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Major Article

Acinetobacter baumannii can be transferred from contaminated nitrile examination gloves to polypropylene plastic surfaces

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Background: Several observational studies suggest that gloves of health care workers are major routes of multidrug-resistant *Acinetobacter baumannii* transmission. However, limited experimental data are available assessing *Acinetobacter* transmission from gloves to environmental surfaces. This study determined whether *A baumannii* was easily transferred from nitrile gloves to polypropylene plastic compared with other gram-negative bacteria that cause health care–associated infections in laboratory-controlled experiments.

Methods: Gloved fingerpad-to-fomite transfer efficiency was determined for drug-resistant and -sensitive strains of *A baumannii, Escherichia coli, Klebsiella pneumoniae, Enterobacter cloacae,* and *Pseudomonas aeruginosa*.

Results: Only *A baumannii* transferred from gloves to fomites 3 minutes after the bacterial transfer event. Transfer efficiency of *A baumannii* was 0.1%-33% at that time point.

Discussion: Bacterial transfer from contaminated gloves to the hospital environment may be related to the type of contaminating bacteria, inoculated bacterial level, fomites, and glove materials. Therefore, it is important to need a comprehensive assessment of the transfer efficiency.

Conclusions: *A baumannii* can transfer easily from nitrile gloves to fomite compared with other gram-negative bacteria that cause health care–associated infections. These findings support data from previous observational studies that gloves of health care workers can be major routes of *A baumannii* transmission in clinical settings.

Acinetobacter baumannii is prevalent among critically ill patients, and infection with this organism is associated with increased morbidity, mortality, and cost.[1] In addition to the reduction of the general spread of health care–associated infections (HCAIs), infection control of *A baumannii* is a priority for all hospitals. In recent decades, outbreak reports of multidrug-resistant (MDR) *A baumannii* are increasing, and control of these MDR *A baumannii* infections are becoming a major public health concern. Many observational studies have revealed contamination of environmental surfaces, such as bed rails and ventilator touch pads, are potential sources of MDR *A baumannii* infections.[2-4] Additionally, several surveys have shown that gloves of health care workers (HCWs) are major fomites in transmission of drug-resistant *A baumannii*.[5-7]

Gloving, one of the most basic and important tools for everyone working in medicine, is recommended as a barrier protection for HCWs to reduce the risk of contamination during contact with infectious sputum, urine, and other body fluids. Contaminated gloves of HCWs potentially increase the risk of cross-transmission of harmful bacteria and other microbes between patients.[5] Failure to change or remove contaminated gloves carries a high risk of pathogen transmission.[8] There are many observational studies that suggest cross-transmission of microorganisms among the gloves of HCWs and hospital environmental surfaces occurs. However, there are limited experimental data that have robustly assessed the ability of *A baumannii* transmission from gloves to environmental surfaces.[9]

The aim of this study was to determine whether *A baumannii* could transfer more easily from nitrile examination gloves to hospital

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surfaces compared with other common gram-negative bacteria that can cause HCAIs, including drug-resistant and -sensitive strains of *Escherichia coli, Klebsiella pneumoniae, Enterobacter cloacae,* and *Pseudomonas aeruginosa*. This study will provide important data on the potential for *A baumannii* to contaminate nitrile gloves, which potentially increases the risk of cross-transmission to the hospital environment and patients.

