Chemical decontamination of Campylobacter jejuni during chicken processing

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Summary

Campylobacter jejuni is among the most frequently reported causes of human bacterial gastrointestinal infection and chicken meat has been identified as a major food source of human campylobacteriosis. Chemical decontamination may be used during processing of chicken carcasses to reduce the level of *C. jejuni*. This intervention strategy is used in the U.S. poultry industry, whereas in the European Union the validity and risk of this procedure is still being evaluated. The assessment of chemical decontamination feasibility is hindered due to limited data regarding, for instance efficacy of chemicals against *C. jejuni* on the chicken carcass and cellular response of *C. jejuni* to chemicals.

The present Phd study demonstrated that nine chemical solutions had superior potency compared to water in reducing *C. jejuni* NCTC11168 on either chicken skin or meat in a 1 min dip treatment. Three chemicals were commercial decontaminants used in U.S. poultry slaughter plants (trisodium phophate 10%, lactic acid 2.5 %, cetylpyridinium chloride 0.5 %), and six compounds had not previously been identified as effective in reducing *C. jejuni* on chicken skin and meat (formic acid 2 %, caprylic acid sodium salt 5 %, capric acid sodium salt 5 %, grapefruit seed extract 1.6 %, chlorhexidine diacetate salt hydrate 1 %, benzalkonium chloride 1 %). Reductions ranged from 0.76 log to > 4.2 log, and for some chemicals the reduction depended on the food sample type: meat or skin. On chicken skin, chlorhexidine diacetate salt hydrate (3.81 log) as well as cetylpyridinium chloride and benzalkonium chloride (> 4.2 log) exhibited superior reduction potential among the nine tested chemicals. On chicken meat, benzalkonium chloride (2.33 log) and caprylic acid sodium salt (2.26 log) were the most efficient of the five tested chemicals. Additionally, an increase in treatment time up to 15 min improved the efficacy of trisodium phosphate and formic acid on chicken skin. The impact of food matrix and treatment time on the reduction potential of *C. jejuni* differed among the tested chemicals, implying the need to carefully evaluate the efficacy of specific chemicals before implementation of this intervention against *C. jejuni*. Interestingly, when chicken skin treated with grape fruit seed extract, cetylpyridinium chloride, or benzalkonium chloride were stored at 5°C for 24 h then the efficacy was lessened, which may point towards recovery of sub-lethally injured cells following the 1 min treatment with these chemicals. This emphasizes the need to further evaluate the extent and impact of recovery of sub-lethally injured *C. jejuni* with regard to chemical decontamination. Collectively, the study identified chemicals with considerable reduction potential against *C. jejuni* on chicken skin and meat and if these chemicals exhibit similar reduction effectiveness on the chicken carcass then application in a slaughter house setting may eventually lead to reduction in the number of human campylobacteriosis cases.

Scientists, legislators, and food processors are concerned that occurrence of sub-lethal chemical treatments during food processing could induce adaptation to the chemical,

cross-protection to other preservation or decontamination treatments, and changes in virulence characteristics of the bacterial populations. This Phd study evaluated the cellular response of *C. jejuni* NCCTC11168 to sub-lethal concentrations of trisodium phosphate (TSP), a highly alkaline decontaminant of chicken carcasses. The stress sensitivity phenotype of *C. jejuni* changed following pre-exposure to TSP, decreasing and increasing hydrogen peroxide and heat sensitivity, respectively. Additionally, exposure to TSP diminished swarming motility. Interestingly, microarray analysis revealed that TSP only elicited a minimal transcriptional response, and tolerance of TSP was instead suggested to depend on the function of specific membrane transporter proteins: NhaA cation/proton antiporters and RND multidrug efflux pumps. *C. jejuni* NCTC11168 orthologues of the *E. coli* NhaA cation/proton antiporter: *nhaA1 (cj1655c)* and *nhaA2 (cj1654c*) were shown to partially complement the TSP sensitive phenotype of an *E. coli* cation/proton antiporter mutant. In addition, inhibition of RND multidrug efflux pumps by inhibitor PaβN (Phe-Arg β-naphtylamide dihydrochloride) abolished growth of *C. jejuni* in the presence of TSP. Understanding these adaptation responses in *C. jejuni* to sub-lethal TSP may assist in the assessment of TSP as a feasible intervention method against *C. jejuni* on chicken meat and may eventually lead to improved strategies against this pathogen on chicken meat.

Dansk resumé

Campylobacter jejuni er en af de hyppigst rapporterede årsager til human bakteriel mave-tarm infektion og kyllingekød er en af de primære smittekilder til infektionen. Kemisk dekontaminering kan anvendes under slagteprocessen af kyllinger til at reducere niveauet af *C. jejuni* på kyllingekroppene. Denne bekæmpelsesstrategi bruges i slagtekyllingeproduktionen i U.S.A., hvorimod anvendeligheden og risici ved denne procedure fortsat er under evaluering i E.U. Vurderingen af hvorvidt kemisk dekontaminering er en anvendelig og udbytterig bekæmpelsesstrategi begrænses i høj grad af manglen på data, som beskriver for eksempel hvor meget kemikalier reducerer *C. jejuni* niveauet på kyllingekroppene og hvilke cellulære respons', som kemikalier udløser i *C. jejuni*.

