Food Safety Briefs
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E. Coli

Prevalence and Characteristics of Shiga Toxin-Producing Escherichia Coli Isolated From Retail Raw Meats in China
X. Bai, H. Wang, Y. Xin, R. Wei, X. Tang, A. Zhao, et al.
doi: 10.1016/j.ijfoodmicro.2015.01.018
Link to full text: Click here

Significance: There is a high genetic diversity of Shiga toxin-producing Escherichia coli in retail raw meats, some of which have potential to cause human diseases.

This study evaluated the prevalence of Shiga toxin-producing Escherichia coli (STEC) from retail raw meats collected from two geographical regions in China. The results revealed that 166 out of 853 samples were stx-positive; 63 STEC isolates were recovered from 58 stx-positive samples including pork (4.4%, 14/318), beef (11.0%, 21/191), mutton (20.6%, 26/126), chicken (0.5%, 1/205), and duck (7.7%, 1/13). Twenty-six O serogroups and 33 O:H serotypes were identified. All three stx, subtypes and five stx, subtypes (2a to 2e) were found in the 63 STEC isolates, among which stx, positive STEC isolates were the most predominant (39.7%), followed by stx, only (20.6%), stx, + stx, (14.3%), and stx, only (9.5%). STEC isolates carried virulence genes eae (6.3%), ehxA (36.5%), katP (4.8%), astA (11.1%), and subA (36.5%). Of the four adherence-associated genes tested, toxB was absent, whereas saa, paa, and efal were present in 28, three, and one STEC isolates respectively. The STEC isolates were divided into 50 PFGE patterns and 33 sequence types. STEC from different sources and geographical regions were separated by PFGE and MLST.

Y.B. Kim, H.W. Kim, M.K. Song, M.S. Rhee
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Link to full text: Click here

Significance: This decontamination method uses only heat and relative humidity without chemicals, and is thus applicable as a general decontamination procedure in spout-producing plants where the use of growth chambers is the norm.

A novel decontamination method was developed to inactivate Escherichia coli O157:H7 on radish seeds without adversely affecting seed germination or product quality. The use of heat (55, 60, and 65 °C) combined with relative humidity (RH; 25, 45, 65, 85, and 100%) for 24 h was evaluated for effective microbial
reduction and preservation of seed germination rates. A significant two-way interaction of heat and RH was observed for both microbial reduction and germination rate (P < 0.0001). Increases in heat and RH were associated with corresponding reductions in E. coli O157:H7 and in germination rate (P < 0.05). The order of lethality for the different treatments was as follows: no treatment < 55 °C/25–65% RH = 60 °C/25–45% RH < 65 °C/25% RH < 55 °C/85% RH = 60 °C/65% RH < 55 °C/100% RH = 60 °C/85–100% RH = 65 °C/45–100% RH. The most effective condition, 65 °C/45% RH, completely inactivated E. coli O157:H7 on the seeds (7.0 log CFU/g reduction) and had no significant effect on the germination rate (85.4%; P > 0.05) or product quality.

G.C. Paoli, C. Wijey, G.A. Uhlich
doi: 10.4315/0362-028X.JFP-14-472
Link to full text: Click here

Significance: Genetically marked strains could be used to enumerate and model the growth of STEC in the presence of foodborne background flora.

In this study, positive control (PC) strains for the detection of Shiga toxin–producing E. coli (STEC) O157:H7 and the six USDA-regulated non-O157 STEC (serogroups O26, O45, O103, O111, O121, and O145) were constructed. To ensure that the food testing samples were not cross-contaminated by the PC sample, it is important that the STEC-PC strains were distinguishable from STEC isolated from test samples. End-point and real-time PCR assays were developed for the specific detection of the PC strains and were tested using 93 strains of E. coli (38 STEC O157:H7, at least 6 strains of each of the USDA-regulated non-O157 STEC, and 2 commensal E. coli) and 51 strains of other bacteria (30 species from 20 genera). The PCR assays demonstrated high specificity for the unique target sequence. The target sequence was detectable by PCR after 10 culture passages (~100 generations). In addition, the strains were tested for their potential use in modeling the growth of STEC. Plating the PC strains mixed with ground beef flora on modified rainbow agar containing spectinomycin (Sp), eliminated the growth of the background flora that grew on modified rainbow agar without Sp.