METHODS

Bacterial strains and preparation of inocula

A baumannii, E coli, K pneumoniae, E cloacae, and *P aeruginosa*, including drug-resistant and -sensitive strains, were used in this study. Three *A baumannii* strains were obtained from American Type Culture Collection (ATCC) (Rockville, MD): ATCC 17978 and 19606 were drug-susceptible and ATCC BAA-1605 was resistant to fluoroquinolones, carbapenems, and aminoglycosides. *A baumannii* BGU 851 was clinically isolated from the hospital environment and was resistant to fluoroquinolones, carbapenems, and aminoglycosides. *E coli* ATCC 25922 and *K pneumoniae* NMS 26548 were clinically isolated from urine, and *E cloacae* ATCC 13047 and *P aeruginosa* PAO 1 were drug-susceptible strains. *E coli* BGU 415 and *K pneumoniae* BGU 352 were clinically isolated from urine and were extended spectrum beta lactamase-producing strains. *E cloacae* BGU 794 was clinically isolated from blood and was resistant to carbapenems. *P aeruginosa* BGU 343 was clinically isolated from sputum and was resistant to fluoroquinolones, carbapenems, and aminoglycosides. The initial inoculum was prepared fresh for each experiment by streaking a frozen aliquot of each strain onto Luria-Bertani agar (Becton, Dickinson and Company, Sparks, MD) and incubating at 37°C for 18 hours. The culture was resuspended in sterile saline solution to 1.5×10^8 colony forming units (CFU)/mL and diluted to 1.5×10^7 and to 1.5×10^5 CFU/mL, respectively.

Nitrile gloved fingerpads-to-polypropylene plastic coupon transfer sampling, and assays

A contact transfer protocol was performed to evaluate the movement of various bacteria between a person wearing nitrile gloves (Ultraform Powder-Free Nitrile Exam Gloves; Microflex Corp, Reno, NV) and touching a polypropylene plastic coupon (5 × 5-cm square) as an exemplar fomite. A gloved forefinger and middle finger were inoculated with 5 μL (from 1.5×10^7 or 1.5×10^5 CFU/mL dilutions, providing a final population at a high level of approximately 1.5×10^5 and a low level of approximately 1.5×10^3 CFU/10 μL) to each finger of various bacteria and allowed to air dry for between 0 and 3 minutes. Once dry, the contaminated gloved forefinger and middle finger were pressed onto the fomite for 10 seconds with an average pressure of 1.0 kg/cm^2 (range, 800-1,200 g/cm^2), as previously described.[10] Immediately after inoculation (0 seconds), 30 seconds, and 3 minutes (by which point the glove surface had dried completely), contaminated fomite and gloved fingerpads were swabbed to remove remaining bacteria using a sterile cotton swab (men-tipR, JCB Industry Ltd, Tokyo, Japan). These were then moistened in 1 mL of sterile saline solution. Excess saline solution was first pressed out of the swab by pressing the tip of the swab against the inside of the tube. Contaminated gloved fingerpads or polypropylene plastic coupons were swabbed in both a forward-back motion and a side-to-side motion while rotating the tip of the swab. The swab was then returned to the sterile saline solution and homogenized in the buffer using a Vortex tube mixer (Hi-Tech Mixer; Bio Medical Science Ltd., Tokyo, Japan) for 30 seconds to remove all cells from the swab. Samples were then serially diluted, plated onto Luria-Bertani agar, and incubated overnight at 37°C for colony enumeration. All strains were assayed in triplicate, and experiments were repeated at least 3 times.

Transfer efficiency calculation and statistical analyses

Bacterial colonies were enumerated, and the transfer efficiencies were calculated using the equation.

$$\text{Percentage transfer efficiency} = [\text{CFU polypropylene plastic coupon}/\text{CFU control-gloved fingerpads}] \times 100$$

where the transfer efficiency is defined as the number of CFU recovered from the polypropylene plastic coupon relative to the CFU recovered from the control-gloved fingerpads.

Polypropylene plastic coupon transfer values greater than 100% were truncated to 100% because, based on this formula, the transfer efficiency could be greater than 100% when the microbial recovery efficiency from the polypropylene plastic coupon is greater than that from the control-gloved fingerpads.[10]

Data were analyzed using Prism 6 software (GraphPad Software, La Jolla, CA) to compute the descriptive statistical measures of mean percent transfer efficiency, the standard deviation, and statistical significance. Differences were considered statistically significant if P value ≤.05.

