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Survival of Bacterial Pathogens on Paper and Bacterial Retrieval from Paper to Hands: Preliminary Results

PAPER MEDICAL RECORDS CAN BE A SOURCE FOR TRANSMISSION OF BACTERIA.

While electronic medical records and information systems are increasingly found in hospitals and other clinical settings, paper may still be one of the most common materials on any hospital unit. Paper is used as a recording medium in medical and nursing charts, patient files, notes, and reports, and may be introduced into the clinical setting by patients and visitors in the form of books, newspapers, magazines, and other items. Paper documents are used every day and in every way, not only by nurses and physicians, but by many other people involved in patient care. Disinfection of paper, unlike most other equipment, is not an easy task because of its porous surface and incompatibility with liquid disinfectants. Evidence is abundant from studies of paper money that paper can transmit pathogens in nonclinical settings.[1-3]

Much research has been conducted on the transmission of pathogens from hands to inanimate surfaces. However, it remains unclear how long bacteria can survive on paper and how many organisms may be transferred in a full hand-to-paper-to-hand transmission cycle.[1, 4-10] Paper documents could be an important vehicle for cross-contamination and infection in clinical settings, but data are scarce. The aim of our study was to investigate how long bacterial pathogens can survive on regular office paper and to quantify the proportion of pathogens transferred from hand to paper and back to another hand.

METHODS

Design. We performed a two-step experimental study of bacterial survivability and transmission under laboratory conditions simulating a "worst-case scenario" (a high number of colony-forming units [CFU] per cm^2, and optimal transmission by wet finger and pressure against paper) for the spread of pathogens.

Preparation of paper swatches. One-centimeter-square swatches were cut from white all-purpose printing paper (80 g/m^2, Future multitech, UPM, Helsinki, Finland) and steam sterilized. The paper was shown to be free of antibacterial properties in an agar diffusion assay in accordance with standard DIN 58940-2-3 of the German Institute for Standardization.[11, 12]

Test of organism survivability on paper. To test the survival of bacterial organisms on paper, we used standard procedures for preparing bacterial cultures. Four organisms—*Escherichia coli*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, and *Enterococcus hirae*—were cultured overnight in tryptic soy broth (TSB, a growth medium commonly used in the cultivation of aerobic bacteria) and prepared to 10^9 CFU/mL. For each strain, 18 swatches were inoculated with 0.25 mL of test suspension and air-dried at room temperature. Immediately after drying, each sample was placed in a vortexer (a device used to agitate microbial samples in solution) with 10 mL of 0.9% saline solution. Volumes of 0.1 mL of undiluted sampling solution and 0.1 mL from 1:10 and 1:100 dilutions in TSB were plated

ABSTRACT

Background: Paper is omnipresent on hospital units, but few studies have examined the possible role of paper in the spread of nosocomial pathogens.

Objective: To determine by laboratory investigation how long bacterial pathogens can survive on office paper and whether bacteria can be transferred from hands to paper and back to hands in a "worst-case scenario."

Methods: Samples of four bacterial pathogens (*Escherichia coli*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, and *Enterococcus hirae*) were prepared according to standard laboratory procedures. Sterile swatches of office paper were inoculated with the pathogens and bacterial survival was tested over seven days. To test the transmission of bacteria from one person's hands to paper and back to another person's hands, the fingertips of volunteers were inoculated with a nonpathogenic strain of *E. coli*; these volunteers then pressed the inoculum onto sterile paper swatches. Another group of volunteers whose hands had been moistened pressed their fingertips onto the contaminated paper swatches. Bacteria transferred to the moistened fingertips were cultivated according to standard laboratory procedures.

Results: The four tested organisms showed differences in length of survival depending on environmental room conditions, but were stable on paper for up to 72 hours and still cultivable after seven days. Test organisms were transferred to paper, survived on it, and were retransferred back to hands.

Conclusion: Paper can serve as a vehicle for cross-contamination of bacterial pathogens in medical settings if current recommendations on hand hygiene aren't meticulously followed.

Keywords: cross-contamination, disinfection, finger pad method, hand antisepsis, hand hygiene, hospital-acquired infection, infection control, nosocomial infection, spread of pathogens, survival on inanimate surfaces

onto Columbia blood agar plates (Becton Dickinson, Heidelberg, Germany), incubated at 36±1°C for 24 hours, and plate counted. Samples were stored, while protected from direct sunlight and contamination, under standard room conditions (23±2°C, 55±5% relative air humidity). They were then sampled and plate counted after 48, 72, 96, 144, and 168 hours, to test for bacterial growth. Tests for bacterial growth were repeated three times.

