ILSI North America

Food Safety Briefs

August 2015

E. coli

Role of Cellulose and Colanic Acid in Attachment of Shiga Toxin–Producing *Escherichia coli* to Lettuce and Spinach in Different Water Hardness Environments

C-C. Lee, J. Chen, J.F. Frank

Journal of Food Protection, Vol. 78, No. 8; pp. 1461–1466, 2015

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Link to full text: Click here

Significance: Attachment of *E. coli* O157:H7 to leafy greens involves multiple mechanisms that are influenced by the type of leafy green, damage to the leaf, and the water hardness environment.

This study investigated the role of extracellular cellulose production by Shiga toxin–producing *Escherichia coli* (STEC) on attachment to lettuce and spinach in different water hardness environments. Two cellulose-producing wild-type STEC strains, 19 (O5:H–) and 49 (O103:H2), and their cellulose-deficient derivatives were used. Strain 49 attached at levels 0.3 and 0.6 log greater to the surface and 0.9 and 0.4 log greater to the cut edges of spinach compared to strain 19 for both wild-type and cellulose-deficient cells ($P > 0.05$). Cellulose-producing cells attached more to the surface of lettuce but not of spinach than did cellulose-deficient cells. However, more cellulose-deficient cells attached (at levels 0.66 and 0.3 log greater) to the cut edge of lettuce (representing damaged tissue) than did cellulose-proficient cells ($P > 0.05$). There was a decreasing level of attachment for the colanic acid–producing strain when water hardness increased from 200 to 1,000 pm on lettuce and spinach leaf surfaces, but no effects were seen for other cells. This decreased attachment was associated with a more negative surface charge. Cells that produced colanic acid were less hydrophobic and exhibited greater attachment to the surface and cut edge of spinach when compared to cells that did not produce colanic acid. Attachment of colanic acid–producing cells to leafy green surfaces was enhanced in higher water hardness environments.

Sequence of Colonization Determines the Composition of Mixed Biofilms by *Escherichia coli* O157:H7 and O111:H8 Strains

R. Wang, N. Kalchayanand, J.L. Bono

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Significance: *E. coli* O157:H7 strains were able to maintain a higher cell percentage in mixed biofilms with the co-inoculated O111:H8 companion strains, even though the results of planktonic growth competition were strain dependent.

This study investigated how the coexistence of Shiga toxin–producing *Escherichia*

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coli (STEC) O157:H7 and O111:H8 serotypes and their sequence of colonization could affect bacterial growth competition and mixed biofilm development. On solid surfaces with preexisting biofilms, the sequence of colonization played a critical role in determining the composition of the mixed biofilms because early stage precolonization significantly affected the competition results between the *E. coli* O157:H7 and O111:H8 strains. The precolonizer of either serotype was able to outgrow the other serotype in both planktonic and biofilm phases. The competitive interactions among the various STEC serotypes would determine the composition and structure of the mixed biofilms as well as their potential risks to food safety and public health, which is largely influenced by the dominant strains in the mixtures.

Foodborne Pathogens

Efficacy of Neutral pH Electrolyzed Water in Reducing *Escherichia coli* O157:H7 and *Salmonella* Typhimurium DT 104 on Fresh Produce Items using an Automated Washer at Simulated Food Service Conditions

G.K. Afari, Y-C. Hung, C.H. King

Journal of Food Science, Vol. 80, No. 8; pp. M1815–M1822, 2015

doi: 10.1111/1750-3841.12936

Link to full text: Click here

Significance: Washing produce with neutral pH electrolyzed water in an automated washer at food service establishments can help reduce the occurrence of foodborne illnesses related to cross-contamination.

This study determined the efficacy of neutral pH electrolyzed (NEO) water (155 mg/L free chlorine, pH 7.5) in reducing *Escherichia coli* O157:H7 and *Salmonella* Typhimurium DT 104 on romaine lettuce, iceberg lettuce, and tomatoes washed in an automated produce washer for different times and washing speeds. Tomatoes and lettuce leaves were spot inoculated with 100 µL of a 5 strain cocktail mixture of either pathogen and washed with 10 or 8 L of NEO water, respectively. Washing lettuce for 30 min at 65 rpm led to the greatest reductions, with 4.2 and 5.9 log CFU/g reductions achieved for *E. coli* O157:H7 and *S.* Typhimurium respectively on romaine, whereas iceberg lettuce reductions were 3.2 and 4.6 log CFU/g for *E. coli* O157:H7 and *S.* Typhimurium, respectively. Washing tomatoes for 10 min at 65 rpm achieved reductions >8 and 6 log CFU/tomato on *S.* Typhimurium and *E. coli* O157:H7 respectively. All pathogens were completely inactivated in NEO water wash solutions. No detrimental effects on the visual quality of the produce studied were observed under all treatment conditions.