Table 1
Transfer efficiency* (%) from contaminated nitrile gloves to the polypropylene plastic coupon

Inoculum level (CFU/fingerpads)	Time after inoculation (min)	*Escherichia coli*		*Klebsiella pneumoniae*		*Enterobacter cloacae*		*Pseudomonas aeruginosa*		*Acinetobacter baumannii*			
										S		R	
		S	R	S	R	S	R	S	R	ATCC 17978	ATCC 19606	ATCC BAA-1605	BGU 851
High (1.59×10^5)	0	45.9	49.0	57.5	36.6	47.3	29.2	43.4	50.5	34.0	32.8	65.5	33.8
	0.5	41.9	34.4	70.9	52.1	34.7	30.7	16.5	62.1	42.1	100.0	16.7	54.7
	3	ND	0	0	0	0	0	ND	ND	0.2	0.59	33.3	0.1
Low (1.39×10^3)	0	40.4	36.5	62.4	86.9	51.8	32.1	60.3	23.8	35.0	1.9	31.6	63.0
	0.5	14.3	47.4	28.6	100.0†	31.1	25.1	41.8	38.7	100.0†	0.9	22.7	29.0
	3	ND	0	ND	ND	0	ND	ND	ND	ND	33.3	0	0

CFU, colony-forming units; *ND*, not detected on the control gloves; *R*, drug-resistant strains; *S*, drug-sensitive strains.
*Transfer efficiency (%) = (average CFU polypropylene plastic coupon /average CFU control-gloved fingerpads) × 100.
†The value was >100% and was truncated to 100%.

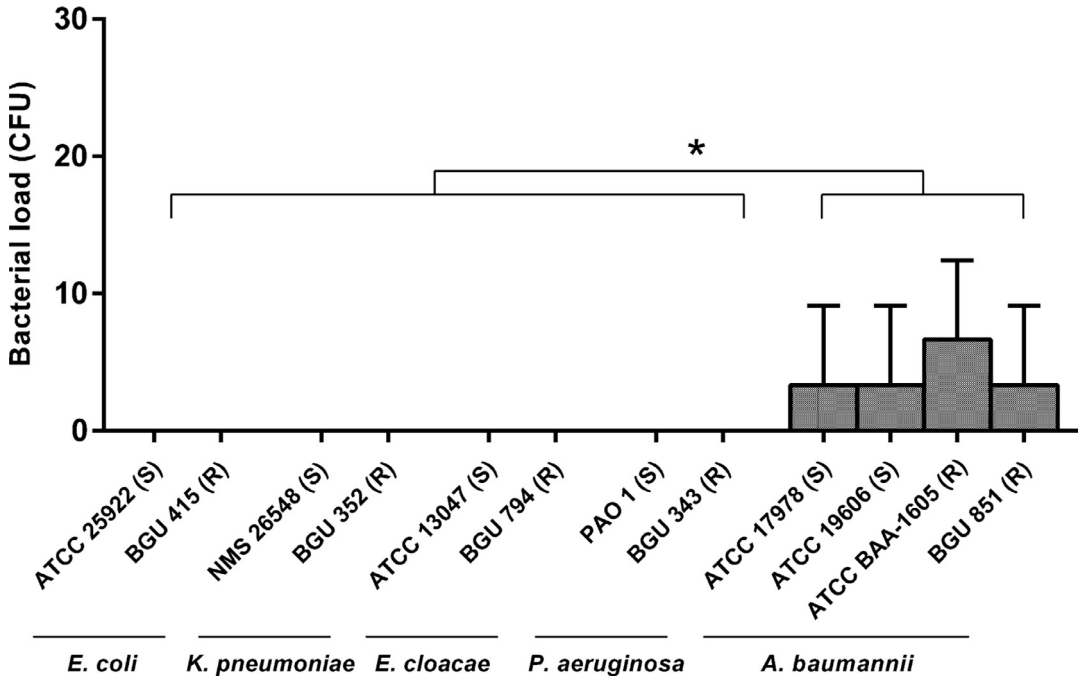

Fig 1. Transfer of bacteria from contaminated nitrile gloves to polypropylene plastic coupon 3 minutes after high-level inoculation of bacteria. Both drug-resistant and -sensitive strains *of Escherichia coli, Klebsiella pneumoniae, Enterobacter cloacae,* and *Pseudomonas aeruginosa* did not transfer from the gloves 3 minutes after high-level (1.59×10^5 CFU) inoculation with bacteria to the polypropylene plastic coupon. In contrast, both multidrug-resistant and drug-sensitive *Acinetobacter baumannii* transferred from the gloves 3 minutes after high-level (1.59×10^5 CFU) inoculation of bacteria to the polypropylene plastic coupon. Asterisk indicates P <.001. *CFU*, colony-forming units; *R*, multidrug-resistant strain; *S*, drug-sensitive strain.

