EXTERNAL SCIENTIFIC REPORT

Escherichia coli and *Enterobacteriaceae* counts on poultry carcasses along the slaughter processing line, factors influencing the counts and relationship between visual faecal contamination of carcasses and counts: a review[1]

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ABSTRACT

A literature review was conducted covering the period 2000-2012 to gather information concerning the presence and counts of *E. coli* and *Enterobacteriaceae* on carcasses during different stages of the slaughter processing line (review question 1); risk factors that could explain the variability of the counts of the indicator organisms (review question 2) and the relationship between the counts of indicator organisms and visual faecal contamination on carcasses (review question 3). In total, 72 papers, providing pertinent data for the scopes of the search, were collected on poultry. A certain level of variability was evidenced among different studies and some variables like the indicator organism considered, the sampling and analytical methods used, the use of chlorine, the setting where the studies were carried out render the available data barely comparable. In relation to review question 1, the steps of the processing line where a decrease of *E. coli* was more evident were scalding, washing and chilling; furthermore as regards *Enterobacteriaceae* counts a decrease was observed at the scalding and washing steps. Considering review question 2, risk factors related to batch and slaughtering process were evaluated. In general when a risk factor was investigated by several studies results were hardly in agreement. Taking into account the slaughter process it is evident that plant features have an influence on indicator bacteria loads but considering each investigated risk factor no reliable conclusions can be drawn. In relation to review question 3, despite the retrieved studies were quite limited, the data obtained suggested that the presence of visible faecal contamination has no predictive value for estimating the microbial quality of the carcasses.

KEY WORDS

Escherichia coli, *Enterobacteriaceae*, counts, poultry carcasses, slaughterhouse, process hygiene criteria

DISCLAIMER

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SUMMARY

A project entitled "Usefulness of *Escherichia coli* and *Enterobacteriaceae* as Process Hygiene Criteria in poultry" was awarded by EFSA to Istituto Zooprofilattico Sperimentale delle Venezie (Legnaro, Padova, Italy) with the purpose to collect available data on the indicator organisms *E. coli* or *Enterobacteriaceae* as Process Hygiene Indicators (PHI) for the main livestock species, based on a literature search and an experimental study, in this case in broiler slaughterhouses, located in the EU. The present document is the report on the extensive literature review on *Escherichia coli* and *Enterobacteriaceae* counts on poultry carcasses. The extensive literature review covering pig and ruminant carcasses and the experimental study in broiler slaughterhouses are published as two separate external scientific reports (Barco et al., 2014; Cibin et al., 2014).

The extensive literature review was conducted to gather information concerning the presence of indicator bacteria, *E. coli* and *Enterobacteriaceae* and their counts on carcasses during different stages in the slaughter processing line (review question 1); risk factors that could explain the variability of the counts of the indicator organisms (review question 2) and the relationship between the counts of indicator organisms and visual faecal contamination on carcasses (review question 3).

The literature search covered the period 2000-2012 and was conducted at worldwide level. Two electronic databases (PubMed and Web of Science) were consulted, in addition also a web-searching, through Google-scholar, was carried out.

The principles of "systematic review methodology" were applied and included the following steps: definition of the review questions and the eligibility criteria, searching for research studies, selecting the studies for inclusion or exclusion in the review, collecting data from the included studies, assessing quality of included studies, synthesising data collected from included studies, presenting data, interpreting results and drawing conclusion.

A total of 72 papers satisfied the eligible criteria considered at the different stages of the screening process and were used to collect data for the three review questions: 30 papers provided pertinent data for review question 1, 41 papers for review question 2 and seven papers for review question 3.

Since treatment of carcasses with chlorine was considered an important factor that likely affects the counts of indicator bacteria, studies were grouped according to whether they were conducted in a European or non-European country, and the use of chlorine. Group 1 included European studies where theoretically chlorine was not used; Group 2 included studies performed in non-European countries and conducted in slaughterhouses where chlorine was not used at any steps of the slaughter processing line; Group 3 included studies performed in slaughterhouses where chlorine was used at least in one step of the slaughter processing line (mainly in non-European countries) and Group 4 included studies performed in non-European countries for which information concerning the use of chlorine was not available.

A certain level of variability among the different studies due to different aspects and to the complexity of the slaughter processing line was evidenced. Some variables like the indicator organism considered, the sampling and analytical method used, the setting where the studies were carried out and the specific step of the slaughter processing line investigated, render the available data barely comparable and could lead to controversial conclusions among studies describing counts at the same stage of the slaughter processing line or investigating the same risk factor.