Control of the Biofilms Formed by Curli- and Cellulose-Expressing Shiga Toxin–Producing Escherichia coli Using Treatments With Organic Acids and Commercial Sanitizers
Y.J. Park, J. Chen
doi: 10.4315/0362-028X.JFP-14-382
Link to full text: Click here

Significance: Bacterial surface components and cell contact surfaces can influence both biofilm formation and the efficacy of sanitizing treatments.

This study was conducted to quantify biofilms formed by different Shiga toxin–producing Escherichia coli (STEC) strains on polystyrene and stainless steel surfaces and to determine the effectiveness of sanitizing treatments in control of these biofilms. STEC producing various amounts of cellulose (n=6) or curli
(n=6) were allowed to develop biofilms on polystyrene and stainless steel surfaces at 28°C for 7 days. The biofilms were treated with 2% acetic or lactic acid and manufacturer-recommended concentrations of acidic or alkaline sanitizers, and residual biofilms were quantified. Treatments with the acidic and alkaline sanitizers were more effective than those with the organic acids for removing the biofilms. Compared with their counterparts, cells expressing a greater amount of cellulose or curli formed more biofilm mass and had greater residual mass after sanitizing treatments on polystyrene than on stainless steel.

**Thermal Inactivation of Shiga Toxin–Producing Escherichia coli Cells Within Cubed Beef Steaks Following Cooking on a Griddle**


doi: 10.4315/0362-028X.JFP-14-454

**Significance:** Cooking single or double cubed steak on a nonstick aluminum griddle heated at 191.5°C for at least 1.25 and 3.0 min/side, respectively, was sufficient to achieve a 5.0log reduction in the levels of the single strains from each of the eight target STEC serogroups tested.

Thermal inactivation of Shiga toxin–producing Escherichia coli (STEC) cells within cubed beef steaks following cooking on a nonstick griddle was quantified. Both faces of each beef cutlet (ca. 64 g; ca. 8.5 cm length by 10.5 cm width by 0.75 cm height) were surface inoculated (ca. 6.6 log CFU/g) with 250 μl of a rifampin-resistant cocktail composed of single strains from each of eight target serogroups of STEC: O26:H11, O45:H2, O103:H2, O104:H4, O111:H2, O121:H19, O145:NM, and O157:H7. Single-cubed steaks (SCS) and double cubed steaks (DCS) were individually cooked for ≤3.5 min/side in 30 ml of extra virgin olive oil heated to 191.5°C on a hard-anodized aluminum nonstick griddle using a flat-surface electric ceramic hot plate. Regardless of single versus double cubed, the longer the cooking time, the higher the final internal temperature, and the greater the inactivation of STEC cells within cubed steaks. The average final internal temperatures of SCS and DCS (cooked for ≤2.5 min and 3.5 min, respectively) ranged from 59.8-94.7°C and 40.3-82.2°C, respectively. Cooking SCS and DCS on an aluminum griddle set at ca. 191.5°C for 0.5 to 2.5 min and 1.0 to 3.5 min per side, respectively, resulted in total reductions in pathogen levels of ca. 1.0 to ≥6.8 log CFU/g.

**Salmonella**

**In-Feed Supplementation of trans-Cinnamaldehyde Reduces Layer-Chicken Egg-Borne Transmission of Salmonella enterica Serovar Enteritidis**


doi: 10.1128/AEM.03809-14

**Significance:** Trans-cinnamaldehyde may potentially be used as a feed additive to reduce egg-borne transmission of S. Enteritidis.