RESULTS

Acinetobacter *transfer to polypropylene plastic coupons 3 minutes after bacterial inoculation of nitrile gloves*

To determine whether *A baumannii* could transfer more easily from nitrile gloves to hospital surfaces, compared with other common gram-negative bacteria that cause HCAIs, we performed transfer assay from contaminated nitrile gloves to polypropylene plastic coupons. All tested bacteria transferred from nitrile gloves immediately after bacterial inoculation of the glove to the fomite (Table 1). Immediately after bacterial inoculation (0 seconds) of the glove, approximately 29%-66% and 24%-87% of inoculated bacteria (high level of 1.59×10^5 and low level of 1.39×10^3 CFU, respectively) were transferred from the glove to the fomite in all tested bacteria. Transfer efficiency varied according to bacterial species. For *A baumannii*, transfer efficiency immediately after high-level inoculation with bacteria on the glove was approximately 33%-66% of inoculated bacteria. Antibiotic sensitivity had no consistent effect on immediate transfer to the fomite. After transfer from the glove to the fomite at 30 seconds and at 3 minutes after bacterial inoculation of the glove, the transferred bacterial loads decreased dose-dependently in all tested bacteria. Additionally, the transferred bacterial loads decreased time-dependently in all tested bacteria except for *A baumannii* BGU 851. Except for *A baumannii*, all bacteria did not transfer from the glove 3 minutes after inoculation with bacteria to the fomite. In contrast, both MDR and drug-sensitive *A baumannii* transferred from the glove 3 minutes after high-level inoculation of bacteria to the fomite. Various *A baumannii* strains more easily transferred to the fomite than the other tested microorganisms at 3 minutes after high-level inoculation of bacteria onto the glove (0 CFU vs 4.2 CFU, respectively, P <.001) (Fig 1). For *A baumannii*, transfer efficiency in 3 minutes after high-level inoculation of bacteria on the glove was approximately 0.1%-33% of inoculated bacteria. Transfer efficiency in 3 minutes after high-level inoculation of *A baumannii* varied greatly and did not correlate with antibiotic sensitivity of *A baumannii. A baumannii* ATCC BAA-1605 strains isolated from sputum could more easily transfer than the other tested *A baumannii*. These data suggest that *A baumannii* has the ability to more easily transfer from contaminated nitrile gloves to fomites and beyond.

DISCUSSION

Our study wanted to determine whether *A baumannii*, a common cause of HCAI, could transfer from contaminated nitrile gloves to the polypropylene plastic coupon in a series of laboratory-controlled experiments. We have shown that *A baumannii* could transfer more easily from the gloves to the fomite, compared with other common gram-negative bacteria that cause HCAI, including drug-resistant and -sensitive strains of *E coli, K pneumoniae, E cloacae,* and *P aeruginosa*. These findings support the data that the contaminated gloves of HCWs could be major routes of *A baumannii* transmission in clinical settings, and our data corroborate previous observational studies.[5-7]

Despite many observational studies that prove cross-transmission of *A baumannii* from the gloves of HCWs to hospital environmental surfaces, there are limited experimental data assessing the ability of transmission from contaminated gloves to environmental surfaces.[9,11] Unfortunately, there are no standard methods to evaluate transfer efficiency of bacteria between gloves to fomites. In addition, several factors can affect contaminated gloves-to-fomite transfer efficiency (eg, types of bacterial species, strains, gloves, and fomites). It is difficult to compare the results from various studies. Therefore, we investigated (1) species and strain variability of *A baumannii* and 4 different, common gram-negative bacteria known to cause HCAI (drug-resistant and -sensitive strains of *E coli, K pneumoniae, E cloacae,* and *P aeruginosa*), (2) the polypropylene plastic as fomites, (3) the nitrile examination gloves as glove material, and (4) inoculum size (approximately 10^3 and 10^5 CFU/fingerpad). Because all of these factors may affect

transfer efficiency, we aimed to be as comprehensive as possible in our assessment.

