

NEWSLETTER

European Union Reference Laboratory for *Salmonella*

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Editorial Note

Bilthoven, 2 October 2017

Dear colleague,

I hope you all enjoyed your summer break and have had a relaxing time!

During the summer we have performed several experiments to test the stability of artificially contaminated hygiene swabs which are used in the **combined interlaboratory comparison study on detection of *Salmonella* in Food and samples from the Primary Production Stage (PPS)**. Last week all samples were prepared and this week they were sent to the participants. The performance of the study will start on 9 October 2017. The time table of the study has been published in the previous Newsletter and is included in this Newsletter again. I would like to wish you much success with the study!

Early September, an interim summary on the overall PFGE typing results of the **21st interlaboratory comparison study on typing of *Salmonella*** (organised fall 2016) was sent to the participants. This interim summary is also available at the EURL-*Salmonella* website:
<http://eurisalmonella.eu/dsresource?type=pdf&disposition=inline&objectid=rivmp:334494&versionid=&subobjectname=>

Currently we are also busy with the preparation of the **22nd interlaboratory comparison study on typing of *Salmonella***. The time table of this study was published in the previous version of the Newsletter, as well as in this version.

During the summer period we usually have to prepare the EURL-*Salmonella* **workplan and budget forecast** for the next year. However, this year the deadline for submitting the plans is changed to 30 October, giving us somewhat more time to finish the documents.

In this Newsletter an update is given of the first **suggested protocol for management of underperformance in comparative testing and/or lack of collaboration of NRLs with EURLs activities**. The first suggested protocol was agreed upon at the EURLs Directors meeting with DG-Sanco in 2007. The updated protocol (provided by DG-Sante) looks very similar to the suggested protocol of 2007, with newly added some guidance for the response time of the NRLs to requests of the EURL. The EURL-*Salmonella* has used the original protocol as guidance on how to proceed in cases described and is intending to use the updated suggested (guidance) protocol from now on.

Best wishes,
Kirsten Mooijman
Coordinator EURL-*Salmonella*

Contribution of the EURL-*Salmonella*

**Time table of the combined interlaboratory comparison study for Food and Primary Production stage (2017)
Detection of *Salmonella* in hygiene swabs**

Week (2017)	Dates	Subject
39	25 – 29 September	Mailing of the protocol and instructions for the web based test report to the NRLs by email. Sending the link and the password for the electronic results form to the participants by email.
40	2 October	Mailing of parcels to the NRLs as Biological Substance Cat. B (UN3373) by door-to-door courier service Preparation of media by the NRLs
41	9 October	Performance of the study
44	<u>2</u> November	Deadline for completing the electronic submission of results: 2 November 2017 (23:59 h CET) After this deadline the electronic submission form will be closed.

Timetable of the 22nd interlaboratory comparison study (2017) on serotyping and optional PFGE typing of *Salmonella* for NRLs-*Salmonella*

Week (2017)	Date	Topic
38	18-22 September	Request for participation PFGE typing and check on contact details (serotyping is obligatory for EU NRLs).
42	16 - 20 October	Emailing of the protocol 2017 to the participants.
44	30 October – 3 November	Shipment of the parcels to the participants as Biological Substance Category B (UN 3373) by door-to-door courier service. Sending the link and the password for the web based test reports to the participants.
44	30 October – 3 November	<i>Upon receipt:</i> Starting the identification of the strains.
50	15 December 2017	Deadline for reporting serotyping results.
51	22 December 2017	Deadline for reporting PFGE typing results.
	January 2018	Serotyping: Reporting of individual laboratory results and Interim Summary Report.
	April 2018	PFGE typing: Reporting of individual laboratory results and Summary Report.
	Summer 2018	Final report.

For Information

Please find below an update of the first suggested protocol for management of underperformance in comparative testing and/or lack of collaboration of NRLs with EURLs activities. The first suggested protocol was agreed upon at the EURLs Directors meeting with DG-Sanco in 2007. The EURL-*Salmonella* has used this protocol as guidance on how to proceed in cases described and is intending to use the updated suggested (guidance) protocol from now on.

Protocol for management of underperformance in comparative testing or lack of collaboration of NRLs

April 2017

According to EU Regulation (EC) 882/2004 (and (EC) 2017/625), the organization and follow up of comparative testing of National Reference Laboratories is a key responsibility for European Union Reference Laboratories. That same regulation states the commitment of collaboration of NRLs with the corresponding designated EURLs.

In order to ensure an appropriate follow up in case of underperformance or lack of collaboration of NRLs, the next two step procedure for EURL actions shall be applied.

In case of underperformance (e.g. proficiency tests)

PHASE 1

1. After publication of the test report (even in working document version), the EURL shall contact the underperforming NRL asking to report on the possible causes of the observed deviations.
2. NRL response shall be transmitted by email to the EURL within 10 working days after request for information.
3. Upon acknowledgment of reception of the explanation, the EURL shall decide if the case can be closed or if further corrective actions need to be taken. Decision will be notified to the NRL. During this first step, strict confidentiality will be kept.
4. In case further action is needed (i.e. repetition of test, on-site visit or training), the EURL shall inform the NRL which action will be decided. After corrective action, a re-assessment of the NRL shall be realised and notified to the NRL.
5. In case of on-site remediation, a dedicated mission report form shall be written by the EURL including, if needed, a "to do" list as well as due dates for the "to do" items. This report shall be transmitted to the NRL as well as to the Commission (for information only). The NRL shall inform the EURL on due dates on the achievement of the "to do" items.

In case of repeated underperformance, or if corrective actions from PHASE 1 still result in an underperforming situation, or if the NRL does not fully collaborate to solve the PHASE 1 requirements, PHASE 2 will be initiated.

PHASE 2

6. The EURL will officially inform the Commission and transmit the dossier to DG SANTE. The Commission shall inform the competent authority of the Member State and require that appropriate actions are taken.

In case of lack of collaboration (e.g. no participation to a workshop)

PHASE 1

1. The EURL shall contact the NRL asking to report on the lack of collaboration.
2. The NRL response shall be transmitted by email to the EURL within 10 working days after request for information.
3. Upon acknowledgment of reception of NRL response, the EURL shall decide if the delivered explanation is justifying the lack of collaboration. The justification of the NRL shall be transmitted to Commission for information.

In case of repetitiveness of lack of collaboration, or in case of absence of response by the NRL, PHASE 2 will be initiated

PHASE 2

4. The EURL will officially inform the Commission on the situation. The Commission shall inform the Competent Authority of the Member State and require that appropriate actions are taken.

From the Literature

Salmonella-related Literature from Scopus: July – September 2017

Laing, C.R., Whiteside, M.D., Gannon, V.P.J.

Pan-genome analyses of the species Salmonella enterica, and identification of genomic markers predictive for species, subspecies, and serovar
(2017) *Frontiers in Microbiology*, 8 (JUL), art. no. 1345, .

ABSTRACT: Food safety is a global concern, with upward of 2.2 million deaths due to enteric disease every year. Current whole-genome sequencing platforms allow routine sequencing of enteric pathogens for surveillance, and during outbreaks; however, a remaining challenge is the identification of genomic markers that are predictive of strain groups that pose the most significant health threats to humans, or that can persist in specific environments. We have previously developed the software program Panseq, which identifies the pan-genome among a group of sequences, and the SuperPhy platform, which utilizes this pan-genome information to identify biomarkers that are predictive of groups of bacterial strains. In this study, we examined the pan-genome of 4893 genomes of *Salmonella enterica*, an enteric pathogen responsible for the loss of more disability adjusted life years than any other enteric pathogen. We identified a pan-genome of 25.3 Mbp, a strict core of 1.5 Mbp present in all genomes, and a conserved core of 3.2 Mbp found in at least 96% of these genomes. We also identified 404 genomic regions of 1000 bp that were specific to the species *S. enterica*. These species-specific regions were found to encode mostly hypothetical proteins, effectors, and other proteins related to virulence. For each of the six *S. enterica* subspecies, markers unique to each were identified. No serovar had pan-genome regions that were present in all of its genomes and absent in all other serovars; however, each serovar did have genomic regions that were universally present among all constituent members, and statistically predictive of the serovar. The phylogeny based on SNPs within the conserved core genome was found to be highly concordant to that produced by a phylogeny using the presence/absence of 1000 bp regions of the entire pan-genome. Future studies could use these predictive regions as components of a vaccine to prevent salmonellosis, as well as in simple and rapid diagnostic tests for both in silico and wet-lab applications, with uses ranging from food safety to public health. Lastly, the tools and methods described in this study could be applied as a pan-genomics framework to other population genomic studies seeking to identify markers for other bacterial species and their sub-groups. ISSN: 1664302X

Jasim, I., Abdullha, A., Shen, Z., Zhang, S., Alalem, M., Dewik, M., Almasri, M.

An impedance biosensor for simultaneous detection of low concentration of Salmonella serogroups in poultry samples

(2017) *TRANSDUCERS 2017 - 19th International Conference on Solid-State Sensors, Actuators and Microsystems*, art. no. 7994151, pp. 726-729.

ABSTRACT: An impedance-based MEMS biosensor was designed, fabricated and tested for the rapid and simultaneous detection of three *Salmonella* serogroups or other pathogens at low concentrations in ready to eat (RTE) Turkey. The important features of the device are the existence of the focusing region and the multi-channels to enable the detection of multiple pathogens or serogroups. The performance of the devices was excellent as evidenced by the focusing capability that increased the strength of the measured signal by a factor between 4 and 18, high sensitivity of 7 cell/ml and detection time of 30 minutes which is significantly quicker than traditional bacterial culture, polymerase chain reaction (PCR) and enzyme-linked immunosorbent assay (ELISA) based detection [1,2].
ISBN: 9781538627310

Giacometti, F., Magarotto, J., Serraino, A., Piva, S.

Highly suspected cases of salmonellosis in two cats fed with a commercial raw meat-based diet: Health risks to animals and zoonotic implications

(2017) *BMC Veterinary Research*, 13 (1), art. no. 224, .

ABSTRACT: Background: Feeding raw meat-based diets (RMBD) to companion animals raises public health concerns for both animals and humans. While considerable attention has been paid to bacterial contamination of commercial pet food, few literature studies have investigated foodborne disease in companion animals. Salmonellosis is reported to be infrequent in cats but no known data or studies estimating feline salmonellosis are available or large-scale epidemiological studies assessing *Salmonella* risk factors. Case presentation: Two highly suspected cases of salmonellosis in two cats fed with a

commercial frozen poultry RMBD are presented, for the first time from the same household. The clinical presentation, diagnostics, treatment and follow-up are reported and the zoonotic implications are discussed. Conclusions: This case highlights the health risks posed to both animals and owners by feeding RMBD to pets, and suggests that these risks should be considered by veterinary practitioners. ISSN: 17466148

Pal, S., Dey, S., Batabyal, K., Banerjee, A., Joardar, S.N., Samanta, I., Isore, D.P.
Characterization of Salmonella Gallinarum isolates from backyard poultry by polymerase chain reaction detection of invasion (invA) and Salmonella plasmid virulence (spvC) genes (2017) Veterinary World, 10 (7), pp. 814-817.

ABSTRACT: Aim: The aim was to characterize *Salmonella enterica* serovar *Gallinarum* isolated from backyard poultry by polymerase chain reaction (PCR) detection of virulence genes invasion (*invA*) and *Salmonella* plasmid virulence C (*spvC*). Materials and Methods: Two strains of *Salmonella* serovar *Gallinarum* isolates used in this study were obtained from an outbreak of fowl typhoid in backyard Vanaraja fowl. PCR technique was used for detection of *invA* and *spvC* genes using standard methodology. The *invA* PCR product from one representative isolate was sequenced and compared with other related *Salmonella* serovars in GenBank data. Results: *Salmonella Gallinarum* produced expected amplicons of *invA* and *spvC* gene products. Nucleotide sequence of 285 bp *invA* gene was deposited in GenBank with accession no. KX788214. Sequence analysis of *invA* gene was found conserved in *Salmonella* serovars and demonstrated 100% homology with closely related serovars of *Salmonella*. Conclusion: Invasion gene (*invA*) was found to be highly conserved in *Salmonella Gallinarum* and highly similar with closely related serovars. The isolates also contained plasmid-mediated *spvC* gene indicating possession of virulence plasmid. ISSN: 09728988

Li, J., Feng, J., Ma, L., de la Fuente Núñez, C., Götz, G., Lu, X.
Effects of meat juice on biofilm formation of Campylobacter and Salmonella (2017) International Journal of Food Microbiology, 253, pp. 20-28.

