for all bacterial species used in this investigation, their source, gram reaction and unique adhesive features. *Streptococcus pneumoniae* was grown for 18 hours in Mueller-Hinton broth (MH) at 37°C with 5% CO_2. All other cultures were grown for 18 hours in MH broth with agitation (125 rpm) at 37°C. Individual species were diluted to 0.5 McFarland Standard (1.5 x 10^8 cells per ml). Because it is debonded so much more readily than other species in this study, *Staphylococcus epidermidis* was used at 0.05 McFarland Standard in mixed cultures to facilitate counting. Colony forming units (CFUs) of *S. epidermidis* were multiplied by 10 to generate comparable data to other species used.

Aliquots were applied to 5 cm x 7.5 cm areas of sterilized fomites (N=3 for screening studies and as indicated for specific studies) and uniformly dispersed with a sterile cell spreader (USA Scientific, 2977-5500) to achieve a concentration of 2.5 x 10^6 cells per surface. After drying, surfaces were swabbed and extracted using the standard protocol.

Handling of swabs

Sterile swabs: nylon (COPAN – FLOQSwab®; flocked), cotton (ULINE - Puritan), rayon (Thermo Fisher Scientific – Remel BactiSwab®), foam (Thermo Fisher Scientific - Puritan), dacron (LOVEDAY – Puritan; knitted) and polyester (LOVEDAY - Puritan) were wetted with MH broth as dry swabbing reduces retrieval (Keeratipibul *et al.,* 2017). Each contaminated area was first sampled using one side of the swab to vertically cover the entire surface, then the swab was flipped and the area was reswabbed horizontally before using

Table 1. Bacterial species used in this investigation

Bacterial species	Source	Identifying code	Gram stain	External feature
Bacillus subtilis	Ward's	470176-524	Positive	Hard, glutamyl polypeptide capsule
Escherichia coli	Coli Genetic Stock Center	BW30388	Negative	Wild type
Escherichia coli	Coli Genetic Stock Center	BW30388 Strain 29667	Negative	Fimbriae free mutant
Escherichia coli	Coli Genetic Stock Center	BW30388 Strain 9024	Negative	Flagella free mutant
Escherichia coli	Coli Genetic Stock Center	BW30388 Strain 9671	Negative	o-oligosaccharide free mutant
Escherichia coli	Coli Genetic Stock Center	BW30388 Strain 11767	Negative	Common pili free mutant
Escherichia coli	Coli Genetic Stock Center	BW30388 Strain 7962	Negative	Type I pili free mutant
Klebsiella pneumoniae	ATCC	13883	Negative	Soft slime layer
Staphylococcus epidermidis	ATCC	12228	Positive	Glycocalyx
Streptococcus pneumoniae	ATCC	49619	Positive	Wild type
Streptococcus pneumoniae	ATCC	39987	Positive	Capsule free mutant

the tip to swab the perimeter. Because swabbing pressure and technique can vary from person to person, one investigator conducted all of the swabbing.

Bacteria were extracted from the swabs using a standardized protocol. Swabs were extracted in 2 ml of MH broth using an Eppendorf Thermoagitator at 37°C and 1,000 rpm for 5 min. Spread plates of MH with 5% sheep red blood cells (SRBC) were inoculated with 10 µl of extract and incubated at 37°C for 24 hours with 5% CO_2. Control plates with 10µl of each culture were prepared to ensure all strains exhibited equivalent growth. CFUs were visually quantified for plates exhibiting low counts and the Flash 'N Go automated colony counter was used for plates with heavy growth to ensure accuracy.

Handling of fomites

For screening studies, 5 cm x 7.5 cm (37.5 cm^2) areas of untreated planed wood, plastic computer tower, acrylic wall paint, glass, aluminum flashing, wrestling mat squares (polyvinyl coated scrim) and ethylene-vinyl acetate (EVA) footwear soles were utilized (Table 2). Interaction of surface

Table 2. Surface energy and topography of sampled fomites

Material	Surface energy (dynes)	Topography	Source
Aluminum	850	smooth	Permabond, 2015
Glass	250	smooth	Permabond, 2015
ABS plastic	42	smooth	Permabond, 2015
Acrylic paint on wall	45	rough	Raghavan, 2018
Poly vinyl	37	rough	Permabond, 2015
EVA	33	rough	Permabond, 2015
Wood	30-50	rough	Jankowska *et al.,* 2018
Plastics	20-50	rough /smooth	Categorizing Surface Energy, 2019

specialization with fomite type was assessed using aluminum (high surface energy (850 dynes); smooth topography), polyvinyl film (low surface energy (30-34 dynes); smooth topography) and polyvinyl coated scrim wrestling mat (low surface energy (30-34 dynes); rough topography) (N=6 for each surface).