Determination of the Thermal Inactivation Kinetics of *Listeria monocytogenes, Salmonella enterica,* and *Escherichia coli* O157:H7 and non-O157 in Buffer and a Spinach Homogenate

E.A. Monu, M. Valladares, D.H. D'Souza, P.M. Davidson

Journal of Food Protection, Vol. 78, No. 8; pp. 1467-1471, 2015

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Link to full text: Click here

Significance: A mild thermal treatment of blended spinach at 70°C for less than 1 min would result in a 6-log reduction of all pathogens tested.

The objectives of this research were to compare the thermal inactivation kinetics of *Listeria monocytogenes, Salmonella enterica,* Shiga toxin–producing *Escherichia*

coli (STEC) O157:H7, and non-O157 STEC in phosphate-buffered saline (PBS; pH 7.2) and a spinach homogenate and to provide an estimate of the safety of mild heat processes for spinach. For *Listeria* and *Salmonella* at 56 to 60°C, *D*-values in PBS ranged from 4.42±0.94 to 0.35±0.03 min and 2.11±0.14 to 0.16±0.03 min, respectively. *D*-values at 54 to 58°C were 5.18±0.21 to 0.53±0.04 min for STEC O157:H7 and 5.01±0.60 to 0.60±0.13 min for non-O157 STEC. In spinach at 56 to 60°C, *Listeria D*-values were 11.77±2.18 to 1.22±0.12 min and *Salmonella D*-values were 3.51±0.06 to 0.47±0.06 min. *D*-values for STEC O157:H7 and non-O157 STEC were 7.21±0.17 to 1.07±0.11 min and 5.57±0.38 to 0.99±0.07 min, respectively, at 56 to 60°C. In spinach, *z*-values were 4.07±0.16, 4.59±0.26, 4.80±0.92, and 5.22±0.20°C for *Listeria, Salmonella,* STEC O157:H7, and non-O157 STEC, respectively.

Prevalence of *Escherichia coli* O157:H7 and *Salmonella* on Inshell California Walnuts

G.R. Davidson, J.C. Frelka, M. Yang, T.M. Jones, L.J. Harris

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Link to full text: Click here

Significance: *Salmonella* but not *E. coli* was present in inshell walnuts from California.

This study evaluated the presence of *Escherichia coli* O157:H7 and *Salmonella* in inshell walnuts collected from California walnut handlers over four harvests. *E. coli* O157:H7 was not detected in any of 2,903 375-g samples evaluated in 2011, 2012, and 2013 (<0.034% prevalence; 95% CI, 0 to 0.13%). *Salmonella* was not isolated from any of the 935 samples in 2010 (100 g evaluated; <0.11% prevalence; 95% CI, 0 to 0.41%) but was isolated from 2 of 905 (375 g; 0.22% prevalence; 95% CI, 0.061 to 0.80%), 1 of 998 (375 g; 0.10% prevalence; 95% CI, 0.018 to 0.56%), and 1 of 1,000 (375 g; 0.10% prevalence; 95% CI, 0.018 to 0.56%) samples in 2011, 2012, and 2013, respectively, for an average annual prevalence of 0.14% (375 g; 95% CI, 0.054 to 0.35%). The levels of *Salmonella* in positive samples determined by a modified most-probable-number (MPN) method were estimated to be 0.32 to 0.42 MPN/100 g (95% CI, 0.045 to 3.6 MPN/100 g).

Effectiveness of Inactivation of Foodborne Pathogens During Simulated Home Pan Frying of Steak, Hamburger or Meat Strips

E. Lahou, X. Wang, E. De Boeck, E. Verguldt, A. Geeraerd, F. Devlieghere, et al.

International Journal of Food Microbiology, Vol. 206, 3 August 2015; pp. 118–129, 2015

doi: 10.1016/j.ijfoodmicro.2015.04.014

Link to full text: Click here

Significance: Consumers could still be exposed to surviving food borne pathogens in pan frying of raw meat and meat preparations at consumer's home.

