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Fomites with Your Coffee: Bacterium Abundance and Transmission on Paper Cup Rims

Open Access **Research Article**

Courtney Engel[2], Ashley Chin[3], Ruby Malik[4], Doc Roc[1]*

***Address for Correspondence**

Doc Roc, Researcher, Stoner Laboratory, McMaster University, Hamilton ON L8S 4K1 Canada

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Abstract

Background

Pathogenic agents, such as rhinoviruses, influenza viruses, and bacteria, are transmitted to human hosts through airborne droplet nuclei, respiratory droplets, direct interpersonal contact, and indirect contact via fomites-inanimate objects that act as vehicles for transmitting pathogens among individuals. Transmission via fomites, hands, and facial mucus membranes are among the most common modes for contraction. A relatively new field in public health sciences consequently has emerged: fomite research. Understanding the causal role that fomites play in pathogen spread is requisite to preventing and controlling pathogenic transmittable diseases. We tested whether paper cups constitute fomites by analysing bacterium abundance on rims under different scenarios.

Materials and Methods

Participants were recruited to act as a worker or consumers in an experimental setting simulating a commercial hot beverage outlet. Bacteria were sampled from paper cup rims and cultured, and colonies were quantified. Bacterium abundances were analysed with conventional statistical protocols to test particular hypotheses.

Results

Increased contact with mouths and hands contaminated through physical interaction between a worker and customers was associated with increased bacterium abundance.

Conclusion

Hot beverage promotional campaigns might enhance infectious disease spread by increasing interactions among human body parts on infected and susceptible individuals via fomites in high human-throughput social settings. Situating hot beverage promotional campaigns outside peak seasons for disease spread and contest vouchers away from paper cup rims or implementing online contests could help minimize pathogen spread.

Keywords: Epidemic; Pathogens; Public Health.

Introduction

Fomites: A Brief History

The contemporary North American hot beverage trade, which burgeoned in the 1960s and 1970s and continues strongly, currently is epitomized by creative caffeinated concoctions, including espressos and their derivatives, often topped with signature barista artworks in foam. Consumers, however, might be receiving more than foam with their takeaway white hot chocolates or cappuccinos-they might be handling fomites, inanimate objects (or parts thereof) that, when contaminated by body contact, act as vehicles for transmitting pathogens between individuals *[1]*. Pathogenic agents are transmitted to human hosts through a variety of routes. Contraction ultimately occurs most commonly via fomites, hands, and facial mucus membranes. The relatively new field in modern medicine 'fomite research' consequently has been heralded. Fomites actually are characterized by a medical history that dates to the origins of infectious disease research. The term 'fomite' was introduced into scientific literature as early as 1546, when Girolamo Fracastoro used the Latin root for 'tinder' (fōmēs) to suggest that inanimate objects could 'spark' epidemics *[2]*. Fracastoro is most-famous for penning the poem Syphilis sive Morbus Gallicus, from which the disease received its name; the Copernican colleague is less famous for the essay De Contagione, which arguably contains the first scientific statements on contagions, disease germs, transmission modes, and infections *[3]*, preceding Pasteur and Koch by three centuries. Fracastoro noted that surface properties often determine whether particular objects function effectively as fomites *[3]*. Fracastoro's research was identified with humoralism by his contemporaries and, so, disappeared as the Galenist movement perished *[2]*. Modern scientists only recently have started confirming and adding to what Fracastoro discovered, showing that nonporous objects generally function more effectively as transmitters than do porous objects, for instance (as pathogens generally remain viable longer on nonporous surfaces) *[1]*. In between, fomites have played substantial roles in human history. Colonizations in the