Det foreliggende Phd studium viste, at ved en behandlingstid på et minut viste ni kemikalier et større reduktionspotentiale end vand overfor *C. jejuni* NCTC11168, enten på kyllingekød eller skind. Tre af disse kemikalier anvendes kommercielt i kyllingeslagterier i U.S.A. (mælkesyre 2,5 %, trinatriumfosfat 10 %, cetylpyridinium klorid 0,5 %), hvorimod seks af kemikalierne ikke tidligere har været afprøvet mht. til reduktionsevnen overfor *C. jejuni* på kyllingekødprodukter (myresyre 2 %, natriumsalt af kaprinsyre 5 %, natriumsalt af kaprylsyre 5 %, ekstrakt af grapefrugtkerner 1.6 %, klorhexidin diacetatsalt 1 %, benzalkonium klorid 1 %). Reduktionerne lå imellem 0,76 log til > 4,2 log og for nogle af kemikalierne afhang reduktionen af fødevaretypen: skind eller kød. På kyllingeskind var klorhexidin diacetatsalt (3,81 log) samt cetylpyridinium klorid og benzalkonium klorid (>4,2 log) de mest effektive iblandt de ni testede kemikalier. Derimod havde benzalkonium klorid (2,33 log) og natriumsalt af kaprylsyre (2,26 log) størst virkningsevne iblandt de fem testede kemikalier på kyllingekød. Desuden medførte en stigning i behandlingstiden op til 15 min en øget reduktionsevne for trinatrium fosfat og myresyre på kyllingeskind. Det viser, at fødevaretype og behandlingstid kan indvirke på kemikaliernes reduktionspotentiale overfor *C. jejuni*, men på forskellig vis for hvert enkelt kemikalie, hvilket indebærer, at hvert specifikt kemikalie skal evalueres grundigt førend det tages i brug, som intervention overfor *C. jejuni*. Det blev yderligere vist, at reduktionen formindskedes for ekstrakt af grapefrugtkerner, cetylpyridinium klorid og benzalkonium klorid, når kyllingeskind blev opbevaret på køl ved 5°C i 24 timer, hvilket antyder, at sub-letalt beskadigede celler kan restituere sig efter 1 minuts behandling med disse kemikalier. Det understreger vigtigheden af at foretage en yderligere evaluering af omfanget og betydningen af restitution af sub-lethalt beskadigede *C. jejuni* i forbindelse med kemisk dekontaminering. Samlet set identificerede dette studium kemikalier med et betragteligt reduktionspotentiale overfor *C. jejuni* på kyllingekød og – skind og såfremt deres effektivitet er tilsvarende på kyllingekroppe i et

slagteri, så kan anvendelsen af disse kemikalier i slagteprocessen medføre et fald i antallet af humane *Campylobacter* infektioner.

Forskere, lovgivere og fødevareproducenter er i stigende grad bekymret for, om forekomsten af sub-letal kemisk behandling i fødevarefremstillingen kan medføre, at bakteriepopulationer adapterer til kemikaliet, opnår krydsbeskyttelse overfor andre konserverings- eller dekontamineringsbehandlinger eller ændrer virulens. Trinatrium fosfat (TSP) er et høj pH kemikalie, som anvendes til dekontaminering af kyllingekroppe.

I dette Phd studium undersøgte vi det cellulære respons hos *C. jejuni* NCCTC11168 overfor sub-letale koncentrationer af TSP. Stressfølsomheden hos *C. jejuni* ændrede sig efter præeksponering med TSP, hvilket medførte mindsket peroxydfølsomhed men øget varmefølsomhed. Desuden observeredes nedsat sværmende bevægelighed, når TSP var til stede. Det var bemærkelsesværdigt, at en microarray analyse viste, at TSP kun udløste et minimalt transkriptionelt respons og tolerance overfor TSP kunne sandsynligvis i stedet tilskrives funktionen af specifikke membrantransportproteiner: NhaA cation/proton antiportere og RND multidrug efflux pumper. Det blev demonstreret, at *C. jejuni* NCTC11168 ortologer til *E. coli* NhaA cation/proton antiporteren: *nhaA1 (cj1655c)* og *nhaA2 (cj1654c*) var i stand til delvist at komplementere den TSP sensitive fænotype i en *E. coli* cation/proton antiporter mutant. Ligeledes, medførte hæmning af RND multidrug efflux pumper med inhibitor PaβN (Phe-Arg β-naphtylamide dihydrochloride), at væksten af *C. jejuni* ophørte under tilstedeværelse af TSP. Denne viden om det adaptive respons i *C. jejuni* overfor sub-letal TSP vil kunne medvirke i vurderingen af, om TSP anvendelse er en udbytterig bekæmpelsesstrategi overfor *C. jejuni* på kyllingekroppe samt på sigt bidrage til udviklingen af forbedrede bekæmpelsesstrategier overfor denne sygdomsfremkaldende bakterie på kyllingekød.