The heat resistance (D-value) of three strains of *Campylobacter jejuni*, *Escherichia coli* O157:H7, *Salmonella* spp., *Listeria monocytogenes* and two strains of generic *E. coli* was validated in BHI and adjusted BHI (i.e., pH 5.6 and 1.5% NaCl) at 60 °C. Heat treatment in adjusted BHI significantly increased heat-resistance of *E. coli* O157:H7 and generic *E. coli*. Subsequently, the thermal inactivation of *L. monocytogenes*, *Salmonella* spp., *C. jejuni* and *E. coli* O157:H7 was evaluated using a standardized procedure simulating commonly used home pan frying of various types of meat including steaks or filets, hamburgers and meat strips

from pork, beef, chicken, lamb and some turkey, horse, kangaroo and crocodile meat. Corresponding F70-values were calculated based upon measured core time/temperature profiles. A core temperature of 70 °C was not always achieved and a heat treatment equivalent to 2 min at 70 °C was also not always obtained. Pan frying of hamburgers yielded the highest number of surviving pathogenic bacteria (46%), followed by well-done filets and steaks (13%) and meat strips (12%). Taking only steaks into account, residual detection of pathogens occurred for all levels of doneness. Numbers of *L. monocytogenes* recovered after heat treatment ranged from < 1 log CFU/g to 2.6 log CFU/g.

Combination Treatment of Chlorine Dioxide Gas and Aerosolized Sanitizer for Inactivating Foodborne Pathogens on Spinach Leaves and Tomatoes

Sang-Hyun Park, Dong-Hyun Kang

International Journal of Food Microbiology, Vol. 207, 17 August 2015; pp. 103–108, 2015

doi: 10.1016/j.ijfoodmicro.2015.04.044

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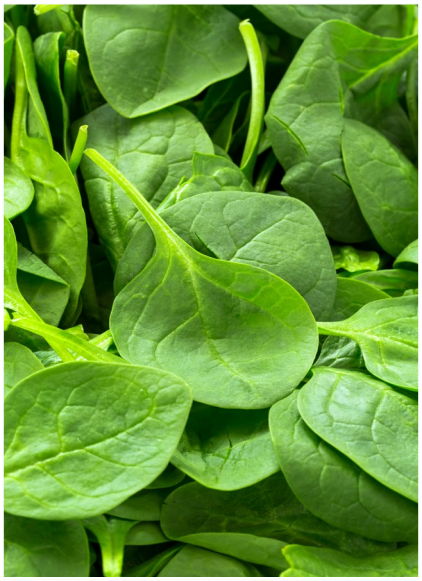

Significance: Combinations of chlorine dioxide gas and aerosolized sanitizer showed additive effects in the inactivation of the three pathogens and did not affect the quality of samples during storage.

The objective of this study was to evaluate the antimicrobial effect of chlorine dioxide (ClO2) gas and aerosolized sanitizer, when applied alone or in combination, on the survival of *Escherichia coli* O157:H7, *Salmonella* Typhimurium, and *Listeria monocytogenes* inoculated onto spinach leaves and tomato surfaces. ClO2 gas (5 or 10 ppmv) and aerosolized peracetic acid (PAA) (80 ppm) were applied alone or in combination for 20 min. Exposure to 10 ppmv of ClO2 gas for 20 min resulted in 3.4, 3.3, and 3.4 log reductions of *E. coli* O157:H7, *S.* Typhimurium, and *L. monocytogenes* on spinach leaves, respectively. Treatment with 80 ppm of aerosolized PAA for 20 min caused 2.3, 1.9, and 0.8 log reductions of *E. coli* O157:H7, *S.* Typhimurium, and *L. monocytogenes*, respectively. Combined treatment of ClO2 gas (10 ppmv) and aerosolized PAA (80 ppm) for 20 min caused 5.4, 5.1, and 4.1 log reductions of *E. coli* O157:H7, *S.* Typhimurium, and *L. monocytogenes*, respectively. *E. coli* O157:H7, *S.* Typhimurium, and *L. monocytogenes* on tomatoes experienced similar reduction patterns to those on spinach leaves.

Listeria

Listeria Monocytogenes-Carrying Consortia in Food Industry. Composition, Subtyping and Numerical Characterisation of Mono-Species Biofilm Dynamics On Stainless Steel

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doi: 10.1016/j.ijfoodmicro.2015.05.003

Link to full text: Click here

Significance: An ecological distribution was demonstrated as *Escherichia coli* appeared to be the most abundant in fish industry and *Carnobacterium* spp. in meat industry.