Surfaces were cleaned and disinfected with acetone, then ethanol and a subsequent 15 min UV light treatment. Because it was determined that this decontaminating process could alter fomite surfaces, follow up studies used fresh 5 cm x 7.5 cm surfaces of wrestling mat, acrylonitrile butadiene styrene (ABS) plastic (McMaster-Carr) (Permabond, 2015) and glass, cleaned by running under DH_2O stream for 60 sec, followed by 15 min exposure to UV radiation.

RESULTS AND DISCUSSION
Initial screening of fomites

S. epidermidis, with no specialized adhesive features, was used to screen fomites. Bacteria were applied to untreated planed wood, plastic computer tower, acrylic painted wall, glass, aluminum flashing, wrestling mat squares, and ethylene-vinyl acetate footwear. Recovery from fomites showed significant variation based on the surface. Greatest retrieval was from the acrylic painted wall and least retrieved was from the porous wood surface (Fig. 1). Similar results were seen by Milks and Goodwin (2018) who inoculated different surface types with a variety of bacteria and found that all species were more easily retrieved from a smooth ethylene vinyl acetate surface than from any other fomite material they tested.

Initial screening of bacteria

Bacillus subtilis, *Streptococcus pneumoniae*, *Streptococcus pneumoniae* capsule free mutant, *Escherichia coli* wild type (with pili, flagella, fimbriae, and capsule), *E. coli*
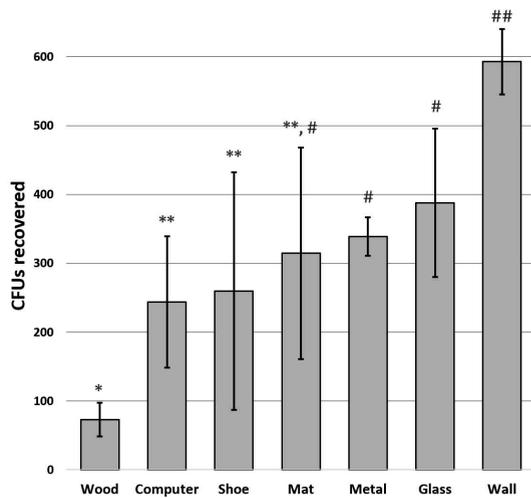

Fig. 1. Retrieval of *S. epidermidis* differed significantly from various surfaces. Graph shows mean CFUs +/- SD. Significance determined by ANOVA $F_{(14,6)}$ = 4.49 p =9.0 x 10^{-3}; asterisk designations indicate significance by t-test at p<0.05.

fimbriae free mutant, *Klebsiella pneumoniae,* and *Staphylococcus epidermidis* were applied to polyvinyl coated scrim wrestling mat (low surface energy (30-34 dynes); rough topography) which tested in the mid-range for adhesion (above). *S. pneumoniae* (both wild type and a capsule free mutant strain) were poorly recovered (less than 0.4%) on all surfaces. *B. subtilis,* with its hard, glutamyl polypeptide capsule (Thorne, 1993), did not differ significantly from *S. pneumoniae.* The large slime layer capsule of *K. pneumoniae* was less likely to remain firmly attached to a test surface as evidenced by approximately 2% retrieval. *S. epidermidis* with no adhesive specializations beyond a glycocalyx demonstrated the greatest retrieval of all bacterial species from the tested surfaces (~2.8%) (Fig. 2). The issue of comparison of bacterial types in mixed cultures is exacerbated by the fact that bacteria grow at different rates (Liang et al., 2017).