Americas and the devastating effects that they imparted to the indigenous populations involved fomites. British colonization in North America in the 18th century particularly involved provisioning blankets from patients to inoculate the indigenous people with smallpox, tantamount to biological warfare from a contemporary perspective *[4]*. Disproving fomites (and implicating mosquitoes) as responsible for spreading yellow fever by US Army physician Walter Reed in 1900-1901 was pivotal to its control and, ultimately, completing construction on the Panama Canal *[3]*. Since the 17th century, when ingredients for hot beverages were brought to Europe through trade, tea and coffee (and hot-chocolate) houses on all continents have become places where people gather together to socialize. Patrons meet to discuss art, philosophy, literature, economics, politics, and entertainment as well as gossip-arguing, bickering, disagreeing, disputing, and quarrelling, all the while interacting indirectly via inanimate objects. From a Habermassean public-sphere perspective, fomites thusly have affected modern lifestyles, especially in Europe and North America. Prior to 1980, infections from pathogens such as norovirus, rotavirus, and Escherichia coli were rare and infections from methicillin-resistant Staphylococcus aureus and Clostridium difficile predominantly were hospital-related, or nosocomial *[5]*. These pathogens, their related infectious diseases, and their spread currently constitute major health concerns in public settings in Europe and North America and, so, have initiated a modern hygienist movement, focusing on hand cleansing and sanitization *[5]*. Given that many inanimate objects come into contact with hands, fomites warrant attention as potential means for pathogen spreading. The SARS outbreak in 2003 famously involved fomites-elevator buttons-as the mostlikely proximate source for the virus spread, starting February 21 from the 9th floor in the Metropole Hotel in Hong Kong and thence to the greater city as well as Singapore, Vietnam, and Canada *[6]*. Fomite research in a modern setting advanced at the same time that the contemporary coffee trade was burgeoning. Harmful microbes such as Escherichia coli have been recovered from fomites with simple culturing experiments for a half century, for instance *[7]*. In the 1970s and 1980s, researchers interested in 'the common cold' sought to test explicitly whether rhinovirus could be transmitted efficiently via direct and indirect contact, with early studies involving objects like coffee cups *[8]*. Eventually, other inanimate objects, like bank notes and coins, keyboards, electronic mobile devices, and even hotel rooms and their contents or sports balls (reviewed herein in the Conclusion section) also have been identified as effective vehicles for pathogen spread, with viability increased greatly when accompanied by respiratory mucus. How can consumers ensure that they are taking-away no more than white hot chocolates and cappuccinos or health care practitioners ensure that they are bringing into contact with patients only equipment, instruments, and electronic mobile devices? Hand hygiene arguably is the most important method for preventing and controlling pathogenic transmittable diseases *[9]*. Prior to the SARS outbreak, the Centers for Disease Control and Prevention published guidelines for hand hygiene in 6 health care settings; the document provides health care practitioners with specific recommendations to promote improved hand hygiene practices and reduce pathogen transmission *[9]*. Following such guidelines will be paramount in ensuring public health practice in the future. Public health thinking and policy must shift to account for fomites and the roles that they play in disease spread, reinvigorating pathogen awareness. Focusing on fomite-based transmission routes and contraction modes for pathogens and their contributions to infections is crucial for providing sustainable preventative and control practices in global healthcare.

Fomites In Public Health Care Research Four Transmission Modes are Recognized Formally In Pathogen Transmission:

Airborne droplet nuclei, respiratory droplets, direct interpersonal contact, and indirect contact via fomites *[10]*. Given that fomites are inanimate objects (or parts thereof) that, when contaminated by body parts (e.g., hands) or bodily fluids (e.g.,saliva), act as vehicles for transmitting pathogens between individuals *[1, 10-11]*, health care researchers should invest increased attention on fomites as sources for human pathogens and vehicles for transmission. Although fomite research still is bourgeoning, studies over the past half century have revealed some general principles, showing that pathogens tend to persist for longer time periods on nonporous objects relative to porous objects *[7,12-14]* and persistence is determined partially by environmental factors *[13-14]*, for instances.

