CA18105

RIBMINS

Risk-based meat inspection and integrated meat safety assurance

EU COST ACTION Ribmins Training school

farm and abattoir interventions

Part I - chemical abattoir interventions

Part II - microbiological validation of interventions

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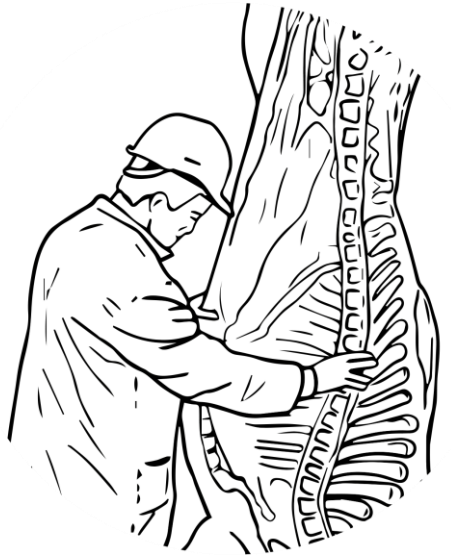
Part I – chemical abattoir interventions

1. concept
2. classes and mode of action
3. way of application
4. some literature data
5. perspectives and critical reflections



Part I – chemical abattoir interventions

– 1. concept

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In the EU, the fundamental principle of controlling microbial contamination during slaughter is based on sanitary and hygienic processes.

Both choosing abattoir technologies and conducting individual operations should be approached with the primary goal of preventing contamination and minimizing microbial load on the carcass.

Part I – chemical abattoir interventions

– 1. concept

Even when best hygienic abattoir practices are applied, complete prevention of all microbial contamination of carcasses is unachievable under commercial conditions.

Therefore, in some situations, it may be considered necessary to further reduce the microbial loads on carcasses through application of additional **interventions**

i.e. decontamination treatments.

(Buncic and Sofos, 2012, Food research international)

Part I – chemical abattoir interventions – 1. concept

Several intervention technologies have been tested to reduce the microbial contamination of carcasses. These can be divided into three major types:

I. physical (e.g. hot water, steam, steam vacuuming),

II. chemical (e.g. organic acids, chlorine, acidified sodium chlorite, polyphosphates)

III. biological (bacteriophages, bacteriocins).

IV. or combinations of the above technologies

(Hugas et. al, 2008, Meat science)

Part I – chemical abattoir interventions

– 1. concept

Chemical decontamination treatments involve

1 an application of a substance

2 at a given step during the slaughter process

3 in order to reduce the microbial contamination level of carcasses.

= reduce in numbers (log / cm^2)

- by detachment (removal)
- by killing (DNA still present)
- by deactivation (bacteria potentially still alive)

- one or more specific hazard(s)
- general microbial reduction

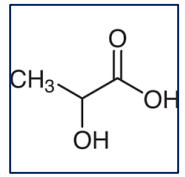
Part I – chemical abattoir interventions – 1. concept

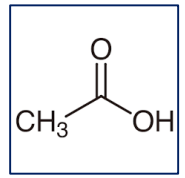
WHO – FAO defined some practical implementation factors to be considered by the establishment manager when choosing an intervention. These vary with the establishment, intervention type and point of application, and include:

- ability to contribute to targeted hazard control objectives
- cost-effectiveness
- reliable and timely supplies of materials or other resources, e.g. chemicals, power
- adequate quantity and reliable supply of potable water
- impact of chemicals on equipment, and effect of accumulation in the establishment environment
- development of resistance in bacterial strains with long-term use of chemicals and biocides
- occupational and safety risks to workers
- acceptance of intervention agents as food additives by regulators in domestic and export markets; and need for labelling.
- technical complexity and ease of use
- cost and availability of infrastructure, with ongoing maintenance
- impact on meat quality
- consumer acceptance
- environmental impact, e.g. waste disposal and pollution

Part I – chemical abattoir interventions – 2. classes and mode of action

1. low-molecule organic acids (e.g. lactic, acetic, citric, fumaric acid)

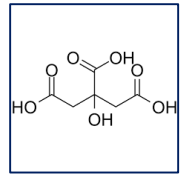


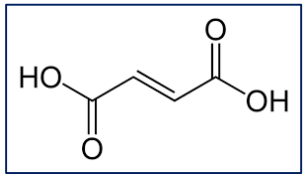


The antibacterial action of low molecule organic acids is

1. due to the lowering of the pH on the surface of the product (e.g. carcass)

2. its ability in the undissociated form to penetrate the cytoplasmic membrane, resulting in reduced intracellular pH and disruption of the transmembrane proton motive force.





Part I – chemical abattoir interventions

– 2. classes and mode of action

2. Chlorine-based treatments

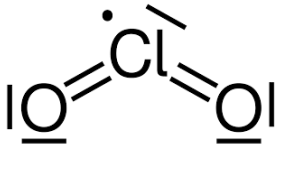
chlorine and chlorine dioxide

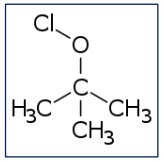
hypochlorite, sodium hypochlorite, sodium chlorite

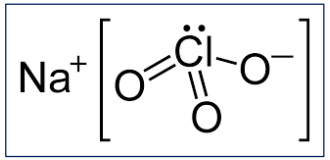
acidified sodium chlorite

cetylpyridinium chloride

monochloramine







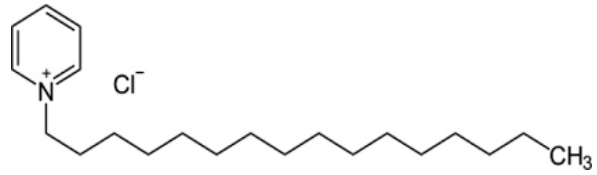
Chlorine compounds destroys microorganisms by chlorinating the lipid protein substance in the bacterial cell wall to form toxic chloro-compounds and induces the leakage of macromolecules from the cells.

Part I – chemical abattoir interventions

– 2. classes and mode of action

2. Chlorine-based treatments

chlorine and chlorine dioxide
hypochlorite, sodium hypochlorite, sodium chlorite
acidified sodium chlorite
cetylpyridinium chloride
monochloramine



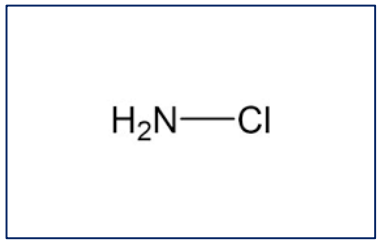
Cetylpyridinium chloride (CPC), first described in 1939, is a *quaternary ammonium*, water-soluble, colorless, broad-spectrum antimicrobial agent. It has been used for over 50 years in oral hygiene products including toothpaste, throat lozenges, and mouthwashes. Because of their low surface tension, hydrophilic, and lipophilic properties, quaternary ammonium compounds absorb into the bacterial cell surface, permeate and destroy the cell wall and cell membrane, and have a direct or indirect lethal effect on the cell. In the specific case of CPC, it has been shown that it interact strongly with negatively charged surfaces, and that the antibacterial activity is related to the hydrophobicity. The degree of damage to the bacterial membrane is however time and concentration dependent.

Part I – chemical abattoir interventions

– 2. classes and mode of action

2. Chlorine-based treatments

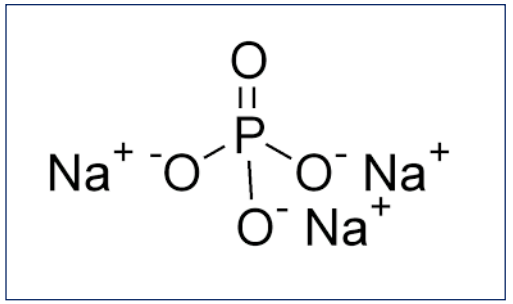
chlorine and chlorine dioxide
hypochlorite, sodium hypochlorite, sodium chlorite
acidified sodium chlorite
cetylpyridinium chloride
monochloramine



Monochloramine is a powerful oxidant that disrupts bacterial protein synthesis.

It has it application as drinking water and wound disinfectants.

In poultry decontamination studies, monochloramine exerted stronger antibacterial activity than NaOCl, probably due to a lesser extent inactivated by organic matter.



3. Trisodium phosphate

Trisodium phosphate (TSP) is an alkaline detergent that removes attached bacteria from carcass surfaces by means of its surfactant properties and high alkalinity (pH about 12.0). In addition, TSP kills bacteria by disrupting the cell membrane and causing leakage of cellular material.