**Table 3.** *E. coli* knockout strains used in this study

Strain	Knockout	Functional change
CGSC:9024	loss of rod assembly protein	non-functional flagella (Moens and Vanderleyden, 1996)
CGSC:9671	no o-oligosaccharide in outer membrane	reduces net negative charge/adhesive ability
CGSC:11767	no common pili	reduces biofilm formation (Stacey *et al.*, 2014)
CGSC:7962	no type 1 pili	lack of associated adhesins (Epler-Barberchek *et al.*, 2018)
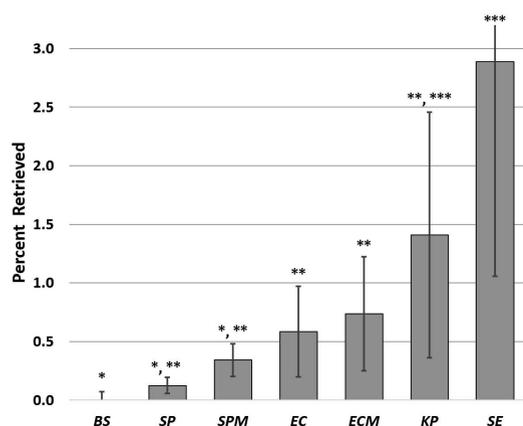
Fig. 2. Comparative retrieval of multiple bacterial species from a polyvinyl surface N≥ 8. Graph shows mean CFUs +/- SD. Significance determined by ANOVA $F_{(97,7)}$ = 9.55, p = 2.6 x 10^{-10}; asterisk designations indicate significance by t-test at p<0.05. *BS* = *B. subtilis*, *SP*= *S. pneumoniae*, *SPM* = *S. pneumoniae* mutant – no capsule*, EC = E. coli, ECM = E. coli* fimbriae free mutant*, KP = K. pneumoniae, SE = S. epidermidis.*

Initial screening of swabs

Cotton, polyester and nylon swabs retrieved significantly greater numbers of wild type *E. coli* from wrestling mats than foam, dacron, or rayon swabs ($F_{17,5}$ = 3.2520, p= 0.043) (Fig. 3). This
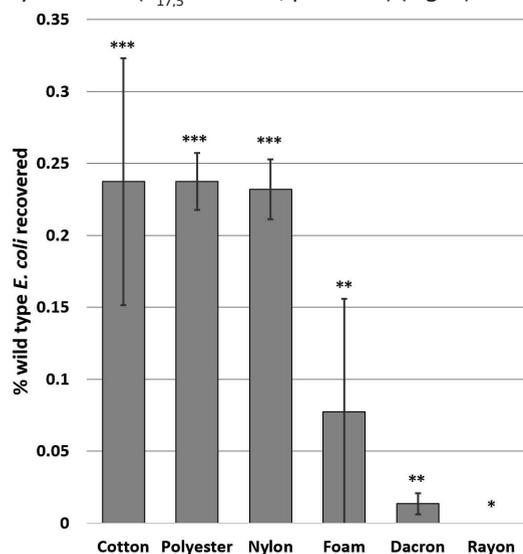
Fig. 3. Significant differences in ability of different swabs to retrieve *E. coli* from a polyvinyl surface with low surface energy and high topography. Graph shows mean CFUs +/- SE. Significance determined by ANOVA $F_{(17,5)}$ = 3.25, p = 4.0 x 10^{-2}; asterisks indicate significant differences by t- test at p<0.05. *SP= S. pneumoniae, BS = B. subtilis, KP = K. pneumoniae.*

is consistent with the findings of Keeratipibul *et al*. (2017) who also found cotton to be the most effective. Cotton swabs of all brands tested had the highest mass of swabbing material, thus the efficacy of the cotton may be due to the amount/ surface area of the swabbing material rather than to the nature of the cotton fiber. It should be noted that swabbing allows for quantification and identification of only culturable bacteria. Young *et al.* (2018) compared retrieval of non-culturable bacteria to culturable species and found a tenfold increase in bacterial types identified with the DNA mi-Seq system over standard swabbed cultures. Since this type of testing is generally cost prohibitive and only culturable species are detected with commonly used methods, it can be presumed that a huge percentage of the bacterial community goes unobserved in the majority of analyses (Paxton, 2004).