REVIEW QUESTION 1. Presence of the indicator organisms *E. coli* and *Enterobacteriaceae* and their counts on carcasses during different stages in the slaughter processing line

The trend of the counts at the different stages of the slaughter process was analysed within the same study and then compared among studies considering the same stage of the slaughter processing line. In particular, evaluations in terms of changes of concentration of the indicator bacteria along the slaughter processing line was limited to studies that sampled carcasses immediately before and after any of the major stages of the slaughtering process, thus allowing to identify the steps of the chain where the counts increase or decrease. Moreover, changes in the concentrations of *E. coli* and *Enterobacteriaceae* on poultry carcasses before and after a specific stage along the slaughter processing line were assessed considering studies (totally 23 studies for *E. coli* and 7 studies for *Enterobacteriaceae*) providing data expressed by using the most commonly used units of enumeration (log cfu/ml; log cfu/cm^2; log cfu/g). At each stage of the slaughter processing line, the change between the two sampling points (before and after) was calculated as the difference between the mean values of the counts at the latter chronological sampling point and the counts at the previous sampling stage.

According to the majority of the retrieved papers, as far as *E. coli* is concerned, the steps of the slaughter processing line where a decrease of the counts was reported were scalding, washing and chilling. Scalding and washing were the stages that contributed to reduce the bacterial loads of *Enterobacteriaceae* on carcasses.

Scalding not only helps the loss of feathers, making defeathering more practical, but also contributes to lower the numbers of bacteria on the external surfaces of broiler carcasses, according to the data obtained from the retrieved studies. The washing step is essential to remove organic debris from carcasses; this practice determines a reduction of the microbial contamination, but the level of reduction depends upon several aspects. The data collected from most of the retrieved studies, demonstrated the importance of the washing step for reducing indicator bacteria loads on carcasses. Chilling is the last step of the process and it is aimed at lowering the temperature of the carcasses in order to control the microbial growth; retrieved studies demonstrated the efficacy of the chilling step in reducing *E coli* counts on carcasses. For *Enterobacteriaceae* the data obtained were quite controversial, and in some cases limited increases of the counts at the end of the air chilling process were reported.

REVIEW QUESTION 2. Risk factors that could explain the variability of the counts of the indicator organisms

According to the defined search process and the established eligibility criteria, a total of 41 papers dealing with risk factors influencing indicator bacteria loads (review question 2) were obtained. Also for papers providing data for review question 2 a certain level of variability was evidenced; different studies considering the same factor were compared in terms of conclusions drawn by authors and not in terms of reported counts.

As far as the batch related risk factors are concerned, the evaluated studies failed to demonstrate any link between counts of indicator bacteria on carcasses and farming practices (e.g. feed withdrawal time, feed regimen before transporting birds to the slaughterhouse) or the health status of the animals. The only exception is reported in one study, which found an increase in counts according to the age of animals at slaughter.

Regarding the slaughter related risk factors, from the analysis of the selected studies, it has not always been possible to understand the effect of the specific practices applied at the different stages of the slaughter processing line on indicator bacteria counts. In fact many different variables, affecting the final bacteria loads, were considered in the different studies. Although slaughterhouses show different

ability in reducing bacterial loads of processed carcasses, neither the dimension of the plant nor the level of mechanization seem to be clearly correlated with the counts of indicator bacteria.

Studies evaluating the effect of each single step of the slaughter processing line on the bacterial loads of carcasses provide various information. The use of physical treatments at the washing step (steam pasteurization, hot water, and high pressure washing treatment) could be a valid solution to reduce carcass bacterial counts. However, the retrieved studies generally refer to pilot studies and reported that even small variations in such treatments are able to produce different results and cause damage to the carcasses. Concerning immersion chilling no significant risks were described in relation to different practices like water renewal time (from 8 to 24 h), treatment and reuse of chilling water, reduction of the ratio of litres of water per Kg of carcass. Moreover chlorine use represents an important factor of variability among the evaluated studies; however, its use was not always linked in a significant way with differences observed during the slaughter operations.

Finally factors related to others steps of the slaughterline were investigated like scalding, defeathering, chilling but the results obtained were in disagreement or provided contrasting results.

REVIEW QUESTION 3. The potential relationship between the counts of indicator organisms and visual faecal contamination on carcasses

Seven papers provided pertinent information on the relationship between faecal contamination of carcasses and their *E. coli* and *Enterobacteriaceae* counts. A different weight was attributed to these papers considering if data were collected in a commercial slaughterhouse instead of a pilot plant and if data were referred to naturally or artificially contaminated carcasses. Among the seven retrieved papers, four papers reported studies considering artificially contaminated carcasses collected on pilot slaughterhouses and three papers illustrated studies providing data on naturally contaminated carcasses obtained in commercial slaughterhouses.

The studies conducted on artificially contaminated carcasses, suggest that the *Enterobacteriaceae* and *E. coli* counts in samples taken at different stages along the slaughter processing line were in general not influenced by the faecal contamination of carcasses, especially when samples were collected at the end of the slaughter processing line. However, the data obtained on artificially contaminated carcasses may not always faithfully mimic natural contamination since bacteria may adhere less persistently on artificially contaminated carcasses than on naturally contaminated ones. The results of the studies conducted on carcasses naturally contaminated with faecal material confirmed that the presence of faecal contamination, in some cases visually evaluated, has no predictive value for estimating the microbial quality of the carcasses.