ABSTRACT: *Campylobacter* and *Salmonella* are leading causes of foodborne illnesses worldwide, vastly harboured by raw meat as their common food reservoir. Both microbes are prevalent in meat processing environments in the form of biofilms that contribute to cross-contamination and foodborne infection. This study applied raw meat juice (chicken juice and pork juice) as a minimally processed food model to study its effects on bacterial biofilm formation. Meat juice was collected during the freeze-thaw process of raw meat and sterilized by filtration. In 96-well polystyrene plates and glass chambers, supplementation of over 25% meat juice (v/v) in laboratory media led to an increase in biofilm formation of *Campylobacter* and *Salmonella*. During the initial attachment stage of biofilm development, more bacterial cells were present on surfaces treated with meat juice residues compared to control surfaces. Meat juice particulates on abiotic surfaces facilitated biofilm formation of *Campylobacter* and *Salmonella* under both static and flow conditions, with the latter being assessed using a microfluidic platform. Further, the deficiency in biofilm formation of selected *Campylobacter* and *Salmonella* mutant strains was restored in the presence of meat juice particulates. These results suggested that meat juice residues on the abiotic surfaces might act as a surface conditioner to support initial attachment and biofilm formation of *Campylobacter* and *Salmonella*. This study sheds light on a possible survival mechanism of *Campylobacter* and *Salmonella* in meat processing environments, and indicates that thorough cleaning of meat residues during meat production and handling is critical to reduce the bacterial load of *Campylobacter* and *Salmonella*. ISSN: 01681605

Hindermann, D., Gopinath, G., Chase, H., Negrete, F., Althaus, D., Zurfluh, K., Tall, B.D., Stephan, R., Nüesch-Inderbilen, M.

Salmonella enterica serovar *infantis* from food and human infections, Switzerland, 2010-2015: Poultry-related multidrug resistant clones and an emerging ESBL producing clonal lineage (2017) *Frontiers in Microbiology*, 8 (JUL), art. no. 1322, .

ABSTRACT: Objectives: The aim of this study was to characterize a collection of 520 *Salmonella enterica* serovar *Infantis* strains isolated from food (poultry meat), human infections and environmental sources from the years 2010, 2013 and 2015 in Switzerland. Methods: We performed antimicrobial susceptibility testing and pulsed-field gel electrophoresis (PFGE) analysis on all 520 *S. Infantis* isolates, and whole genome sequencing (WGS) on 32 selected isolates. Results: The majority (74.8%) of the isolates was multidrug resistant (MDR). PFGE analysis revealed that 270 (51.9%) isolates shared an identity of 90%. All isolates subjected to WGS belonged to sequence type (ST) 32 or a double-locus variant thereof (one isolate). Seven (21.9%) of the sequenced isolates were

phylogenetically related to the broiler-associated clone B that emerged in Hungary and subsequently spread within and outside of Europe. In addition, three isolates harboring blaCTX-M-65 on a predicted large (~320 kb) plasmid grouped in a distinct cluster. Conclusion: This study documents the presence of the Hungarian clone B and related clones in food and human isolates between 2010 and 2015, and the emergence of a blaCTX-M-65 harboring MDR *S. serovar Infantis* lineage. ISSN: 1664302X

Medrano-Félix, A., Estrada-Acosta, M., Peraza-Garay, F., Castro-del Campo, N., Martínez-Urtaza, J., Chaidez, C.

Differences in carbon source utilization of Salmonella Oranienburg and Saintpaul isolated from river water

(2017) *International Journal of Environmental Health Research*, 27 (4), pp. 252-263.

ABSTRACT: Long-term exposure to river water by non-indigenous micro-organisms such as *Salmonella* may affect metabolic adaptation to carbon sources. This study was conducted to determine differences in carbon source utilization of *Salmonella Oranienburg* and *Salmonella Saintpaul* (isolated from tropical river water) as well as the control strain *Salmonella Typhimurium* exposed to laboratory, river water, and host cells (Hep-2 cell line) growth conditions. Results showed that *Salmonella Oranienburg* and *Salmonella Saintpaul* showed better ability for carbon source utilization under the three growth conditions evaluated; however, *S. Oranienburg* showed the fastest and highest utilization on different carbon sources, including D-Glucosaminic acid, N-acetyl-D-Glucosamine, Glucose-1-phosphate, and D-Galactonic acid, while *Salmonella Saintpaul* and *S. Typhimurium* showed a limited utilization of carbon sources. In conclusion, this study suggests that environmental *Salmonella* strains show better survival and preconditioning abilities to external environments than the control strain based on their plasticity on diverse carbon sources use. ISSN: 09603123

Royan, M.

The immune-genes regulation mediated mechanisms of probiotics to control salmonella infection in chicken

(2017) *World's Poultry Science Journal*, 73 (3), pp. 603-610.

ABSTRACT: Probiotics are live microorganisms with confirmed beneficial effects on poultry health, growth performance, immune system and gut microbial population. A better perception of the mechanisms underlying the immunomodulatory effects of probiotic bacteria is usually needed to give a superior direction to the development and administration of probiotics. The oral administration of probiotic bacteria influence host cytokine levels and therefore, alters both innate and adaptive host immune responses. Selected probiotics, including some lactobacillus isolates and enterococcal strains, have been considered to prevent salmonella colonisation. Part of the effect of probiotic bacteria may be mediated through changes in the immune system related genes, including cytokine expression. Administration of probiotics in chickens could moderate salmonella mediated changes in genes, including encoding pro-inflammatory cytokines, T helper (Th) 1 cytokines, and Th2 cytokines. This review summarises the findings on the mechanisms of salmonella inhibition by using probiotic bacteria at the molecular level. ISSN: 00439339

Vuthy, Y., Lay, K.S., Seiha, H., Kerleguer, A., Aidara-Kane, A.

Antibiotic susceptibility and molecular characterization of resistance genes among Escherichia coli and among Salmonella subsp. in chicken food chains

(2017) *Asian Pacific Journal of Tropical Biomedicine*, 7 (7), pp. 670-674.

ABSTRACT: Objective To investigate the occurrence of resistance genes among *Escherichia coli* (*E. coli*) and *Salmonella subsp.* isolated in chicken food chains in Phnom Penh, 2012–2013. Methods Six hundred eighty two *E. coli* and 181 *Salmonella Albany*, *Corvallis*, and *Kentucky* strains were examined for susceptibilities to eight antimicrobials and following resistance genes were identified by PCR: blaTem, StrA, aadA, sul1, sul2, gyrA, Tet (A), and Tet (B). Results *E. coli* presented high resistances to tetracycline, amoxicillin, and sulfamethoxazole (63.1%–76.1%). *Salmonella Albany* and *Salmonella Kentucky* traduced high resistance percentages to amoxicillin, tetracycline, sulfamethoxazole, and nalidixic acid (84.6%–100%). Among amoxicillin-resistant isolates, blaTem genes were observed for 62% of *E. coli* isolates and 20% of 65 *Salmonella Kentucky*. The StrA gene was prevalent in 36% of 331 aminoglycoside-resistant *E. coli* and 90% of 40 aminoglycoside-resistant *Salmonella Corvallis*. The sul2 gene was predominant among sulfamethoxazole-resistant isolates, for 56% of 431 *E. coli* and 53% of 66 *Salmonella Corvallis*; the sul1 gene was observed in 54% of *Salmonella Albany*. The Tet (A) resistance gene was prevalent in *E. coli* (86%), *Salmonella Corvallis* (82%), *Salmonella Kentucky* (84%). High percentages of gyrA genes observed among nalidixic-acid resistant *E. coli* (91%), *Salmonella Albany* (92%), *Salmonella Corvallis* (75%) and *Salmonella Kentucky* (85%).

Conclusions Important occurrences of resistance gene were observed among *E. coli* and *Salmonella* in chicken food chains in Cambodia. ISSN: 22211691

Bang, J., Choi, M., Jeong, H., Lee, S., Kim, Y., Ryu, J.-H., Kim, H.

Heat tolerances of salmonella, cronobacter sakazakii, and pediococcus acidilactici inoculated into galactooligosaccharide

(2017) *Journal of Food Protection*, 80 (7), pp. 1123-1127.

ABSTRACT: Food-grade galactooligosaccharide (GOS) with low water activity (a_w of ca. 0.7) is used as an ingredient in various foods. We evaluated heat tolerances of *Salmonella*, *Cronobacter sakazakii*, and *Pediococcus acidilactici* at temperatures (70 to 858C) used during the saturation process of GOS by comparing decimal reduction time (D-values) and thermal resistance constants (z-values). To determine the D- and z-values, GOS containing *Salmonella* (5.1 to 5.8 log CFU/g) or *C. sakazakii* (5.3 to 5.9 log CFU/g) was heat treated at 70, 77.5, or 858C for up to 40, 25, or 15 s, respectively, and GOS containing *P. acidilactici* (6.1 to 6.5 log CFU/g) was heat treated at 70, 77.5, or 858C for up to 150, 75, or 40 s, respectively. The D-values were calculated using a linear model for heating time versus microbial population for each bacterium. When the D-values for *Salmonella*, *C. sakazakii*, and *P. acidilactici* in GOS were compared, the thermal resistance of all bacteria decreased as the temperature increased. Among the three bacteria, *P. acidilactici* had higher D-values than did *Salmonella* and *C. sakazakii*. The z-values of *Salmonella*, *C. sakazakii*, and *P. acidilactici* were 30.10, 33.18, and 13.048C, respectively. Overall order of thermal resistance was *P. acidilactici* > *Salmonella* > *C. sakazakii*. These results will be useful for selecting appropriate heat treatment conditions for the decontamination of pathogenic microorganisms during GOS manufacturing. ISSN: 0362028X

Lee, S.-K., Song, K.-Y., Chon, J.-W., Kim, D.-H., Seo, K.-H.

Evaluation of Selective-Enrichment and Chromogenic Media for Salmonella Detection in Raw Shell Egg Contents with a Low Microbial Load

(2017) *Foodborne Pathogens and Disease*, 14 (7), pp. 414-418.

ABSTRACT: The current study was conducted to evaluate the ability to recover *Salmonella* from shell egg contents by culture methods. A total of 4,000 eggs were obtained from a grading and packing center located in the Gyeonggi Province of South Korea, and 200 samples were created by pooling 20 broken eggs. The pooled samples were held at room temperature for 4 d before a 25-mL aliquot of each pool was added to 225 mL of modified trypticase soy broth (mTSB) and incubated at 35°C for 24 ± 2 h. A loopful of the culture was streaked onto chromogenic Druggan-Forsythe-Iversen (DFI) agar and incubated at 36 ± 1°C for 18-24 h. In addition, 1 mL and/or 0.1 mL of the mTSB cultures were added to 10 mL of Muller-Kauffmann tetrathionate with novobiocin (MKTn) or Rappaport-Vassiliadis (RV) broth, and they were incubated for 24 ± 2 h at 35 ± 2°C or 42 ± 0.2°C, respectively. A loopful from these cultures was streaked onto Brilliant Green (BG), xylose lysine deoxycholate (XLD), and bismuth sulfite (BS) agar plates, respectively. Directly streaking onto DFI agar revealed the presence of *Salmonella* in 14 out of the 200 pooled samples (7%); whereas the combination of RV medium and BG, XLD, and BS agar detected the pathogen in only 9 (4.5%), 7 (3.5%), and 3 (1.5%) of the pooled samples, respectively. When MKTn broth was used, *Salmonella* was detected in 7 (3.5%), 2 (1%), and 0 (0%) of the samples when streaked onto BG, XLD, and BS agar, respectively. The results indicate that direct plating onto DFI agar without enrichment was the most suitable among the methods evaluated in this study for detecting *Salmonella* in raw shell egg contents with a low microbial load. ISSN: 15353141

Kasturi, K.N., Drgon, T.

Real-time PCR method for detection of Salmonella spp. in environmental samples (2017) *Applied and Environmental Microbiology*, 83 (14), art. no. e00644-17, .

ABSTRACT: The methods currently used for detecting *Salmonella* in environmental samples require 2 days to produce results and have limited sensitivity. Here, we describe the development and validation of a real-time PCR *Salmonella* screening method that produces results in 18 to 24 h. Primers and probes specific to the gene *invA*, group D, and *Salmonella enterica* serovar Enteritidis organisms were designed and evaluated for inclusivity and exclusivity using a panel of 329 *Salmonella* isolates representing 126 serovars and 22 non-*Salmonella* organisms. The *invA*- and group D-specific sets identified all the isolates accurately. The PCR method had 100% inclusivity and detected 1 to 2 copies of *Salmonella* DNA per reaction. Primers specific for *Salmonella*-differentiating fragment 1 (*Sdf-1*) in conjunction with the group D set had 100% inclusivity for 32 *S. Enteritidis* isolates and 100% exclusivity for the 297 non-*Enteritidis* *Salmonella* isolates. Single-laboratory validation performed on 1,741 environmental samples demonstrated that the PCR method detected 55% more positives than the Vitek immunodiagnostic assay

system (VIDAS) method. The PCR results correlated well with the culture results, and the method did not report any false-negative results. The receiver operating characteristic (ROC) analysis documented excellent agreement between the results from the culture and PCR methods (area under the curve, 0.90; 95% confidence interval of 0.76 to 1.0) confirming the validity of the PCR method. ISSN: 00992240

Anukampa, Shagufta, B., Sivakumar, M., Kumar, S., Agarwal, R.K., Bhilegaonkar, K.N., Kumar, A., Dubal, Z.B.

Antimicrobial resistance and typing of Salmonella isolated from street vended foods and associated environment

(2017) *Journal of Food Science and Technology*, 54 (8), pp. 2532-2539.