Fomites abound in daily cosmopolitan city life, including inanimate objects found in tea houses, coffee shops, and cafés. After transfer on fomites, pathogens enter hosts by self-inoculation via conjunctival mucosae or upper respiratory tracts *[15]*. High hand to facial membrane contact rates have been observed in contemporary settings: 0.37 episodes per hour 'conjunctivally' and 0.33 episodes per hour nasally *[16]* as well as 2.5 instances per hour finger contact with eyes, 5.3 instances per hour finger contact with nostrils, and 7.9 instances per hour finger contact with lips *[17]*. Self-inoculation thus is achieved through common habits such as eye rubbing, nose picking, and nail biting, which promote contact between pathogen-contaminated hand and facial membranes *[18]*. Commercially available paper cups constitute underappreciated fomites in contemporary modern social settings. During some hot beverage promotional campaigns in Canada, consumers are enticed to roll-up rims on paper cups to reveal whether prizes have been won, then remit the rims to cash handlers who interact subsequently with many other consumers. Some campaigns coincide temporally with peaks in seasonal human pathogenicity patterns, like influenza virus *[19]*. Evaluating whether paper cups are fomites therefore could have significant implications for appropriately timing and implementing hot beverage promotional campaigns.

Indirect transmission, via fomites, for pathogens between humans is common *[20-21]*; rhinoviruses, responsible for 30-50% common colds, for instance, are the most commonly transmitted pathogen by indirect contact with contaminated environmental surfaces *[22]*. Bacteria such as Staphylococcus also are transmitted commonly via fómites *[23-24]*. We

therefore tested whether a relationship existed between human contact, as would be experienced during hot beverage promotional campaigns, and bacterium abundance on paper cup rims. We hypothesised that increased contact between fingers and facial membranes within and between participants would correlate with greater bacterium abundance on paper cup rims, suggesting that paper cup rims may function as fomites in indirect human pathogen transmission. We predicted that paper cup rims that have contacted mouths, and possibly saliva, would have greater bacterium abundances than would nonexposed paper cup rims. We also predicted that paper cup rims handled by consumers and a cashier would yield greater bacterium abundances than would paper cup rims handled only by consumers. Testing these predictions may have application to timing and design in hot beverage promotional campaigns, especially those that coincide with seasonal infection peaks for human pathogens.

Materials and Methods

Participant Recruitment

Potential study participants were informed about the experiment through poster advertisements and an email message circulated to the Department of Biology at McMaster University (Hamilton, Ontario, Canada) in 2012 January. Thirty-eight participants were recruited, including 15 undergraduate students, 15 graduate students, and staff or faculty members. Recruitment and experimental procedures had been reviewed and approved by the McMaster Research Ethics Board. Appropriate standards for human experimentation subsequently were followed, and subjects were provided with informed consent prior to participation. Simulation Experiment Three scenarios mimicking different stages in hot beverage promotional campaigns were simulated in a laboratory setting. The first participant to arrive was designated as a 'worker' (used with group HUH, as explained subsequently). All other participants, upon arrival, were provided with an identification number and were assigned to one among three groups. Each participant was asked to sign a consent form in duplicate; one copy was kept by the participant, the other was kept by the experimenters. Each participant also was asked to complete a brief questionnaire inquiring about demographic background-including age, gender identification, and academic status-as well as general hand hygiene practice, current respiratory or virus-related symptoms, and tendency to participate in hot beverage promotional campaigns. Group H ('Handled;' n=13) 'customers' were asked to 'retract the rim' on an empty paper cup, tear off the rim, and store it in a sterile specimen bag. Group HU ('Handled & Used;' n=12) customers were asked to drink coffee from a paper cup, retract the rim, tear off the rim, and store it in a sterile specimen bag. Group HUH 10 ('Handled & Used & Handled;' n=12) customers were asked to drink coffee from a paper cup, retract the rim, tear off the rim, and pass the rim to the worker; the worker was asked to store each rim in a separate sterile specimen bag. All specimen bags were labeled with the participant identification number and group designation. The specimen bags collected from group HUH included additional lower case letter labels (from 'a' to 'k') to denote the chronological order in which the worker collected them.