This study investigated the efficacy of in-feed supplementation with trans-cinnamaldehyde (TC) in reducing Salmonella enterica serovar Enteritidis cecal
colonization and systemic spread in layers. Additionally, the effect of TC on S. Enteritidis virulence factors critical for macrophage survival and oviduct colonization was investigated in vitro. Supplementation of TC in feed for 66 days at 1 or 1.5% (vol/wt) for 40- or 25-week-old layer chickens decreased the amounts of S. Enteritidis on eggshell and in yolk (P<0.001). Additionally, S. Enteritidis persistence in the cecum, liver, and oviduct in TC-supplemented birds was decreased compared to that in controls (P<0.001). In vitro cell culture assays revealed that TC reduced S. Enteritidis adhesion to and invasion of primary chicken oviduct epithelial cells and reduced S. Enteritidis survival in chicken macrophages (P 0.001). Follow-up gene expression analysis using real-time quantitative PCR (qPCR) showed that TC downregulated the expression of S. Enteritidis virulence genes critical for chicken oviduct colonization (P<0.001).

**Foodborne Pathogens**

*Previous Physicochemical Stress Exposures Influence Subsequent Resistance of Escherichia Coli O157:H7, Salmonella Enterica, and Listeria Monocytogenes to Ultraviolet-C in Coconut Liquid Endosperm Beverage*

A.A. Gabriel

doi: 10.1016/j.ijfoodmicro.2015.02.003

Link to full text: Click here

**Significance:** The D and D$_{UV-C}$ values of *S. enterica* after previous exposure to sequential acid and desiccation stresses were found significantly greatest, making the organism and physiological state an appropriate reference organism for the establishment of UV-C pasteurization process for the beverage.

This study investigated the influences of prior exposures to common physicochemical stresses encountered by microorganisms in food and food processing ecologies on their subsequent susceptibility towards UV-C treatment in coconut liquid endosperm beverage. Cocktails of *Escherichia coli* O157:H7, *Salmonella enterica*, and *Listeria monocytogenes* were separately subjected to gradually acidifying environment (final pH 4.46), exposed to abrupt desiccation by suspension in saturated NaCl solution (aw = 0.85) for 4, 8, and 24 h, and sequential acidic and desiccated stresses before suspending in the coconut beverage for UV-C challenge. Non-stressed cells had exposure time (D) values of 3.2–3.5 s, and corresponding UV-C energy dose values (D$_{UV-C}$) values of 8.4–9.1 mJ/cm$^2$. Cells exposed to previous acid stress had D values of 4.1–4.8 s and corresponding D$_{UV-C}$ values of 10.7–12.5 mJ/cm$^2$. Prior exposure to desiccation resulted in D values of 5.6–7.9 s and D$_{UV-C}$ values of 14.7–20.6 mJ/cm$^2$, while exposure to combined acid and desiccation stresses resulted in D values of 6.1–8.1 s and D$_{UV-C}$ values of 15.9–21.0 mJ/cm$^2$.

**Reduction of Surrogates for Escherichia coli O157:H7 and Salmonella During the Production of Nonintact Beef Products by Chemical Antimicrobial Interventions**