ABSTRACT: The present study was carried out to find out the occurrence and types of *Salmonella* present in street vended foods and associated environment, and their resistance pattern against various antibiotics. About 1075 street vended food and associated environment samples were processed for isolation and confirmation of different *Salmonella* spp. by targeting gene specific *invA* gene and serotype specific *Sdf I*, *Via B* and *Spy* genes by PCR. Selected *Salmonella* isolates were screened for antibiotic resistance by using Baur–Kirby disk diffusion test. Out of 1075 samples, only 31 (2.88%) isolates could be amplified the *invA* gene of which 19 could be recovered from meat vendors; 8 from egg vendors while remaining 4 from milk vendors. Though, majority of *Salmonella* recovered from raw foods the ready-to-eat food like chicken gravy and rasmalai also showed its presence which pose a serious public health threat. Overall, 19, 6 and 1 isolates of *S. Typhimurium*, *S. Enteritidis* and *S. Typhi* could be detected by PCR while remaining 5 isolates could not be amplified suggesting other type of *Salmonella*. Selected *Salmonella* isolates were completely resistance to Oxacillin (100%) followed by Cefoxitin (30.43%) and Ampicillin (26.10%). Thus, it is observed that the street vended foods of animal origin and associated environment play an important role in transmission of food borne pathogens including *Salmonella*. ISSN: 00221155

Fei, X., He, X., Guo, R., Yin, C., Geng, H., Wu, K., Yin, K., Geng, S., Pan, Z., Li, Q., Jiao, X.

Analysis of prevalence and CRISPR typing reveals persistent antimicrobial-resistant Salmonella infection across chicken breeder farm production stages

(2017) *Food Control*, 77, pp. 102-109.

ABSTRACT: *Salmonella* is considered one of the most important foodborne pathogens, and is commonly associated with the consumption of poultry meat and eggs. Multidrug-resistant (MDR) *Salmonella* strains are highly adaptive and have been responsible for foodborne disease outbreaks with high mortality worldwide. Therefore, we investigated *Salmonella* prevalence and antimicrobial resistance at different production stages in a chicken breeder farm in Jiangsu Province, China. A total of 115 *Salmonella* isolates were recovered from 638 samples. Interestingly, serotyping revealed all of these to be *Salmonella enterica* subsp. *enterica* serovar *Enteritidis* (*S. Enteritidis*). Prevalence was highest at the laying stage, with 29.17% of samples containing *Salmonella*, followed by the hatching phase (21.56%). Tests of susceptibility to 16 antimicrobial agents using a disk diffusion assay showed that all isolates were resistant to at least one compound, and 31.30% exhibited MDR phenotypes, covering all five production stages. Of the *Salmonella* isolates recovered during the rearing and laying periods, a large proportion (>50%) were MDR (100 and 71.43%, respectively). Our results imply that the laying period constitutes a high-risk stage in breeder farms. Clustered regularly interspaced short palindromic repeat (CRISPR) sequence typing identified three CRISPR patterns among the isolates. Two of these were detected in samples from four of the five production stages, suggesting that certain genetically similar strains may have spread across the farm. Our study provides data concerning *Salmonella* epidemiology and antimicrobial resistance in breeder farms, which may aid the optimization of hazard analysis and critical control point strategies for such sites. ISSN: 09567135

Pucciarelli, M.G., García-del Portillo, F.

Salmonella intracellular lifestyles and their impact on host-to-host transmission

(2017) *Microbiology Spectrum*, 5 (4), art. no. MTBP-0009-2016, .

ABSTRACT: More than a century ago, infections by *Salmonella* were already associated with foodborne enteric diseases with high morbidity in humans and cattle. Intestinal inflammation and diarrhea are hallmarks of infections caused by nontyphoidal *Salmonella* serovars, and these pathologies facilitate pathogen transmission to the environment. In those early times, physicians and microbiologists also realized that typhoid and paratyphoid fever caused by some *Salmonella* serovars could be transmitted by "carriers," individuals outwardly healthy or at most suffering from some minor chronic complaint. In

his pioneering study of the nontyphoidal serovar Typhimurium in 1967, Takeuchi published the first images of intracellular bacteria enclosed by membrane-bound vacuoles in the initial stages of the intestinal epithelium penetration. These compartments, called Salmonella-containing vacuoles, are highly dynamic phagosomes with differing biogenesis depending on the host cell type. Single-cell studies involving real-time imaging and gene expression profiling, together with new approaches based on genetic reporters sensitive to growth rate, have uncovered unprecedented heterogeneous responses in intracellular bacteria. Subpopulations of intracellular bacteria displaying fast, reduced, or no growth, as well as cytosolic and intravacuolar bacteria, have been reported in both in vitro and in vivo infection models. Recent investigations, most of them focused on the serovar Typhimurium, point to the selection of persisting bacteria inside macrophages or following an autophagy attack in fibroblasts. Here, we discuss these heterogeneous intracellular lifestyles and speculate on how these disparate behaviors may impact host-to-host transmissibility of Salmonella serovars. ISSN: 21650497

Pablos, C., Fernández, A., Thackeray, A., Marugán, J.

Effects of natural antimicrobials on prevention and reduction of bacterial cross-contamination during the washing of ready-to-eat fresh-cut lettuce
(2017) *Food Science and Technology International*, 23 (5), pp. 403-414.

ABSTRACT: Microbiological safety of the fresh-cut produce may not be guaranteed if the quality of wash water is not maintained. The use of natural antimicrobials as alternative to chlorine may offer interesting possibilities for disinfecting wash water. Antimicrobial properties of allyl- and benzyl-isothiocyanates, respectively, and chitosan against *Salmonella* spp. were evaluated by standard plate count. Minimal inhibitory concentration values were observed for benzyl-isothiocyanate and chitosan, corresponding to 50 and 1000 mg l⁻¹, respectively. A 5 min washing of 25 g fresh-cut lettuce was performed. Transfer of *Salmonella* from the water to the produce was observed. Benzyl-isothiocyanate addition of 75 mg l⁻¹ before starting the washing process gave rise to a complete removal of total bacteria and *Salmonella* in the wash water after 24 h before starting the second cycle. Antimicrobial benzyl-isothiocyanate effects have been demonstrated to persist after 48 h. ISSN: 10820132

Bullard, B., Stumpf, C.H., Zhao, W., Kuzenko, S., Niehaus, G.D.

Crystal Diagnostics Xpress S Kit for the rapid detection of Salmonella spp. in selected food matrixes
(2017) *Journal of AOAC International*, 100 (4), pp. 1038-1050.

ABSTRACT: The Crystal Diagnostics (CDx) Xpress S Kit is a rapid screening assay for *Salmonella* spp. in whole raw tomatoes, whole chicken carcasses, raw ground beef, raw beef trim, and whole liquid pasteurized eggs with citric acid when present at levels of 1 CFU/portion size. The Xpress S system comprises an automatic CDx Xpress Reader and a single-use CDx BioCassette that incorporates antibody-coupled microspheres and liquid crystal for the selective identification of the intended microbe. In internal evaluations, the CDx Xpress S Kit detected all 142 *Salmonella* strains tested, including non-enterica subspecies, and excluded all non-*Salmonella* species assayed. Method-developer studies, as well as a third-party evaluation, demonstrated that 15 h single-stage enrichment permits rapid detection equivalent to the U.S. Department of Agriculture and U.S. Food and Drug Administration reference methods. The results demonstrate that the CDx Xpress S Kit is one of the fastest, most sensitive, and most accurate methods for detecting *Salmonella* in food matrixes. ISSN: 10603271

Smith, R.P., Andres, V., Dormer, L., Gosling, R., Oastler, C., Davies, R.H.

Study of the impact on Salmonella of moving outdoor pigs to fresh land
(2017) *Epidemiology and Infection*, 145 (10), pp. 1983-1992.

ABSTRACT: Anecdotal evidence has suggested that outdoor-kept pigs show an improvement to health and productivity after being moved to a new site. This study explores whether *Salmonella* occurrence reduced and was sustained after moving to a new site. Nine farms were followed for a year in which four sampling visits were completed. The highest detection of *Salmonella* was from pooled faecal dropping from pigs, run-off/ pooled water, rodents and wild birds. Descriptive summaries showed that the prevalence of both all *Salmonella* and serovars of public health importance were lower at all visits after the move. Some variability was shown in results from individual farms, but a year after the move, six farms still maintained a lower prevalence. A risk factor model showed that the prevalence at visits 2 and 3 after the move was significantly lower than baseline, after accounting for a number of significant factors that were included in the model. These were sample type and seasonality (included as a priori), presence of coughing in the sampled group and Glasser's disease on the farm, and the use of tent or kennel accommodation.

This finding provides important evidence that more frequent site moves may help reduce *Salmonella* prevalence in outdoor herds. ISSN: 09502688

Thompson, C.K., Wang, Q., Bag, S.K., Franklin, N., Shadbolt, C.T., Howard, P., Fearnley, E.J., Quinn, H.E., Sintchenko, V., Hope, K.G.

Epidemiology and whole genome sequencing of an ongoing point-source Salmonella Agona outbreak associated with sushi consumption in western Sydney, Australia 2015 (2017) *Epidemiology and Infection*, 145 (10), pp. 2062-2071.

ABSTRACT: During May 2015, an increase in *Salmonella* Agona cases was reported from western Sydney, Australia. We examine the public health actions used to investigate and control this increase. A descriptive case-series investigation was conducted. Six outbreak cases were identified; all had consumed cooked tuna sushi rolls purchased within a western Sydney shopping complex. Onset of illness for outbreak cases occurred between 7 April and 24 May 2015. *Salmonella* was isolated from food samples collected from the implicated premise and a prohibition order issued. No further cases were identified following this action. Whole genome sequence (WGS) analysis was performed on isolates recovered during this investigation, with additional *S. Agona* isolates from sporadic-clinical cases and routine food sampling in New South Wales, January to July 2015. Clinical isolates of outbreak cases were indistinguishable from food isolates collected from the implicated sushi outlet. Five additional clinical isolates not originally considered to be linked to the outbreak were genomically similar to outbreak isolates, indicating the point-source contamination may have started before routine surveillance identified an increase. This investigation demonstrated the value of genomics-guided public health action, where near real-time WGS enhanced the resolution of the epidemiological investigation. ISSN: 09502688

Chaabna, K., Alali, W.

Enteric Salmonella in humans and food in the Middle East and North Africa: Protocol of a systematic review (2017) *BMJ Open*, 7 (7), art. no. e017399, .

ABSTRACT: Introduction Non-typhoidal *Salmonella* is considered one of the leading causes of foodborne disease worldwide. This protocol provides methods that will be used to synthesise available epidemiological data on non-typhoidal enteric *Salmonella* in humans and food in Middle East and North Africa (MENA) region and to characterise the morbidity of human salmonellosis in this region. Methods and analysis A systematic review will be conducted based on the Cochrane Collaboration handbook and will be reported following the items outlined in the Preferred Reporting Items for Systematic Reviews and Meta-Analyses guidelines. We will search PubMed, Embase, CAB Direct and Global Health Library (WHO) databases in order to identify relevant reports. Additionally, the literature search will be supplemented by checking references of the included reports and the identified reviews. Furthermore, we will hand-search conference proceedings and Ministry of Health's website of each country of the MENA region. We will use comprehensive search criteria with no time and no language restrictions. We will extract data on report and study characteristics, biological assay characteristics, individuals' demographic characteristics and on primary and secondary outcomes of interest. If appropriate, meta-analysis will be conducted in order to estimate pooled prevalence measures using DerSimonian and Laird random-effects models. We will conduct meta-regression analysis to explore the effect of study-level characteristics as potential sources of heterogeneity. Ethics and dissemination The results of the systematic review will be disseminated in a peer-reviewed journal and presented at relevant conferences. Trial registration number The trial registration number is CRD42016046360. ISSN: 20446055

Keeratipibul, S., Laovittayanurak, T., Pornruangsarp, O., Chaturongkasumrit, Y., Takahashi, H., Techaruvichit, P.

Effect of swabbing techniques on the efficiency of bacterial recovery from food contact surfaces (2017) *Food Control*, 77, pp. 139-144.

ABSTRACT: Four types of swab (cotton, gauze, polyurethane foam (PU foam) and cellulose sponge) were used to recover four food-borne pathogens (*Salmonella* Typhimurium, *Staphylococcus aureus*, *Escherichia coli* and *Listeria monocytogenes*) from stainless steel and polyester urethane (old and new) surfaces under wet and dry surface conditions. Characteristics of swabs and swab surfaces were analyzed. The cellulose sponge swab showed the highest bacterial release efficiency, followed by the PU foam, gauze and cotton swabs. The bacterial Gram type affected the efficiency of bacterial recovery on dry surfaces, but the surface type had no apparent effect on the swab efficiency. Swabbing on wet surfaces using PU foam or cellulose sponge yielded a higher efficiency than with gauze

or cotton swabs. Swabbing on dry surfaces with cellulose sponge and cotton swabs showed the highest and lowest swab efficiency, respectively. Swabbing on a dry surface decreased the efficiency of all swab types to 30%. For recovery from bacterial biofilms, the swab efficiency was 40% lower than those of wet surfaces. The cellulose sponge and PU foam swabs had a higher percentage recovery of biofilm than gauze and cotton swabs. Thus, the swab type and surface condition can affect the swab efficiency, and choosing the appropriate type of swab for the surface condition will increase the swab efficiency. ISSN: 09567135

Colás-Medà, P., Viñas, I., Alegre, I., Abadías, M.

