Mean Difference of Coliform Counts in Relation to Sanitation Frequencies at the Simon Fraser University Childcare Society

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ABSTRACT:

Background: Young children in child care facilities are more likely to contract communicable diseases than if they are cared for at home. The relationship between pathogen presence and frequency of toy sanitation at these facilities is not well studied. Thus, the discrepancies currently seen in the hygiene guidelines between health authorities in British Columbia, Canada. Most childcare facility studies in the current literature focus on gastrointestinal outbreak situations or the sanitation of multiple surfaces. The focus of this project is on toys only. Toys made out of wood were selected because research shows that this material is more susceptible to harboring bacteria on it. Microbiological swabbing was performed to measure the effectiveness of the sanitation schedule of a child care facility in Burnaby.

Method: Twenty-four wooden blocks were randomly selected for surface sampling. The 3M™ Quick Swabs were used to collect the bacterial coliforms before and after sanitizing the blocks, whereas, the 3M™ Petrifilm™ E. coli/Coliform Count Plates were used to enumerate the bacteria. The last time the facility had cleaned the blocks was 1.5 weeks prior to sampling.

Results: There were 0 CFU/cm^2 for before and after sanitizing the blocks, therefore, the mean difference was also 0 CFU/cm^2. Inferential statistics could not be conducted.

Conclusion: The results can be interpreted several ways. One interpretation is that the current toy sanitation frequency at the facility is good. It could also mean that, the methodology used was not able to detect any coliforms. In combination with the conclusions from the different studies discussed in the evidence review, the development of a prescriptive toy sanitation schedule for child care facilities would not be a high priority for health authorities.

Keywords: child care facility, sanitation, frequency, colony-forming unit (CFU), coliforms, *E. coli* gastroenteritis

INTRODUCTION:

A little over a century ago, children under the age of five accounted for 30% of all mortalities in the United States of America. Gastroenteritis was also one of the top three causes of death. By 1997, this rate had decreased to 1.4% and the leading causes of deaths were due to heart disease and cancer. The prevention of infectious diseases has been the main contributing factor to reducing mortality rates, which is why it has been considered one of the top ten greatest achievements in public health in the 20th century (Morbidity and Moratality Weekly Report, 1999). Although morbidity and mortality rates have significantly declined, children at child care facilities remain at an elevated risk for contracting a communicable

disease compared to children who are cared for at home (Enserink et al., 2015; Ibfelt, Engelund, Schultz, & Andersen, 2015; Lee & Greig, 2008).

In the literature review conducted by Lee and Greig (2008), it was found that between January 1996 and November 2006, 1806 children contracted gastroenteritis from the child care facility that they attended. One hundred four of these children were hospitalized, and two deaths were reported. The authors identified person-to-person contact as the leading mode of transmission for bacterial outbreaks (43%) in child care facilities.

There were an array of factors that could have contributed to the transmission of pathogens in those facilities, but the risk level for each factor had varied (Gudnason, Hrafnkelsson, Laxdal, & Kristinsson, 2012). An example where there was a low risk of disease transmission was handwashing (BC Centre for Disease Control, 2011; Lee & Greig, 2008). Facilities with written handwashing procedures overall had higher handwashing compliance rates (Zomer et al., 2016). An example of where the significance of disease transmission is not well-known is the frequency of cleaning and sanitizing toys.

This knowledge gap is reflected by the variation seen in the recommendations outlined in the cleaning and sanitizing guidelines for toys across the health authorities in British Columbia. The Vancouver Coastal Health Authority suggests child care facilities clean toys when they are either soiled, frequently used or at least once a week (Vancouver Coastal Health, 2014). In comparison, the Fraser Health Authority has created a self-inspection checklist approach for their child care facilities (Fraser Health, 2013). The form has a close-ended question format that asks whether the toys are cleaned "often" without defining the subjective term. Furthermore, the British Columbia Centre for Disease Control (BCCDC) recommends cleaning soft and hard toys at least daily (BC Centre for Disease Control, 2011). In addition, mouthing toys are recommended to be cleaned and sanitized prior to use.

Studies on the optimal frequency of toy sanitation at child care facilities remain limited and in dissonance. Overall, most of the conclusions suggest that the frequency of toy sanitation was not a significant contributor in disease transmission at child care facilities. This suggestion may be confounded by other factors such as the facility type, cleaning procedures, and type of toy. Hence, this research project used a microbiological approach in an attempt to help develop a standardized toy sanitation guideline across all child care facilities in British Columbia. The number of coliforms on toys before and after sanitation was measured at the Simon Fraser University (SFU) Childcare Society. The difference between the mean CFU levels before and after sanitation was analyzed in relation to the facility's sanitation schedule.