Part I – chemical abattoir interventions

– 2. classes and mode of action

Occasionally, the antibacterial activity of other chemicals such as:

- peroxides
- sulfate-based compounds
- sodium hydroxide (NaOH)

Based on the evaluated studies,

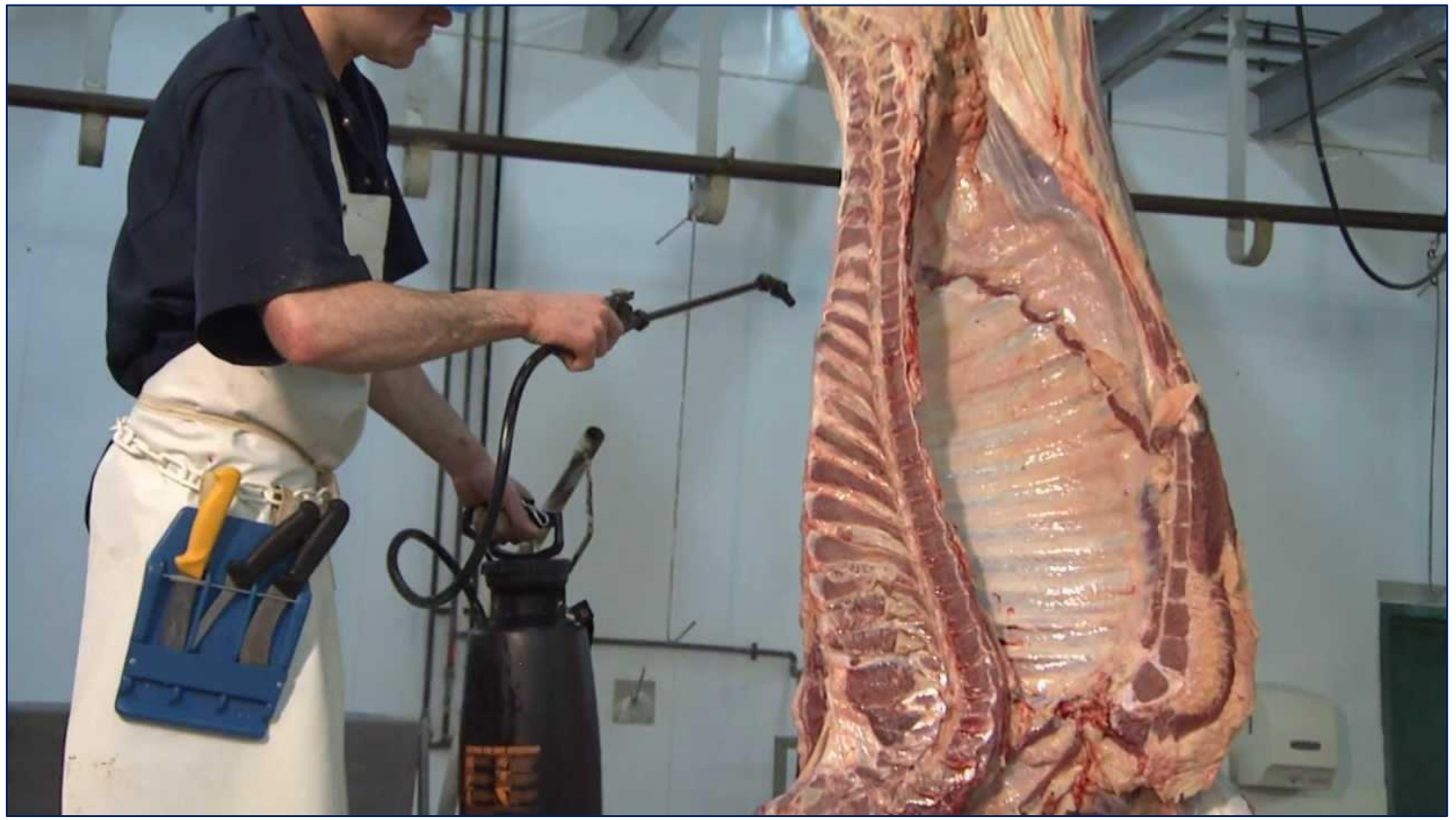
acetic and lactic acid, acidified sodium chlorite (ASC), and trisodium phosphate (TSP)

in particular proved to be effective for reducing the bacterial load

Part I – chemical abattoir interventions – 3. application

Spraying

by hand



Part I – chemical abattoir interventions – 3. application

Spraying

by hand

cabinet

Part I – chemical abattoir interventions – 3. application

Spraying

by hand

cabinet

Part I – chemical abattoir interventions – 3. application

Spraying

by hand

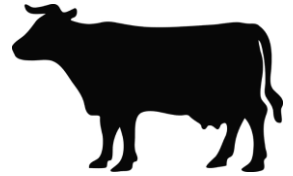
cabinet

Immersion (dipping)

Part I – chemical abattoir interventions

– 4. some literature data

Cattle hide treatments



Chemical washes

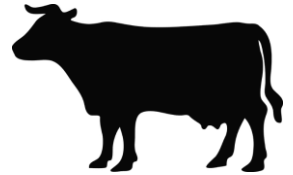
1. Oxidisers (peroxyacetic acid (PAA), hypobromous acid, hydrogen peroxide, …)
2. Quaternary ammonium compounds
3. Other chemicals (chlorine solutions, cetylpyridinium chloride (CPC), sodium hydroxide, sodium metasilicate, trisodium phosphate (TSP)

chemical dehairing process

process of applying successive water and chemical washes (sodium sulphide followed by a neutralizing solution of hydrogen peroxide) in a cabinet to remove hair and improve visible cleanliness and reduce microbial loads on animal hides.

Part I – chemical abattoir interventions

– 4. some literature data



Microbial immobilization treatment ('shellac hide coating')

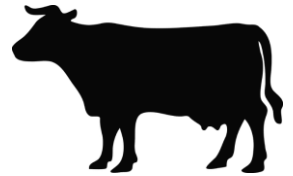
Spray treatment of cattle hides with natural resin (shellac), to form a protective coating as a barrier to microorganisms resulting in the reduction in their transfer to beef carcasses

Antic D. *et al.* 2010. Meat Science, 85, (1):77-81

Summary of findings for cattle hide interventions: studies under commercial conditions measuring bacteria counts.

Intervention[‡]	No. studies/ design	Intervention/outcome surface	Microorganism	Log_{10} CFU reduction[a]	Reference
Pre-exsanguination interventions					
Water wash & CPC (1%)	1/CT	Live animal hide/ carcass*	Aerobic bacteria	1.5	Bosilevac, Arthur, et al. (2004)
			Enterobacteriaceae	1.1	
Post-exsanguination washing/clipping					
Water wash/manual curry comb	1/BA	Veal calf hide	Aerobic bacteria	0.8	Wang et al. (2014)
			Enterobacteriaceae	3.5	
			E. coli	1.6	
Warm water wash	1/BA	Hide cut lines	Aerobic bacteria	0.1	Scanga et al. (2011)
Hide clipping (dirty hides)	2/CT	Hide/carcass*	Aerobic bacteria	0.1–0.3	Van Donkersgoed et al. (1997), McCleery et al. (2008)
			E. coli	0.3	
Organic acids					
Acetic acid (5%)	1/BA	Hide cut lines	Aerobic bacteria	2.6	Scanga et al. (2011)
			E. coli	3.7	
Lactic acid (6%)	1/BA	Hide cut lines	Aerobic bacteria	2.3	
			E. coli	3.7	
Other chemicals					
Chlorine/ASC (200 ppm)	1/BA	Veal calf hide	Aerobic bacteria	1.3	Wang et al. (2014)
			Enterobacteriaceae	1.5	
			E. coli	1.0	
Water wash & sodium hydroxide (1.5%)	1/CT	Hide/carcass*	Aerobic bacteria	0.8	Bosilevac, Nou, et al. (2005)
			Enterobacteriaceae	0.8	
Water wash & sodium hydroxide (1.5%)	2/BA	Hide	Aerobic bacteria	1.5–2.1	Bosilevac, Nou, et al. (2005), Yang, Badoni, Tran, and Gill (2015)
			Enterobacteriaceae	3.4	
Sodium hydroxide (3%)	1/BA	Hide cut lines	Aerobic bacteria	1.6	Scanga et al. (2011)
			E. coli	3.5	
TSP (20%)	1/BA	Hide	Aerobic bacteria	1.8	Çalicioğlu et al. (2010)
Ethanol (75%)	1/BA	Hide	Aerobic bacteria	1.2	
A proprietary QAC sanitiser & vacuuming	1/CT	Hide/carcass*	Aerobic bacteria	1.0	Antic et al. (2011)
			Enterobacteriaceae	1.3	
			E. coli	1.2	
Chemical dehairing and thermal interventions					
Chemical dehairing	1/CT	Hide/carcass*	Aerobic bacteria	2.0	Nou et al. (2003)
			Enterobacteriaceae	1.8	
Hot water wash	1/BA	Hide cut lines	Aerobic bacteria	3.6	Çalicioğlu et al. (2010)
Chlorine spray & hot water rinse	1/BA	Veal calf hide	Aerobic bacteria	2.1	Wang et al. (2014)
			Enterobacteriaceae	2.7	
			E. coli	2.6	
Microbial immobilisation treatments					
Shellac in ethanol hide coating	1/CT	Hide/carcass*	Aerobic bacteria	1.7	Antic et al. (2011)
			Enterobacteriaceae	1.4	
			E. coli	1.3	
Aqueous shellac hide coating	1/CT	Hide/carcass*	Aerobic bacteria	0.3–1.1	Antic et al. (2018)
			Enterobacteriaceae	0.1–0.7	

‡ Acidified sodium chlorite (ASC), trisodium phosphate (TSP), cetylpyridinium chloride (CPC), quaternary ammonium compounds (QAC).
* Reduction in hide-to-carcass transfer.
CT: Controlled trial; BA: Before-and-after trial.
[a] The comparison group was in all cases "no treatment" (controlled trials) and "pre-treatment" (before-and-after trials).