Test of bacterial transmissibility. To test the transmissibility of bacteria from one hand to paper and back to another hand, we adapted the classic finger-pad method developed by Ansari and Sattar and specified in the American Society for Testing and Materials (ASTM) Standards E-1838-96 and E-1838-02 for testing virucidal activity of hand antiseptics.[5, 13-15] The nonpathogenic *E. coli* strain NCTC 10538 (from the National Collection of Type Cultures [NCTC], a part of the Health Protection Agency of the United Kingdom) was used as the test organism. Volunteers washed their hands in tap water without soap, dried them with single-use paper towels, and waited 10 minutes to ensure that they were dry.[16] The tip of each volunteer's index finger was inoculated with 25 microliters of test suspension (10^9 CFU/mL) and air-dried.

After drying, volunteers pressed the inoculated fingertips on paper swatches for 30 seconds. The index fingertips of another group of volunteers were then irrigated with sterile 0.9% saline (to simulate the common bad habit of licking the finger before turning pages or going through files) and pressed on the contaminated swatches for 30 seconds to simulate cross-contamination. A sterile Eppendorf tube filled with 1 mL of saline solution was then pressed to the fingertip of each of the second volunteers and shaken for one minute; volumes of 0.1 mL of this undiluted sampling solution were plated onto Columbia blood agar and incubated as described above. Tests were repeated six times.

Bacteria can be transferred to paper, survive on it, and subsequently contaminate hands.

RESULTS

Survival of test organisms on paper over time. All test strains survived on the inoculated paper. Figure 1 shows the changes in recoverable organisms for each individual organism. There were notable differences in the survival of different pathogens over time. *E. coli* was reduced by almost 5 $\log_{10}$ in 24 hours (a reduction

of 5 $\log_{10}$ is equivalent to a 99.999% reduction in recoverable organisms, the minimum reduction required for a surface in a clinical setting to be considered disinfected). Other organisms, including *P. aeruginosa* and *E. hirae*, were quite resistant to room conditions and were reduced by 3 $\log_{10}$ (99.9%) only after seven days; therefore the paper wasn't disinfected within the test period, and was still a potential source of infection.

Transmissibility of bacteria from hand to paper and back. We demonstrated that test organisms were transferred from hands to paper and back to hands (see Table 1). A transmission was detected in all six experiments. Although the mean bacterial transfer rate (from one volunteer's finger to the next volunteer's finger) was relatively low (0.009%), quantities of bacteria sufficient to cause infection or disease were resampled from the second volunteer's fingertip. (An inoculum of 5 $\log_{10}$ organisms—that is, an inoculum containing 100,000 organisms—would still be enough for cross-contamination. The initial quantity of bacteria in the inoculum was 2.75×10^7 CFU/mL, corresponding to a total of 7.44 $\log_{10}$ or 74,400,000,000 bacteria in the sample solution.)

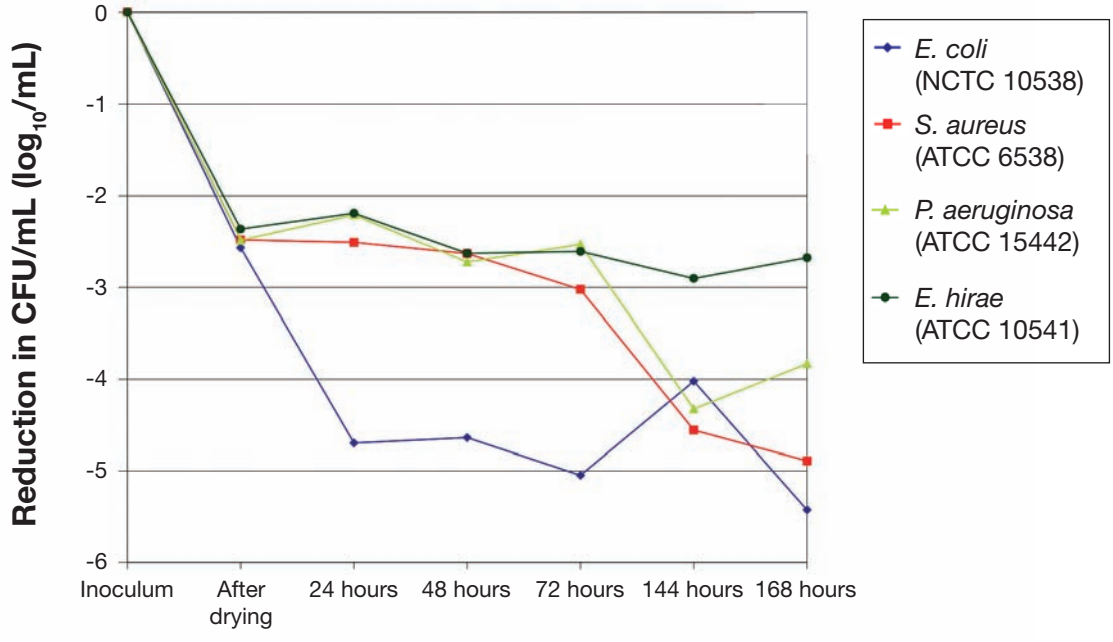
DISCUSSION

Because of paper's omnipresence on hospital units, the question if and to what extent it can play a role as a vehicle for bacterial pathogens and promote cross-infection is of great importance. It has been repeatedly shown that medical records can be heavily contaminated with pathogens, including multidrug-resistant bacteria like methicillin-resistant *S. aureus* (MRSA), extended-spectrum β-lactamase (ESBL)–producing enterobacteria, or vancomycin-resistant *Enterococcus* (VRE).[17,18] However, data on the survival of bacteria on paper and other porous surfaces are scarce.