doi: 10.4315/0362-028X.JFP-14-344

Link to full text: Click here
The efficacy of chemical antimicrobials for controlling Escherichia coli O157:H7 and Salmonella during production of marinated non-intact beef products was evaluated using nonpathogenic surrogates. Boneless beef strip loins were inoculated with either approximately 5.8 (high) or 1.9 (low) log CFU/cm² of non-pathogenic rifampin-resistant E. coli, chilled at 2°C for 24 h, vacuum packaged, and aged for 7-24 days at 2°C. After aging, strip loins received no treatment (control) or one of five antimicrobial spray treatments: 2.5% L-lactic acid (pH 2.6), 5.0% L-lactic acid (pH 2.4), 1,050 ppm of acidified sodium chlorite (pH 2.8), 205 ppm of peroxyacetic acid (pH 5.2), or tap water (pH 8.6). Mean application temperatures were 53, 26, 20, and 18°C for lactic acid, water, peroxyacetic acid, and acidified sodium chlorite treatments, respectively. For high-inoculation strip loins, the 5.0% L-lactic acid treatment was most effective for reducing surrogates on meat surfaces before marination, producing a 2.6-log mean reduction. Peroxyacetic acid treatment resulted in the greatest reduction of surface-located surrogate microorganisms in marinated product. Water treatment resulted in greater internalization of surrogate microorganisms compared with the control, as determined by enumeration of surrogates from cored samples.

**Norovirus**

A Comparative Study of Digital RT-PCR and RT-qPCR for Quantification of Hepatitis A Virus and Norovirus in Lettuce and Water Samples


doi: 10.1016/j.ijfoodmicro.2015.02.006

Link to full text: Click here

The performance of microfluidic digital RT-PCR (RT-dPCR) was compared to RT-qPCR for detecting the main viruses responsible for foodborne outbreaks (human Noroviruses (NoV) and Hepatitis A virus (HAV)) in spiked lettuce and bottled water. Two process controls (Mengovirus and Murine Norovirus) were used and external amplification controls (EAC) were added to examine inhibition of RT-qPCR and RT-dPCR. For detecting viral RNA and cDNA, the sensitivity of the RT-dPCR assays was either comparable to that of RT-qPCR (RNA of HAV, NoV GI, Mengovirus) or slightly (around 1 log₁₀) decreased (NoV GII and MNV-1 RNA and of HAV, NoV GI, NoV GII cDNA). The number of genomic copies determined by dPCR was always from 0.4 to 1.7 log₁₀ lower than the expected numbers of copies calculated by using the standard qPCR curve. Viral recoveries calculated by RT-dPCR were found to be significantly higher than by RT-qPCR for NoV GI, HAV and Mengovirus in water, and for NoV GII and HAV in lettuce samples. The RT-dPCR assay proved to be more tolerant to inhibitory substances present in lettuce samples.
Listeria

Development of Predictive Models for the Growth Kinetics of Listeria monocytogenes on Fresh Pork Under Different Storage Temperatures

K. Luo, S-S. Hong, J. Wang, M-J. Chung, D-H. Oh

doi: 10.4315/0362-028X.JFP-14-428

Link to full text: Click here

**Significance:** The model developed in this study was capable of predicting the growth of *L. monocytogenes* under various isothermal conditions.

This study was conducted to develop a predictive model to estimate the growth of *Listeria monocytogenes* on fresh pork during storage at constant temperatures (5, 10, 15, 20, 25, 30, and 35°C). The Baranyi model was fitted to growth data (log CFU/gram) to calculate the specific growth rate (SGR) and lag time (LT) with a high coefficient of determination (R² > 0.98). As expected, SGR increased with a decline in LT with rising temperatures in all samples. Secondary models were then developed to describe the variation of SGR and LT as a function of temperature. Subsequently, the developed models were validated with additional independent growth data collected at 7, 17, 27, and 37°C and from published reports using proportion of relative errors and proportion of standard error of prediction. The proportion of relative errors of the SGR and LT models developed herein were 0.79 and 0.18, respectively. In addition, the standard error of prediction values of the SGR and LT of *L. monocytogenes* ranged from 25.7 to 33.1% and from 44.92 to 58.44%, respectively.

Comparative Efficacy of Potassium Levulinate With and Without Potassium Diacetate and Potassium Propionate Versus Potassium Lactate and Sodium Diacetate for Control of Listeria monocytogenes on Commercially Prepared Uncured Turkey Breast


doi: 10.4315/0362-028X.JFP-14-467

Link to full text: Click here

**Significance:** Potassium levulinate is at least as effective as potassium propionate and potassium diacetate as an antilisterial agent.