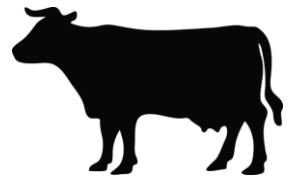


Antic D. *et al.* Meat Science, 2021, v.182

Summary of findings for cattle hide interventions: studies under commercial conditions measuring prevalence reductions.

Intervention[‡]	No. studies/ design	Intervention/outcome surface	Microorganism	% of samples positive in study population		Reference
				No treatment[a]	Treatment	
Pre-exsanguination interventions						
Water wash	1/BA	Live animal hide/hide	*Salmonella*	36–58%	40–72%	Mies et al. (2004)
Lactic acid (0.5%)	1/BA			50.0%	52.2%	
Chlorine	1/BA			60.0%	55.6%	
Water wash & CPC (1%)	1/CT	Live animal hide/hide	*E. coli* O157	56%	34%	Bosilevac, Arthur, et al. (2004)
	1/CT	Live animal hide/carcass*	*E. coli* O157	23%	3%	
Bacteriophage Finalyse® spray	1/CT	Live animal hide/hide	*E. coli* O157:H7	57.6	51.8	Arthur et al. (2017)
	1/CT	Live animal hide/carcass*	*E. coli* O157:H7	17.6	17.1	
Post-exsanguination washing/clipping						
Water wash	1/BA	Hide	*E. coli* O157:H7	62.5%	38.4%	Arthur et al. (2008)
			Salmonella	88.1%	24.3%	
Water wash & chlorine	2/BA	Hide	*E. coli* O157:H7[b]	4–35%	1–13%	Arthur et al. (2007), Bosilevac et al. (2009)
			Salmonella[b]	27–40%	7-13%	
Water wash/manual curry comb	1/BA	Veal calf hide	*E. coli* O103	26%	17%	Wang et al. (2014)
			E. coli O111	23%	17%	
Warm water wash	1/BA	Hide cut lines	*E. coli* O157:H7	78.0%	84.0%	Scanga et al. (2011)
			Salmonella	68.0%	88.0%	
Organic acids						
Acetic acid (5%)	1/BA	Hide cut lines	*E. coli* O157:H7	76%	30%	Scanga et al. (2011)
Lactic acid (6%)	1/BA		*E. coli* O157:H7	84%	56%	
			Salmonella	74%	50%	
Other chemicals						
Water wash/sodium hydroxide	1/CT	Hide/carcass*	*E. coli* O157	17%	2%	Bosilevac, Nou, et al. (2005)
(1.5%)	1/BA	Hide		44%	16%	
Sodium hydroxide (3%)	1/BA	Hide cut lines	*E. coli* O157:H7	94%	41%	Scanga et al. (2011)
			Salmonella	60%	43%	
Chemical dehairing						
Chemical dehairing	1/CT	Hide/carcass*	*E. coli* O157:H7	50%	1%	Nou et al. (2003)

‡ Cetylpyridinium chloride (CPC).
* Reduction in hide-to-carcass transfer.
CT: Controlled trial; BA: Before-and-after trial.
[a] The comparison group was in all cases "no treatment" (controlled trials) and "pre-treatment" (before-and-after trials).
[b] Percentage of total samples that had *E. coli* O157:H7 and *Salmonella* spp. counts at or above the detection limit of 40 CFU/100 cm^2 after enumeration.



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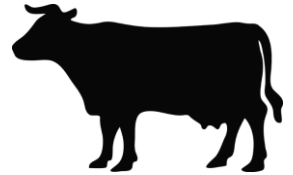
Part I – chemical abattoir interventions

– 4. some literature data



Chemical washes of carcasses

washes with antimicrobials such as **lactic, acetic and citric acids** that affect microbial growth through disruptions to nutrient transport and energy generation and can cause injury to microbial cells through their low pH.

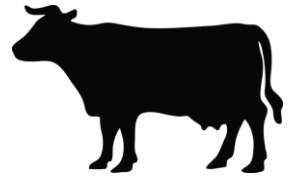
Washes containing **other chemicals and oxidizers**

Include washes containing products that destroy bacteria through various actions, such as oxidation and disruption of cellular functions, or that prevent bacterial attachment to meat.

Examples include: peroxyacetic acid (PAA), acidified sodium chlorite (ASC), hydrogen peroxide, trisodium phosphate (TSP)

Part I – chemical abattoir interventions

– 4. some literature data



Summary of findings for beef carcass interventions: studies under commercial conditions measuring bacteria counts.

Intervention[1]	No. studies/ design	Intervention/ outcome surface	Microorganism	Log_{10} CFU reduction[a]	Reference
Organic acid washes					
Lactic acid	5/BA 2/CT	Carcass	Aerobic bacteria *Enterobacteriaceae* *E. coli*	0.9–3.8 0.4–1.0 0.1–1.8	Bosilevac et al. (2006), De Martinez et al. (2002), Dormedy, Brashears, Cutter, and Burson (2000), Rodriguez (2007), Ruby and Ingham (2007), Signorini et al. (2018), Wright (2011)
Acetic acid	2/BA 1/CT	Carcass	Aerobic bacteria *Enterobacteriaceae* *E. coli*	0.4–0.6 1.0 0.5–0.7	Algino et al. (2007), Carranza et al. (2013), Signorini et al. (2018)
Citric acid	1/BA	Carcass	Aerobic bacteria *E. coli*	0.8 0.4	Signorini et al. (2018)
Organic acid mixtures	2/BA	Carcass	Aerobic bacteria *Enterobacteriaceae* *E. coli*	0.2 0.6 0.1–0.9	Algino et al. (2007), Signorini et al. (2018)

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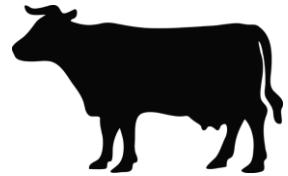
Part I – chemical abattoir interventions

– 4. some literature data

Summary of findings for beef carcass interventions: studies under commercial conditions measuring prevalence reductions.

Intervention‡	No. studies/ design	Intervention/ outcome surface	Microorganism	% of samples positive in study population		Reference
				No treatment[a]	Treatment	
Standard processing procedures and GHP						
Downward hide pulling	1/CT	Carcass*	*Enterobacteriaceae*	83%	94%	Kennedy et al. (2014)
Bung bagging	1/CT	Carcass*	VTEC non-O157	58%	35%	Stopforth et al. (2006)
			E. coli O157:H7	5%	1.7%	
			Salmonella	8.3%	0.0%	
Thermal interventions						
Hot water	2/BA	Carcass	*Enterobacteriaceae*	19–27%	12–15%	Algino et al. (2007), Bosilevac et al. (2006)
			E. coli	18–24%	3%	
			E. coli O157:H7	27%	5%	
Steam pasteurisation	2/BA	Carcass	*Enterobacteriaceae*	46%	3%	Corantin et al. (2005), Nutsch et al. (1997)
			E. coli	14–16%	0–1.8%	
			Salmonella	0.7%	0%	
Organic acid washes						
Lactic acid	3/BA	Carcass	*E. coli* O157:H7	31%	20%	Bosilevac et al. (2006), Chaves et al. (2013), Ruby and Ingham (2007)
			VTEC non-O157	6.7%	0%	
			Salmonella	45%	28%	
Acetic acid	1/BA	Carcass	*Enterobacteriaceae*	58%	30%	Algino et al. (2007)
			E. coli	47%	13%	
Organic acid mixtures	1/BA	Carcass	*Enterobacteriaceae*	28%	22%	
			E. coli	24%	7%	
Hot water/LA	2/BA	Carcass	*E. coli* O157:H7	19%	4%	Bosilevac et al. (2006), Ruby and Ingham (2007)
			Salmonella	28%	2.3%	
Multiple interventions applied at multiple steps						
Steam vacuum, PAA & organic acid washes, thermal interventions	5/BA	Carcass	*E. coli* O157:H7	4–43%	0–17%	Arthur et al. (2004), Elder et al. (2000), Kanankege et al. (2017), Rekow et al. (2011), Ruby and Ingham (2007)
			VTEC non-O157	70–79%	14–62%	
			Salmonella	45%	2%	

‡ Lactic acid (LA), peroxyacetic acid (PAA).
* Reduction in transfer to carcass.
CT: Controlled trial; BA: Before-and-after trial.
[a] The comparison group was in all but one case "no treatment" (controlled trials) and "pre-treatment" (before-and-after trials); In the "downward hide pulling" a comparison group was "upward hide pulling".