A total of 270 environmental samples belonging to work surfaces from fish (n=123), meat (n=75) and dairy industries (n=72) were analyzed in order to

detect *Listeria monocytogenes*. Twelve samples were positive for *L. monocytogenes* and a total of 18 different species were identified as accompanying microbiota in fish and meat industry. Molecular characterisation combining results of *Asc*I and *Apa*I macrorestriction PFGE assays yielded 7 different subtypes of *L. monocytogenes* sharing in 71.43% of cases the same serogroup (1/2a–3a). Results from dynamic numerical characterisation between *L. monocytogenes* monospecies biofilms on stainless steel (SS) demonstrated that except in isolate A1, average diffusion distance (ADD) and maximum diffusion distance (MDD) was observed after 120 h of culture. Quantitative dual-species biofilm association experiments performed on SS indicated that *L. monocytogenes* cell counts presented lower values in mixed-species cultures with certain species at 24 and 48 h compared with mono-species culture.

Salmonella

Acid Environments Affect Biofilm Formation and Gene Expression in Isolates Of *Salmonella Enterica* Typhimurium DT104

D. O'Leary, E.M. McCabe, M.P. McCusker, M. Martins, S. Fanning, G. Duffy

International Journal of Food Microbiology, Vol. 206, 3 August 2015; pp. 7–16, 2015

doi: 10.1016/j.ijfoodmicro.2015.03.030

Link to full text: Click here

Significance: Lactic acid has the potential to inhibit *Salmonella* biofilms.

This study examined the survival and potential virulence of biofilm-forming *Salmonella* Typhimurium DT104 under mild acid conditions. The cells were acid-adapted by culturing them in 1% glucose and their ability to form biofilms on stainless steel and on the surface of Luria Bertani (LB) broth at pH 7 and pH 5 was examined. Of the 4 isolates that were examined only one (1481) that produced a rigid biofilm in LB broth at pH 7 also formed this same structure at pH 5. This indicated that the lactic acid severely impeded the biofilm producing capabilities of the other isolates examined under these conditions. Isolate 1481 also had higher expression of genes associated with virulence (*hilA*) and invasion (*invA*) with a 24.34-fold and 13.68-fold increase in relative gene expression respectively at pH 5 compared to pH 7. Although genes associated with biofilm formation had increased expression in response to acid stress for all the isolates this only resulted in the formation of a biofilm by isolate 1481.

Salmonella Isolated From Ready-To-Eat Pasteurized Liquid Egg Products: Thermal Resistance, Biochemical Profile, and Fatty Acid Analysis

J.B. Gurtler, A. Hinton Jr., R.B. Bailey, W.C. Cray Jr., R.J. Meinersmann, T.A. Ball, et al.

International Journal of Food Microbiology, Vol. 206, 3 August 2015; 109–117, 2015

doi: 10.1016/j.ijfoodmicro.2015.04.010

Link to full text: Click here

Significance: *Salmonella* contamination in pasteurized egg products may be the result of either thermally-resistant isolates or post-processing contamination.

The Egg Products Inspection Act of 1970 requires that egg products in the U.S. must be pasteurized prior to release into commerce. The USDA Food Safety and Inspection Service (FSIS) is responsible for regulating egg products. Salmonellae are infrequently isolated from pasteurized egg products by food manufacturers or the FSIS and may be present as a result of either pasteurization-resistant bacteria

or post-processing contamination. In this study, 17 strains of *Salmonella* isolated from pasteurized egg products and three heat-resistant control strains were compared for thermal resistance in liquid whole egg (LWE) at 60 °C, enzymatic profiles, and serotyping and phage typing, antibiotic susceptibility, fatty acid analysis and strain morphological variation. Isolates were serotyped as Heidelberg (4 isolates), Widemarsh, Mbandaka, Cerro, Thompson, 4,12:i:-, and Enteritidis (8 isolates). All 20 isolates were sensitive to all 14 antibiotics tested for. The D60 values in LWE ranged from 0.34 to 0.58 min. All 20 strains were recovered from LWE inoculated with 8.5 log CFU/mL of *Salmonella* and pasteurized at 60 °C for 3.5 min; however, some isolates were not recovered from pasteurized LWE that had been inoculated with only 4.5 log CFU/mL *Salmonella* and treated at 60 °C for 3.5 min. Furthermore, fatty acid analysis revealed that differences insaturate/unsaturated profiles may be correlated with differences in heat resistance, in two instances. One heat resistant strain (#13, Enteritidis) had the statistically lowest unsaturated/saturate ratio at 39%. However, one heat sensitive strain (#3, serovar 4,12:i:-) had the highest unsaturated/saturate ratio at 81%, and also the lowest concentration of stearic acid.