The impact of a cold chain break on the survival of Salmonella enterica and Listeria monocytogenes on minimally processed 'Conference' pears during their shelf life (2017) Journal of the Science of Food and Agriculture, 97 (9), pp. 3077-3080.

ABSTRACT: BACKGROUND: In recent years, improved detection methods and increased fresh-cut processing of produce have led to an increased number of outbreaks associated with fresh fruits and vegetables. During fruit and vegetable processing, natural protective barriers are removed and tissues are cut, causing nutrient rich exudates and providing attachment sites for microbes. Consequently, fresh-cut produce is more susceptible to microbial proliferation than whole produce. RESULTS: The aim of this study was to examine the impact of storage temperature on the growth and survival of *Listeria monocytogenes* and *Salmonella enterica* on a fresh-cut 'Conference' pear over an 8 day storage period. Pears were cut, dipped in antioxidant solution, artificially inoculated with *L. monocytogenes* and *S. enterica*, packed under modified atmospheric conditions simulating commercial applications and stored in properly refrigerated conditions (constant storage at 4 °C for 8 days) or in temperature abuse conditions (3 days at 4 °C plus 5 days at 8 °C). After 8 days of storage, both conditions resulted in a significant decrease of *S. enterica* populations on pear wedges. In contrast, when samples were stored at 4 °C for 8 days, *L. monocytogenes* populations increased 1.6 logarithmic units, whereas under the temperature abuse conditions, *L. monocytogenes* populations increased 2.2 logarithmic units. CONCLUSION: *Listeria monocytogenes* was able to grow on fresh-cut pears processed under the conditions described here, despite low pH, refrigeration and use of modified atmosphere. © 2016 Society of Chemical Industry. ISSN: 00225142

Cinti, S., Volpe, G., Piermarini, S., Delibato, E., Palleschi, G.

Electrochemical biosensors for rapid detection of foodborne Salmonella: A critical overview (2017) Sensors (Switzerland), 17 (8), art. no. 1910, .

ABSTRACT: *Salmonella* has represented the most common and primary cause of food poisoning in many countries for at least over 100 years. Its detection is still primarily based on traditional microbiological culture methods which are labor-intensive, extremely time consuming, and not suitable for testing a large number of samples. Accordingly, great efforts to develop rapid, sensitive and specific methods, easy to use, and suitable for multi-sample analysis, have been made and continue. Biosensor-based technology has all the potentialities to meet these requirements. In this paper, we review the features of the electrochemical immunosensors, genosensors, aptasensors and phagosensors developed in the last five years for *Salmonella* detection, focusing on the critical aspects of their application in food analysis. ISSN: 14248220

Grim, C.J., Daquigan, N., Lusk Pfefer, T.S., Ottesen, A.R., White, J.R., Jarvis, K.G.

High-resolution microbiome profiling for detection and tracking of Salmonella enterica (2017) Frontiers in Microbiology, 8 (AUG), art. no. 1587, .

ABSTRACT: 16S rRNA community profiling continues to be a useful tool to study microbiome composition and dynamics, in part due to advances in next generation sequencing technology that translate into reductions in cost. Reliable taxonomic identification to the species-level, however, remains difficult, especially for short-read sequencing platforms, due to incomplete coverage of the 16S rRNA gene. This is especially true for *Salmonella enterica*, which is often found as a low abundant member of the microbial community, and is often found in combination with several other closely related enteric species. Here, we report on the evaluation and application of Resphera Insight, an ultra-high resolution taxonomic assignment algorithm for 16S rRNA sequences to the species level. The analytical pipeline achieved 99.7% sensitivity to correctly identify *S. enterica* from WGS datasets extracted from the FDA GenomeTrakr Bioproject, while demonstrating 99.9% specificity over other Enterobacteriaceae members. From low-diversity and low-complexity samples, namely ice cream, the algorithm achieved 100% specificity and sensitivity for *Salmonella* detection. As demonstrated using cilantro and chili powder, for highly complex and diverse samples, especially those that contain closely related species, the detection threshold will likely have to be adjusted higher to account for

misidentifications. We also demonstrate the utility of this approach to detect *Salmonella* in the clinical setting, in this case, bloodborne infections. ISSN: 1664302X

Young, A.M., Palmer, A.E.

Methods to illuminate the role of Salmonella effector proteins during infection: A review (2017) Frontiers in Cellular and Infection Microbiology, 7 (AUG), art. no. 363, .

ABSTRACT: Intracellular bacterial pathogens like *Salmonella enterica* use secretion systems, such as the Type III Secretion System, to deliver virulence factors into host cells in order to invade and colonize these cells. *Salmonella* virulence factors include a suite of effector proteins that remodel the host cell to facilitate bacterial internalization, replication, and evasion of host immune surveillance. A number of diverse and innovative approaches have been used to identify and characterize the role of effector proteins during infection. Recent techniques for studying infection using single cell and animal models have illuminated the contribution of individual effector proteins in infection. This review will highlight the techniques applied to study *Salmonella* effector proteins during infection. It will describe how different approaches have revealed mechanistic details for effectors in manipulating host cellular processes including: the dynamics of effector translocation into host cells, cytoskeleton reorganization, membrane trafficking, gene regulation, and autophagy. ISSN: 22352988

Mohammed, M., Hello, S., Leekitcharoenphon, P., Hendriksen, R.

The invasome of Salmonella Dublin as revealed by whole genome sequencing (2017) BMC Infectious Diseases, 17 (1), art. no. 544, .

ABSTRACT: Background: *Salmonella enterica* serovar Dublin is a zoonotic infection that can be transmitted from cattle to humans through consumption of contaminated milk and milk products. Outbreaks of human infections by *S. Dublin* have been reported in several countries including high-income countries. A high proportion of *S. Dublin* cases in humans are associated with invasive disease and systemic illness. The genetic basis of virulence in *S. Dublin* is not well characterized. Methods: Whole genome sequencing was applied to a set of clinical invasive and non-invasive *S. Dublin* isolates from different countries in order to characterize the putative genetic determinants involved in the virulence and invasiveness of *S. Dublin* in humans. Results: We identified several virulence factors that form the bacterial invasome and may contribute to increasing bacterial virulence and pathogenicity including mainly Gifsy-2 prophage, two different type 6 secretion systems (T6SSs) harbored by *Salmonella* pathogenicity islands; SPI-6 and SPI-19 respectively and virulence genes; *ggt* and *PagN*. Although Vi antigen and the virulence plasmid have been reported previously to contribute to the virulence of *S. Dublin* we did not detect them in all invasive isolates indicating that they are not the main virulence determinants in *S. Dublin*. Conclusion: Several virulence factors within the genome of *S. Dublin* might contribute to the ability of *S. Dublin* to invade humans' blood but there were no genomic markers that differentiate invasive from non-invasive isolates suggesting that host immune response play a crucial role in the clinical outcome of *S. Dublin* infection. ISSN: 14712334

Tsai, M.-H., Liu, Y.-Y., Soo, V.-W.

PathoBacTyper: A web server for pathogenic bacteria identification and molecular genotyping

(2017) Frontiers in Microbiology, 8 (AUG), art. no. 1474, .

ABSTRACT: With the decline in the cost of whole-genome sequencing because of the introduction of next-generation sequencing (NGS) techniques, many public health and clinical laboratories have started to use bacterial whole genomes for epidemiological surveillance and clinical investigation. For epidemiological and clinical purposes in this "NGS era," whole-genome-scale single nucleotide polymorphism (wgSNP) analysis for genotyping is considered suitable. In this paper, we present an online service, PathoBacTyper (<http://halst.nhri.org.tw/PathoBacTyper/>), for pathogenic bacteria identification and genotyping based on wgSNP analysis. More than 400 pathogenic bacteria can be identified and genotyped through this service. Four data sets containing 59 *Salmonella* Heidelberg isolates from three outbreaks with the same pulsed-field gel electrophoresis pattern, 34 *Salmonella* Typhimurium isolates from six outbreaks, 103 isolates of hospital-associated vancomycin-resistant *Enterococcus faecium* and 15 *Legionella pneumophila* isolates from clinical and environmental samples in Israel were used for demonstrating the operation and testing the performance of the PathoBacTyper service. The test results reveal the applicability of this service for epidemiological typing and clinical investigation. ISSN: 1664302X

Zhang, X., Guo, L., Ma, R., Cong, L., Wu, Z., Wei, Y., Xue, S., Zheng, W., Tang, S.
Rapid detection of Salmonella with Recombinase Aided Amplification

(2017) *Journal of Microbiological Methods*, 139, pp. 202-204.

ABSTRACT: Rapid *Salmonella* detection using Recombinase Aided Amplification was established. The reaction completes in 20 min at 39 °C and can be performed with a portable device. Once further improved, this method should be a great choice for monitoring contamination, such as foodborne *Salmonella* or for similar purposes.
ISSN: 01677012

Ramachandran, G., Panda, A., Higginson, E.E., Ateh, E., Lipsky, M.M., Sen, S., Matson, C.A., Permala-Booth, J., DeTolla, L.J., Tennant, S.M.

Virulence of invasive Salmonella Typhimurium ST313 in animal models of infection
(2017) *PLoS Neglected Tropical Diseases*, 11 (8), art. no. e0005697, .

ABSTRACT: *Salmonella* Typhimurium sequence type (ST) 313 produces septicemia in infants in sub-Saharan Africa. Although there are known genetic and phenotypic differences between ST313 strains and gastroenteritis-associated ST19 strains, conflicting data about the in vivo virulence of ST313 strains have been reported. To resolve these differences, we tested clinical *Salmonella* Typhimurium ST313 and ST19 strains in murine and rhesus macaque infection models. The 50% lethal dose (LD50) was determined for three *Salmonella* Typhimurium ST19 and ST313 strains in mice. For dissemination studies, bacterial burden in organs was determined at various time-points post-challenge. Indian rhesus macaques were infected with one ST19 and one ST313 strain. Animals were monitored for clinical signs and bacterial burden and pathology were determined. The LD50 values for ST19 and ST313 infected mice were not significantly different. However, ST313-infected BALB/c mice had significantly higher bacterial numbers in blood at 24 h than ST19-infected mice. ST19-infected rhesus macaques exhibited moderate-to-severe diarrhea while ST313-infected monkeys showed no-to-mild diarrhea. ST19-infected monkeys had higher bacterial burden and increased inflammation in tissues. Our data suggest that *Salmonella* Typhimurium ST313 invasiveness may be investigated using mice. The non-human primate results are consistent with clinical data, suggesting that ST313 strains do not cause diarrhea. ISSN: 19352727

Seys, S.A., Sampedro, F., Hedberg, C.W.

Assessment of meat and poultry product recalls due to salmonella contamination: Product recovery and illness prevention

(2017) *Journal of Food Protection*, 80 (8), pp. 1288-1292.

ABSTRACT: Data from the recalls of meat and poultry products from 2000 through 2012 due to *Salmonella* contamination were used to assess the factors associated with the recovery of the recalled product and to develop quantitative models to estimate the number of illnesses prevented by recalls. The percentage of product recovered following a recall action was not dependent on establishment size, recall expansions, complexity of the distribution chain, type of distribution, amount of time between the production and recall dates, or number of pounds of product recalled. However, illness-related recalls were associated with larger amounts of recalled product, smaller percentages of recalled product recovered, a greater number of days between the production date and recall date, and nationwide distribution than were recalls that were not illness related. In addition, the detection of recall-associated illnesses appeared to be enhanced in states with strong foodborne illness investigation systems. The number of *Salmonella* illnesses prevented by recalls was based on the number of illnesses occurring relative to the number of pounds consumed, which was then extrapolated to the number of pounds of recalled product recovered. A simulation using a program evaluation and review technique probability distribution with illness-related recalls from 2003 through 2012 estimated that there were 19,000 prevented *Salmonella* illnesses, after adjusting for underdiagnosis. Recalls not associated with illnesses from 2000 through 2012 prevented an estimated additional 8,300 *Salmonella* illnesses, after adjusting for underdiagnosis. Although further improvements to ensure accurate and complete reporting should be undertaken, our study demonstrates that recalls are an important tool for preventing additional *Salmonella* illnesses. Moreover, additional training resources dedicated to public health agencies for enhancing foodborne illness detection, investigations, and rapid response and reporting would further prevent illnesses. ISSN: 0362028X

Grant, A., Parveen, S., Schwarz, J., Hashem, F., Vimini, B.

Reduction of Salmonella in ground chicken using a bacteriophage

(2017) *Poultry Science*, 96 (8), pp. 2845-2852.