Antic D. *et al.* Meat Science, 2021, v.182

Part I – chemical abattoir interventions

– 4. some literature data

pre-chilling carcass interventions

Only a few studies investigated the antibacterial efficacy of organic acids on naturally contaminated pig carcasses under commercial conditions.

lactic acid
acetic acid
citric acid

acidified sodium chlorite (ASC)

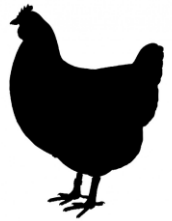
washing
spraying before - after - before and after evisceration

Zdolec N. *et al.*, 2022, MDPI submitted
Loretz M. *et al.*, 2011, Food Control, 22, 1121-1125

Part I – chemical abattoir interventions

– 4. some literature data

chemical decontamination treatments for poultry carcasses



Organic acids

1. acetic acid
2. lactic acid
3. citric acid

chlorine-based treatments

1. chlorine and chlorine dioxide
2. hypochlorite, sodium hypochlorite, and sodium chlorite .
3. acidified sodium chlorite
4. cetylpyridinium chloride
5. monochloramine

Phosphate-based treatments

1. trisodium phosphate
2. other phosphate-based compounds

Other chemical treatments

Antibacterial activity of acetic and lactic acid treatments on the surface of poultry carcasses and parts.

Agent/ microorganism	Reduction (log CFU)	Treated material	Application[a]	Sampling point	Concentration	Contamination	Temperature (°C)	Exposure time (min)	Reference
Acetic acid									
Aerobic bacteria	2.0 ml^{-1}	Carcass	IC	During slaughter	20 ppm	Artificial	4	45	Fabrizio et al. (2002)
	0.9–1.7 ml^{-1}	Carcass	IC	After slaughter	0.6%	Natural	Ice water	60	Dickens and Whittemore (1995)
	0.6 ml^{-1}	Carcass	SP	During slaughter	1%	Natural	23	0.8	Dickens and Whittemore (1997)
	0.5–0.8 cm^{-2}	Carcass	SP	During slaughter	0.5%	Natural	NA[b]	NA	Sakhare et al. (1999)
	0.4 ml^{-1}	Carcass	SP	During slaughter	20 ppm	Artificial	NA	0.3	Fabrizio et al. (2002)
	0.3–0.4 ml^{-1}	Carcass	IM	After slaughter	0.3–0.6%	Natural	10	10	Dickens and Whittemore (1994)
	0.2–0.7 cm^{-2}	Carcass	IM	During slaughter	0.5%	Natural	NA or 58	1 or 2	Sakhare et al. (1999)
Campylobacter jejuni	1.2–1.4 g^{-1}	Wing	IM	At retail	2%	Artificial	4	0.3–0.8	Zhao and Doyle (2006)
Coliforms	3.0 ml^{-1}	Carcass	IC	During slaughter	20 ppm	Artificial	4	45	Fabrizio et al. (2002)
	0.9–2.2 cm^{-2}	Carcass	IM	During slaughter	0.5%	Natural	NA	1	Sakhare et al. (1999)
	0.1–1.0 cm^{-2}	Carcass	SP	During slaughter	0.5%	Natural	NA	NA	Sakhare et al. (1999)
Enterobacteriaceae	2.3 ml^{-1}	Carcass	IM	After slaughter	0.6%	Natural	10	10	Dickens and Whittemore (1994)
	0.9 ml^{-1}	Carcass	IM	After slaughter	0.3%	Natural	10	10	
	0.6–1.8 ml^{-1}	Carcass	IC	After slaughter	0.6%	Natural	Ice water	60	Dickens and Whittemore (1995)
Escherichia coli	2.8 ml^{-1}	Carcass	IC	During slaughter	20 ppm	Artificial	4	45	Fabrizio et al. (2002)
	0.5–1.2/10 cm^2	Breast	SP	At processing	0.1–0.3%	Artificial	20–55	0.1–0.3	Jiménez, Destefanis, Salsi, Tiburzi, and Pirovani (2005)
Salmonella Hadar	1.8–2.0/10 cm^2	Breast	SP	At processing	2.5%	Artificial	55	0.5	Jiménez, Caliusco, Tiburzi, Salsi, and Pirovani (2007)
	1.2–1.8/10 cm^2	Breast	SP	At processing	1–2.5%	Artificial	25	0.2–0.4	Jiménez et al. (2007)
Salmonella Typhimurium	1.4 ml^{-1}	Carcass	IC	During slaughter	20 ppm	Artificial	4	45	Fabrizio et al. (2002)
	0.8 ml^{-1}	Carcass	SP	During slaughter	20 ppm	Artificial	NA	0.3	Fabrizio et al. (2002)
Staphylococcus aureus	1.3–1.8 cm^{-2}	Carcass	SP	During slaughter	0.5%	Natural	NA	NA	Sakhare et al. (1999)
	0.5–0.7 cm^{-2}	Carcass	IM	During slaughter	0.5%	Natural	NA or 58	1 or 2	Sakhare et al. (1999)

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Review

Antimicrobial activity of decontamination treatments for poultry carcasses: A literature survey

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Antibacterial activity of chlorinated water, acidified sodium chlorite, and cetylpyridinium chloride on the surface of poultry carcasses and parts.