The efficacy of potassium levulinate (KLEV; 0.0, 1.0, 1.5, and 2.0%) with and without a blend of potassium propionate (0.1%) and potassium diacetate (0.1%) (KPD) versus a blend of potassium lactate (1.8%) and sodium diacetate (0.125%) (KLD) were evaluated for inhibiting *Listeria monocytogenes* on commercially prepared, uncured turkey breast during refrigerated storage. Product formulated with KLD or KLEV (1.5%) was also subsequently surface treated with 44 ppm of a solution of lauric arginate (LAE). Without inclusion of antimicrobials in the formulation, pathogen levels increased by ca. 5.2 log CFU/slice, whereas with the inclusion of 1.0-2.0% KLEV pathogen levels increased by only ca. 2.9 to 0.8 log CFU/slice after 90 days at 4°C. When 1.0% KLEV and KPD were included as ingredients, pathogen levels increased by ca. 0.8 log CFU/slice after storage at 4°C for 90 days, whereas a decrease of ca. 0.7 log CFU/slice was observed when 1.5 or 2.0% KLEV and KPD were included as ingredients. KLD was effective at suppressing *L. monocytogenes* in uncured turkey breast. When uncured turkey
breast was formulated with KLD or KLEV (1.5%) or without antimicrobials and subsequently surface treated with LAE, pathogen levels decreased by ca. 1.0 log CFU/package within 2 h. Results validate the use of KLEV to inhibit outgrowth of L. monocytogenes during refrigerated storage of uncured turkey breast.

**Acrylamide**

**Effect of Pretreatments and Air-Frying, a Novel Technology, on Acrylamide Generation in Fried Potatoes**

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Link to full text: Click here

**Significance:** Air-frying reduced acrylamide content by about 90% compared with conventional deep-oil-frying.

This paper investigated the effect of air-frying technology, in combination with a pretreatment based of soaking the samples in different chemical agent solutions (citric acid, glycine, calcium lactate, sodium chloride, or nicotinic acid [vitamin B3]), on the generation of acrylamide in fried potatoes. The influence of reducing sugars on the development of surface’s color was also analyzed. The experiments were conducted at 180 °C by means of air-frying and deep-oil-frying, as a reference technology. Based on the evolution of color crust with frying time, it could be concluded that the rate of Maillard reaction decreased as the initial reducing sugars content increased in the raw material, and was also lower for deep-oil-frying than for air-frying regardless of pretreatments applied. Air-frying reduced acrylamide content by about 90% compared with conventional deep-oil-frying without pretreatment. However, deep-oil fried potatoes pretreated with solutions of nicotinic acid, citric acid, glycine at 1%, and NaCl at 2% presented much lower acrylamide levels (up to 80% to 90% reduction) than nonpretreated samples.

**Food Allergy**

**Sublingual Immunotherapy for Peanut Allergy: Long-Term Follow-Up of a Randomized Multicenter Trial**


*Journal of Allergy and Clinical Immunology*, Vol. 135, No. 5; pp. 1240–1248.e3, 2015

Link to full text: Click here

**Significance:** Peanut sublingual immunotherapy induced a modest level of desensitization, decreased immunologic activity over 3 years in responders, and had an excellent long-term safety profile.

This study sought to provide long-term (3-year) clinical and immunologic outcomes for the peanut sublingual immunotherapy (SLIT) trial. Key end points were (1) percentage of responders at 2 years (ie, could consume 5 g of peanut powder or a 10-fold increase from baseline), (2) percentage reaching desensitization at 3 years, (3) percentage attaining sustained unresponsiveness after 3 years, (4) immunologic end points, and (5) assessment of safety parameters.
Response to treatment was evaluated in 40 subjects aged 12-40 years by performing a 10-g peanut powder oral food challenge after 2 and 3 years of daily peanut SLIT therapy. At 3 years, SLIT was discontinued for 8 weeks, followed by another 10-g oral food challenge and an open feeding of peanut butter to assess sustained unresponsiveness. Approximately 98% of the 18,165 doses were tolerated without adverse reactions beyond the oropharynx, with no severe symptoms or uses of epinephrine. A high rate (>50%) discontinued therapy. By study's end, 4 (10.8%) of 37 SLIT-treated participants were fully desensitized to 10 g of peanut powder, and all 4 achieved sustained unresponsiveness. Responders at 2 years showed a significant decrease in peanut-specific basophil activation and skin prick test titration compared with nonresponders.

