Change in attachment of pathogenic bacteria to decontaminated pig carcasses.

Krag, R.∗(1,2), Olsen, J.E.(1) and Aabo, S.(2)

(1)Department of Veterinary Disease Biology, Faculty of Life Sciences, University of Copenhagen, Stügebyglen 4, Dk-1870 Frederiksberg C, Denmark
(2)Department of Microbiology and Risk Assessment, National Food Institute, Technical University of Denmark, Mørkhøj Bygade 19, Dk-2860 Søborg, Denmark

*corresponding author: rikra@food.dtu.dk

Abstract

Yersinia enterocolitica, Salmonella Typhimurium and Listeria monocytogenes are food born human pathogenic bacteria, which are associated with consumption of raw pork. At the slaughterhouses decontamination of the pig carcasses has shown to be effective to reduce the number of pathogenic bacteria and may improve food safety. However, decontamination could change the meat surface properties, and thereby also the ability of bacteria to attach, with a potential impact on cross- and recontamination during handling at slaughterhouses and at meat processing. The purpose of the study was to investigate the attachment of Yersinia enterocolitica, Salmonella Typhimurium and Listeria monocytogenes to surfaces of pig skin and muscle after decontamination with 80°C water or 55°C 1% lactic acid. Loosely attached bacteria were removed by irrigation, and the number of firmly attached bacteria was counted after detachment by swabbing and stomaching. Bacterial location was studied by Confocal Laser Scanning Microscopy. The results show that bacteria were associated both superficially and in deep structures and that significant more bacteria adhered to the meat surfaces compared to the skin surfaces. Also, bacteria were more easily swabbed off the skin surfaces than off the meat surfaces. Whether this was due to enhanced binding or better physical entrapment of the bacteria at the meat surface could not be clarified by this study. At decontaminated skin surfaces bacterial attachment increased compared to non-decontaminated surfaces. In contrast, fewer bacteria attached to lactic acid treated meat surfaces compared to the non-treated surfaces. The conclusion is that decontamination affect bacterial tissue adhesion, which may impact re- and cross contamination properties through later processing steps.

Introduction

Yersina enterocolitica, Salmonella Typhimurium, and Listeria monocytogenes are food born human pathogenic bacteria, associated with consumption of contaminated raw pork. Contamination of the pig carcass typically occurs during slaughter and subsequent meat processing and therefore it is important to establish intervention procedures for minimizing contamination and improving the food safety. Beside optimal slaughter hygine, a promising approach seems to be physical or chemical carcass decontamination at the end of the slaughter line (Dickson et al., 1992, Dorsa et al., 1998, Eggenberger-Solorzago et al., 2002). However, side effects of decontamination should be evaluated. In addition to organoleptic changes and possible changes in bacterial growth and survival, decontamination could alter the meat surface properties, which again could influence bacterial attachment. This may be of importance for cross- and recontamination during subsequent handling of the meat. Therefore the purpose of the study was to investigate if decontamination with hot water and lactic acid would change the ability of pathogenic bacteria to adhere to pork surfaces. Additionally, differences in adhesion between bacteria species and surface types were studied, together with the location of Yersinia enterocolitica at decontaminated and non-decontaminated pig carcasses.

Material and methods

In the study Yersinia enterocolitica O:3 (isolate from a pig slaughterhouse, Denmark), Salmonella Typhimurium 4/74 (Watson et al., 1995) and Listeria monocytogenes strain 6895 (Larsen et al., 2002) were
used. All strains were maintained on brain heart infusion (BHI) agar (Oxoid), and inoculum was prepared by growing cells in BHI broth (Oxoid) O/N at 37°C.

Fresh pig skin and muscle samples (5x5cm) were decontaminated with 80°C water or 55°C 1% Lactic acid for 5 and 15 sec, and subsequently inoculated with approximately 7.5 log_{10} CFU per cm² of either Yersinia enterocolitica or Salmonella Typhimurium or Listeria monocytogenes. To allow attachment, samples were left at room temperature for 30 min. Loosely attached bacteria were removed by water irrigation for 15 sec. Firmly attached, superficially located bacteria, were counted after swabbing the surface with gauze, and stomaching the gauze in 25 ml sterile saline for 120 sec. The deeply located bacteria were then stomaching off the surfaces in 100 ml sterile saline for 120 sec. Dilutions of the two different stomachates was made, plated on species selective media and incubated O/N at 30°C (Yersinia and Listeria) and at 37°C (Salmonella). Finally, species suspected colonies were counted.