Agent/ microorganism	Reduction (log CFU)	Treated material	Application[a]	Sampling point	Concentration	Contamination	Temperature (°C)	Exposure time (min)	Reference
Chlorine									
Aerobic bacteria	2.1–2.3 ml^{-1}	Carcass	SP	During slaughter	55 ppm	Artificial	21–54	0.1	Northcutt et al. (2005)
	0.9 cm^{-2}	Carcass	IM or SP	During slaughter	50 ppm	Natural	NA[b]	5	Sinhamahapatra et al. (2004)
	0.5 ml^{-1}	Carcass	IC	After slaughter	20–50 ppm	Natural	NA	NA	Stopforth et al. (2007)
	0.1–0.3 ml^{-1}	Carcass	SP	During slaughter	20–50 ppm	Natural	NA	NA	Stopforth et al. (2007)
Campylobacter spp.	2.5–2.6 ml^{-1}	Carcass	SP	During slaughter	55 ppm	Artificial	21–54	0.1	Northcutt et al. (2005)
Campylobacter jejuni	2.6–3.0 g^{-1}	Wing	IM	At retail	50 ppm	Artificial	4 or 23	10 or 30	Park et al. (2002)
	2.3 g^{-1}	Carcass	SP/IM	During slaughter	73 ppm	Artificial	NA/3	0.03/40	Kim et al. (2005)
	2.1 g^{-1}	Carcass	IM	During slaughter	73 ppm	Artificial	3	40	Kim et al. (2005)
	1.9–2.5/carcass	Carcass	SP/IC	During slaughter	50 ppm	Artificial	20–60/NA	0.2/50	Li et al. (2002)
	1.8–2.2/carcass	Carcass	SP	During slaughter	50 ppm	Artificial	55–60	0.2	Li et al. (2002)
	1.3–1.7 cm^{-2}	Carcass	IM	During slaughter	50 ppm	Artificial	60–65	0.3	Li et al. (2002)
	1.2/carcass	Carcass	SP	During slaughter	50 ppm	Artificial	20	0.2	Li et al. (2002)
	0.5–0.7 cm^{-2}	Carcass	IM	During slaughter	50 ppm	Artificial	20–55	0.3	Li et al. (2002)
Coliforms	0.7 cm^{-2}	Carcass	IM	During slaughter	50 ppm	Natural	NA	5	Sinhamahapatra et al. (2004)
	0.6 cm^{-2}	Carcass	SP	During slaughter	50 ppm	Natural	NA	5	
	0.4 ml^{-1}	Carcass	IC	After slaughter	20–50 ppm	Natural	NA	NA	Stopforth et al. (2007)
	0.2–0.3 ml^{-1}	Carcass	SP	During slaughter	20–50 ppm	Natural	NA	NA	Stopforth et al. (2007)
Escherichia coli	1.9–2.1 ml^{-1}	Carcass	SP	During slaughter	55 ppm	Artificial	21–54	0.1	Northcutt et al. (2005)
	0.3 ml^{-1}	Carcass	IC	After slaughter	20–50 ppm	Natural	NA	NA	Stopforth et al. (2007)
	0.2–0.3 ml^{-1}	Carcass	SP	During slaughter	20–50 ppm	Natural	NA	NA	Stopforth et al. (2007)
Salmonella spp.	0.9–1.1 ml^{-1}	Carcass	SP	During slaughter	55 ppm	Artificial	21–54	0.1	Northcutt et al. (2005)
Acidified sodium chlorite									
Aerobic bacteria	2.0 g^{-1}	Leg	IM	During slaughter	1200 ppm	Natural	18	15	Del Río et al. (2007)
	1.6 cm^{-2}	Wing	IM	After chilling	900 ppm	Artificial	NA	0.3	Sexton et al. (2007)
	1.1 cm^{-2}	Carcass	IM	After slaughter	1200 ppm	Natural	20	0.1	Sinhamahapatra et al. (2004)
	0.8 cm^{-2}	Carcass	SP	During slaughter	1200 ppm	Natural	20	0.1	
	0.8–1.0 ml^{-1}	Carcass	IM	After slaughter	500–1200 ppm	Natural	12–14	0.1	Kemp et al. (2000)
Campylobacter spp.	2.6 ml^{-1}	Carcass	SP	After slaughter	1100 ppm	Natural	14–18	0.3	Kemp, Aldrich, Guerra, and Schneider (2001)
Coliforms	1.5–2.0 ml^{-1}	Carcass	IM	After slaughter	850–1200 ppm	Natural	12–14	0.1	Kemp et al. (2000)
	1.4 g^{-1}	Leg	IM	After slaughter	1200 ppm	Natural	18	15	Del Río et al. (2007)
	1.4 cm^{-2}	Carcass	IM	After slaughter	1200 ppm	Natural	20	0.1	Sinhamahapatra et al. (2004)
	1.1 cm^{-2}	Carcass	SP	After slaughter	1200 ppm	Natural	20	0.1	
	0.9 ml^{-1}	Carcass	IM	After slaughter	500 ppm	Natural	12–14	0.1	Kemp et al. (2000)
Enterobacteriaceae	1.5 g^{-1}	Leg	IM	During slaughter	1200 ppm	Natural	18	15	Del Río et al. (2007)
Escherichia coli	2.3 ml^{-1}	Carcass	SP	After slaughter	1100 ppm	Natural	14–18	0.3	Kemp et al. (2001)
	2.3 ml^{-1}	Carcass	IM	After slaughter	500–1200 ppm	Natural	12–14	0.1	Kemp et al. (2000)
Cetylpyridinium chloride									
Aerobic bacteria	2.2/carcass	Carcass	SP	During slaughter	0.5%	Artificial	35	0.28	Yang et al. (1998)
	1.6–2.0 ml^{-1}	Breast	SP	During slaughter	0.1–0.5%	Artificial	20	0.5	Xiong et al. (1998)
Campylobacter jejuni	>4.2 ml^{-1}	Carcass	IM	At retail	0.5%	Arificial	20	1	Riedel et al. (2009)
Salmonella Typhimurium	2.0/carcass	Carcass	SP	During slaughter	0.5%	Artificial	35	0.28	Yang et al. (1998)
	1.5–2.5/38.5 cm^2	Breast	SP	After chilling	0.1%	Artificial	10–60	0.5	Wang et al. (1997)
	1.5–1.9 ml^{-1}	Breast	SP	During slaughter	0.1–0.5%	Artificial	20	0.5	Xiong et al. (1998)
	1.0–1.6 cm^{-2}	Breast	IM	After chilling	0.1%	Artificial	NA	1–3	Kim and Slavik (1996)
	0.9–1.7 cm^{-2}	Breast	SP	After chilling	0.1%	Artificial	15 or 50	1	Kim and Slavik (1996)

[a] IM, immersion; SP, spraying; IC, immersion chilling.
[b] NA, not available.

Part I – chemical abattoir interventions - 5. perspectives

There are three main aspects to be considered with chemical interventions:

i) safety of the intended substance itself,
ii) Its effect as to the development of antimicrobial resistance and
iii) the efficacy i.e. does the use of the substance in practice decrease the level of contamination of pathogenic microorganisms.

For this purpose,

EFSA issued a guidance document (EFSA, 2006) which points out the major components and data that a dossier/application should contain in order to demonstrate that the substance intended to be used for the removal of microbial surface contamination of foods of animal origin is both safe and efficacious.

3.5. Methods of analysis
All methods used for the **microbial analyses** and for the analysis of the substance(s), its (their) degradation products and major reaction by-products should be provided by the applicant (including detailed protocols, validity and performance parameters, etc.).

Part I – chemical abattoir interventions - 5. perspectives

EFSA The BIOHAZ Panel concluded that the use of substance(s) for decontaminating treatments will be regarded efficacious

- when any reduction of the prevalence and/or numbers of pathogenic target pathogenic microorganisms is statistically significant when compared to the control (e.g. water)

  and, at the same time

- this reduction has a positive impact on reduction of human illness cases (EFSA, 2008a).

The efficacy depends on a range of factors such as

- concentration
- contact time
- temperature
- mode of application
- Initial microbial load of the surface
- other conditions of application.

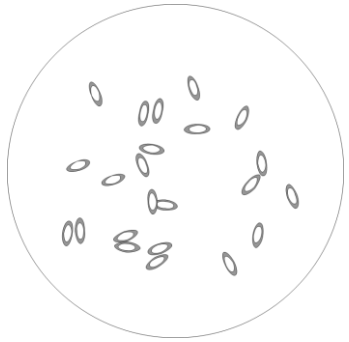
Part II – microbiological validation of interventions

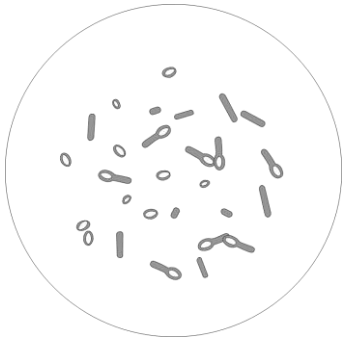
Factors with an impact or causing bias on microbiological results

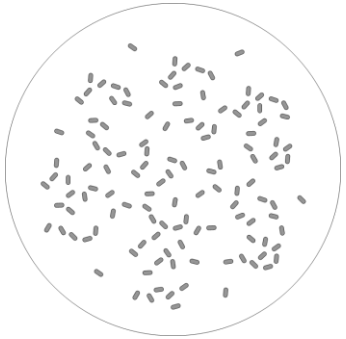
Intrinsic factors

Extrinsic factors

Part II – microbiological validation of interventions





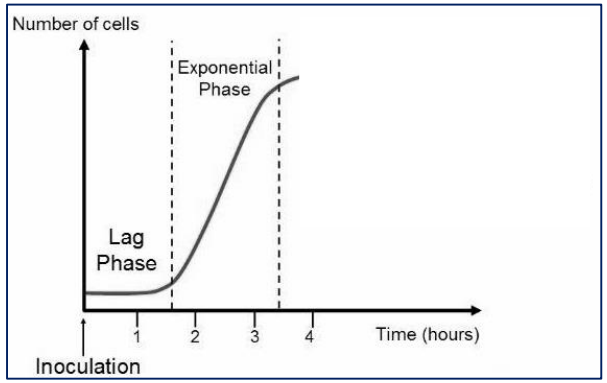


alive - vegetative

(endo)spores

death

VBNC



Mini-Review | Open Access | Published: 26 November 2019

Microbial response to acid stress: mechanisms and applications

acid tolerance

Ningzi Guan & Long Liu

Applied Microbiology and Biotechnology **104**, 51–65 (2020) | Cite this article

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Part II – microbiological validation of interventions

Factors with an impact or causing bias on microbiological results

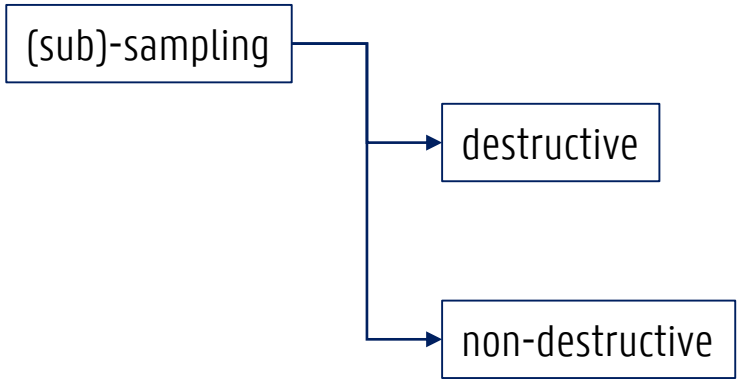
Intrinsic factors

1. metabolic state and bacterial strain heterogeneity => *important when using inoculum testing*