EVIDENCE REVIEW:

Toy sanitation frequency during gastrointestinal outbreaks:

The number of studies that conducted microbiological sampling of toys at child care facilities is limited. A majority of the literature focused on gastroenteritis outbreaks at child care facilities as a whole and looked at toys as a potential source of indirect disease transmission (Cozad & Jones, 2003; Enserink et al., 2015; Lee & Greig, 2008).

Lee and Greig (2008) conducted a systematic review of gastroenteritis outbreaks in child care facilities that occurred between January 1996 and November 2008. In the 75 journal articles and documents that they reviewed, the involvement of toys in indirect transmission was briefly discussed. Interestingly, there were some discrepancies between implemented effective practices and perceived effective practices during an outbreak. Cleaning and sanitizing toys were grouped under environmental cleaning along with other cleaning duties. Increased environmental cleaning was reported as the second most common implemented practice but was only the fourth in terms of perceived effectiveness by child care staff.

In the Netherlands, a microbiological surveillance was conducted over a period of three years (Enserink et al., 2015). Over 1800 gastroenteritis episodes were recorded and 5100 fecal samples were collected. The fecal samples were used to calculate the weekly incidence of five pathogens of interest: norovirus, rotavirus, astrovirus, cryptosporidium and giardia. Different areas and practices of the child care facilities were assessed for their risk and association with increased gastroenteritis. For example, aspects that were analyzed included the presence or absence of: sandpits, dedicated sinks for potty cleaning, and child care capacity. The authors found that cleaning linens and toys on a daily basis was an important means to reducing gastroenteritis episodes.

Toy sanitation frequency during non-gastrointestinal outbreaks:

A medical doctor, who was studying illnesses at child care facilities, referenced hand washing to be an effective practice in reducing the number illness cases. In her article, she advised that "toys are [to be] vigorously cleaned at least weekly" (Bailey, 2013). It is noteworthy that the author did not reference any articles for this recommendation. This makes a weekly toy sanitation schedule debatable.

In the study conducted by Ibfelt et al. (2015), the researchers implemented a two-week frequency cleaning program for selected child care facilities located in Denmark. The number of absences was counted as a means to measure the effectiveness of the intervention. During the facility selection process, it was confirmed that all participating facilities did not have a cleaning schedule for toys nor a methodical approach for cleaning them. Twelve participating child care facilities were then randomly divided into a control group and an intervention group. Sampling of toys and other surfaces were conducted before and after implementing the intervention program. The intervention involved a commercial company picking up linens and toys that were suitable for machine washing. The company came every two weeks over a three month period. If the toys could not be washed by a washing machine, the toy was either submerged into a disinfectant, or wiped clean and sanitized, and then rinsed with water and air dried. Staff were asked to record the reason of absences into the categories predefined by the researchers. The level of respiratory viruses as well as the level of bacteria on the linens and toys were measured. The results showed no significant difference between the intervention group and control group, nor any difference before and after intervention. Most importantly, the number of absences did not decline after the intervention. Although the study by Ibfelt et al. (2015) had a different sanitation schedule, the conclusion of their study was similar to a cross-sectional and longitudinal study performed in Iceland (Gudnason et al., 2012).

In the Icelandic study, some facilities cleaned their toys more than once a week and others less than once a week (Gudnason et al., 2012). The authors could not find any elevated risk with sanitation practices that could contribute to illnesses at child care facilities. This suggested that during non-outbreak situations, the length of time since a toy was last sanitized is irrelevant to gastroenteritis prevention.

Microbiological studies that focused on bacterial counts:

Cosby et al. (2008) used aerobic plate counts to measure coliform and *E. coli* counts at child care facilities at three different times of the day: preopening, lunch and post-cleanup. Overall, the diapering stations had the lowest percentages of positive counts of coliforms and *E. coli*. Between the food serving area and the preparation area, the former had lower counts than the latter. Preopening, lunch and post-cleanup coliform percentages for the serving area were 5.5%, 12.5%, and 8.3% respectively, whereas, the percentages were 0%, 0% and 0.3% for *E.coli.* The amount found post-cleanup was alarming because it was even higher than preopening. While these counts may not have been reflective of the bacterial counts on toys, it makes daily sanitation of toys to be a worthwhile consideration.