ABSTRACT: This study's goal was to ascertain the effectiveness of a commercially available *Salmonella* bacteriophage during ground chicken production focusing on: water source, different *Salmonella* serovars, and time. *Salmonella*-free boneless, skinless chicken meat was inoculated with 4.0 Log CFU/cm² of either a cocktail of 3 *Salmonella* isolates derived

from ground chicken (GC) or a cocktail of 3 *Salmonella* strains not isolated from ground chicken (non-GC). Bacteriophages were spread onto the chicken using sterile tap or filtered water for 30 min or 8 h. *Salmonella* was recovered using standard plating method. Greater *Salmonella* reduction was observed when the bacteriophage was diluted in sterile tap water than in sterile filtered water: 0.39 Log CFU/cm² and 0.23 Log CFU/cm² reduction after 30 min, respectively (P < 0.05). The non-GC isolates showed reductions of 0.71 Log CFU/cm² and 0.90 Log CFU/cm² after 30 min and 8 h, respectively (P < 0.05). The GC isolates were less sensitive to the bacteriophage: 0.39 Log CFU/cm² and 0.67 Log CFU/cm² reductions after 30 min and 8 h, respectively (P < 0.05). In conclusion, bacteriophage reduction was dependent on water used to dilute the bacteriophage, *Salmonella*'s susceptibility to the bacteriophage, and treatment time. ISSN: 00325791

Fujikawa, H.

Estimation of microbial concentration in food products from qualitative, microbiological test data with the MPN technique

(2017) *Journal of the Food Hygienic Society of Japan*, 58 (4), pp. 173-179.

ABSTRACT: Microbial concentration in samples of a food product lot has been generally assumed to follow the log-normal distribution in food sampling, but this distribution cannot accommodate the concentration of zero. In the present study, first, a probabilistic study with the most probable number (MPN) technique was done for a target microbe present at a low (or zero) concentration in food products. Namely, based on the number of target pathogen-positive samples in the total samples of a product found by a qualitative, microbiological examination, the concentration of the pathogen in the product was estimated by means of the MPN technique. The effects of the sample size and the total sample number of a product were then examined. Second, operating characteristic (OC) curves for the concentration of a target microbe in a product lot were generated on the assumption that the concentration of a target microbe could be expressed with the Poisson distribution. OC curves for *Salmonella* and *Cronobacter sakazakii* in powdered formulae for infants and young children were successfully generated. The present study suggested that the MPN technique and the Poisson distribution would be useful for qualitative microbiological test data analysis for a target microbe whose concentration in a lot is expected to be low. ISSN: 00156426

Barba, F.J., Koubaa, M., do Prado-Silva, L., Orlien, V., Sant'Ana, A.D.S.

Mild processing applied to the inactivation of the main foodborne bacterial pathogens: A review

(2017) *Trends in Food Science and Technology*, 66, pp. 20-35.

ABSTRACT: Background *Salmonella*, *Listeria monocytogenes*, *Escherichia coli* O157:H7 and *Campylobacter* are the major bacterial pathogens associated with foodborne diseases and their inactivation is fundamental to ensure microbiologically safe products. Although efficient in generating safe foods with proper shelf-lives, pasteurization and commercial sterilization may result in numerous nutritional and sensory changes in foods. To address these disadvantages, mild processing methods (i.e., processing technologies for food preservation that apply mild temperature; <40 °C) aiming to destroy microbial food contaminants have been developed. Scope and approach This review emphasizes the main applications of mild technologies aiming to the inactivation of the four main pathogenic bacteria of relevance for food safety as well as their mechanisms of action. Key findings and conclusions Mild processing technologies such as high pressure processing, ultrasounds, pulsed electric fields, UV-light, and atmospheric cold plasma may serve, in some conditions, as useful alternatives to commercial sterilization and pasteurization aiming to destroy foodborne pathogens. Each of these mild technologies has a specific mode of microbial inactivation and their knowledge is of foremost importance in the design and practical application aiming to produce high quality and safe foods. This is necessary to ensure that mild technologies are highly advantageous in comparison to conventional technologies not only for preservation of nutritional and sensorial aspects of foods but also to ensure their safety throughout shelf-life. ISSN: 09242244

Abay, S., Irkin, R., Aydin, F., Müştak, H.K., Diker, K.S.

The prevalence of major foodborne pathogens in ready-to-eat chicken meat samples sold in retail markets in Turkey and the molecular characterization of the recovered isolates

(2017) *LWT - Food Science and Technology*, 81, pp. 202-209.

ABSTRACT: The aims of the present study were to evaluate the prevalence of *Arcobacter* spp., *Campylobacter* spp., *Listeria* spp., and *Salmonella* spp. in heat-processed ready-to-eat (RTE) chicken products manufactured by various companies using bacterial culture methods and to perform virulence gene analysis, serotyping, genotyping, and antibacterial susceptibility tests on the isolated strains. For this purpose, 50 packages of chicken

convenience products were used as the study material. Phenotypic tests and a molecular method (Polymerase Chain Reaction, PCR) were used to identify the isolated bacteria. All samples examined were negative for *Arcobacter* spp., *Campylobacter* spp., and *Salmonella* species. *Listeria* species were isolated from 12 (24%) of the examined samples. Among the *Listeria* species isolated, 9 were identified as *L. monocytogenes*, 2 were identified as *L. innocua*, and one was identified as *L. welshimeri*. All isolates were susceptible to the antibiotics tested. A detailed molecular analysis of the *Listeria* spp. revealed that the examined food products posed a significant public health hazard. Considering the presence of different genotypes of *L. monocytogenes* in RTE food production facilities, all the steps of food production must be reviewed in terms of conformity with sanitation and hygiene rules, and necessary measures must be set in place. ISSN: 00236438

Ziebell, K., Chui, L., King, R., Johnson, S., Boerlin, P., Johnson, R.P.

Subtyping of Canadian isolates of Salmonella Enteritidis using Multiple Locus Variable Number Tandem Repeat Analysis (MLVA) alone and in combination with Pulsed-Field Gel Electrophoresis (PFGE) and phage typing
(2017) *Journal of Microbiological Methods*, 139, pp. 29-36.

ABSTRACT: *Salmonella enterica* subspecies *enterica* serovar *Enteritidis* (SE) is one of the most common causes of human salmonellosis and in Canada currently accounts for over 40% of human cases. Reliable subtyping of isolates is required for outbreak detection and source attribution. However, Pulsed-Field Gel Electrophoresis (PFGE), the current standard subtyping method for *Salmonella* spp., is compromised by the high genetic homogeneity of SE. Multiple Locus Variable Number Tandem Repeat Analysis (MLVA) was introduced to supplement PFGE, although there is a lack of data on the ability of MLVA to subtype Canadian isolates of SE. Three subtyping methods, PFGE, MLVA and phage typing were compared for their discriminatory power when applied to three panels of Canadian SE isolates: Panel 1: 70 isolates representing the diversity of phage types (PTs) and PFGE subtypes within these PTs; Panel 2: 214 apparently unrelated SE isolates of the most common PTs; and Panel 3: 27 isolates from 10 groups of epidemiologically related strains. For Panel 2 isolates, four MLVA subtypes were shared among 74% of unrelated isolates and in Panel 3 isolates, one MLVA subtype accounted for 62% of the isolates. For all panels, combining results from PFGE, MLVA and PT gave the best discrimination, except in Panel 1, where the combination of PT and PFGE was equally as high, due to the selection criteria for this panel. However, none of these methods is sufficiently discriminatory alone for reliable outbreak detection or source attribution, and must be applied together to achieve sufficient discrimination for practical purposes. Even then, some large clusters were not differentiated adequately. More discriminatory methods are required for reliable subtyping of this genetically highly homogeneous serovar. This need will likely be met by whole genome sequence analysis given the recent promising reports and as more laboratories implement this tool for outbreak response and surveillance. ISSN: 01677012

Pärn, T., Dahl, V., Lienemann, T., Perevosčikovs, J., De Jong, B., Raska, K., Rimhanen-Finne, R., Huusko, S., Salmenlinna, S., Halkilahti, J., Ollgren, J., Pesola, A.K., Feher, A., Lucenko, I., Korotinska, R., Kantson, I., Marcenkova, T., Storozenko, J., Aleksiene, G., Lange, H., Vold, L., Löf, E., Jernberg, C., Alm, E., Awofisayo, A., Gossner, C., Westrell, T., Korhonen, T., Danielsson, N., Takkinen, J., Niskanen, T., Muehlen, M.

Multi-country outbreak of Salmonella enteritidis infection linked to the international ice hockey tournament

(2017) *Epidemiology and Infection*, 145 (11), pp. 2221-2230.

ABSTRACT: In April 2015, Finnish public health authorities alerted European Union member states of a possible multi-country *Salmonella enteritidis* outbreak linked to an international youth ice-hockey tournament in Latvia. The European Centre for Disease Prevention and Control (ECDC), Finnish and Latvian authorities initiated an outbreak investigation to identify the source. The investigation included a description of the outbreak, retrospective cohort study, microbiological investigation and trace-back. We identified 154 suspected and 96 confirmed cases from seven countries. Consuming Bolognese sauce and salad at a specific event arena significantly increased the risk of illness. Isolates from Finnish, Swedish and Norwegian cases had an identical multiple-locus variable-number of tandem repeats analysis-profile (3-10-6-4-1). Breaches in hygiene and food storing practices in the specific arena's kitchen allowing for cross-contamination were identified. Riga Cup participants were recommended to follow good hand hygiene and consume only freshly cooked foods. This investigation demonstrated that the use of ECDC's Epidemic Intelligence Information System for Food- and Waterborne Diseases and Zoonoses platform was essential to progress the investigation by facilitating information

exchange between countries. Cross-border data sharing to perform whole genome sequencing gave relevant information regarding the source of the outbreak.
ISSN: 09502688

Meidinger, K., Schellenberg, S., Brawand, S.G.

Persistent bacteriuria in a dog caused by Salmonella enterica subspecies enterica serovar Indiana

(2017) *Veterinary Record Case Reports*, 5 (3), art. no. e000470, .

ABSTRACT: An 11-year-old male intact Kromfohrlander dog was presented with vomiting, inappetence and progressive lethargy. Polypoid cystitis and acute prostatitis were suspected. Bacterial culture of the urine revealed a bacteriuria with *Salmonella enterica subspecies enterica serovar Indiana* (referred to as S Indiana). Although the general condition improved with antibiotic treatment (enrofloxacin; 10 mg/kg/day orally and osaterone acetate; 3.75 mg/kg/day orally), S Indiana was still present in the dog's urine four weeks later. Repeated treatment with antibiotics (amoxicillin-clavulanic acid, 18 mg/kg orally) resulted in full recovery of the dog but S Indiana was still present in the urine. Clinical improvement under antibiotic treatment indicates that S Indiana was probably the causative agent of the diagnosed cystitis and prostatitis, but long-known chronic diseases may have favoured the colonisation with this pathogen and its persistence. To the authors' knowledge, this is the first report of bacteriuria associated with S Indiana in a dog. ISSN: 20526121

Loddeke, M., Schneider, B., Oguri, T., Mehta, I., Xuan, Z., Reitzer, L.

Anaerobic cysteine degradation and potential metabolic coordination in Salmonella enterica and Escherichia coli

(2017) *Journal of Bacteriology*, 199 (16), art. no. e00117-17, .

ABSTRACT: *Salmonella enterica* has two CyuR-activated enzymes that degrade cysteine, i.e., the aerobic CdsH and an unidentified anaerobic enzyme; *Escherichia coli* has only the latter. To identify the anaerobic enzyme, transcript profiling was performed for *E. coli* without *cyuR* and with overexpressed *cyuR*. Thirty-seven genes showed at least 5-fold changes in expression, and the *cyuPA* (formerly *yhaOM*) operon showed the greatest difference. Homology suggested that *CyuP* and *CyuA* represent a cysteine transporter and an iron-sulfur-containing cysteine desulfidase, respectively. *E. coli* and *S. enterica* Δ *cyuA* mutants grown with cysteine generated substantially less sulfide and had lower growth yields. Oxygen affected the CyuR-dependent genes reciprocally; *cyuP-lacZ* expression was greater anaerobically, whereas *cdsH-lacZ* expression was greater aerobically. In *E. coli* and *S. enterica*, anaerobic *cyuP* expression required *cyuR* and cysteine and was induced by L-cysteine, D-cysteine, and a few sulfur-containing compounds. Loss of either *CyuA* or *RidA*, both of which contribute to cysteine degradation to pyruvate, increased *cyuP-lacZ* expression, which suggests that *CyuA* modulates intracellular cysteine concentrations. Phylogenetic analysis showed that *CyuA* homologs are present in obligate and facultative anaerobes, confirming an anaerobic function, and in archaeal methanogens and bacterial acetogens, suggesting an ancient origin. Our results show that *CyuA* is the major anaerobic cysteine-catabolizing enzyme in both *E. coli* and *S. enterica*, and it is proposed that anaerobic cysteine catabolism can contribute to coordination of sulfur assimilation and amino acid synthesis. ISSN: 00219193

Kim, S.A., Park, S.H., Lee, S.I., Ricke, S.C.