We demonstrated that bacteria not only survive on paper but can also be transferred from one person's hands to paper and back to another person's hands. This is congruent with the results of studies of bacterial growth and survival on other inanimate surfaces and observational evidence.[10,19-21] Interestingly, the transmission rate found in a full hand-to-paper-to-hand cycle fits well with published data on transmission rates to and from other inanimate surfaces.[19]

Organisms show differences in resistance to room conditions, but most of the tested pathogens were quite stable on paper for up to 72 hours and still cultivable

FIGURE 1. Survival of Test Organisms on Paper Over Time[a,b]



CFU = colony-forming units.

[a] Initial quantity of bacteria in the inoculum was 2.75×10^7 CFU/mL, corresponding to a total of 7.44 $\log_{10}$ organisms.

[b] Specific bacterial strains are identified by the alphanumeric designations in parentheses. The American Type Culture Collection (ATCC) and the National Collection of Type Cultures (NCTC) are repositories and distributors of standard reference microorganisms, cell lines, and other biological materials.

TABLE 1. Transmissibility of *Escherichia coli* (NCTC 10538)[a] from Hand to Paper to Another Hand[b]

Test Number	Recovered Bacteria (CFU/mL)[c]	Average Bacterial Transfer Rate, %[d]
1	1.91E+03 [1,910]	0.007
2	4.00E+02 [400]	0.001
3	5.07E+03 [5,070]	0.018
4	3.87E+03 [3,870]	0.014
5	9.20E+02 [920]	0.003
6	3.44E+03 [3,440]	0.013
Mean	**2.60E+03 [2,600]**	**0.009**
SD	1.82E+03 [1,820]	0.007

CFU = colony-forming units; NCTC = National Collection of Type Cultures.

[a] Designates a nonpathogenic strain of *E. coli* from the National Collection of Type Cultures.

[b] Initial quantity of bacteria in the inoculum was 2.75×10^7 CFU/mL, corresponding to a total of 7.44 $\log_{10}$ organisms.

[c] Quantities of bacteria recovered at the end of the transmission cycle, expressed in (scientific) E notation, with whole number quantities in brackets.

[d] Designates the rate of transmission in a full hand-to-paper-to-hand transmission cycle.

after seven days. Thus, hands can become contaminated by these pathogens when paper is handled.

Limitations. Our study has several limitations. Designed as a pilot study, we wanted to assess whether paper can promote cross-infection. We therefore created conditions that are considered "worst case" in terms of hygiene and optimal in terms of pathogen transmission (high inoculum, wet finger) but with a very small contaminated spot (just one fingertip) to test transmissibility, as previously described by other authors.[19] This may not well represent real-world conditions, but our results are supported by those of other studies.[18] suspension, implying a higher transmission rate. Further research should be focused on whether our results are reproducible under real-world conditions. More investigation is also needed to explore whether handwashing with soap and water or hand disinfection with alcohol-based hand rubs (hand sanitizers) more effectively decreases transmission from hand to paper and vice versa in health care settings.

Conclusion. Our research shows that bacteria can be transferred to paper, survive on it, and subsequently contaminate hands. Paper, therefore, can serve as a vehicle for the cross-contamination of bacterial pathogens

The best way to minimize the spread of pathogens is proper hand hygiene, because the transiently contaminated hands of health care workers are known to be the most important route of transmission of pathogenic bacteria.

The uncoated paper used in this study bound most of the bacterial suspension by adsorption and absorption, thus reducing transfer. Coated paper, on the other hand, which is often used for printed material including paper currency, adsorbs and absorbs less bacterial if current recommendations on hand hygiene aren't meticulously followed. Once contaminated, paper is hard to disinfect, because it cannot be disinfected by chemical means, as other inanimate surfaces can. Thus, the best way to minimize the spread of pathogens is

proper hand hygiene, because the transiently contaminated hands of health care workers are known to be the most important route of transmission of pathogenic bacteria.[22-24] Alcohol-based hand rubs have repeatedly been shown to help improve compliance with hand hygiene and reduce transmission of pathogens[25-27] and could therefore help to reduce bacterial transfer from hands to inanimate surfaces. Paper should also be considered as a possible reservoir for cross-contamination of resistant organisms, especially in outbreak situations that involve different hospital units and floors, and whenever routes of transmission are unclear. Introduction of electronic health records, while reducing the use of paper, doesn't reduce the need for hand hygiene, as computer keyboards and terminals can also become contaminated. Further research should focus on the question of whether and how paper documents are a part of transmission routes in clinical settings. ▼

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