Bacterial Culturing

Immediately following the experiment, liquid Stuart and rayon swabs were used to sample bacteria from each rim, carefully ensuring that swabbed surface areas were identical across samples. The swabs then were streaked across agar plates premade with a Lysogeny Broth (LB) medium. The plates were sealed immediately with Parafilm wax and incubated at 37°C for approximately 30 hours. After incubation, the plates were stored at 4°C (the swabs and rims also were stored at 4°C immediately after use). Images containing the plates were captured and used to count (manually) bacteria colonies present. Experimental Design Interpretation Bacterial colonies present on the agar plates from group H derived from participant fingers and paper cup rims. Bacteria observed on the agar plates from group HU derived from participant saliva, coffee, participant fingers, and paper cup rims. Bacteria on agar plates from group HUH derived from worker fingers, participant 11 saliva, coffee, participant fingers, and paper cup rims. Contributions by saliva and facial mucus membranes to bacterium abundances were analyzed by comparing data from group H with data from group HU. Contributions by indirect human contact to bacterium abundances were analyzed by comparing data from group HU with data from group HUH. Contributions by saliva, facial mucous membranes, and indirect human contact to bacterium abundances were analyzed by comparing data from group H with data from group HUH.

Statistical Analysis

Data were analysed according to conventional statistical protocols with the software JMP (Version <9.0>. SAS Institute Inc., Cary, NC, 1989-2007). Outlying data first were identified with the quartile method and omitted from analysis. A one-way analysis of variance (ANOVA) then was performed to test for differences in mean

values among groups. The post-hoc Tukey-Kramer Honestly Significant Difference (HSD) test then was performed thrice (i.e., for all possible pairwise comparisons), to test for differences between groups.

Results

Two outliers were removed from group H, one outlier was removed from group HU, and two outliers were removed from group HUH. Mean bacterial colony count was greatest in group HUH, intermediate in group HU, and least in group H (Figure 1). The one-way ANOVA indicated that the null hypothesis 'no differences among groups' was falsified according to the data (P=0.0108). The Tukey-Kramer HSD test 12 revealed that null hypothesis 'no difference between groups' was falsified according to data for group H and group HUH (P=0.0077; corresponding tests for group H and group HU as well as group HU and group HUH failed to falsify the null hypothesis 'no

difference between groups,‘ returning respectively P=0.2605 and 0.2235). The chronological order in which the worker participant handled paper cup rims produced no effect on bacterium abundances.

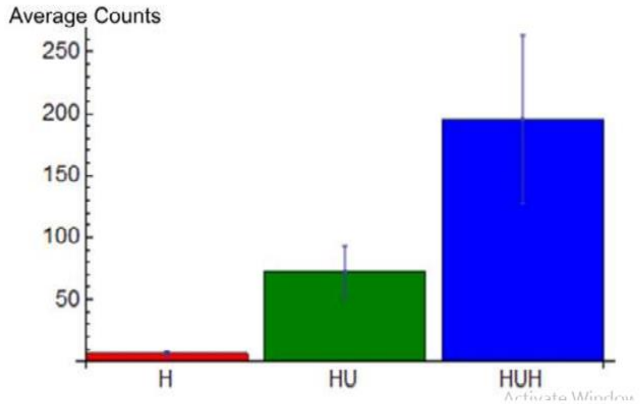

Figure 1: Mean counts for bacterium colonies on coffee cup rims in a simulated coffee shop setting during a hot beverage promotional campaign. Letter abbreviations for categories represent groups in which rims were handled only by customers (H); handled and used by customers (HU); and handled and used by customers, then handled by cashiers (HUH). Whiskers represent standard errors.

