MARIN BIOLOGIC
CONTRACT RESEARCH LABORATORIES

Quantitation of Growth of Shigella Dysenteriae, Salmonella choleraesuis, Listeria monocytogenes, Esherichia coli on Cardboard and ABS Plastic Surfaces

Final Report Submitted to:

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SUMMARY:

Five infectious bacteria commonly found as kitchen/food contamination were tested to determine whether growth on ABS plastic was inhibited compared to cardboard and whether washing the plastic with kitchen antibacterial soap inhibited bacterial growth. The experimental design was to transfer an array of bacteria as discrete drops on to the plastic or cardboard. The plastic and cardboard materials were incubated overnight then blotted onto a sterile agar plate that would support bacterial growth. Data show that although the bacteria grew little on plastic, if at all, they were still present. Even though the bacteria were dried on the plastic, when the plastic was blotted onto sterile agar growth medium, the inoculate was able to sustain growth. However, data show that the antibacterial soap reduced/eliminated the bacterial contamination on the plastic, so that when plastic was blotted onto sterile agar growth medium, there was no bacterial growth. Data also show that moist/wet cardboard is a rich medium for bacterial growth. The results demonstrate that a "lawn" of bacteria grew on the sterile agar growth medium when the cardboard was blotted on the surface.

METHODS and RESULTS:

1 . Organisms:

Shigella dysenteriae	ATCC #13313
Escherichia coli	ATCC #15922
Salmonella choleraesuis	ATCC #13311
Listeria monocytogenes	ATCC #51414
Campylobacter jejuni	ATCC #49943

2. Lyophilized bacteria were resuspended in LB broth and incubated at 37°C overnight to achieve a growing culture. The Campylobacter did not grow. To determine the concentration of bacteria which would grow as discrete, individual colonies, serial dilutions of the E. coli were made (1 /10, 1 /100, 1 /1000, 1 /10,000, 1 /100,000, 1 /1,000,000) and 1 00ul of culture were plated onto 100CM^2 agar plates. The plates were incubated overnight at 37°C. The two concentrations resulting in individual colonies were 1/100,000 and 1/1,000,000. The optimum concentration was approximately 1/500,000.

3. All the bacteria were diluted to 1/500,000 and 1 /1,000,000 and 100ul of culture were transferred to the agar plates and incubated overnight at 37°C. Depending upon the bacterial strain, the concentration was appropriate, but there was uneven distribution of colonies. Some colonies were very close together.

A blot of the bacteria colonies was made by laying the plastic or cardboard upon the agar plate in order to transfer the bacteria to the test material. The cardboard was pretreated by applying water to all surfaces including between the two faces in the corrugated portion. The plastic and cardboard were grown in a moist atmosphere, at 37°C overnight. The bacteria were present on the plastic but did not grow. The cardboard contained a lawn of bacteria. These plates were kept at 4°C until they were digitally imaged.

4. A different approach was used to minimize the visual bacterial detection on the plastic. Using a multichannel pipettor, less than I ul was applied as individual spots directly onto the plastic and dried cardboard. The plastic was incubated overnight at 37°C, in a moist atmosphere that was allowed to dry out. The cardboard was "floated" on water, and incubated in a moist atmosphere overnight at 37°C. The bacteria appeared as dried spots on the plastic. On some of the cardboard pieces, the bacteria could not be visualized. On others, the drops of bacteria were remained as drops rather than soak into the cardboard. One replicate set of plastic samples was washed with a dilute antibacterial soap. The washing process included squirting the samples five times with soap then squirting with purified water to rinse off the soap. The samples were air dried before blotting onto the agar plates.

   The plastic and cardboard pieces were blotted onto a sterile agar plate. The plates were incubated at 37°C overnight and imaged using a digital camera imaging system. The photos were touched-up in Adobe Photoshop and replicated in floppy discs and hard copy. The agar plates blotted with the unwashed plastic materials resulted in the growth of small individual spots of bacteria replicating the pattern of the bacterial application to the plastic. The agar plates blotted with the washed plastic materials supported no growth of bacteria. The agar plates blotted with some of the cardboard showed a "lawn" of bacteria. In agar plates blotted with other cardboard samples a replicate pattern of the bacterial application to the plastic was observed, however, the colonies were larger than that on plastic indicating growth.

CONCLUSIONS

The ABS plastic did not support the growth of the various bacteria, however, the bacteria remained viable after 24 hours even after the medium in the drop dried out. The wet cardboard is a rich medium supporting vigorous growth of the bacteria. Washing the ABS plastic with antibacterial soap was an effective means to reduce the organisms. In this case, all the bacteria were eliminated. In the cardboard samples where the bacteria were applied to wet cardboard rather than dry cardboard, and incubated in a moist atmosphere overnight at 37°C, a thicker "lawn" of bacteria was observed. This suggests that either wet cardboard supports better growth than moist cardboard or the results may be due to the fact that the bacteria spread out during the application process, rather than soak into the cardboard as discrete spots.