Natural History of Peanut Allergy and Predictors of Resolution in the First 4 Years of Life: A Population-Based Assessment
Journal of Allergy and Clinical Immunology, Vol. 135, No. 5; pp. 1257–1266.e2, 2015
doi: 10.1016/j.jaci.2015.01.002
Link to full text: Click here

Significance: In this study, thresholds for SPT and sIgE levels were generated in which all participants underwent oral food challenges at both diagnosis and follow-up, irrespective of SPT and sIgE results.

This study sought to describe the natural history of peanut allergy between 1 and 4 years of age and develop thresholds for skin prick test (SPT) results and specific IgE (sIgE) levels measured at age 1 and 4 years that have 95% positive predictive value (PPV) or negative predictive value for the persistence or resolution of peanut allergy. One-year-old infants with challenge-confirmed peanut allergy (n=156) were followed up at 4 years of age with repeat oral food challenges, SPTs, and sIgE measurements (n=103). Peanut allergy resolved in 22% (95% CI, 14% to 31%) of children by age 4 years. Decreasing wheal size predicted tolerance, and increasing wheal size was associated with persistence. Thresholds for SPT responses and sIgE levels at age 1 year with a 95% PPV for persistent peanut allergy are an SPT-induced response of ≥13 mm and an sIgE level of ≥5.0 kU/L. Thresholds for SPT and sIgE results at age 4 years with a 95% PPV for persistent peanut allergy are an SPT response of ≥8 mm and an sIgE level of ≥2.1 kU/L. Ara h 2, tree nut, and house dust mite sensitization; coexisting food allergies; eczema; and asthma were not predictive of persistent peanut allergy.

A Randomized, Double-Blind, Placebo-Controlled Pilot Study of Sublingual Versus Oral Immunotherapy for the Treatment of Peanut Allergy
Journal of Allergy and Clinical Immunology, Vol. 135, No. 5; pp. 1275–1282.e6, 2015
doi: 10.1016/j.jaci.2014.11.005
Link to full text: Click here

Significance: Oral immunotherapy appeared far more effective than sublingual immunotherapy for the treatment of peanut allergy, but was also associated with significantly more adverse reactions and early study withdrawal.
This double-blind study compared the safety, efficacy, and mechanistic correlates of peanut oral immunotherapy (OIT) and sublingual immunotherapy (SLIT) in children with peanut allergy (PA). Twenty-one subjects (7 to 13 years) were randomized to receive active SLIT/placebo OIT or active OIT/placebo SLIT. Doses were escalated to 3.7 mg/d (SLIT) or 2000 mg/d (OIT), and subjects were rechallenged after 6 and 12 months of maintenance. After unblinding, therapy was modified per protocol to offer an additional 6 months of therapy. Subjects who passed challenges at 12 or 18 months were taken off treatment for 4 weeks and rechallenged. Five subjects discontinued therapy; of the remaining 16, all had a >10-fold increase in challenge threshold after 12 months. The increased threshold was significantly greater in the active OIT group (141- vs 22-fold, P=.01). Significant within-group changes in skin test results and peanut-specific IgE and IgG4 levels were found, with overall greater effects with OIT. Adverse reactions were generally mild but more common with OIT (P<.001), including moderate reactions and doses requiring medication. Four subjects had sustained unresponsiveness at study completion.