Polypropylene plastic was the environmental fomite model used in this study. The polypropylene plastic surfaces are found in various locations in hospitals, including disposable plastic aprons, basins, gowns, and shoe covers, all of which are frequently touched by HCWs. We have shown that *A baumannii* could transfer easily from the glove to the polypropylene plastic coupon compared with other common gram-negative bacteria. Surprisingly, *A baumannii* could transfer from the gloves 3 minutes after inoculation of bacteria (glove surface dried completely) to the polypropylene plastic coupon. There is only 1 experimental study that has previously assessed transfer efficiency using *A baumannii* and the latex glove. Greene et al[9] evaluated fingerpad-fomite transfer of *A baumannii* ATCC 17978 efficiencies using 6 materials, including glass, stainless steel, porcelain, polypropylene, polycarbonate, and rubber, with and without latex gloves. For *A baumannii* ATCC 17978, the latex glove-to-polypropylene transfer efficiency was approximately 1.6% in that study, which exceeded the transfer efficiency result in our study (0.2%). This difference in transfer efficiency may depend on the level of initial inoculated bacteria and the type of glove material. We, therefore, evaluated the transfer assay using 2 different initial inoculum sizes of bacteria: a high inoculum of approximately 1.5×10^5 CFU/10 μL/ fingerpads and a low inoculum of 10^3 CFU/10 μL/fingerpads. We chose more realistic conditions in this study because our inoculated bacterial level estimated respiratory tract infection (growth $\geq 10^7$ CFU/mL of bacteria) and urinary tract infection (growth $\geq 10^5$ CFU/mL of bacteria). The bacterial inoculum size used by Greene et al (10^6 CFU/ 20 μL/fingerpads) was 10-fold higher than that of this study. This difference in the initial inoculated bacterial load affects transfer efficiency and likely explains the disparity noted between Greene et al and our findings.

Glove material is also an important factor influencing bacterial transfer. Medical gloves are usually made from polymers such as latex, nitrile, and vinyl. Both nitrile and latex gloves are commonly used in hospitals. In recent years, nitrile glove use has increased because it avoids the issue of allergies associated with latex.[12] Therefore, we used nitrile gloves instead of latex gloves in this study. Greene et al[9] assessed latex glove-to-polypropylene transfer efficiency of *A baumannii* and was higher than that of our study using nitrile gloves (1.6% vs 0.2%, respectively). This disparity between Greene et al and our study may depend on the difference in glove material. There is an interesting outbreak report of *Acinetobacter calcoaceticus* var *anitratus*, which was directly attributed to contaminated latex gloves.[13] In addition, Moore et al[11] demonstrated that glove material influenced bacterial transfer of methicillin-resistant *Staphylococcus aureus* (MRSA) and nitrile gloves were associated with the lowest transfer rates.*A baumannii* has similar characteristics to MRSA because of their propensity for nosocomial cross-transmission, multidrug resistance, and capacity for long-term survival in the hospital environment. These characteristics have led to the designation of *A baumannii* as the gram-negative "MRSA." This may be owing to lower transfer efficiency of nitrile gloves compared with latex gloves. Further study is warranted to assess the association between bacterial transfer efficiency and the types of glove materials used within health care.

