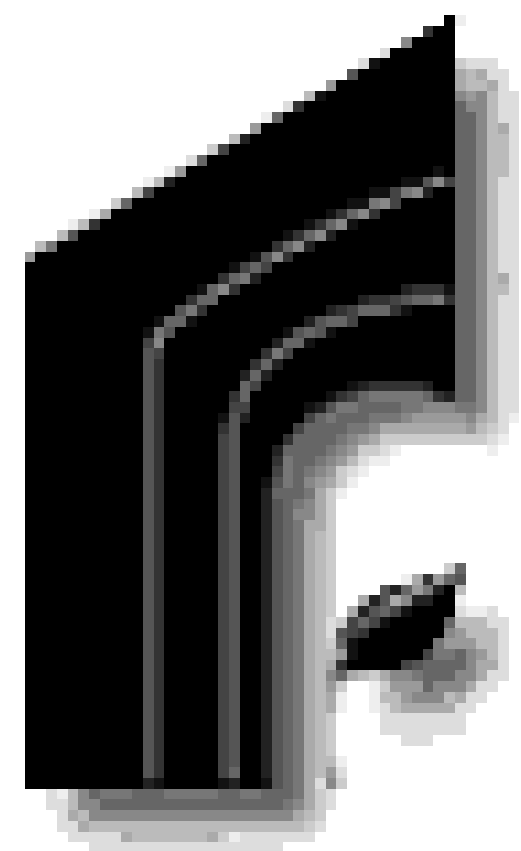
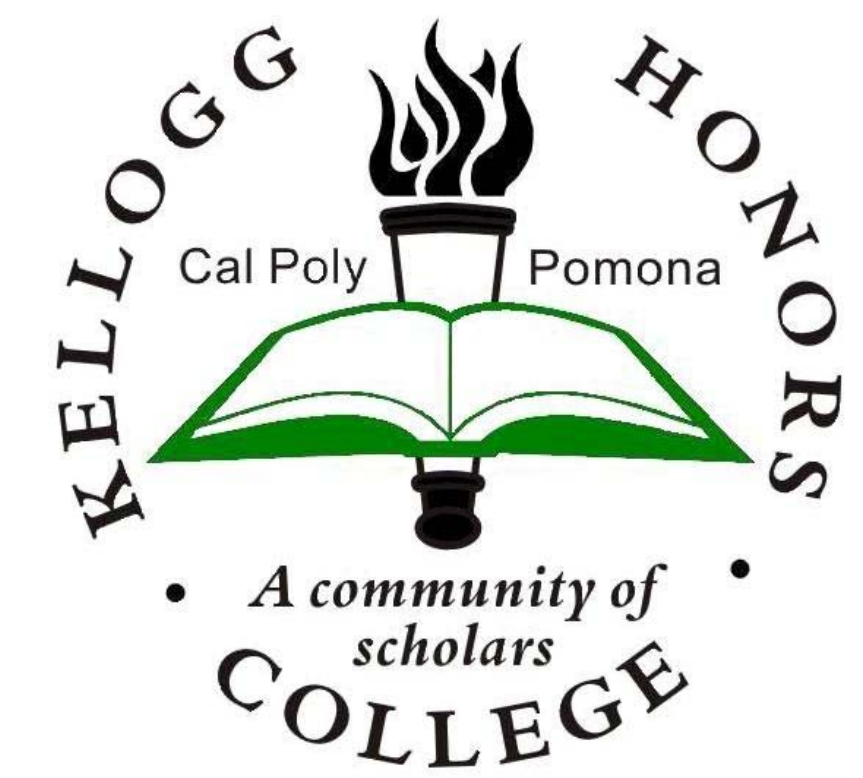


# The Microbial Bioburden of Bathroom Door Handles at California State Polytechnic University, Pomona



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## Why Handwashing?

- A study performed at a small private liberal arts college found that 63% of their female college students washed their hands after using the restroom, though only 2% washed for 10 or more seconds (Drankiewicz and Dundes, 2003).
- Although there are flyers encouraging handwashing post in California State Polytechnic University, Pomona's bathrooms across campus, I was curious to determine how many students, staff, and faculty were heeding their advice and what sort of health risks could be caused by their non-compliance.
- I decided to focus on the detection of coliforms.
- I also decided to further analyze the samples to determine if there was pathogenic *E. coli* present on the door handles. The 6 clones of pathogenic *E. coli* that I decided to test for were Shiga toxin-producing, enteropathogenic, enterotoxigenic, enteroinvasive, enteroaggregative, and diffuse adherent *E. coli*.

## What are Coliforms?

- Coliforms are a class of bacteria that "live in the intestine of warm blooded animals" and are commonly used as an indicator of fecal contamination (coliform).

## Hypothesis:

- When I started this project, I hypothesized that I would find a high count of coliforms and pathogenic *E. coli* on the bathroom door handles.