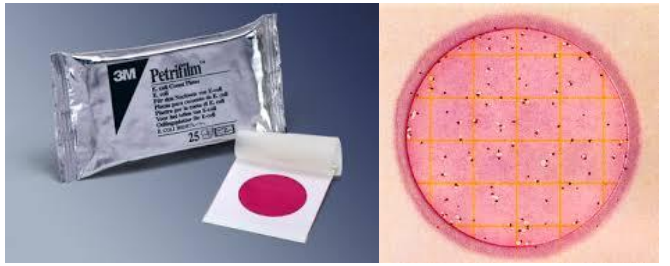
Extrinsic factors

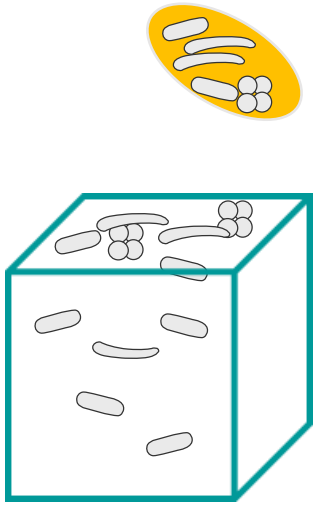
1. sampling method

Part II – microbiological validation of interventions



Part II – microbiological validation of interventions





before and after study

sampling the same place only once

Part II – microbiological validation of interventions

Factors with an impact or causing bias on microbiological results

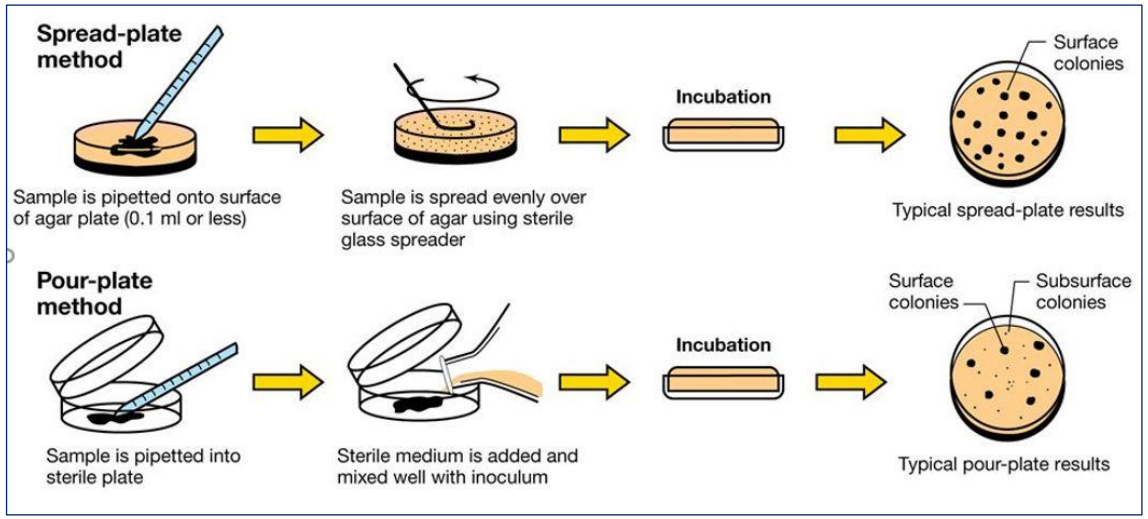
Intrinsic factors

1. metabolic state and bacterial strain heterogeneity => *important when using inoculum testing*

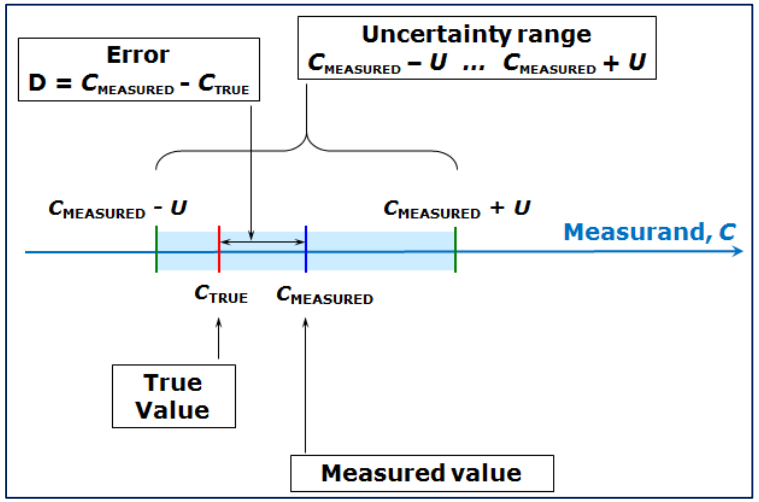
Extrinsic factors

1. sampling method
2. analysis method

Part II – microbiological validation of interventions



- "1" colony represents "1" cell
- with normalized methods (e.g. ISO): awareness of standard error and uncertainty of measurement
- there is a (technical) detection limit

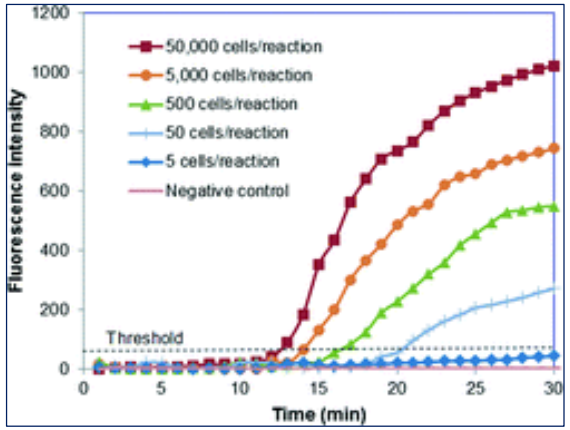


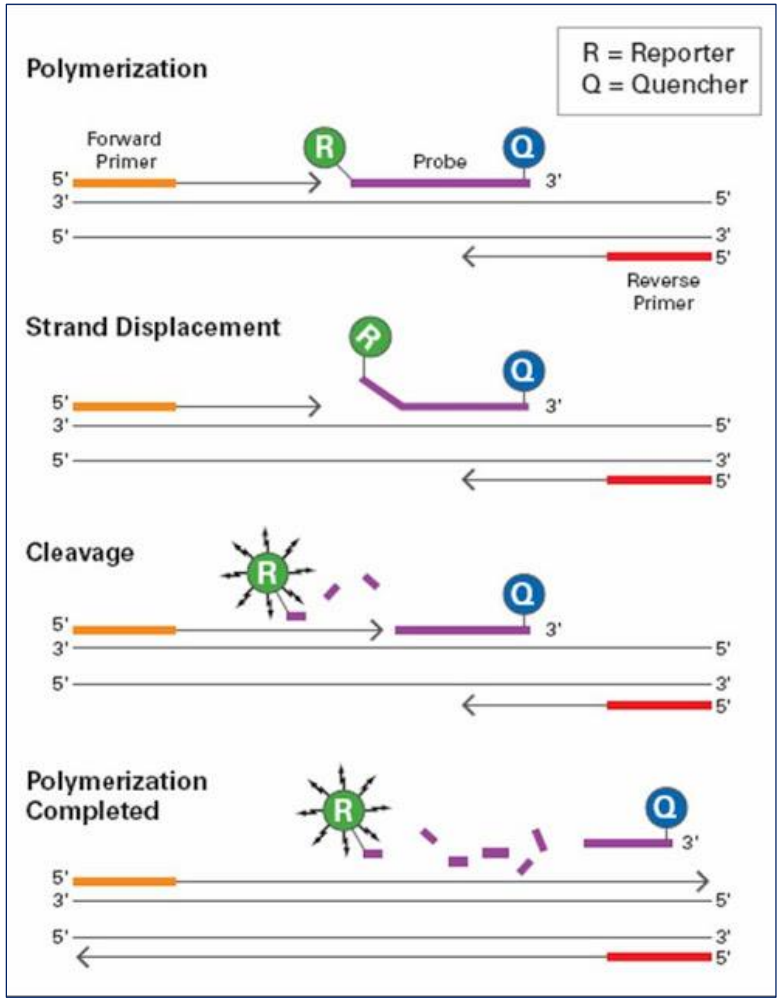
Part II – microbiological validation of interventions