The difference in transfer efficiency may also depend on inoculated bacterial strains. We found no studies assessing transfer efficiencies using MDR *A baumannii*. Therefore, we evaluated the transfer assay using various *A baumannii* strains, including drug-resistant and -sensitive strains. All tested *A baumannii* strains, including both drug-resistant and -sensitive transferred from the glove to the fomite 3 minutes after high-level inoculation with bacteria on the gloves. However, transfer efficiency over a period of 3 minutes after high-level inoculation of *A baumannii* varied greatly (approximately 0.1%-33%), and antibiotic sensitivity of *A baumannii* had no consistent effect on transfer to the fomite. *A baumannii* ATCC BAA-1605 transferred more easily than the other tested *A baumannii* in this transfer study, and was isolated from sputum. Wendt et al[14] studied the ability of different strains of *A baumannii* to survive and to recover under dry conditions. They showed that prolonged survival and recoverability on dry surface associated with sources of bacterial isolation. They also demonstrated that strain isolated dry surfaces could survive better than those isolated from wet surfaces. In contrast, Jawad et al[15] noted that there was no statistically significant difference between the survival times of sporadic strains of *A baumannii* and outbreak strains, including some recovered from urinary tract infections, for a long time on dry surfaces.These findings suggest that the relationship with *A baumannii* survival, recoverability, and the sources of bacterial isolation are controversial. It is difficult to compare our results and the results from the 2 previous studies. Our study demonstrated transfer efficiency of bacteria between gloves to fomites and recoverability from the fomite in the short term over a period of just 3 minutes, although the previous studies evaluated the abilities of *A baumannii* strains to survive for a long time. In addition, we evaluated only 4 strains of *A baumannii*. Our data suggest that bacterial transfer from contaminated gloves to the hospital environment may be related to the type of contaminating bacteria, inoculated bacterial level, fomites, and glove materials. However, there are some limitations to this study, especially the variability in transfer efficiency, among *A baumannii* strains. Further studies are needed to clarify the variability in transfer efficiency among *A baumannii* strains.

One reason for this variability in transfer efficiency of *A baumannii* may be related to the adhesion forces between cells and surface(s). *A baumannii* can also attach to and form biofilm structures on plastic and glass surfaces.[16] Several studies have documented the ability of *A baumannii* to adhere to epithelial cells and to form biofilms on glass and plastic surfaces.[16,17] Therefore, we also used a biofilm formation assay and bacterial cell adhesion assay using preliminary lung epithelial cells (Supplementary Fig S1). All tested *A baumannii* strains showed greater ability to form biofilms and bacterial cell adhesion compared with *E coli*. Interestingly, MDR *A baumannii* strains constantly exhibited high ability to produce biofilm and demonstrate bacterial adhesion even though drug-sensitive *A baumannii* strains varied by strain. Similar trends were reported in a recent study in which clinical isolates of MDR *A baumannii* can form large amounts of biofilm, and this ability was shown to significantly correlate with epithelial cell adherence.[17] Greene et al[18] showed that biofilm formation capacity and MDR phenotype, a highly antibiotic-resistant *A baumannii* with a distinct Rep-PCR banding pattern, were statistically significant and each had statistically significant interaction terms with clinical or environmental status.These findings suggest that the ability to produce biofilm and adhere to lung epithelial cells may affect transfer of MDR *A baumannii* from the gloves to the hospital surfaces. Further experiments are required to evaluate the association between the variability in transfer efficiency of *A baumannii* and bacterial characteristics using a large number of *A baumannii* strains.

CONCLUSIONS

Gloving, one of the most basic and important tools for everyone working in medicine, is recommended as a barrier protection for HCWs for reducing the risk of contamination during contact with infectious sputum, urine, and other body fluids. However, gloves are often misused in clinical practice. Failure to change or remove contaminated gloves carries a high risk of pathogen transmission. This study demonstrates that *A baumannii* could transfer more easily from nitrile gloves to the polypropylene plastic coupon 3 minutes after inoculation compared with other common gram-negative bacteria that cause HCAI. These data support previous findings that

contaminated gloves of HCWs are major routes of transmission of *A baumannii* in clinical settings, as was observed in previous observational studies. This study also suggests that it is important to establish a basis for a risk assessment and a management approach to each type of bacteria owing to transfer efficiency rates being different from types of bacteria. Improving glove use compliance can decrease the risk of cross-contamination via contaminated gloves.

SUPPLEMENTARY MATERIALS

Supplementary material associated with this article can be found in the online version at https://doi.org/10.1016/j.ajic.2019.04.009.

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