For microscopy observations the tissue samples was fast frozen and cut vertically and horizontally into 40 μm sections by a cryostat at -25°C (CM1900, Leica, Wetzlar, Germany). A TCS SP1 three channel Confocal Laser Scanning Microscope (Leica Microsystems Heidelberg GmbH, Germany) was used to visualize the in situ location of gfp-tagged Yersinia enterocolitica. Laser excitation at 458 and 488 nm was applied and the emitted light (495 to 530 nm) was assigned a green colour for the GFP image. The Leica Confocal Software TCS SP/NT version 2.5.1347 was used to process images.

Results

The numbers of bacteria adhering firmly to the surfaces are presented in table 1. Since the population of bacteria attached to surfaces decontaminated with water for 5 sec and for 15 sec did not differ significantly, the results are demonstrated together. For the same reason are the results for lactic acid treated surfaces at 5 sec and at 15 sec merged. A comparison of the three bacterial species shows that there were no markedly differences in the attachment pattern between them.

Table 1: The total number of firmly attached bacteria retained from the non-treated and treated skin and meat surfaces (logCFU/cm²) by swabbing and stomaching.

<table>
<thead>
<tr>
<th>Skin</th>
<th>No treatment</th>
<th>80°C Water</th>
<th>55°C Water</th>
<th>1% Lactic acid</th>
</tr>
</thead>
<tbody>
<tr>
<td>Yersinia enterocolitica</td>
<td>5.90 (0.15)</td>
<td>6.7 (0.03)</td>
<td>6.56 (0.07)</td>
<td></td>
</tr>
<tr>
<td>Salmonella Typhimurium</td>
<td>6.4 (0.19)</td>
<td>6.99 (0.04)</td>
<td>6.9 (0.16)</td>
<td></td>
</tr>
<tr>
<td>Listeria monocytogenes</td>
<td>6.12 (0.21)</td>
<td>6.94 (0.06)</td>
<td>6.84 (0.02)</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Meat</th>
<th>No treatment</th>
<th>80°C Water</th>
<th>55°C Water</th>
<th>1% Lactic acid</th>
</tr>
</thead>
<tbody>
<tr>
<td>Yersinia enterocolitica</td>
<td>6.85 (0.1)</td>
<td>6.76 (0.06)</td>
<td>6.55 (0.04)</td>
<td></td>
</tr>
<tr>
<td>Salmonella Typhimurium</td>
<td>7.02 (0.07)</td>
<td>6.84 (0.04)</td>
<td>6.46 (0.27)</td>
<td></td>
</tr>
<tr>
<td>Listeria monocytogenes</td>
<td>6.88 (0.03)</td>
<td>6.84 (0.06)</td>
<td>6.68 (0.07)</td>
<td></td>
</tr>
</tbody>
</table>

* Mean Log_{10}CFU per cm² of 3 (Salmonella and Listeria) or 6 (Yersinia) replicates.

The total number of bacteria firmly attached to both water and lactic acid decontaminated skin surfaces was significantly higher than the number of bacteria firmly attached to non-decontaminated skin surfaces (Figure 1a). At the meat surfaces the tendency was different, since fewer bacteria were attached to the lactic acid treated surface compared to the non-treated surfaces (Figure 1b). Though, the differences were
smaller and only significant when pooling the data for all three bacteria species. The number of bacteria attached to water treated meat surfaces did not changes significantly from the number attached to non-decontaminated surfaces or lactic acid treated surfaces.

A comparison of the total number of bacteria attached to the skin and meat surfaces shows significant more bacteria attached to the non-decontaminated meat surfaces compared to the non-decontaminated skin surfaces. However, at the treated surfaces the bacteria attached to the same extent to the meat and skin (table 1).

The firmly adhering bacteria were categorized into a population superficially located (removed by swabbing) and a population of more deeply located (removed by stomaching). As demonstrated in figure 2, significantly more bacteria are superficial located at the skin surfaces compared to deeply located and compared to meat surfaces. However, at the meat surfaces significantly more bacteria are located in relation to deeper tissue structures.

![Graph](image)

**Figure 1a and b:** The total number of bacteria firmly attached to non-decontaminated (light grey bar), 80°C water (dark grey bar) and 55°C 1% Lactic acid (black bar) decontaminated skin (a) and meat (b) surfaces in Log_{10} CFU per cm^2. Mean with SEM n=12 (non-treated) n=24 (treated). Bars with the same letter are not significant different.

![Graph](image)

**Figure 2:** The total number of superficially (light grey) and deeply (dark grey) located, firmly attached bacteria to skin and meat surfaces (Log_{10} CFU per cm^2). Mean with SEM n=60. Bars with the same letter are not significant different (α>0.05).

