Microbicidal efficacy of the Optima Steamer™: *Salmonella enterica subsp. enterica* (ex Kauffman and Edwards)

Provided by:
Steamericas™

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INTRODUCTION

Cross-contamination of food substances with pathogenic microbes during processing poses a significant health risk for the U.S. population and liability for food industries. *Salmonella spp.* can survive on hands, clothes, and utensils for hours or days after initial contact with the microorganisms and transfer rates of cross-contamination among hands, foods, and kitchen surfaces were found to be highly variable (0.0005% to 100%, Chen Y 2000). *Salmonella enteritides*, survive on stainless steel surfaces at room temperature. The transfer rate from sponges to stainless steel surfaces was studied and it was found that microbes can remain viable on dry stainless steel surfaces and present a contamination hazard for long periods of time. (Kusumaningrum 2003). In addition, *Salmonella enterica* can adapt to acidic environments. Table 1 (below) is an overiew of *Salmonella*, nontyphoidal and includes basic information such as foods they contaminate, illnesses they cause and annual number of cases of hospitalizations and deaths.

Table 1. Food Pathogen Studied

Organism Name	Description
Salmonella, nontyphoidal	• gram negative food borne bacterium • contaminates dairy, meat, poultry • causes diarrhea, fever, abdominal cramps • number cases illness per year: 1,027,561 • number hospitalizations per year: 19,336 • number deaths per year: 378 (CDC 2011)

Legend: Basic information about the four food pathogens that were studied. References provided in lower right hand corners.

MATERIALS AND METHODS

Microbe:

Salmonella enterica subsp. enterica (ex Kauffman and Edwards) was selected for efficacy studies (see Table 1 above for descriptions and relevance): *Salmonella enterica subsp. enterica* (*ex* Kauffman and Edwards) Le Minor and Popoff serovar Choleraesuis (ATCC® 10708D-5). Bacteria were purchased form the ATCC (Manassas, VA) and maintained and tested for the ability to generate colony forming units (CFU) following procedures and using growth and test Agar plates as described in the respective product sheets found on the ATCC® website (*Salmonella enterica subsp. enterica*, ex Kauffman and Edwards, Le Minor and Popoff serovar Choleraesuis, ATCC® 10708D-5, http://www.atcc.org/Products/All/10708D-5.asp#documentation).

Test Surfaces:

One food-like substance was selected: sterile organic chicken broth (purchased from VONS, Anaheim, CA). The selected test surface was 4" x 4" custom stainless steel plates with holes drilled at each corner for mounting (Stainless Supply, Monroe, NC). A "target zone" of 5.1 cm^2 was drawn with a waterproof marking pen (Sharpie™) using a circular template. Plates were enclosed in tin foil and autoclaved for 35 minutes at a pressure and temperature of 1.0 – 1.5 bars and ~125 °C that resulted in sterilization, as indicated by autoclave indicator tape. Stainless steel plates were washed and reused for multiple test runs and sterile conditions were

maintained throughout all procedures. At room temperature, sterile stainless steel plates were transferred to a Class II biohazard hood. An aliquot of late log phase culture was diluted in culture medium and an estimated 5,000 cells were transferred to 500 µl of matrix solution (sterile drinking water) and mixed by pipette re-pipette 5 times. A sterile swab was immersed in the solution and used to swab the target area 5 times. The swab was then re-introduced into the solution and the plate was turned 90 degrees and swabbed again 5 times. Inoculated plates were transferred to the exposure chamber in their tinfoil wrappers.

Test Armature:

The exposure chamber consisted of a modified glove box (Plas-Labs, Lansing, MI) with pass through chamber. Operators wore a biohazard suit, full-face respirator and double gloves during operations and the study was conducted in a room with HEPA filtration designed for sterile and biohazard workflow. A 0.22 µm filter (OptiScale Capsule Durapore 0.22 µm filter; Millipore, Billerica, MA) was added to the exposure chamber air input port. The output ports were fitted with tubing, which passed through a 4 liter and then 2 liter vacuum flasks prior to vacuum pumps. The 4 liter vacuum flask was filled with a 50% bleach solution and constructed such that air coming from the chamber bubbled through the bleach solution prior to the second dry vacuum flask. One output port was a general exhaust that could be toggled to clear the pass-through chamber or the chamber itself. The second output port was fitted with a large diameter funnel, which was used to clear the line and ensure a steady flow of steam prior to exposures (described below). The chamber was fitted with a dehumidifier (Frigidaire), which operated continuously during and after exposures. Routine clean up was conducted after each exposure using 70% isopropanol. A squeegee and sponge were used for cleaning between test runs. Excess liquid was treated with bleach to create a 10% solution and then transferred to bottles and then autoclaved prior to disposal. The dry steam generator (Steamericas Optima Steamer™) was in an adjacent room with a hose that entered through the wall and then into the sealed exposure chamber. Deionized water was used in the Optima Steamer™ eliminating the need for an anti-scaling agent that could potentially contaminate food or introduce additional variables to the study. The dry steam generator nozzle ("gun") clamped to the test armature. The test armature was constructed using standard lab clamps, stands and bars; on one side the nozzle was mounted and the other side was the mounting hardware for the stainless steel plates. For all conditions, the end of the nozzle was 15 cm from the stainless steel plate. The nozzle rotated to one side to allow pre-clearing by directing the vapor jet into a vacuum driven funnel to clear the Optima Steamer™ hose of cool water or rotation to inline with the center of the test target. When the nozzle valve was manually opened, the dry hot steam vapor jet extended the entire length of the exposure chamber, approximately 3 feet, and when directed at the target 15 cm from the end of the nozzle resulted in significant reflection of the vapor jet; filling the entire enclosure with steam. The vapor jet had a significant force and in several cases the target was displaced. After longer exposures, stainless steel plates were noticeably hot to touch by gloved hand. Even after a 2 second exposure, careful inspection indicated that the target was free of any color or visible residue (for example, no red color was present anywhere on the steel plates after two seconds of cleaning blood from the target) indicating a high cleaning power for these food matrices from stainless steel.

During preliminary testing, it was found that the efficacy of the Optima Steamer™ was best when the steam hose was first cleared of condensation and the Optima Steamer™ was run continuously until the steam was increased in temperature and was dry. Therefore, the nozzle valve was manually opened for 5 minutes (or longer) during exposures, until a dry, hot steam

jet was produced, at which point, the nozzle was then redirected towards the target and a timer was set. In some cases, there was as much as a 3 second timing error due to the movement and direction of the steam jet or reduced visibility of the target. When visibility was reduced, the operator had to rely on the mechanics of the armature for targeting, which may explain some of the variability in the data. Also of note, a dehumidifier and vacuum pumps were run for 5-20 minutes to clear the chamber before removal of the test plate for analysis.

After exposure, the plates were transferred to sterile tinfoil stored in the pass-through chamber and then transferred to a Class II biosafety cabinet. A sterile swab was immersed in sterile phosphate buffered saline (PBS) and was used to remove surface microbes by swabbing 10 times horizontally, starting at the top and working downward, swabbing 10 times vertically moving left to right. The swab was then immersed in 500 µl of PBS and vigorously shaken. A ten-fold serial dilution series was performed by transferring 50ul from the highest concentration to sterile tubes with 450 µl of PBS, mixed by pipette re-pipette and so forth. 100 µl of serial diluent was transferred to appropriate Agar media (as described by the ATCC® Product Sheets). The plates were labeled, placed in an incubator and monitored for colony development. When colonies emerged, they were counted and recorded. Values were expressed as the number of colony forming units ("CFU") or as a percent survival as compared to control. The number of cells detected was quite variable and ranged from too numerous to count (TNTC) to 0; which was not expected. Variability may have resulted from recovery procedures or variability in steam temperature. CFU counts spanned a relatively broad range. CFU counts should be regarded as estimates in all cases.

*RESULTS

Table 2 (below) shows results for 5 repeated tests (some performed on the same day, others repeated on separate days, all with fresh bacterial cultures).

Table 2. Comparison at recovery from stainless steel for *Salmonella enterica subsp. enterica* (ex Kauffman and Edwards) (CFU = colony forming units)

Organism	Repeat	Control (CFU/5.1 cm^2)	2 second clean (CFU/5.1 cm^2)	4 second clean (CFU/5.1 cm^2)	8 second clean (CFU/5.1 cm^2)	16 second clean (CFU/5.1 cm^2)	32 second clean (CFU/5.1 cm^2)
***Salmonella enterica subsp. enterica* (ex Kauffman and Edwards) Le Minor and Popoff serovar Choleraeuis** Matrix: Sterile, Organic Chicken Broth	1	18800	210	58	ND	ND	ND
	2	1000	1000	28	8	ND	ND
	3	10000	62	ND	ND	ND	ND
	4	50000	1800	1000	118	ND	ND
	5	500	9	91	1	ND	2