Reduction of *Salmonella* on Chicken Meat and Chicken Skin by Combined or Sequential Application of Lytic Bacteriophage with Chemical Antimicrobials

A.T. Sukumaran, R. Nannapaneni, A. Kiess, C.S. Sharma

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doi: 10.1016/j.ijfoodmicro.2015.04.025

Link to full text: Click here

Significance: The sequential application of chlorine, peracetic acid, and phage can provide additional hurdles to reduce *Salmonella* on fresh poultry carcasses or cut up parts.

The effectiveness of recently approved *Salmonella* lytic bacteriophage preparation (SalmoFresh™) in reducing *Salmonella in vitro* and on chicken breast fillets was examined in combination with lauric arginate (LAE) or cetylpyridinium chloride (CPC). In another experiment, a sequential spray application of this bacteriophage (phage) solution on *Salmonella* inoculated chicken skin after a 20 s dip in chemical antimicrobials (LAE, CPC, peracetic acid, or chlorine) was also examined in reducing *Salmonella* counts on chicken skin. The application of phage in combination with CPC or LAE reduced *S.* Typhimurium, *S.* Heidelberg, and *S.* Enteritidis up to 5 log units *in vitro* at 4 °C. On chicken breast fillets, phage in combination with CPC or LAE resulted in significant reductions of *Salmonella* ranging from 0.5 to 1.3 log CFU/g as compared to control up to 7 days of refrigerated storage. When phage was applied sequentially with chemical antimicrobials, all the treatments resulted in significant reductions of *Salmonella*. The application of chlorine (30 ppm) and PAA (400 ppm) followed by phage spray (109 PFU/ml) resulted in highest *Salmonella* reductions of 1.6–1.7 and 2.2–2.5 log CFU/cm2, respectively.

Food Allergy

Consensus Communication on Early Peanut Introduction and the Prevention of Peanut Allergy in High-Risk Infants

D.M. Fleischer, S. Sicherer, M. Greenhawt, D. Campbell, E. Chan, A. Muraro, et al.

Journal of Allergy and Clinical Immunology, Vol. 136, No. 2; pp. 258–261, 2015

doi: 10.1016/j.jaci.2015.06.001

Link to full text: Click here

Significance: This paper highlights emerging evidence for existing allergy prevention guidelines regarding potential benefits of supporting early rather than delayed peanut introduction during the period of complementary food introduction in infants.

Based on data generated in the Learning Early About Peanut Allergy (LEAP) trial, the following interim guidance is suggested to assist the clinical decision making of health care providers. Health care providers should recommend introducing peanut-containing products into the diets of "high-risk" infants between 4 and 11 months of age in countries where peanut allergy is prevalent because delaying the introduction of peanut can be associated with an increased risk of peanut allergy. Infants with early-onset atopic disease, such as severe eczema, or egg allergy in the first 4 to 6 months of life, might benefit from evaluation by an allergist or physician trained in management of allergic diseases in this age group to diagnose any food allergy and assist in implementing these suggestions regarding the appropriateness of early peanut introduction. Without intervention by health care providers, there is the potential that such high-risk infants will remain at risk for delayed introduction of solids and allergenic foods into their diet because of the widespread belief that such foods can exacerbate eczema.

Norovirus

Postharvest Survival of Porcine Sapovirus, a Human Norovirus Surrogate, on Phytopathogen-Infected Leafy Greens

M.A. Esseili, A. Chin, L. Saif, S.A. Miller, F. Qu, M.L. Lewis Ivey, et al.

Journal of Food Protection, Vol. 78, No. 8; pp. 1472–1480, 2015

doi: 10.4315/0362-028X.JFP-14-518

Link to full text: Click here

Significance: Phytopathogen-induced necrotic lesions may enhance the postharvest survival of human noroviruses on lettuce leaves.