Development of a rapid method to quantify Salmonella Typhimurium using a combination of MPN with qPCR and a shortened time incubation

(2017) *Food Microbiology*, 65, pp. 7-18.

ABSTRACT: A novel method was developed for the specific quantification of *S. Typhimurium* using a most-probable-number (MPN) combined with qPCR and a shortened incubation time (MPN-qPCR-SIT). For *S. Typhimurium* enumeration, dilutions of samples were transferred into three wells on a microtiter plate and the plate was incubated for 4 h. The *S. Typhimurium* presence in the wells was identified using a qPCR and populations were determined based on an MPN calculation. The R² between the MPN-qPCR-SIT and conventional MPN exhibited a high level of correlation (0.9335–0.9752), suggesting that the MPN-qPCR-SIT offers a reliable alternative method for *S. Typhimurium* quantification. Although plating and qPCR were limited in their ability to detect low levels of *S. Typhimurium* (e.g. 0.18 log MPN/ml), these levels could be successfully detected with the MPN-qPCR-SIT. Chicken breast samples inoculated with *S. Typhimurium* were incubated at 0, 4, and 24 h and incubated samples were subjected to microbiome analysis. Levels of *Salmonella* and *Enterobacteriaceae* increased significantly with incubation time. The obvious benefits of the MPN-qPCR-SIT are: 1) a further confirmation step is not required,

2) the detection limit is as low as conventional MPN, but 3) is more rapid, requiring approximately 7 h to simultaneously complete quantification. ISSN: 07400020

Gkana, E.N., Doulgeraki, A.I., Nychas, G.-J.E.

Survival and transfer efficacy of mixed strain Salmonella enterica ser. Typhimurium from beef burgers to abiotic surfaces and determination of individual strain contribution (2017) Meat Science, 130, pp. 58-63.

ABSTRACT: The aim of the study was to evaluate the survival and transfer efficacy of 3 *Salmonella* Typhimurium strains from beef burgers to abiotic surfaces and determine the individual strain distribution. *S. Typhimurium* population on beef burgers during incubation remained constant at initial levels of contamination approximately 3 and 5 log CFU/g. Additionally, the survival of pathogens on soiled HDPE surfaces was significant during incubation at both initial inocula, while ca 1.5 log CFU/cm² reduction was observed at 168h. The log transformed transfer rate (log₁₀Tr) was -1.86 ± 0.23 and -1.75 ± 0.40 for high and low inoculum. The level of initial contamination did not have any statistical important impact on bacterial transfer ($P > 0.05$). In addition, the results regarding the strain contribution revealed rather random individual proportion of each strain, recovered from HDPE, SS surfaces and beef burgers. However, the dominance of each strain was strongly dependent on surface at low inoculum and time in case of high inoculum. This observed strain variability during survival and transfer of *S. Typhimurium* might be of great importance in order to understand and consequently limit the possibility of cross contamination during food processing in a common household. ISSN: 03091740

Vaz, C.S.L., Voss-Rech, D., De Avila, V.S., Coldebella, A., Silva, V.S.

Interventions to reduce the bacterial load in recycled broiler litter (2017) Poultry Science, 96 (8), pp. 2587-2594.

ABSTRACT: Two experiments were undertaken to evaluate the bacterial load in recycled litter between broiler flocks following addition of quicklime (T1), windrowing (T2), shallow fermentation (T3), and control (no intervention, T4). The first experiment was developed in field conditions in which the broiler houses were accompanied by 6 consecutive flocks and the effect of the treatments was assessed on enterobacteria and aerobic mesophiles. The second experiment was conducted in an experimental broiler house with recycled litter for assessment of *Salmonella* Enteritidis phage type 4 (SE PT4). In the field study, T3 presented the best results in reducing enterobacteria in broiler litter in relation to the other treatments, with the highest reduction occurring in the first 3 flocks, tending to stabilization from the fourth flock onward for all the treatments assessed. From the third to sixth flocks, enterobacteria level at the end of the treatments (d 12) was lower than the average in the fresh litter, except in T4. All treatments reduced aerobic mesophiles throughout the flocks, where T2 showed the highest reduction. The percentage of dry matter in the broiler litter diminished in T4 and increased in T3 over the course of the flocks. In the second experiment, the drop in the SE PT4 level in the broiler litter first occurred in T2 and T3. However, all the treatments except for T4 eliminated SE PT4 within 12 d. The temperature of the broiler litter in T2 was higher in relation to the other treatments. The results show that litter treatment prior to reutilization by the successive broiler flock is required to reduce the level of residual bacteria. The fermentative treatments (T2 and T3) were found to be superior to the others in terms of reducing the bacterial load, with shallow fermentation standing out with the highest reduction of enterobacteria and equivalent SE PT4 elimination when compared to windrowing. ISSN: 00325791

Jung, L.-S., Ding, T., Ahn, J.

Evaluation of lytic bacteriophages for control of multidrug-resistant Salmonella Typhimurium

(2017) Annals of Clinical Microbiology and Antimicrobials, 16 (1), art. no. 66, .

ABSTRACT: Background: The emergence of antibiotic-resistant bacteria can cause serious clinical and public health problems. This study describes the possibility of using bacteriophages as an alternative agent to control multidrug-resistant *Salmonella* Typhimurium. Methods: The potential lytic bacteriophages (P22-B1, P22, PBST10, PBST13, PBST32, and PBST 35) were characterized by morphological property, heat and pH stability, optimum multiplicity of infection (MOI), and lytic activity against *S. Typhimurium* KCCM 40253, *S. Typhimurium* ATCC 19585, ciprofloxacin-induced antibiotic-resistant *S. Typhimurium* ATCC 19585, and *S. Typhimurium* CCARM 8009. Results: P22-B1 and P22 belong to Podoviridae family and PBST10, PBST13, PBST32, and PBST 35 show a typical structure with polyhedral head and long tail, belonging to Siphoviridae family. *Salmonella* bacteriophages were highly stable at the temperatures (< 60 °C) and pHs (5.0-11.0). The reduction rates of host cells were increased at the MOI-dependent manner, showing the

highest reduction rate at MOI of 10. The host cells were most effectively reduced by P22, while P22-B1 showed the least lytic activity. The ciprofloxacin-induced antibiotic-resistant *S. Typhimurium* ATCC 19585, and clinically isolated antibiotic-resistant *S. Typhimurium* CCARM 8009 were resistant to ciprofloxacin, levofloxacin, norfloxacin, and tetracycline. P22 showed the highest lytic activity against *S. Typhimurium* KCCM 40253 (> 5 log reduction), followed by *S. Typhimurium* ATCC 19585 (4 log reduction) and ciprofloxacin-induced antibiotic-resistant *S. Typhimurium* ATCC 19585 (4 log reduction). Conclusion: The results would provide vital insights into the application of lytic bacteriophages as an alternative therapeutics for the control of multidrug-resistant pathogens. ISSN: 14760711

Wilhelm, B.J., Young, I., Cahill, S., Desmarchelier, P., Nakagawa, R., Rajić, A.
Interventions to reduce non-typhoidal Salmonella in pigs during transport to slaughter and lairage: Systematic review, meta-analysis, and research synthesis based infection models in support of assessment of effectiveness
(2017) *Preventive Veterinary Medicine*, 145, pp. 133-144.

ABSTRACT: A systematic review of the effectiveness of interventions to reduce *Salmonella* prevalence or concentration in pork was undertaken. A broad search was conducted in two electronic databases. Each citation was appraised using screening tools designed and tested a priori. Level 1 relevance screening excluded irrelevant citations; level 2 confirmed relevance and categorized. Data were then extracted, and intervention categories were descriptively summarized. Meta-analysis was performed to provide a summary estimate of treatment effect where two or more studies investigated the same intervention in comparable populations. The Grading of Recommendation, Assessment, Development and Evaluation (GRADE) approach was used to assess the confidence in the estimated summary measures of intervention effect for each data subgroup. Data were also extracted from the control groups of 25 challenge trials captured by the review, to fit logistic regression models of *Salmonella* infection in pigs, using odds of infection as the outcome measure. The only intervention captured by the review which was significantly associated with reduced risk of *Salmonella* in field settings, was elimination of lairage, which is not currently feasible commercially. The logistic regression model for fecal *Salmonella* shedding in pigs with a random intercept for trial yielded the following predictors significantly associated with increased odds of infection: oral challenge route relative to intra-nasal, log increase in challenge dose, and elapsed time post-challenge. Univariable exact logistic regression modeling lymph node contamination post-challenge yielded the following predictors significantly associated with increased odds of *Salmonella* infection: younger animals relative to older ones; intra-nasal challenge route relative to oral route; and animals sampled within the first 7 days post-challenge relative to those sampled at 14 or 21 days. We hypothesize that the presence of absence of one or more of these predictors across studies could help to explain the inconsistent and/or non-significant findings reported for some interventions applied at lairage. ISSN: 01675877

Stokar-Regenscheit, N., Overesch, G., Giezendanner, R., Roos, S., Gurtner, C.
Salmonella enterica subsp. diarizonae serotype 61:k:1,5,(7) associated with chronic proliferative rhinitis and high nasal colonization rates in a flock of Texel sheep in Switzerland
(2017) *Preventive Veterinary Medicine*, 145, pp. 78-82.

ABSTRACT: *Salmonella* (*S.*) *enterica* subspecies *diarizonae* (IIIb) serovar 61:(k):1,5,(7) (*S.* IIIb 61:(k):1,5,(7)) is considered to be host adapted to sheep and is found regularly in feces of healthy carriers and of sheep with salmonellosis. A few cases of chronic proliferative rhinitis (CPR) in sheep have been described as a new disease in association with *S.* IIIb 61:k:1,5,(7) in the USA, in Spain and now for the first time in Switzerland. Three animals of a flock of Texel sheep suffering from chronic nasal discharge and dyspnea with subsequent death were necropsied. The pathological lesions are consistent with a severe proliferation of the nasal mucosae of the turbinates in association with severe chronic inflammation. *S.* IIIb 61:(k):1,5,(7) was isolated from the lesions by direct bacteriological culture and the presence of *Salmonella* spp. was confirmed by immunohistochemistry. The affected flock was systematically tested after the first occurrence of the disease. Clinical examination of the flock revealed approx. 20% of the adult sheep to show nasal discharge, approx. 5% having severe dyspnea and approx. 5% having chronic intermittent diarrhea. Lambs ($n = 28$) showed no clinical signs at all. High positivity of nasal mucosa (46%), but low prevalence in feces (6%) for *S.* IIIb 61:k:1,5,(7) was found. The results lead to the assumption of a direct animal to animal transmission by nasal discharge followed by a chronic disease leading to death after several months to years. Animals tested positive for *S.* IIIb 61:k:1,5,(7) were all >1 year old. CPR represents a chronic disease in adult sheep posing a risk for spreading *S.* IIIb 61:k:1,5,(7) between flocks and with a zoonotic potential. ISSN: 01675877

Mandal, R.K., Kwon, Y.M.

Global screening of Salmonella enterica serovar Typhimurium genes for desiccation survival

(2017) *Frontiers in Microbiology*, 8 (SEP), art. no. 1723, .

ABSTRACT: *Salmonella* spp., one of the most common foodborne bacterial pathogens, has the ability to survive under desiccation conditions in foods and food processing facilities for years. This raises the concerns of *Salmonella* infection in humans associated with low water activity foods. *Salmonella* responds to desiccation stress via complex pathways involving immediate physiological actions as well as coordinated genetic responses. However, the exact mechanisms of *Salmonella* to resist desiccation stress remain to be fully elucidated. In this study, we screened a genome-saturating transposon (Tn5) library of *Salmonella* Typhimurium (S. Typhimurium) 14028s under the in vitro desiccation stress using transposon sequencing (Tn-seq). We identified 61 genes and 6 intergenic regions required to overcome desiccation stress. *Salmonella* desiccation resistance genes were mostly related to energy production and conversion; cell wall/membrane/envelope biogenesis; inorganic ion transport and metabolism; regulation of biological process; DNA metabolic process; ABC transporters; and two component system. More than 20% of the *Salmonella* desiccation resistance genes encode either putative or hypothetical proteins. Phenotypic evaluation of 12 single gene knockout mutants showed 3 mutants (atpH, atpG, and corA) had significantly ($p < 0.02$) reduced survival as compared to the wild type during desiccation survival. Thus, our study provided new insights into the molecular mechanisms utilized by *Salmonella* for survival against desiccation stress. The findings might be further exploited to develop effective control strategies against *Salmonella* contamination in low water activity foods and food processing facilities. ISSN: 1664302X

Palacios, O.A., Zavala-Díaz de la Serna, F.J., de Lourdes Ballinas-Casarrubias, M., Espino-Valdés, M.S., Nevárez-Moorillón, G.V.

Microbiological impact of the use of reclaimed wastewater in recreational parks

(2017) *International Journal of Environmental Research and Public Health*, 14 (9), art. no. 1009, .