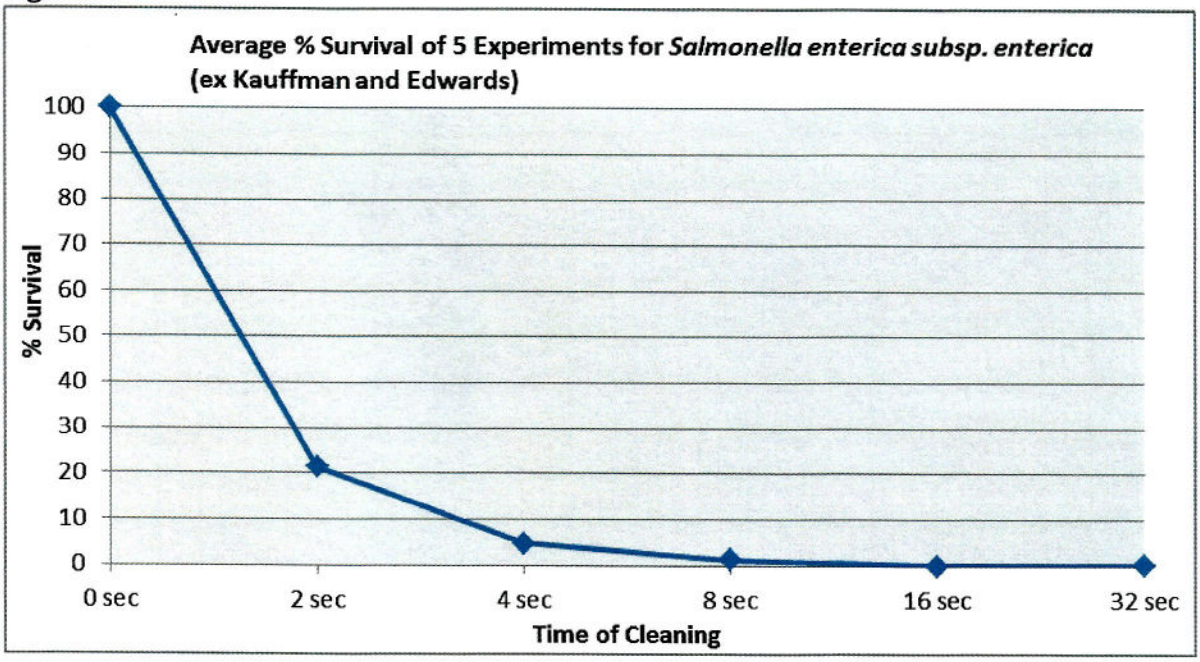
Table 3 (below) shows the data in Table 2 converted to percent not detected (%K). %K = [1 minus (number of CFU in test condition divided by number of CFU for the corresponding control)] times 100.

Table 3. Percent not detected (%K) on stainless steel for *Salmonella enterica subsp. enterica* (ex Kauffman and Edwards)

Organism	Repeat	Control (%K/5.1 cm^2)	2 second clean (%K/5.1 cm^2)	4 second clean (%K/5.1 cm^2)	8 second clean (%K/5.1 cm^2)	16 second clean (%K/5.1 cm^2)	32 second clean (%K/5.1 cm^2)
***Salmonella enterica subsp. enterica* (ex Kauffman and Edwards) Le Minor and Popoff serovar Choleraeuis** Matrix: Sterile, Organic Chicken Broth	1	0	98.9	99.7	100	100	100
	2	0	0	97.2	99.2	100	100
	3	0	99.4	100	100	100	100
	4	0	96.4	98.0	99.8	100	100
	5	0	98.2	81.8	99.8	100	99.6

Figure 1 (below) is a graphical representation of the data converted to average percent survival (Average % Survival). % Survival = number of CFU in each test condition divided by number of CFU for the corresponding control times 100. Cleaning times ranged from 0 (control) to 32 seconds.

Figure 1.



These studies suggest that controlled use of the Optima Steamer™ is an effective method to significantly decreasing food pathogens from stainless steel under the optimized laboratory conditions studied here.

* Disclaimer: These results are for a research study intended to explore microbicidal efficacy using optimized laboratory conditions; results may not be representative of field conditions. For additional limitations see: http://www.lebrunlabs.com/condi.pdf

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