Conclusion

Mean bacterial colony count was greatest in group HUH, followed by group HU, and then group H. This pattern is consistent with the prediction that scenarios involving more human body parts and more human contact would yield the greatest bacterium abundances. The consistency suggests that saliva on paper cup rims and subsequent rim transferring between individuals, specifically consumers and workers, can increase pathogen abundances in public circulation. That mean values differed significantly (i.e., according to conventional statistical protocols, given the data) only between groups HUH and H might be attributed to effect size, (small) sample size, and, especially, individual variation. Rim retracting technique, tear location, and torn rim size undoubtedly varied among participants and might have contributed to variation within groups. Using teeth to rim-retract and fingers to tear, tearing sections that had been in contact with saliva, and tearing relatively large rim pieces would associate positively with increased bacterium abundance, for instances. Bacteria were transferred to and remained viable on 13 paper cup rims in each group. This suggests that paper cup rims can act as fomites and promote indirect human pathogen transmission. Future paper cup rim fomite research should standardise rim retracting and tearing; weighing then resuspending samples in fixed-volume Phosphate Buffered Saline followed by vortexing and serial dilutions would enhance quantitative analysis. We anticipated that colony counts would increase in chronological order, from sample ‘a’ to ‘k,’ in group HUH. The prediction was formulated on the basis of the rationale that the lone worker participant handled, in increasing numbers, rims that had been in contact with customer participant mouths and hands. We therefore expected to observe bacteria accumulating on the worker’s hands and partial transfer to each subsequent rim. This prediction was refuted. The same factors that might have increased variation within groups – rim retracting technique, tear location, and torn rim size-might have confounded identifying statistically such an increase. Handling alternatively might have imparted little to bacterium abundances, as the worker’s technique could have involved microbe transfer between each rim and other inanimate objects during the associated interactions (e.g., when the cashier adjusted clothing) and, so, dilution into the surroundings. Persistence also might constitute an influential factor in pathogen transmission via paper cup rims. The time period between drinking a hot beverage and remitting a winning rim in a commercial setting is longer than the time period used in our simulated, experimental setting, and rims would be stored in pockets and money-14 holders. Future paper cup rim fomite research should standardize worker technique and identify how long pathogens can persist under different storage conditions. Interpretation and Recommendations for Future Research and Public Health Policy We interpreted our results within a ‘seasonality’ context. Some human infectious diseases are known to be seasonal in temperate regions, circulating from November to March. Sporadic and outbreak-associated influenza cases recorded in nearby, Toronto hospitals have followed this pattern, for instance *[25] (Figure 2).*

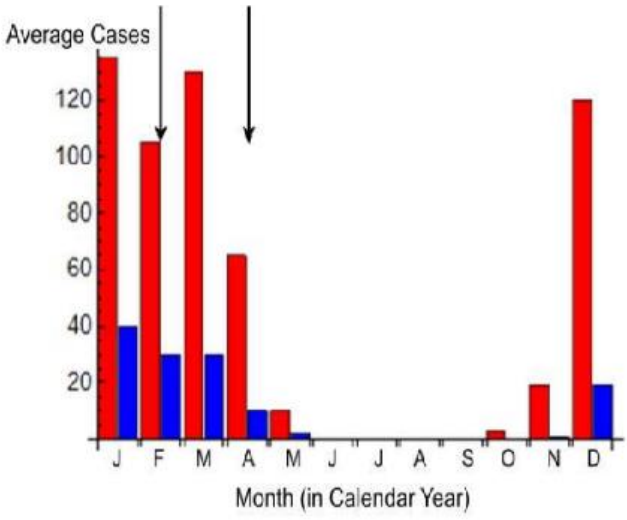

Figure 2: Mean sporadic (red) and outbreak-associated (blue) influenza cases recorded in Toronto hospitals from 2004 to 2011 (with months represented by first letter, presented in order). Data from 2009 were omitted due to differences in categories resulting from a H1N1 influenza virus outbreak. More-recent data (unshown) also indicate that most 2015 influenza cases arise between February and March (City of Toronto. Communicable Diseases in Toronto. 2015; accessed at Hot beverage promotional campaigns typically run from February to April (indicated with arrows).