ABSTRACT: Reclaimed wastewater for irrigation is an opportunity for recovery of this natural resource. In this study, microbial risk from the use of treated wastewater for irrigation of recreational parks in the city of Chihuahua, evaluating the effect of distribution distance, season, and presence of storage tanks, was analyzed. *Escherichia coli*, *Salmonella*, and multidrug-resistant bacteria were recovered from samples of reclaimed water and soils at recreational parks in Chihuahua by the membrane filtration method, using selected agars for microbial growth. Samples were taken at three different seasons. No correlation in the presence of microbial indicators and multidrug-resistant bacteria ($p > 0.05$) was found between the distance from the wastewater treatment plant to the point of use. Presence of storage tanks in parks showed a significant effect ($p < 0.05$) with a higher level of *E. coli*. The highest count in wastewater occurred in summer. We isolated 392 multidrug-resistant bacteria from water and soil; cluster analysis showed that the microorganisms at each location were of different origins. Irrigation with reclaimed wastewater did not have a negative effect on the presence of microbial indicators of the quality of soils in the parks. However, the prevalence of multidrug-resistant bacteria still represents a potential risk factor for human health. ISSN: 16617827

Guo, R., Li, Z., Jiao, Y., Geng, S., Pan, Z., Chen, X., Li, Q., Jiao, X.

O-polysaccharide is important for Salmonella Pullorum survival in egg albumen, and virulence and colonization in chicken embryos

(2017) *Avian Pathology*, 46 (5), pp. 535-540.

ABSTRACT: The pathogen *Salmonella* Pullorum is the causative agent of persistent systemic infection of poultry, leading to economic losses in developing countries due to morbidity, mortality and reduction in egg production. These infections may result in vertical transmission to eggs or progeny. Limited information is available regarding the mechanisms involved in the survival of *Salmonella* Pullorum in egg albumen and developing chicken embryos. Hence, we investigated the role of O-polysaccharide in the contamination of eggs and the colonization of chicken embryos. Compared with the wild-type strain, the isogenic waaL mutant exhibited an O-antigen-deficient rough phenotype, and increased sensitivity to egg albumen and chicken serum, as well as reduced adherence to DF-1 cells. Infection with *Salmonella* Pullorum lacking O-polysaccharide resulted in significantly reduced embryo lethality and bacterial colonization. These results suggest that O-polysaccharide is essential for *Salmonella* Pullorum colonization in eggs, both post-lay and developing embryos. The chicken embryo infection model could be used to characterize the interaction between *Salmonella* Pullorum and developing embryos, and it

will also contribute to the development of more rational vaccines to protect laying hens and embryos. ISSN: 03079457

Ives, A.-K., Antaki, E., Stewart, K., Francis, S., Jay-Russell, M.T., Sithole, F., Kearney, M.T., Griffin, M.J., Soto, E.

Detection of Salmonella enterica Serovar Montevideo and Newport in Free-ranging Sea Turtles and Beach Sand in the Caribbean and Persistence in Sand and Seawater Microcosms

(2017) *Zoonoses and Public Health*, 64 (6), pp. 450-459.

ABSTRACT: Salmonellae are Gram-negative zoonotic bacteria that are frequently part of the normal reptilian gastrointestinal flora. The main objective of this project was to estimate the prevalence of non-typhoidal *Salmonella enterica* in the nesting and foraging populations of sea turtles on St. Kitts and in sand from known nesting beaches. Results suggest a higher prevalence of *Salmonella* in nesting leatherback sea turtles compared with foraging green and hawksbill sea turtles. *Salmonella* was cultured from 2/9 and identified by molecular diagnostic methods in 3/9 leatherback sea turtle samples. *Salmonella* DNA was detected in one hawksbill turtle, but viable isolates were not recovered from any hawksbill sea turtles. No *Salmonella* was detected in green sea turtles. In samples collected from nesting beaches, *Salmonella* was only recovered from a single dry sand sample. All recovered isolates were positive for the *wzx* gene, consistent with the O:7 serogroup. Further serotyping characterized serovars Montevideo and Newport present in cloacal and sand samples. Repetitive-element palindromic PCR (rep-PCR) fingerprint analysis and pulsed-field gel electrophoresis of the 2014 isolates from turtles and sand as well as archived *Salmonella* isolates recovered from leatherback sea turtles in 2012 and 2013, identified two distinct genotypes and four different pulsotypes, respectively. The genotyping and serotyping were directly correlated. To determine the persistence of representative strains of each serotype/genotype in these environments, laboratory-controlled microcosm studies were performed in water and sand (dry and wet) incubated at 25 or 35°C. Isolates persisted for at least 32 days in most microcosms, although there were significant decreases in culturable bacteria in several microcosms, with the greatest reduction in dry sand incubated at 35°C. This information provides a better understanding of the epizootiology of *Salmonella* in free-ranging marine reptiles and the potential public health risks associated with human interactions with these animals in the Caribbean. ISSN: 18631959

Pang, X.Y., Yang, Y.S., Yuk, H.G.

Biofilm formation and disinfectant resistance of Salmonella sp. in mono- and dual-species with Pseudomonas aeruginosa

(2017) *Journal of Applied Microbiology*, 123 (3), pp. 651-660.

ABSTRACT: Aims: This study aimed to evaluate the biofilm formation and disinfectant resistance of *Salmonella* cells in mono- and dual-species biofilms with *Pseudomonas aeruginosa*, and to investigate the role of extracellular polymeric substances (EPS) in the protection of biofilms against disinfection treatment. Methods and Results: The populations of *Salmonella* in mono- or dual-species biofilms with *P. aeruginosa* on stainless steel (SS) coupons were determined before and after exposure to commercial disinfectant, 50 µg ml⁻¹ chlorine or 200 µg ml⁻¹ Ecolab® Whisper™ V (a blend of four effective quaternary ammonium compounds (QAC)). In addition, EPS amount from biofilms was quantified and biofilm structures were observed using scanning electron microscopy (SEM). Antagonistic interactions between *Salmonella* and *P. aeruginosa* resulted in lower planktonic population level of *Salmonella*, and lower density in dual-species biofilms compared to mono-species biofilms. The presence of *P. aeruginosa* significantly enhanced disinfectant resistance of *S. Typhimurium* and *S. Enteritidis* biofilm cells for 2 days, and led to an average of 50% increase in polysaccharides amount in dual-species biofilms than mono-species biofilms of *Salmonella*. Microscopy observation showed the presence of large microcolonies covered by EPS in dual-species biofilms but not in mono-species ones. Conclusion: The presence of *P. aeruginosa* in dual-species culture inhibited the growth of *Salmonella* cells in planktonic phase and in biofilms, but protected *Salmonella* cells in biofilms from disinfection treatment, by providing more production of EPS in dual-species biofilms than mono-species ones. Significance and Impact of the Study: This study provides insights into inter-species interaction, with regard to biofilm population dynamics and disinfectant resistance. Thus, a sanitation protocol should be designed considering the protective role of secondary species to pathogens in biofilms on SS surface which has been widely used at food surfaces and manufacturers. ISSN: 13645072

Feng, K., Hu, W., Jiang, A., Saren, G., Xu, Y., Ji, Y., Shao, W.

Growth of Salmonella spp. and Escherichia coli O157:H7 on Fresh-Cut Fruits Stored at Different Temperatures

(2017) *Foodborne Pathogens and Disease*, 14 (9), pp. 510-517.

ABSTRACT: The objective of this work was to determine the growth potential of *Salmonella* spp. and *Escherichia coli* O157:H7 on fresh-cut honeydew melon, cantaloupe, watermelon, pitaya, mango, papaya, and pineapple stored at 5°C, 13°C, and 25°C. The results showed that both pathogens were able to grow on fresh-cut fruits except fresh-cut pineapple at 13°C and 25°C. *Salmonella* spp. grew more rapidly on fresh-cut honeydew melon, cantaloupe, watermelon, and mango than did *E. Coli* O157:H7 at 13°C. The growth of both species was inhibited on fresh-cut pineapple, with that of *Salmonella* spp. being particularly pronounced. Naturally occurring microbiota populations on fresh-cut fruits increased significantly at 13°C and 25°C, but no significant changes in growth were observed for *Salmonella* spp., *E. Coli* O157:H7, or natural microbiota species at 5°C. The study therefore emphasizes the importance of strict temperature control from processing to consumption, including transportation, distribution, storage, and handling in supermarkets and by consumers. ISSN: 15353141

Oscar, T.P.

Risk of salmonellosis from chicken parts prepared from whole chickens sold in flow pack wrappers and subjected to temperature abuse

(2017) *Journal of Food Protection*, 80 (9), pp. 1496-1505.

ABSTRACT: The flow pack wrapper is a popular packaging choice for retail sale of whole chickens. However, it may provide a favorable environment for growth and spread of *Salmonella* within the package, leading to an outbreak of salmonellosis. To investigate this possibility, a process risk model was developed that predicted the risk of salmonellosis from chicken parts prepared from whole chickens sold in flow pack wrappers and subjected to proper storage (6 h at 4°C) or improper storage (72 h at 15°C) before preparation. The model had four unit operations (pathogen events): (i) preparation (contamination), (ii) cooking (death), (iii) serving (cross-contamination), and (iv) consumption (dose-response). Data for prevalence, number, and serotype of *Salmonella* on chicken parts were obtained by whole sample enrichment, real-time PCR. Improper storage increased ($P < 0.05$) prevalence of *Salmonella* on raw chicken parts from 10.6% (17 of 160) to 41.2% (66 of 160) and incidence of cross-contamination of cooked chicken from 10% (4 of 40) to 52.2% (24 of 46). Improper storage also increased ($P < 0.05$) the number (mean \pm standard deviation) of *Salmonella* from 0.017 ± 0.030 to 3.51 ± 1.34 log per raw chicken part and from 0.048 ± 0.089 to 3.08 ± 1.50 log per cooked chicken part. The predominant serotypes isolated ($n = 111$) were Typhimurium (34.2%), Typhimurium var 5- (20.7%), Kentucky (12.6%), Enteritidis (11.7%), and Heidelberg (8.1%). When chicken was properly stored before preparation, the model predicted that risk of salmonellosis was low and sporadic with only six cases per 100 simulations of 105 chicken parts. However, when 0.1 to 1% of chickens were improperly stored before preparation, the model predicted that salmonellosis would increase ($P < 0.05$) linearly from a median of 7 (range, 1 to 15) to a median of 72 (range, 52 to 93) cases per 105 chicken parts. These results indicated that the flow pack wrapper provided a favorable environment for growth and spread of *Salmonella* within the package and that even when only a small percentage of packages were subjected to improper storage before preparation, the risk and size of an outbreak of salmonellosis from chicken parts increased significantly. ISSN: 0362028X

Beuchat, L.R., Mann, D.A., Kelly, C.A., Ortega, Y.R.

Retention of viability of salmonella in sucrose as affected by type of inoculum, water activity, and storage temperature

(2017) *Journal of Food Protection*, 80 (9), pp. 1408-1414.

ABSTRACT: Outbreaks of salmonellosis have been associated with consumption of high-sugar, low-water activity (aw) foods. The study reported here was focused on determining the effect of storage temperature (5 and 25°C) on survival of initially high and low levels of *Salmonella* in dry-inoculated sucrose (aw 0.26 ± 0.01 to 0.54 ± 0.01) and wet-inoculated sucrose (aw 0.24 ± 0.01 to 0.44 ± 0.04) over a 52-week period. With the exception of dry-inoculated sucrose at aw 0.26, *Salmonella* survived for 52 weeks in dry- and wet-inoculated sucrose stored at 5 and 25°C. Retention of viability was clearly favored in sucrose stored at 5°C compared with 25°C, regardless of level or type of inoculum or aw. Survival at 5°C was not affected by aw. Initial high-inoculum counts of 5.18 and 5.25 log CFU/g of dry-inoculated sucrose (aw 0.26 and 0.54, respectively) stored for 52 weeks at 5°C decreased by 0.56 and 0.53 log CFU/g; counts decreased by >4.18 and >4.25 log CFU/g in samples stored at 25°C. Inactivation rates in wet-inoculated sucrose were similar to those in dry-inoculated sucrose; however, a trend toward higher persistence of

Salmonella in dry- versus wet-inoculated sucrose suggests there was a higher proportion of cells in the wet inoculum with low tolerance to osmotic stress. Survival patterns were similar in sucrose initially containing a low level of *Salmonella* (2.26 to 2.91 log CFU/g). The pathogen was recovered from low-inoculated sucrose stored at 5°C for 52 weeks regardless of type of inoculum or *aw* and from dry-inoculated sucrose (*aw* 0.54) and wet-inoculated sucrose (*aw* 0.24) stored at 25°C for 12 and 26 weeks, respectively. Results emphasize the importance of preventing contamination of sucrose intended for use as an ingredient in foods not subjected to a treatment that would be lethal to *Salmonella*.
ISSN: 0362028X

Gayet, R., Bioley, G., Rochereau, N., Paul, S., Corthésy, B.

Vaccination against Salmonella infection: The mucosal way

(2017) *Microbiology and Molecular Biology Reviews*, 81 (3), art. no. e00007, .