## Materials and Methods

- Starting on February 15, 2012 and continuing every week until May 10, 2012, I swabbed the door handles leading out of both the men's and women's restrooms of eighteen buildings at California State Polytechnic University, Pomona:  
**Buildings 1, 2, 3, 4, 5, 6, 7, 8, 9, 13, 15, 24, 35, 79a, 97, 98, the 24-hour computer lab, and the Los Olivos Dining Commons.**
- I was unable to collect on February 22, 2012, which gave me 12 days of collections.

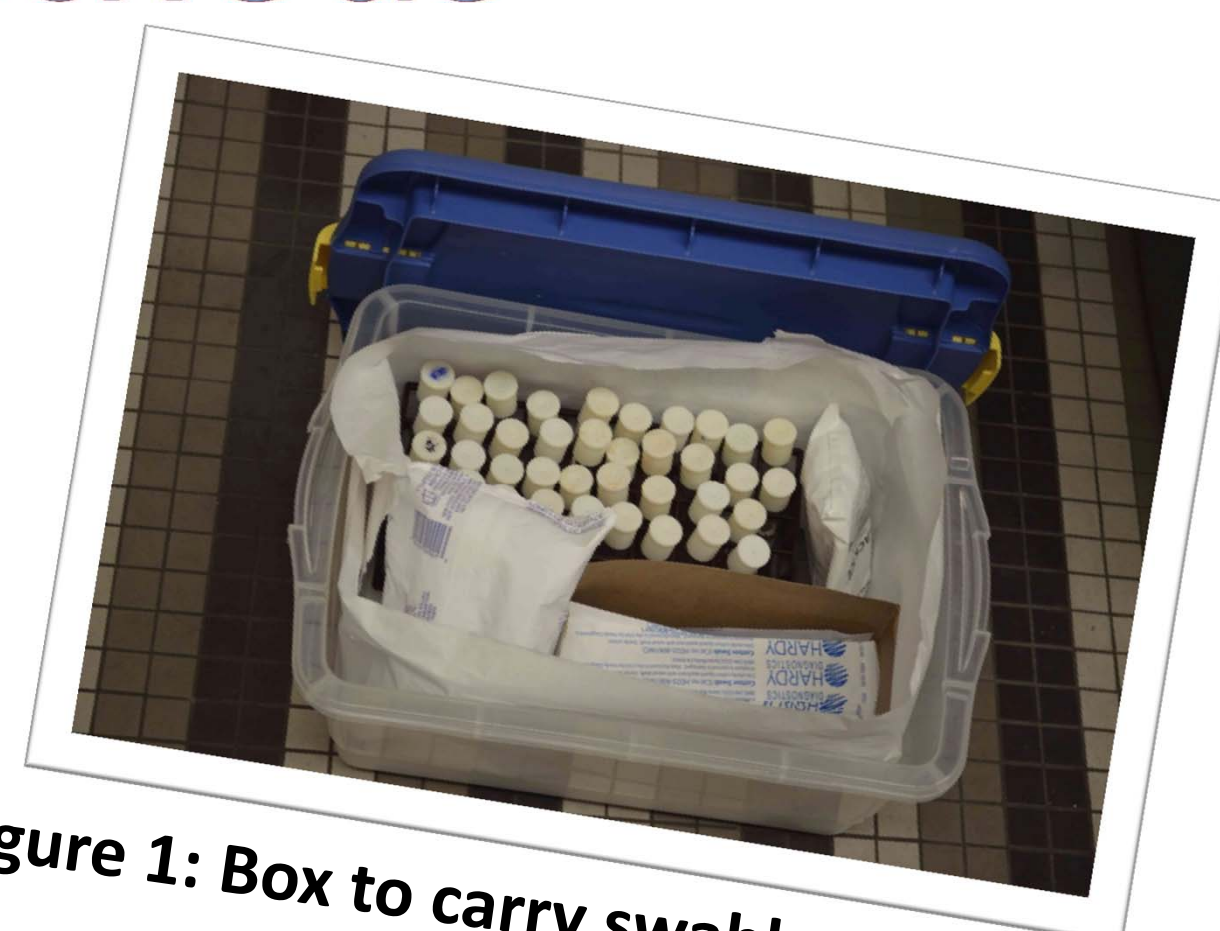


Figure 1: Box to carry swabbed samples

- After collecting the samples, I incubated them at 37°C for 24 hours. I observed which samples contained growth by looking for turbidity, then I streaked for isolation on McConkey Lactose agar plates.
- After obtaining growth on McConkey, I streaked those colonies for isolation on Tryptic Soy Agar, then I placed the colonies that grew in Tryptic Soy Broth and incubated them at 37°C for 24 hours.
- After incubating, I placed 500µL of the growth into a cryopreservation tube containing 500µL of 30% glycerol Tryptic Soy Broth and stored the samples at -20°C for preservation in order to use them for polymerase chain reaction (PCR) analysis at a later date.
- Materials that were used for sample collection were sterile cotton tipped applicators, buffered peptone water, McConkey lactose agar, Tryptic Soy Agar, and 30% glycerol Tryptic Soy Broth.
- The buffered peptone water was used as a carrier media to transport the samples and prevent them from becoming contaminated or drying out.
- McConkey lactose agar was used as a differential media in order to determine lactose fermentation, which is a characteristic of coliforms.