In a study that combined microbiological swabbing and surveys, the authors found a statistically significant association between group child-care facilities and family child-care homes in terms of the number of aerobic coliform colonies (Li et al., 2014). Family child-care homes were found to have higher coliform counts. It was also found that facilities with no written cleaning schedule and food preparation procedure had significantly higher counts. It is important to note that the coliform counts of swabbed toys were combined together with irregular surfaces, therefore, a conclusive statement about toy sanitation alone cannot be made from this study.

Strengths and limitations of the evidence review:

Although the focus of this project is on the frequency of toy sanitation, the information from the literature pointed to a conclusion that the causes of illness at child care facilities was multifactorial. It can be agreed that the sanitation frequency was influenced by the type of child care facility (group or family homes), sanitation method, and time of day for sanitation. This agreement was a strength to reassuring the significance in conducting more research on the relationship between illness and child care facilities.

A major limitation in this review was the variation in methodology. This made it difficult to directly compare the varying sanitation frequencies that was studied. Another limitation is the current number of conducted studies on toy cleaning frequency. Many past studies focused on different areas of the facility such as the diapering or food preparation area. The limited number of toy sanitation studies makes it difficult to create policies that are based on well-accepted evidence. Furthermore, in the study conducted by Ibfelt et al. (2015), the effectiveness of bacterial and viral load reduction between groups that had a two-week cleaning schedule and those without, revealed that there was no significance in implementing such a schedule. The authors found that the background levels of selected pathogenic bacteria were lower than the ones done in other studies. The validity of the methods that Ibfelt et al. (2015) used to detect the pathogens is questionable. In their study, 16S rRNA gene sequencing analysis was used to identify the different bacteria in a given area swabbed. The current literature does mention how this technology is limited by the databases that are used and that 5% of the information in the databases may be incorrect (Janda & Abbott, 2007).

Gaps in policy:

The differences in the current toy sanitation frequency requirements between health authorities is an exact reflection of the knowledge gap in the science. Some studies showed that daily sanitation was important during an outbreak situation. In studies that focused on routine sanitation, some facilities had sanitation frequencies of less than one week, others had greater than one week. A two-week frequency was also studied and found that cleaning helped reduced the amount of microorganisms, but illness was not directly associated with how often that occurred.

METHODOLOGY:

Materials:

One container had blocks with a plastic window surrounded by a wooden frame, while the other container had blocks that were solid wood. Twelve blocks with the same or different size and shape were randomly pulled out of each container. A ruler was used to measure the length and width of the swabbed area. The standard methods and interpretation guides of the 3M™ Quick Swabs and Petrifilm™ E. coli/Coliform Count Plates were followed (3M, n.d.-a, n.d.-b). In total, forty-eight swabs and forty-eight Petrifilms™ plates were used for the actual sampling, while two swabs and two plates were used for the pilot test. They were all stored in a refrigerator prior to sampling. An insulated cooler box with ice packs was used during the transportation of the swabs from the child care facility to the lab located at the British Columbia Institute of Technology (BCIT). A 3M™ Petrifilm™ Spreader was used to ensure even distribution of the inoculum. Furthermore, an

incubator in the lab was maintained at 35°C. For the statistical analysis part of this project, a computer with Microsoft Excel was used to tabulate the descriptive statistics of the collected data. The statistical package, NCSS, was installed on the same computer in order to perform paired t-tests (NCSS, 2017). The chemical agents used by the child care facility included: Dawn® Ultra dishwashing liquid, Keystone™ Orange Multi-Surface Cleaner and Keystone™ Food Contact Surface Sanitizer.

Standard Methods:

Sampling Preparation:

The researcher was referred to a Program Director of the SFU Childcare Society by her research supervisor. The director's contact information was also available on the facility's website (SFU Childcare, n.d.). Confirmation of research participation was obtained after a description of the purpose, materials, methods and tentative sampling date was described via email. In mid-January 2017, the researcher toured the facility with the help of the office assistant. The toddler program called Les Petits was selected for the project. Upon coordination with Les Petits and the BCIT lab technician, the sampling date was arranged for a date two weeks from the initial tour.