ABSTRACT: *Salmonella enterica* subspecies *enterica* includes several serovars infecting both humans and other animals and leading to typhoid fever or gastroenteritis. The high prevalence of associated morbidity and mortality, together with an increased emergence of multidrug-resistant strains, is a current global health issue that has prompted the development of vaccination strategies that confer protection against most serovars. Currently available systemic vaccine approaches have major limitations, including a reduced effectiveness in young children and a lack of crossprotection among different strains. Having studied host-pathogen interactions, microbiologists and immunologists argue in favor of topical gastrointestinal administration for improvement in vaccine efficacy. Here, recent advances in this field are summarized, including mechanisms of bacterial uptake at the intestinal epithelium, the assessment of protective host immunity, and improved animal models that closely mimic infection in humans. The pros and cons of existing vaccines are presented, along with recent progress made with novel formulations. Finally, new candidate antigens and their relevance in the refined design of anti-*Salmonella* vaccines are discussed, along with antigen vectorization strategies such as nanoparticles or secretory immunoglobulins, with a focus on potentiating mucosal vaccine efficacy.
ISSN: 10922172

Devleesschauwer, B., Marvasi, M., Giurcanu, M.C., Hochmuth, G.J., Speybroeck, N., Havelaar, A.H., Teplitski, M.

High relative humidity pre-harvest reduces post-harvest proliferation of Salmonella in tomatoes

(2017) *Food Microbiology*, 66, pp. 55-63.

ABSTRACT: Outbreaks of human illness caused by enteric pathogens such as *Salmonella* are increasingly linked to the consumption of fruits and vegetables. Knowledge on the factors affecting *Salmonella* proliferation on fresh produce therefore becomes increasingly important to safeguard public health. Previous experiments showed a limited impact of pre-harvest production practices on *Salmonella* proliferation on tomatoes, but suggested a significant effect of harvest time. We explored the data from two previously published and one unpublished experiment using regression trees, which allowed overcoming the interpretational difficulties of classical statistical models with higher order interactions. We assessed the effect of harvest time by explicitly modeling the climatic conditions at harvest time and by performing confirmatory laboratory experiments. Across all datasets, regression trees confirmed the dominant effect of harvest time on *Salmonella* proliferation, with humidity-related factors emerging as the most important underlying climatic factors. High relative humidity the week prior to harvest was consistently associated with lower *Salmonella* proliferation. A controlled lab experiment confirmed that tomatoes containing their native epimicrobiota supported significantly lower *Salmonella* proliferation when incubated at higher humidity prior to inoculation. The complex interactions between environmental conditions and the native microbiota of the tomato crop remain to be fully understood. ISSN: 07400020

Kovačić, A., Huljev, Ž., Sušić, E.

Ground water as the source of an outbreak of Salmonella Enteritidis

(2017) *Journal of Epidemiology and Global Health*, 7 (3), pp. 181-184.

ABSTRACT: In September 2014, an outbreak of gastroenteritis was reported to the Public Health Institute of Šibenik and Knin County in Croatia. The outbreak occurred in the County center of Šibenik, a town with 50,000 inhabitants, and it lasted for 12 days. An epidemiological investigation suggested a nearby water spring as the source of the outbreak. Due to the temporary closure of the public water supply system, the inhabitants started to use untreated water from a nearby spring. Microbiological analysis revealed that the outbreak was caused by *Salmonella enterica* subsp. *enterica* serovar *Enteritidis* that was isolated from stool samples of the patients and ground water. The isolates were

further analysed with pulsed-field gel electrophoresis using XbaI, which revealed an identical macrorestriction profile. Although 68 cases were reported, it was estimated that the actual number of affected persons was more than several hundred. In order to prevent further spread of disease, public advice was released immediately after the first epidemiological indication and a warning sign was placed at the incriminated water source, after microbiological confirmation. It is necessary to regularly monitor microbiological quality of ground water especially in urban areas and provide adequate education and awareness to the inhabitants regarding the risk of using untreated ground water.
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Wang, F., Li, J., Li, Q., Liu, R., Zheng, M., Wang, Q., Wen, J., Zhao, G.

Changes of host DNA methylation in domestic chickens infected with Salmonella enterica (2017) *Journal of Genetics*, 96 (4), pp. 545-550.

ABSTRACT: Cytosine methylation is an effective way to modulate gene transcription. However, very little is known about the epigenetic changes in the host that is infected with *Salmonella enterica*. In this study, we used methylated DNA immunoprecipitation sequencing to analyse the genomewide DNA methylation changes in domestic chickens after infected with *Salmonella*. The level of DNA methylation was slightly higher in the genomic regions around the transcription start termination sites in a *Salmonella*-infected group compared to the controls. Overall, 879 peaks were differentially methylated between *Salmonella*-infected and control groups, among which 135 were located in the gene promoter regions. Genes including MHC class IV antigen, GABARAPL1, MR1 and KDM1B were shown to be methylated more heavily after infected with *Salmonella*, whereas DYNLRB2, SEC14L3 and ANKIB1 tended to have fewer methylated cytosine residues in the promoter regions. Gene interaction network analysis of differentially methylated genes in the promoter regions revealed extensive connections with immune-related genes, indicating the possible impact of infection with *Salmonella* on the epigenetic status of the host. ISSN: 00221333

Zhu, J., Bai, Y., Wang, Y., Song, X., Cui, S., Xu, H., Jiao, X., Li, F.

A risk assessment of salmonellosis linked to chicken meals prepared in households of China (2017) *Food Control*, 79, pp. 279-287.

ABSTRACT: A quantitative microbiological risk assessment model was used to quantify the risk of salmonellosis caused by bacterial growth and cross-contamination of chicken meals prepared in households of China. Chinese data on initial loads of *Salmonella* in chicken carcasses sold at retail, storage time and handling of raw chicken meat in household kitchens and confirmatory transfer rates of *Salmonella* among different kitchen objects were collected. Only one third of Chinese families in our sample separated the cutting board between raw and ready-to-eat foods. The cross-contamination of ready-to-eat foods from chicken meals via the cutting board, the knife and cooks' hands increased the frequency of pathogen ingestion and the risk of salmonellosis. A significant decrease in the risk of salmonellosis could be achieved by reducing the cross-contamination when handling raw chicken meat and ready-to-eat foods. Decreasing the prevalence of *Salmonella* contamination to 8.8% or removing chicken carcasses with contamination densities higher than 100 MPN/100 g at retail was less effective. Using transfer rates of *Salmonella* from raw chicken meat to the wooden cutting board instead of that from references, a statistically higher risk of salmonellosis per serve due to the cross-contamination in households was observed. The present study validated values of hygiene practices in China to reduce the risk of salmonellosis from contaminated raw chicken meat at retail. Deliberate surveys for cooking behaviors and transfer rates of *Salmonella* from and to different objects including wooden cutting boards were needed. ISSN: 09567135

Puri, M.A.A., Joelsson, A.C., Terkhorn, S.P., Brown, A.S., Gaudio, Z.E., Siciliano, N.A.

Comparative evaluation of veriflow® salmonella species to USDA and FDA culture-based methods for the detection of salmonella spp. in food and environmental samples (2017) *Journal of AOAC International*, 100 (5), pp. 1445-1457.

ABSTRACT: Veriflow® *Salmonella* species (Veriflow SS) is a molecular-based assay for the presumptive detection of *Salmonella* spp. from environmental surfaces (stainless steel, sealed concrete, plastic, and ceramic tile), dairy (2% milk), raw meat (20% fat ground beef), chicken carcasses, and ready-to-eat (RTE) food (hot dogs). The assay utilizes a PCR detection method coupled with a rapid, visual, flow-based assay that develops in 3 min post-PCR amplification and requires only an 18 h enrichment for maximum sensitivity. The Veriflow SS system eliminates the need for sample purification, gel electrophoresis, or fluorophore-based detection of target amplification and does not require complex data analysis. This Performance Tested Method SM validation study demonstrated the ability of

the Veriflow SS method to detect low levels of artificially inoculated or naturally occurring *Salmonella* spp. in eight distinct environmental and food matrixes. In each reference comparison study, probability of detection analysis indicated that there was no significant difference between the Veriflow SS method and the U.S. Department of Agriculture Food Safety and Inspection Service Microbiology Laboratory Guidebook Chapter 4.06 and the U.S. Food and Drug Administration Bacteriological Analytical Manual Chapter 5 reference methods. A total of 104 *Salmonella* strains were detected in the inclusivity study, and 35 nonspecific organisms went undetected in the exclusivity study. The study results show that the Veriflow SS method is a sensitive, selective, and robust assay for the presumptive detection of *Salmonella* spp. sampled from environmental surfaces (stainless steel, sealed concrete, plastic, and ceramic tile), dairy (2% milk), raw meat (20% fat ground beef), chicken carcasses, and RTE food (hot dogs). ISSN: 10603271

Chen, J., Park, B., Eady, M.

Simultaneous Detection and Serotyping of Salmonellae by Immunomagnetic Separation and Label-Free Surface-Enhanced Raman Spectroscopy
(2017) *Food Analytical Methods*, 10 (9), pp. 3181-3193.

ABSTRACT: Current detection and characterization techniques for *Salmonellae* are time consuming, and rapid methods could benefit investigation and control of foodborne outbreaks. In this study, the potential of surface-enhanced Raman spectroscopy (SERS) in label-free detection and serotyping of *Salmonella* was evaluated. After immunomagnetic separation (IMS) and overnight culture, SERS spectra were collected from multiple replicates and experiments and analyzed by chemometrics. The detection/characterization accuracies were evaluated in real unknown mixture samples, which were confirmed by plating on selective agar plates and anti-sera agglutination tests. Prediction accuracies were found between 93 and 100%, 87 and 100%, and 67 and 100% for detecting *Salmonella* from other species, characterization of *Salmonella* serotypes, and simultaneous detection and characterization, respectively. When validated in mixture samples consisting of six bacteria, accuracies were 65–100% with increased misclassification. Overall, the approach may provide an inexpensive alternative within similar or slightly longer periods of time, but further improvement in spectral reproducibility and accuracy is needed.
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Tadapaneni, R.K., Syamaladevi, R.M., Villa-Rojas, R., Tang, J.

Design of a novel test cell to study the influence of water activity on the thermal resistance of Salmonella in low-moisture foods
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ABSTRACT: A novel test cell was developed to study the influence of water activity (a_w) on thermal inactivation of food pathogens in low-moisture foods (LMF). The cell consisted of multiple wells with a shared headspace; a_w of the inoculated food sample was controlled by lithium chloride (LiCl) solution of selected molality during heating. The performance of the test cell was evaluated by studying sample heating uniformity using finite element simulation and by comparing measured headspace relative humidities (RH) with predicted RH provided by LiCl solutions. The new cells were used to determine thermal resistance (D-value) of *Salmonella* at 80 °C in organic wheat flour (OWF) maintained at a_w of 0.45. The D-value of *Salmonella* was also determined in conventional thermal death time test cells in which a_w of OWF samples increased from 0.45 at room temperature to 0.73 at 80 °C according to the measured isotherm. The results demonstrated that a_w significantly influenced the D-values of *Salmonella*, and the new test cells can be used to directly relate thermal resistance of food pathogens to water activities of LMF at processing temperatures. ISSN: 02608774

Ferrario, C., Lugli, G.A., Ossiprandi, M.C., Turrone, F., Milani, C., Duranti, S., Mancabelli, L., Mangifesta, M., Alessandri, G., van Sinderen, D., Ventura, M.

Next generation sequencing-based multigene panel for high throughput detection of foodborne pathogens
(2017) *International Journal of Food Microbiology*, 256, pp. 20-29.

ABSTRACT: Contamination of food by chemicals or pathogenic bacteria may cause particular illnesses that are linked to food consumption, commonly referred to as foodborne diseases. Bacteria are present in/on various food products, such as fruits, vegetables and ready-to-eat products. Bacteria that cause foodborne diseases are known as foodborne pathogens (FBPs). Accurate detection methods that are able to reveal the presence of FBPs in food matrices are in constant demand, in order to ensure safe foods with a minimal risk of causing foodborne diseases. Here, a multiplex PCR-based Illumina sequencing method for FBP detection in food matrices was developed. Starting from 25 bacterial targets and 49 selected PCR primer pairs, a primer collection called foodborne

pathogen – panel (FPP) consisting of 12 oligonucleotide pairs was developed. The FPP allows a more rapid and reliable identification of FBPs compared to classical cultivation methods. Furthermore, FPP permits sensitive and specific FBP detection in about two days from food sample acquisition to bioinformatics-based identification. The FPP is able to simultaneously identify eight different bacterial pathogens, i.e. *Listeria monocytogenes*, *Campylobacter jejuni*, *Campylobacter coli*, *Salmonella enterica* subsp. *enterica* serovar *enteritidis*, *Escherichia coli*, *Shigella sonnei*, *Staphylococcus aureus* and *Yersinia enterocolitica*, in a given food matrix at a threshold contamination level of 101 cell/g. Moreover, this novel detection method may represent an alternative and/or a complementary approach to PCR-based techniques, which are routinely used for FBP detection, and could be implemented in (parts of) the food chain as a quality check.
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