Figure 2: Set up for Gel Electrophoresis

Figure 3: Primers used for PCR

Gene:	Primer sequence (5'-3')	Size of product (bp) and reference
lacZ	ATGAAAGCTGGCTACAGGAAGGCC CACCATGCCGTGGGTTCAATATT	876 (Bej, et al, 1990)
uidA	GTG TGA TAT CTA CCC GCT TCG C AGA ACG GTT TGT GGT TAA TCA GGA	82 (Frahm and Obst, 2003)
Stx1	CAG TTA ATG TGG TGG CGA AGG CAC CAG ACA ATG TAA CCG CTG	348(Vidal, et al,2005)
Stx2	ATC CTA TTC CCG GGA GTT TAC G GCG TCA TCG TAT ACA CAG GAG C	584(Vidal, et al,2005)
Eae	TCA ATG CAG TTC CGT TAT CAG TT GTA AAG TCC GTT ACC CCA ACC TG	482(Vidal, et al, 2005)
Bfp	GGA AGT CAA ATT CAT GGG GGT AT GGA ATC AGA CGC AGA CTG GTA GT	300(Vidal, et al,2005)
It	GCA CAC GGA GCT CCT CAG TC TCC TTC ATC CTT TCA ATG GCT TT	218(Vidal, et al,2005)
stII	AAA GGA GAG GTT CGT CAC ATT TT AAT GTC CGT CTT GCG TTA GGA C	129(Vidal, et al,2005)
virF	AGC TCA GGC AAT GAA ACT TTG AC TGG GCT TGA TAT TCC GAT AAG TC	618(Vidal, et al,2005)
ipaH	CTC GGC ACG TTT TAA TAG TCT GG GTG GAG AGC TGA AGT TTC TCT GC	933(Vidal, et al,2005)
daaE	GAA CGT TGG TTA ATG TGG GGT AA TAT TCA CCG GTC GGT TAT CAG T	542(Vidal, et al,2005)
aafII	CAC AGG CAA CTG AAA TAA GTC TGG ATT CCC ATG ATG TCA AGC ACT TC	378 (Vidal, et al,2005)

## What is PCR?

- Polymerase Chain Reaction
- Method of making amplifying DNA
- Focus on a particular gene

## How is it done?

- Combine Taq Polymerase, Nucleotides, Sample, and primers
- Place into a thermocycler
- Denaturing
- Annealing
- Elongation

## Gel Electrophoresis

- Method for analyzing DNA
- Separates DNA based on fragment length
- Gel can then be stained
- Bands can then be seen

## Results:

- Once I performed PCR using the primers for the *lacZ* gene, I confirmed that only 17 samples were coliforms, meaning that only 0.04% samples were confirmed coliforms.
- The bathrooms that had contained confirmed coliforms were in the following buildings: 4 men's, 5 women's, 6 women's (two different times), 9 men's and women's, 13 women's, 15 men's, 24 men's and women's, 79a men's and women's, 97 men's, 98 women's, and at the Los Olivos Commons men's and women's.
- I performed PCR using the primers for the *uidA* gene on the confirmed coliforms to determine if any of them were *E. coli*. Only 1 of the 17 coliforms tested came up positive for *E. coli*: Building 4 men's restroom.
- After running a multiplex PCR in order to determine if the sample was a pathogenic clone of *E. coli*, I determined that it was not pathogenic.

Figure 5: A Map of Campus



Yellow indicates coliforms were found.  
Red indicates *E. coli* was found.

## What does this mean for Cal Poly Pomona?

- While we did confirm that there were coliforms including *E. coli* on the bathroom door handles of California State Polytechnic University, Pomona, no pathogenic *E. coli* was found. This disproves our hypothesis, but it does not mean that there is no risk to students. It is still noteworthy that coliforms were found on the handles at all and still poses a significant public health hazard if not addressed.
- One of the most pressing concerns was the discovery of coliforms on the bathroom door handles of building 79a, which is a part of the Collins College of Hospitality Management. One of the main focuses of the Collins College is the operation of the Restaurant at Kellogg Ranch, a full service restaurant located on campus. This finding shows that more education about the importance of hand washing and the proper way to wash your hands needs to be provided to these students to prevent and outbreak of foodborne illness.
- There were limitations to this study that could have affected the results. In order to get a more accurate estimation of the abundance of coliforms, I could have collected more days a week, as well as for a longer period of time. There is also a possibility that the magnitude of coliforms could have been decreased due to the desiccant environment of the handle.
- I propose a new advertising campaign to be developed with the help of a committee of students, student health services, and the wellness center. It could be modeled off of a campaign that was done at the University of Texas. It featured fliers posted in the stalls with the statement that "2 out of 5 people don't wash their hands after using the restroom. Is your neighbor one of them?" While it was not successful in getting more students to wash their hands, it did increase the use of soap on campus (Mackert, et. al. 2012).

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