Leafy greens are increasingly being recognized as an important vehicle for human noroviruses (HuNoV). Leafy greens often become infected by phytopathogens in the field. Lettuce and spinach plants were infected with a bacterium, *Xanthomonas campestris* pv. *vitians* strain 701a, and with *Cucumber mosaic virus* strain Fny, respectively. The survival rate of porcine sapovirus (SaV), a HuNoV surrogate, on infected and noninfected postharvest leaves was then assessed. In addition, acibenzolar-*S*-methyl, a commercial chemical elicitor of plant systemic defense, was used to assess whether stimulating the plant host defense affects the postharvest survival of SaV. Leaves harvested from control and treated plants were inoculated with SaV and incubated for 7 days at 4°C. The infectivity and RNA titers of SaV were assayed using immunohistochemistry staining and SaV-specific TaqMan real-time reverse transcription PCR. Cucumber mosaic virus Fny induced mild, nonnecrotic symptoms on spinach leaves and had no effect on SaV survival. In contrast, *X. campestris* pv. *vitians* 701a induced small localized necrotic lesions and significantly enhanced SaV survival on lettuce leaves. Treatment with acibenzolar-*S*-methyl was effective in reducing *X. campestris* pv. *vitians* 701a–induced lesions on infected lettuce plants but had no direct effect on SaV survival when used on healthy lettuce plants.

Inactivation of Human Norovirus and Its Surrogates on Alfalfa Seeds by Aqueous Ozone

Q. Wang, S. Markland, K.E. Kniel

Journal of Food Protection, Vol. 78, No. 8; pp. 1586-1591, 2015
doi: 10.4315/0362-028X.JFP-15-029
Link to full text: Click here

Significance: The significant inactivation by aqueous ozone indicates that ozone may be a plausible substitute for chlorine as an alternative treatment for seeds.

This study assessed aqueous ozone for the disinfection of alfalfa seeds contaminated with human norovirus (huNoV) and its surrogates. The inactivation of viruses without a food matrix was also investigated. Alfalfa seeds were inoculated with huNoV genogroup II, Tulane virus (TV), and murine norovirus (MNV); viruses alone or inoculated on seeds were treated in deionized water containing 6.25 ppm of aqueous ozone with agitation at 22°C for 0.5, 1, 5, 15, or 30 min. Aqueous ozone resulted in reductions of MNV and TV infectivity from 1.66±1.11 to 5.60±1.11 log PFU/g seeds; for all treatment times, significantly higher reductions were observed for MNV ($P<0.05$). Viral genomes were relatively resistant, with a reduction of 1.50±0.14 to 3.00±0.14 log genomic copies/g seeds; the reduction of TV inoculated in water was similar to that of huNoV, whereas MNV had significantly greater reductions in genomic copies ($P<0.05$). Similar trends were observed in ozone-treated viruses alone, with significantly higher levels of inactivation ($P<0.05$), especially with reduced levels of infectivity for MNV and TV.

Destruction of the Capsid and Genome of GII.4 Human Norovirus Occurs during Exposure to Metal Alloys Containing Copper

C.S. Manuel, M.D. Moore, L.A. Jaykus

Applied and Environmental Microbiology, Vol. 81, No. 15; pp. 4940-4946, 2015
doi: 10.1128/AEM.00388-15
Link to full text: Click here

Significance: Copper surfaces destroy human noroviruses and may be useful in preventing environmental transmission of the virus in at-risk settings.

This study characterized the destruction of GII.4 human norovirus (HuNoV) and virus-like particles (VLPs) during exposure to copper alloy surfaces. Fecal suspensions positive for a GII.4 HuNoV outbreak strain or GII.4 VLPs were exposed to copper alloys or stainless steel for 0 to 240 min and recovered by elution. HuNoV genome integrity was assessed by reverse transcription-quantitative PCR (RT-qPCR) (without RNase treatment), and capsid integrity was assessed by RT-qPCR (with RNase treatment), transmission electron microscopy (TEM), SDS-PAGE/Western blot analysis, and a histo-blood group antigen (HBGA) binding assay. Exposure of fecal suspensions to pure copper for 60 min reduced the GII.4 HuNoV RNA copy number by ~3 log10 units when analyzed by RT-qPCR without RNase treatment and by 4 log10 units when a prior RNase treatment was used. The rate of reduction of the HuNoV RNA copy number was approximately proportional to the percentage of copper in each alloy. Exposure of GII.4 HuNoV VLPs to pure-copper surfaces resulted in noticeable aggregation and destruction within 240 min, an 80% reduction in the VP1 major capsid protein band intensity in 15 min, and a near-complete loss of HBGA receptor binding within 8 min. In all experiments, HuNoV remained stable on stainless steel.

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