Protective external structures: capsules and glycocalyx

To compare the effect of capsule type, *S. pneumoniae* (gram positive; encapsulated wild type), *B. subtilis* (gram positive; large capsule), and *K. pneumoniae (*gram negative; soft slime layer) were inoculated onto wrestling mat surfaces and retrieved using rayon swabs (Thermo Fisher Scientific – Remel BactiSwab). The soft slime layer of *K. pneumoniae* composed of loosely bound exopolymeric substances (Hori and Matsumoto, 2010) appears to confer less adhesion than harder encapsulated species which are composed of more tightly bound exopolymeric substances. (Fig. 4). The glutamyl polypeptide found in the capsule of *B. subtilis* (Liu *et al.,* 2013) can be degraded by glutamyl hydrolase also found in *B. subtilis* (Ashiuchi *et al.,* 2003). This natural capsule degradation increases hydrophobicity which has been shown to increase bacterial adhesion (Bartley *et al*., 2013). The ability of bacteria to form biofilms is similarly influenced by the composition of the exopolymeric substances secreted (Danese *et al.,* 2000). Thus, species specific capsule composition must be considered when evaluating retrieval of different encapsulated bacteria under the same conditions.

External cell wall structures : flagella, pili and o-oligosaccharides

The impact of attachment features was differentiated utilizing a single species, *E. coli*
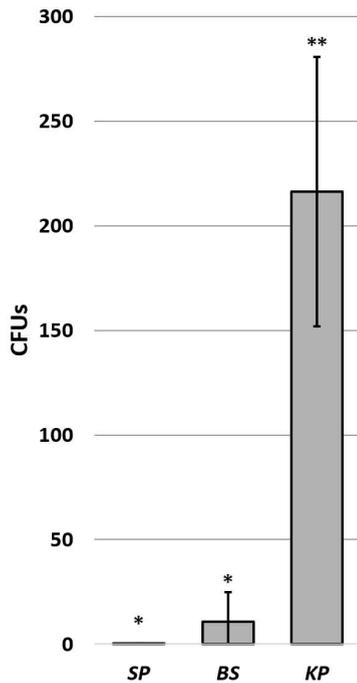
Fig. 4. Effect of capsule type on retrievability of bacteria from a mat surface. Graph shows mean CFUs +/- SD. Significance determined by ANOVA $F_{(20,3)}$ = 52.08, p= 1.24 x 10^{-9}; asterisks indicate significance at p< 2.0 x 10^{-4}; N=6.

(Coli Genetic Stock Center; CGSC BW30388) with associated knockout strains (Table 3). Fig. 5.

Bacteria were inoculated onto wrestling mats (low surface energy (30-34 dynes); rough topography), aluminum (high surface energy (850 dynes); smooth topography), and ABS plastic (low surface energy (42 dynes); smooth topography). There was a significant reduction in surface adhesion of mutant *E. coli* when compared to the wild type (Fig. 5). Different types of pili have different structures and functions (Proft and Baker, 2009). On all surfaces tested, the trend observed indicates that loss of common pili causes the greatest decrement in adhesion. As expected, microbes lacking functional flagella have decreased adhesive properties, confirming findings of Giron *et al.,* 2002 and Duan *et al*., (2012). Type I pili have relatively weaker receptor bonding, unless subjected to tensile stressors like urine flow (Hospenthal *et al.,* 2017) thus mutations of type I pili have less impact on adhesion. Although total CFUs varied, the trend

in retrievability of mutants was not significantly different between the fomites.

**Combined effects of fomite and swab type on retrieval of *E. coli***

For all strains of *E. coli*, retrieval from aluminum (very high surface energy (850 dynes); smooth topography) had a similar adhesive pattern to the ABS (low surface energy (30-34 dynes); smooth topography) (p=0.81) which differed significantly from retrieval from the mat (low surface energy (30-34 dynes); rough topography) (p=0.02).