RT-PCR or Quantitative PCR

Commercial kits available

- cells: death or alive?
- inhibition by matrix (fat)
- detection limit

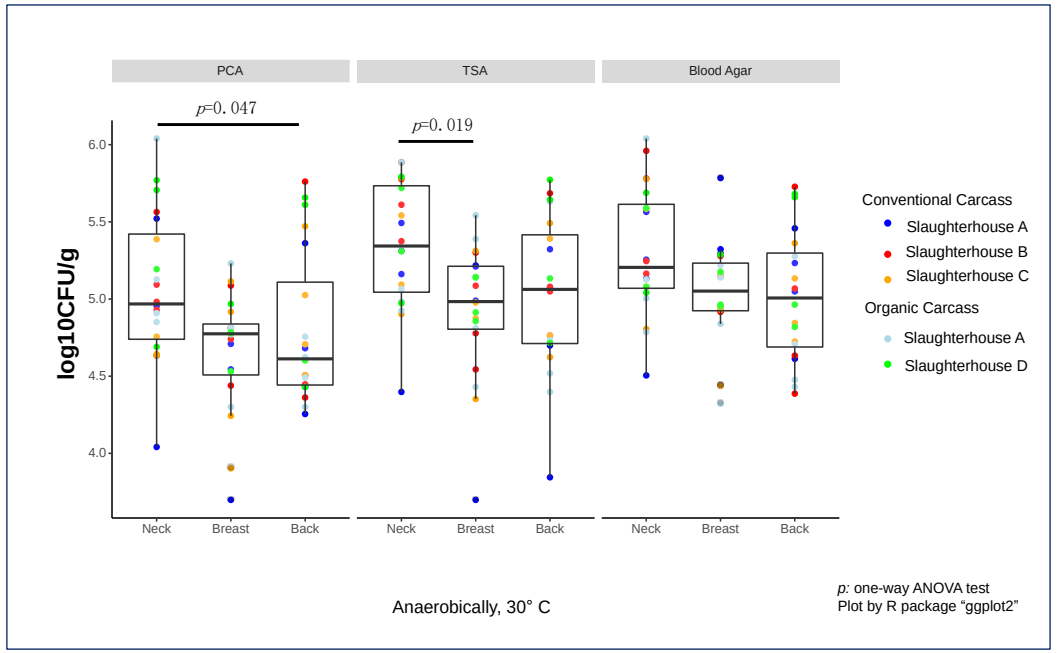


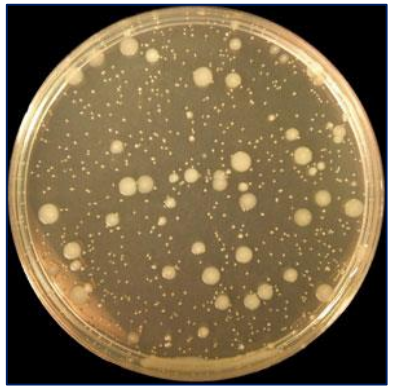


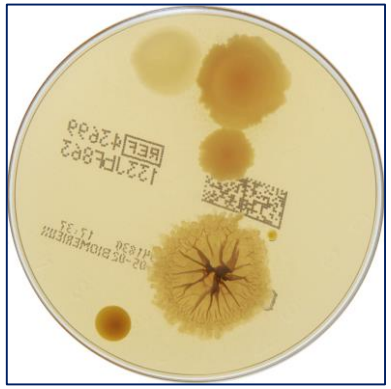
Part II – microbiological validation of interventions

Microbial validation of the intervention

- general viable counts
  - total aerobic bacteria
    - media
- semi-general viable counts
- Indicator or index organisms
- specific pathogens (hazards)





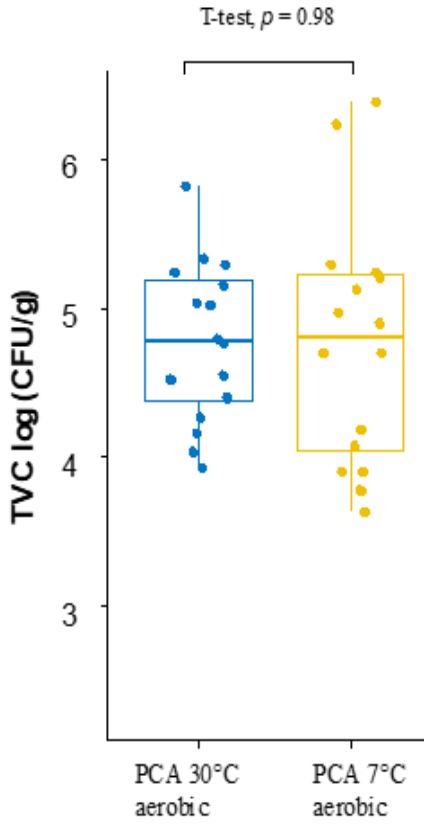


PCA Plate Count Agar
TSA Tryptone Soya Agar
BA Blood agar

Yu Z. *et al.* Front. microbiol, 2020, V 82

Part II – microbiological validation of interventions

- **Microbial validation of the intervention**
  - general viable counts
    - **total aerobic bacteria**
      - 7°C
      - 20 °C
      - **30°C**
      - 37°C
  - semi-general viable counts
  - Indicator or index organisms
  - specific pathogens (hazards)



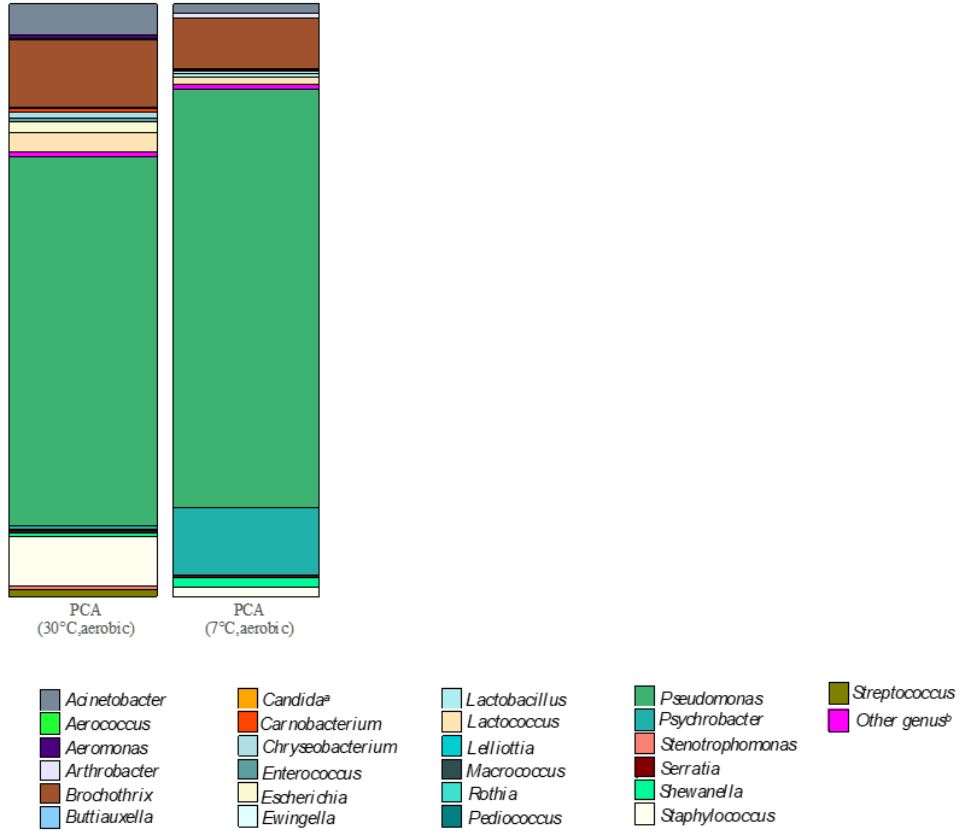
Yu Z. *et al.* Food microbiology, 2019, V 82

Part II – microbiological validation of interventions

Microbial validation of the intervention

- general viable counts
  - total aerobic bacteria
    - 7°C
    - 20 °C
    - **30°C**
    - 37°C
- semi-general viable counts
- Indicator or index organisms
- specific pathogens (hazards)

Impact of intervention may not be at the quantitative level but in the diversity level



Yu Z. *et al.* Food microbiology, 2019, V 82

Part II – microbiological validation of interventions

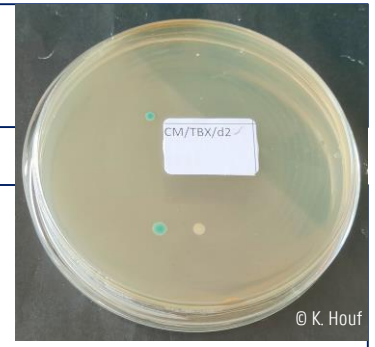
Microbial validation of the intervention

- general viable counts
- semi-general viable counts
- Indicator or index organisms
- specific pathogens (hazards)

The term was suggested by Ingram in 1977 for a marker whose presence indicated the possible presence of an ecologically similar pathogen.