Swabbing Procedures:

The Early Childhood Educators (ECEs) confirmed that the last routine sanitation of the blocks was a week and a half prior. The blocks were randomly pulled out from their respective containers and were numbered off. All swabs were labelled accordingly to easily identify whether they were part of the before or after sanitation group, and to identify which block they corresponded to. The length and width of the swabbed surface was used to calculate surface area in centimetres. Steps 1 to 6 of the 3M™ Quick Swab method were used on all blocks before getting sanitized (3M, 2013). Next, the researcher used the facility sanitation procedures as instructed by an ECE to sanitize all the blocks. This involved scrubbing the block with a scrubber in warm soapy water. After that, the blocks were sprayed with Keystone™ Orange Multi-Surface Cleaner and Keystone™ Food Contact Surface Sanitizer. The same six steps from above were used once the blocks were dried after being sanitized. All swabs were then transported back to the BCIT lab in a cooler box with ice packs.

Table 1. Microsoft Excel Descriptive Statistics

	Before Sanitation (CFU/cm^2)	*After Sanitation (CFU/cm^2)*	*Difference Between Before and After Sanitation (CFU/cm^2)*
Mean	0	0	0
Standard Error	0	0	0
Median	0	0	0
Mode	0	0	0
Standard Deviation	0	0	0
Sample Variance	0	0	0
Range	0	0	0
Minimum	0	0	0
Maximum	0	0	0
Sum	0	0	0
Count	24	24	24

Table 2. Null and Alternate Hypotheses

H_o	Mean CFU/cm^2 after sanitizing toys ≥ mean CFU/cm^2 before sanitizing toys
H_a	Mean CFU/cm^2 after sanitizing toys < mean CFU/cm^2 before sanitizing toys

Inoculating and Incubating the Plates:

The subsequent inoculation and incubation procedures followed steps 7 to 13 of the 3M™ E.coli/Coliform Count Interpretation Guide (3M, n.d.-a). All Petrifilms™ plates were labelled so that CFUs would be correctly recorded. During incubation, the plates were stacked into four groups of twelve because the maximum number in a stack was twenty as per the guide. The plates were incubated for 24 hours at 35°C for coliforms. *E. coli* CFUs required an incubation time of 48 hours at 35°C, however, due to schedule conflicts between the researcher and the lab, it was not possible to achieve *E. coli* incubation requirements as originally planned.

RESULTS:

Description of Data:

Total surface areas and CFUs are both numerical data. The former is continuous while the latter is discrete numeric. The data that was used for statistical analysis was the difference between the mean CFU/cm^2 before and after sanitation.

CFU Counts:

The number of CFUs was zero for all the forty-eight plates. Should there have been any CFUs, the 3M™ E.coli/Coliform Count Interpretation Guide would have been used to determine which formations were considered proper CFUs (3M, n.d.-a). According to the guide, only CFUs with a gas-bubble formation would have been counted as a true CFU.

Descriptive Statistics:

As outlined in Table 1, it was found that the mean difference before and after sanitation, median, mode, and range was 0 CFU/cm^2. Standard deviation was 0. Because all the differences before and after sanitation were 0 CFU/cm^2, there was no normal distribution.

Inferential Statistics:

Due to the CFU counts being 0 for both before and after sanitation for all samples, inferential statistics was not possible. Could the results have been analyzed with inferential statistics, a one-tail paired t-test would have been used because the null and alternate hypotheses were testing for a difference in one direction (Table 2).

DISCUSSION:

From the results, it can be inferred that the wooden blocks were either free of coliforms, or that the methodology failed to enumerate the coliforms. If the swabbed surfaces were truly free of coliforms, those surfaces would be considered clean according to the guidelines published by the British Columbia Centre for Disease Control (McIntyre, 2010). It is noteworthy that the same guideline indicated that it is acceptable to have background levels of bacteria on cleaned surfaces in food service establishments, but none for sanitized surfaces.

There are several speculations as to why this project had no CFUs. There is the possibility that the general sanitation practices at Les Petits were effective in leaving little to no food residue on surfaces for microbes to proliferate and to cross-contaminate other toys. Another possibility could be that the blocks were cleaned recently by another ECE but the other ECEs were unaware. There is also the chance that microbes did not survive long enough on the wooden blocks to become ingested by the toddlers at infectious doses. Given that other

microbiological studies did have some microbial growth, it is possible that the methodology used in this project was the reason as to why there were no coliforms detected. Human error during the swabbing process cannot be disregarded. The 3M Petrifilm™ Coliform/E. coli plates showed no bacteria, however, when in fact it might just be that the media could not effectively enumerate the type of bacteria that was swabbed. A previous student project used the 3M Petrifilm™ Aerobic Count plate and the researcher was able to enumerate bacteria, however, toys were swabbed at medical clinics instead (Kira Jang, 2010).