When all bacteria were considered together, cotton swabs retrieved 3.4 times more bacteria than rayon swabs. However, the cotton swab tips weighed an average of 0.034 $\pm$ 0.006 g, which is 4.25 times the weight of the rayon tips (0.008 $\pm$ 0.001g). When swab weight differential is factored out, there is no significant difference between the swab materials on retrievability for cotton vs rayon (p = 0.13). Potentially surface area of swabs may alter results but was deemed unquantifiable for this study.
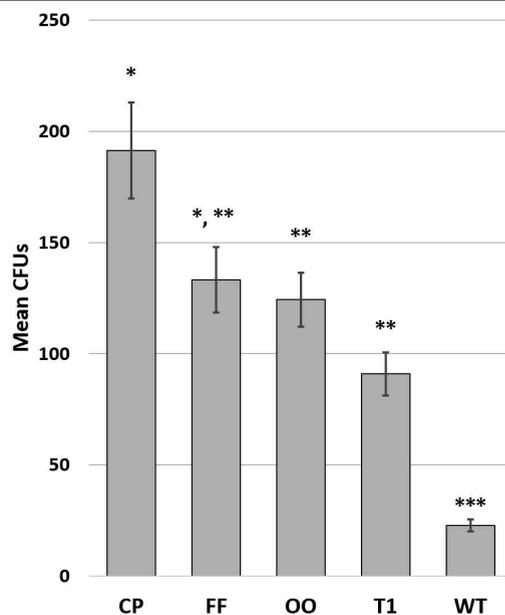
Fig. 5. Combined counts of *E. coli* mutant counts for 3 fomite surfaces. Graph shows mean +/- SE. Significance determined by ANOVA $F_{(44,4)}$ = 6.099; p = 6.0 x 10^{-4}; asterisks indicate significant differences by t-test at p<0.05; N=18 per species. CP = no common pili, FF = no functional flagella, OO = no o-oligosaccharides, T1 = no type 1 pili, WT = wild type.

Both the trend in adhesion to surfaces and the insignificant variation in swab type was confirmed using wild type *E. coli* collected with nylon (flocked COPAN – FLOQSwab®) and cotton (ULINE - Puritan) swabs (p = 0.455 by paired t-test) and replacing the high surface energy, smooth topography aluminum with glass which has similar high surface energy, smooth topography surface characteristics (Fig. 6). This change was necessary as metals are susceptible to surface change during chemical decontamination and bacterial adhesion to metals appears to depend on the terminating polyelectrolyte layer (Kovacevic *et al.,* 2016). Fig. 6.
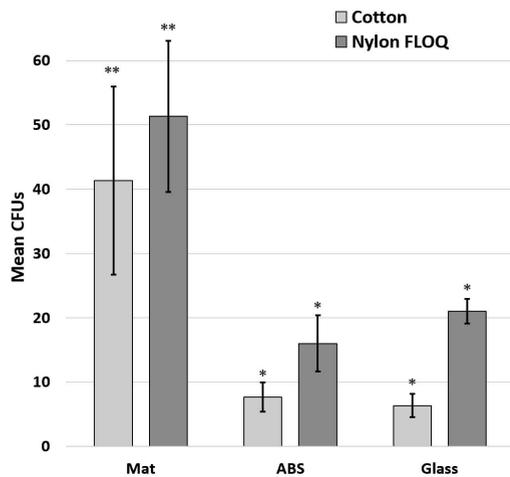

Fig. 6. Retrieval of wild type *E. coli* from different fomites using two swab types. Graph shows mean +/- SE. Significance determined by ANOVA $F_{(20,3)}$ = 5.23, p= 7.8 x 10^{-3}; asterisks indicate statistical significances at p<0.05 by t-test. N=6 for each fomite.

### Effects of fomite and swab type on retrieval of mixed cultures

Mixed cultures of wild type *E. coli,* wild type *S. pneumoniae* and *S. epidermidis* were inoculated onto mat, ABS, and glass surfaces and retrieved with cotton and nylon FLOQswabs®. Swab type made no significant difference in retrieval of different bacteria from varied surfaces (p = 0.455 by paired t-test). However, there was a distinct difference in collection of bacterial species ($F_{51,2}$ = 4.91, p=0.011) that varies with fomite surface ($F_{(15,2)}$ = 17.60, p=0.0001). *S. epidermidis* (no attachment specializations) was more efficiently retrieved from ABS and mat surfaces which