Indicator organism can be applied to any taxonomic, physiological or ecological group of organisms whose presence or absence provides indirect evidence concerning either a particular feature in the past history of the sample, or the contemporary presence of a feature not directly investigated.

Part II – microbiological validation of interventions



Microbial validation of the intervention

- general viable counts
- semi-general viable counts
- Indicator or index organisms
- specific pathogens (hazards)

The same … but not the same …

Enterobacteriaceae

There are about 20 genera in the family Enterobacteriaceae, which include *E. coli* and the group of coliform bacteria. Members of the family are Gram-negative, facultative anaerobes and rod-shaped. Numerous Enterobacteriaceae are found in the intestines of humans and other animals, some occur in water or soil whereas others are parasites on animals and plants.

coliform bacteria

are common in the feces of warm-blooded animals and can be found in aquatic environments, in soil and on vegetation. Coliform bacteria do not usually trigger serious illnesses. Due to the fact that they are easy to culture, their presence can used to indicate that more pathogenic organisms of fecal origin may be present.

Escherichia coli

is common in the lower intestine of warm-blooded animals. Most species of *E. coli* are harmless. However, some strains can cause serious food poisoning in humans. Fecal-oral transmission is the most common route through which pathogenic organisms cause disease.

Part II – microbiological validation of interventions

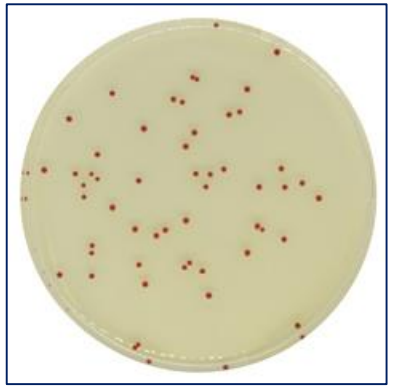
Microbial validation of the intervention

- general viable counts
- semi-general viable counts
- Indicator or index organisms
- specific pathogens (hazards)

From qualitative to quantitative analysis

use of chromogenic media

Listeria chromogenic agar plate



Campylobacter chromogenic agar plate

but hazards often under the detection limit in commercial abattoir setting

Part II – microbiological validation of interventions

Factors with an impact or causing bias on microbiological results

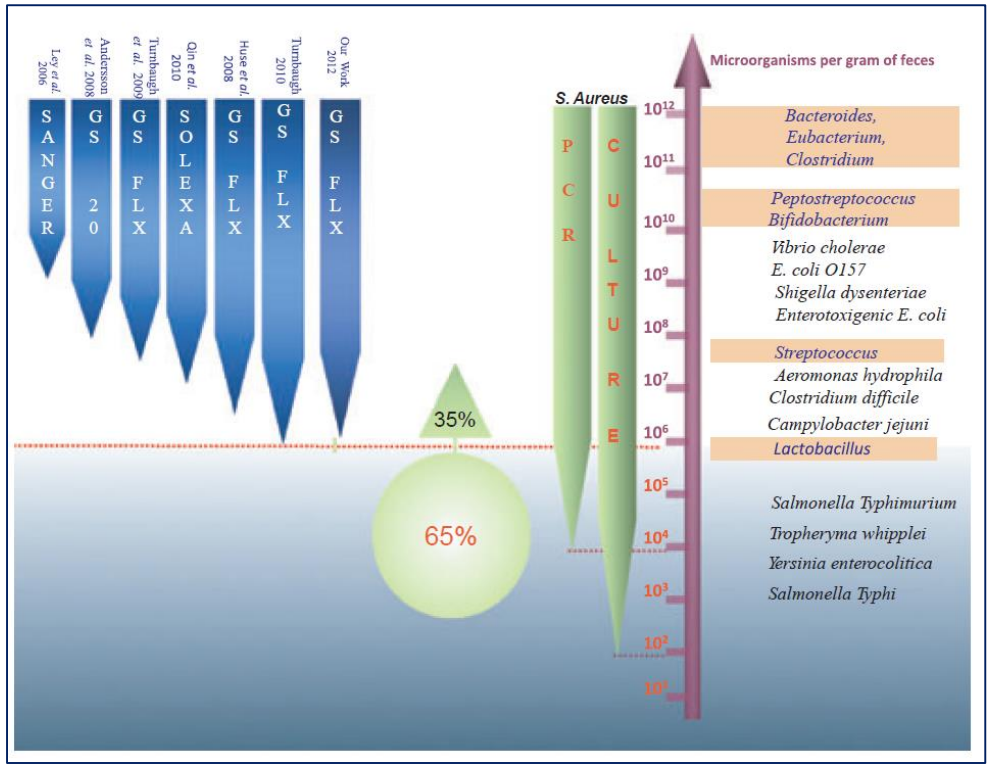
Intrinsic factors

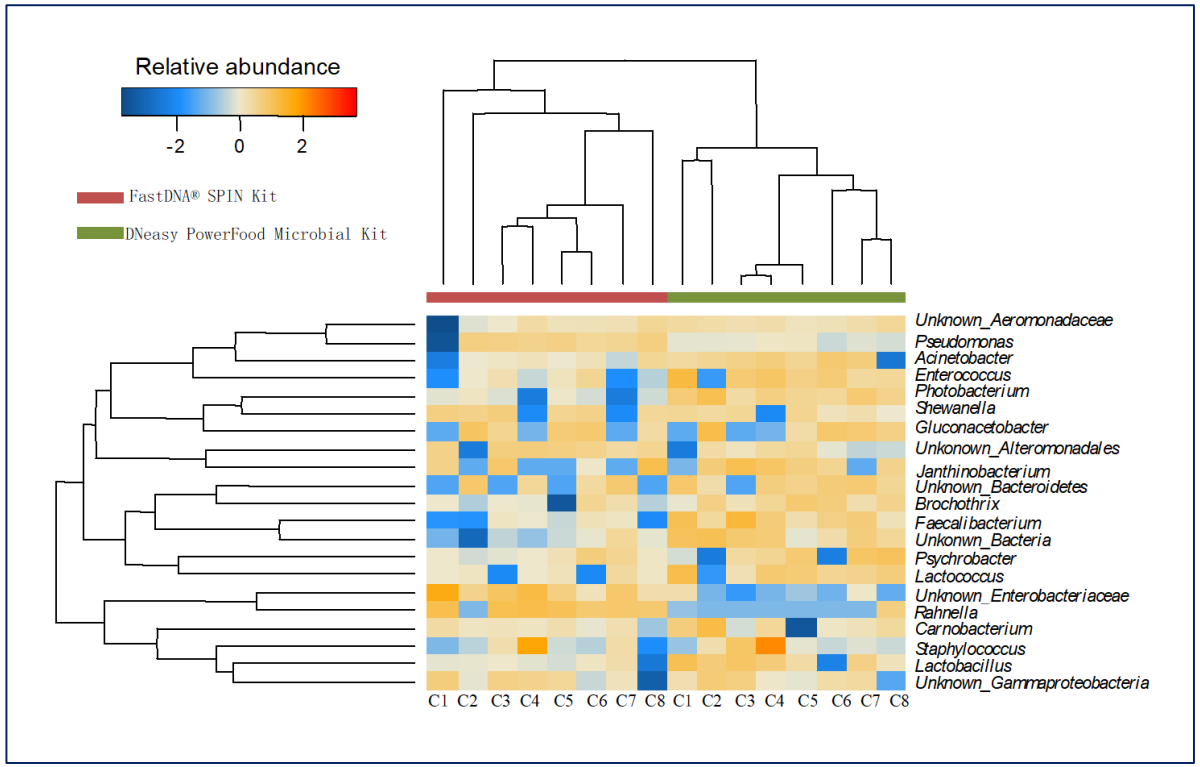
1. metabolic state and bacterial strain heterogeneity

Extrinsic factors

1. sampling method
2. analysis method
    1. culture depended
        1. quantitative
        2. qualitative
    2. culture independed

Part II – microbiological validation of interventions





Lagier JC *et al.* Microbial culturomics: paradigm shift in the human gut microbiome Study. Clin Microbiol Infect 2012; 18: 1185–1193

Yu Z. *et al.* Food microbiology, 2019, V 82

CA18105

RIBMINS

Risk-based meat inspection and integrated meat safety assurance

Thank you for your attention

Kurt Houf

Faculty veterinary medicine
Department of veterinary and biosciences

Ghent University
University of Antwerp

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