The methodologies and purposes of previous microbiological studies from the evidence review were different from this study, however, the results from those studies can still be used as an indirect comparison. All the other studies had some microbial growth, unlike the results from this project. For this project, it was anticipated that nearly all Petrifilms™ plates in the after sanitation group would have zero coliforms. It was not expected that both before and after sanitation groups would have zero growth. In contrast, Cosby et al. (2008) found some growth for aerobic coliforms and a couple CFUs for *E. coli* at all three set sampling times and areas. What was unexpected from that study was that there was an overall higher percentage of growth post-sanitization. In the study conducted by Ibfelt et al. (2015), the effectiveness of scheduled toy sanitation was analyzed in terms of number of absences due to illness. The study found no statistically significant difference in the number of illness absences between facilities with a cleaning schedule and those without. As a part of the research design, the authors also measured background bacterial and viral levels. The study did have growth but the prevalence rates were lower than similar studies. In comparison, inferential statistics could not be conducted for this project. This is because of the 0 CFU before and after sanitation, thus, a conclusion with statistically significant evidence could not be given. However, the results from this study may be supportive of the conclusions made by Ibfelt et al. (2015) and Gudnason et al. (2012). The cross-sectional and longitudinal study conducted by Gudnason et al. (2012) also measured the number of illness absences. Similar to Ibfelt et al. (2015), Gudnason et al. (2008) concluded that the risk of contracting a communicable disease was not any higher for facilities with no sanitation schedules compared to those with a one-week sanitation schedule.

LIMITATIONS:

The major limitation to this project was the amount of funding provided. If there was more funding, a pilot study involving more samples could have been incorporated in order to better determine the most effective enumeration method. Additional funds could have been used to increase the sampling size. At least thirty blocks would have been needed to get a normal distribution and to make the power of the results higher. Another limitation was the researcher's constrained schedule. This made scheduling multiple sampling days between the sampling location and the lab difficult. Ideally, sampling on set intervals around the facility's sanitation schedule would have increased the validity of the study. Furthermore, both funding and time has affected the generalizability. If this project could be extended to other child care facilities of different sizes, geographic location and types (e.g. family child care), then the validity would have been increased.

KNOWLEDGE TRANSLATION:

Although a statistically significant claim could not be made for the hypotheses of this project, the results could possibly be used to direct health authorities as to what health protection areas they should focus on. The results suggest that the varying toy sanitation frequencies guidelines produced by the different

health authorities may be sufficient. It was speculated that the enumeration methodology used was not the most suitable, however, it would be difficult to not generate any visible coliforms if the toy blocks were severely soiled. Therefore, there is a possibility that the normal amount of bacteria found on wooden blocks is not at a level in which health authorities need to prescribe mandatory sanitation frequencies. Yet it would be advisable for the health authorities that do not have defined sanitation frequency recommendations to consider developing some. The participating facility in this project was a group child-care facility. The vast majority of the facilities in the literature review were also group child-care facilities. The results from this project and the ones from the literature review cannot be applied to small family-owned child care facilities. It would not be cost-effective to do the same research project for smaller child care facilities. This is because even the studies that looked at group child-care facilities without sanitation schedules did not find an elevated risk for not having a schedule (Gudnason et al., 2012; Ibfelt et al., 2015). By having a defined sanitation frequency in the guidelines, it would reduce any disputes over objective vocabulary such as "often" when child care facility licensing officers do their inspections. It would also be good guidance for maintaining general sanitation.

FUTURE RESEARCH:

Potential areas of research:

- Microbiological sampling on other toy materials (i.e. plastic and soft material)
- Microbial levels on toys from family-owned childcare facilities

CONCLUSION:

The exact timeframe when a toy was last cleaned and sanitized at a child care facility may not be a critical control point in terms pathogen transmission. That does not imply it is irrelevant when it comes to disease prevention. The sanitization of toys mostly becomes a concern when there is a gastrointestinal outbreak within the facility. Even then, research findings have not been able to give a threshold on the number of hours, days or weeks in which toys need to be sanitized in order to stop an outbreak. Moreover, the current literature in this area is lacking. There are few studies that look at different sanitation frequencies or toy materials, whether it be during outbreak or non-outbreak situations. The findings from this project would not recommend health authorities or other government bodies to pursue additional research in these areas. Results from this research study suggests that the frequency of toy sanitation is not a priority for the health of children at child care facilities.

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COMPETING INTERESTS:

The authors declare that they have no competing interests.

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