have low surface energy than the *E. coli* or the *S. pneumoniae* (Fig. 7). *S. pneumoniae* (negatively charged capsule) was most easily retrieved from the high surface energy glass. *E. coli* with its many adhesive factors was most difficult to remove from smooth surfaces (Fig. 8).
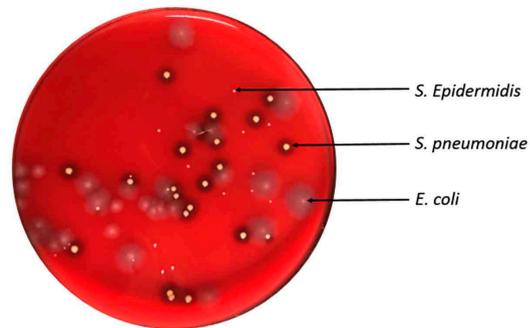
Fig. 7. Mixed culture retrieved from ABS with cotton swab. *E. coli* and *S. pneumoniae* at 0.5 McFarland Standard, *S. epidermidis* at 0.05 McFarland Standard.
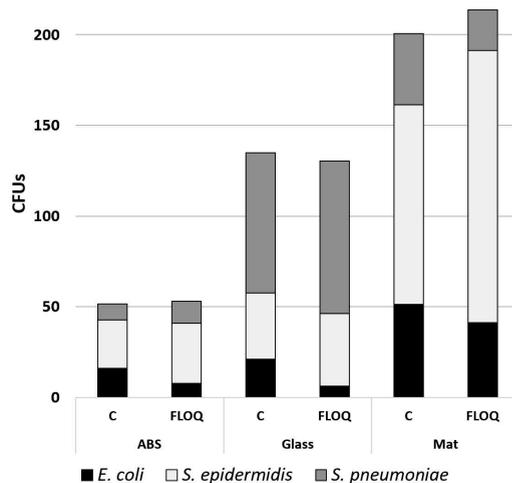
Fig. 8. Effects of surface and swab adhesive properties on retrieval of bacterial species with different adhesive features. N=6. C. cotton, FLOQ – nylon FLOQSwabs®.

CONCLUSIONS

Swab composition appears to have a minimal effect on microbial retrieval, with swab mass and surface area having greater importance to the total number of microbes retrieved. However, importantly, proportion of different species retrieved does not appear to be skewed by swab type. Bacterial surface specializations, fomite topography and fomite surface energy appear to

interact in bacterial adhesion to and retrieval from surfaces. In species relying on adhesive structures such as pili and flagella, rough topography decreases adhesion and also decreases the portions of the surfaces contacted by swabs. Even if a bacterium in a surface depression could debond easily, a swab might be unable to contact it for removal. In species for which bonding is related to microbial surface chemistry and features such as charged capsules, the fomite surface energy appears to be a significant factor in adhesion and retrieval. The surface energy of the bacteria themselves was not considered and measuring bacterial surface energy has proven difficult (Hori and Matsumoto, 2010). Of clinical note, the tendency to develop smooth surfaces for objects, such as medical equipment and surgical implants with the goal of reducing adhesion may, in practice, be counter to that objective as smooth, defect-free surfaces increase bacterial adhesion (Bazaka *et al.,* 2018).

Although swabbing is the traditional method of sampling in both clinical and commercial settings, this study indicates that results obtained in this manner do not accurately reflect contaminating microbial communities. Characteristics of bacterial surfaces, fomites, and swabs, all appear to affect bacterial retrieval. **Consequently, the standard method of directly comparing CFUs of retrieved bacteria to one another appears to be invalid.**

CONFLICT OF INTEREST

The authors declare that there is no conflic of interest.

AUTHOR'S CONTRIBUTION

VAM, LMY, MEM and SCY designed research. VAM, LMY and SCY performed experiment and obtained data. VAM, LMY and MEM analed data. VAM, LMY, MEM and SCY wrote the manuscript and apporved it for publication.

DATA AVAILABILITY

We have all of the raw data in spread sheets should someone need to see it.

ETHICS STATEMENT

All text, data, figures and tables are completely original. All supportive information from sources other than this laboratory has been cited in the text and included in the literature cited section. None of the authors have any conflict of interest or any affiliation or involvement which could be a potential source of bias, nor would any of us benefit financially from its publication. No animals or human subjects were used in this research.

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