Some hot beverage promotional campaigns indeed run coincidentally within this peak *(Figure 2)*. Paper cup rims situated in typical Canadian winter environments particularly are conducive to pathogen persistence. Pathogenic viruses, for instance, thrive in mucus-rich, low humidity, and low temperature settings. Although sunlight acts as a natural primary germicide, with ultraviolet (UV) radiation wavelength ranging from 100 nm to 400 nm *[26]*, UV light is limited between November and March in Canadian cities. Paper cup rims thus might act as environmental vehicles underlying seasonal pathogen (i.e., virus as well as bacteria) transmission. Hand hygiene arguably is the most important method for preventing and controlling pathogenic transmittable diseases *[9]*. Following prescribed guidelines will be important in ensuring public health in the future, as pathogens like Staphylococcus aureus can remain infectious on hands for at least 150 minutes and S. aureus as well as methicillin-resistant S. aureus can remain infectious on fomites for 7 months *[9]*. Relatively short survival times on hands together with relatively long survival times 15 on fomites suggest that contaminated inanimate objects are sources for transient colonization, transmission, and spread *[9]*. Other commercial inanimate objects, like money, 15 constitute common and effective vehicles for influenza viruses, with viability increased greatly when accompanied by respiratory mucus. Harmful microbes such as pathogenic Staphylococcus strains, for instance, have been recovered from fomites with simple culturing experiments for almost a half-century *[27]*. We therefore propose that, as paper cup rims and bank notes are handled contemporaneously by customer and cashier hands during hot beverage promotional campaigns and often are stored in pockets, money-holders, and registers, the interaction among these fomites may increase opportunistic transfer for human pathogens. As with our study on paper cup rims, studies on bank notes as fomites have used culture-dependent techniques to document microbial contamination *[28-29]*. These methods greatly limit identification, especially for unculturable microorganisms, thereby preventing researchers from documenting the true microbiome and its diversity on fomites. Contemporary high-throughput sequencing technology and bioinformatic techniques, however, now provide researchers with versatile, powerful methods. Metagenomic sequencing has become the standard for characterising and identifying constituents in microbial communities in environmental samples *[30-31]*; 16S rRNA sequencing particularly has become popular, as it provides information about taxonomic identities in samples, while 16 shotgun sequencing has increased resolution, as it provides data on gene repertoires without bias *[32-33]*. Future paper cup rim fomite research should involve these technologies and techniques to include microorganism identification and diversity quantification.

In addition to paper cup rims and money, telephones *[23]*, sports balls *[24]*, keyboards *[34]*, and even hotel rooms and their contents *[35]* could be among many fomites on which infectious diseases could spread and should be investigated for abundance and composition. Public health thinking and policy ought to shift to account for fomites and the roles that they play in disease spread, revising pathogen awareness. For paper cup rims, displacing promotional hot beverage campaigns to periods that lie outside the peak seasons for known infectious diseases would be warranted. Companies alternatively might consider revising campaigns to promote tearing off bottom or side portions from paper cups (perhaps revealing codes that could be certified online), thereby minimizing pathogenic microorganism transfer from mouth to hand, or transforming the promotion to an online campaign, eliminating at least some fomite exchange. As prevention is the hallmark for reducing morbidity and mortality associated with many diseases, health awareness campaigns should be arranged to educate consumers specifically and the public generally about hazards from contaminated fomites in disease transmission.

Prospectus

At least one hot beverage promotional campaign offered by a Canadian retailer has been modified in response to the 2019-2020 SARS-CoV2 pandemic. The contest involves consumers downloading an application and interacting with their mobile devices to vie for prizes. This proverbially represents a step in the right direction. Mobile devices are fomites, of course *[36]* caveat player.

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