By visualization it was demonstrated that *Yersinia enterocolitica*, applied to the skin and muscle surface in a liquid suspension did float on the surface and was drawn into hair follicles, cleft and crevices by capillary like forces. Skin cryosections illustrated a tendency that firmly attached bacteria, both on the decontaminated and the non-decontaminated surfaces, cluster in high number in rough areas at the surfaces, in clefts and other deeper tissue structures, like hair follicles. At the muscle surfaces the population of firmly attached bacteria “hide” in the small cleft between muscle myo-fibres, both superficially and in the depth.

**Discussion**

Previous studies on bacterial attachment at decontaminated meat are by our knowledge limited to a study by Warriner et al., (2001) on steam-pasteurize lactic acid treated beef. They observed the same amount of *Salmonella Typhimurium*, *Listeria monocytogenes*, *Pseudomonas* and *E.coli* attached to treated and not-treated surfaces. Their results are supported by the current experiment, because it was not possible to identify a difference in attachment to the 80°C water treated and non-treated meat surfaces. Likewise, only a weak significant difference in the bacterial number attached to lactic acid treated meat surfaces compared to non-treated surfaces, was identified. However, at the skin surfaces the result was not reproduced, since water and lactic acid decontamination seems to induce surface changes, which alter the bacterial ecology and the ability for bacterial attachment at recontamination. However, it is not possible to conclude how the increased adhesion, will affect the cross contamination properties of the decontaminated meat.
The present study shows, that the bacterial attachment to surface seems to be independent of the bacterial species. That result, together with the tendency that attachment seems to be greater on meat surfaces compared to skin surfaces, support the theory of Fratamico et al., (1996), Lillard et al. (1986), and Thomas and McMeekin (1984); that surface characteristics and environmental factors are more important in defining cell attachment, than the bacterial properties. However, it is mentionable that other investigations are able to demonstrate significant changes in the surface attachment between different spoilage and pathogenic bacteria (Binito et al., 1997, Butler et al., 1979, Faber and Idziak 1984).

Fewer bacteria are swabbed off the meat surfaces compared to the skin surfaces and by microscopy it was shown that meat surfaces contain a lot of small cleft and gaps between the muscle myofibers where bacteria could “hide”. This could explain why Christensen et al., (2009) found a lower bacterial reduction at meat surfaces compared to the skin surfaces after water and lactic acid decontamination of Yersina, Salmonella and E.coli inoculated pork. The result also leads to speculations that the main part of inoculated bacteria actually is physical entrapped in stead of strongly attachment at specific surface site. However, the experimental design is not appropriate to prove that, but the idea get along with Li et al., (1999), who were not able to saturate muscle surfaces with bacteria, which indicate that attachment is not alone due to specific attachment. On the other hand, conclusions by Benedict et al., (1991), Fratamico et al., (1996) and Prachaiyo et al., (2000), indicate that surface located bacteria properly is a mix of entrapped and attached cells, because they found that surface bacterial adhesion at the meat surfaces was related to connective tissue protein and collagen fibres.

Conclusion

Decontamination may favour bacterial adhesion to and recontamination of decontaminated pork, as demonstrated by a significant increase in attachment of bacteria to decontaminated skin surfaces. However, no differences adhesion between species was demonstrated. Bacteria are able to penetrate deep tissue structure both on non-decontaminated and decontaminated skin and meat surfaces, but especially at the meat surfaces, bacteria tend to “hide” in the deep. The adhesion is properly both due to specific surface attachment sites and physical entrainment.

References

Dickson JS., Anderson ME., 1992. Microbiological decontamination of food animal carcasses by washing and sanitizing systems. A review. Journal of food protection, 55, 133-140
Dorsa WJ., et al., 1998. Bacterial profile of ground beef made from carcass tissue experimentally contaminated with pathogenic and spoilage bacteria before being washed with hot water, alkaline solution, or organic acid and then stored at 4 or 12 degrees. Journal of food protection, 61, 1109-1118
Faber JM., and Idziak ES., 1984. Attachment of psychotropic meat spoilage bacteria to muscle surfaces. Journal of food protection, 47, 92-95
Larsen CN., et al., 2002. In vitro and in vivo invasiveness of different pulsed field gel electrophoresis types of Listeria monocytogenes. Applied and environmental microbiology. 68, 5698-5702
Lillard HS., 1986. Role of fimbriae and flagella in the attachment of *Salmonella typhimurium* to poultry skin. Journal of food science, 51, 54-56

Safe pork 2009 - Québec city, Québec, Canada