Despite the retrieved studies providing relevant data for review question 3 were quite limited, both the data obtained on artificially and naturally contaminated carcasses suggest that there is no correlation between the bacteria loads of carcasses (*E.coli* and *Enterobacteriaceae*) and their faecal contamination.

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BACKGROUND AS PROVIDED BY EFSA

Regulation (EC) No 854/2004 of the European Parliament and of the Council lays down specific rules for the organization of official controls on products of animal origin intended for human consumption. Among others, inspection tasks within this Regulation include checks and analysis of food chain information, ante-mortem inspection and post-mortem inspection.

EFSA received a mandate from the Commission in May 2010 on the modernization of meat inspection, requesting a series of scientific opinions. The main scope of these opinions was to identify and rank the most relevant meat safety risks, to assess the strengths/weaknesses of the current meat inspection system, to propose alternative approaches for addressing current meat-safety risks, and to outline a generic framework for inspection, prevention and control (including related methodology) for the prioritized hazards that are not (sufficiently) covered by the current system.

Several species were to be considered. The scientific opinions on the public health hazards to be covered by inspection of swine meat (EFSA-Q-2010-00886) and poultry meat (EFSA-Q-2010-01469) were published in 2011 and 2012. Four more opinions concerning the inspection of meat from bovines/cattle (EFSA-Q-2011-00365), farmed game (EFSA-Q-2011-00366), small ruminants (EFSA-Q-2011-00365) and solipeds (EFSA-Q-2011-00367) were published in 2013.

In the scientific opinion on meat inspection of poultry, the BIOHAZ Panel concluded that *Campylobacter* spp. and *Salmonella* spp. are considered of high public health relevance for poultry meat inspection. Currently in the EU, the use of the food chain information for microbial food safety purposes is limited to *Salmonella* control, leading to *Salmonella*-positive flocks being slaughtered at the end of the day. In addition, samples of neck skin on broiler carcasses after chilling are used for the Process Hygiene Criteria laid down in Regulation No 2073/2005[3] as amended in Regulation 1086/2011[4].

Current post-mortem visual inspection is not able to detect any of the public health hazards identified as the main concerns for food safety. Visual detection of faecal contamination of carcasses at post-mortem inspection can be an indicator of slaughter hygiene. However, the high speed of the slaughter lines reduces the sensitivity of detection of carcass contamination by visual inspection and there is not a direct association with the occurrence of pathogens. Hence, other approaches to verify slaughter hygiene were considered as more appropriate by the BIOHAZ Panel.

The BIOHAZ Panel proposed recommending that the current visual inspection process is replaced by the establishment of targets for the main biological hazards on the carcass and by verification of the food business operators own hygiene management through the use of Process Hygiene Criteria (PHC). A potential approach for the latter is measuring *E. coli* or *Enterobacteriaceae* on poultry carcasses after chilling.

SPECIFIC OBJECTIVES AS PROVIDED BY EFSA

The purpose of the Service Contract is to provide EFSA with the available data on the indicator organisms *E. coli* or *Enterobacteriaceae* as Process Hygiene Indicators (PHI) for the main livestock species. Based on this literature search, an experimental study in broiler slaughterhouses located in the EU should be designed and carried out to collect relevant data on these two indicator organisms. The ultimate aim is to support the purpose of potential PHC for evaluating process control in EU broiler slaughterhouses.

[3] OJ L 338, 22.12.2005, p. 26.
[4] OJ L 281, 28.10.2011, p. 7.

According to the Technical Specifications of the Service Contract CFT/EFSA/BIOHAZ/2012/03-CT1, the tasks to be covered are as follows:

- To carry out literature searches for data related to the main livestock species on (i) the presence of the indicator organisms *E. coli* and *Enterobacteriaceae* and their counts on carcasses during different stages in the slaughter processing line; (ii) information that could explain the variability of the counts of the indicator organisms and (iii) the potential relationship between the counts of indicator organisms and visual faecal contamination on carcasses;

- To perform an experimental study in broiler slaughterhouses located in the EU in order to (i) collect relevant data on the variability of the counts of *E. coli* and *Enterobacteriaceae* on broiler carcasses after chilling; (ii) collect information that could lead to interpretation of the variability of these counts and (iii) compare *E. coli* and *Enterobacteriaceae* counts on carcasses with and without visual faecal contamination.

The present document is the report on the extensive literature search for available data on *E. coli* and *Enterobacteriaceae* on carcasses of poultry. The extensive literature review covering pig and ruminant carcasses and the experimental study in broiler slaughterhouses are published as two separate external scientific reports (Barco et al., 2014; Cibin et al., 2014).

This contract was awarded by EFSA to:

Contractor: Istituto Zooprofilattico Sperimentale delle Venezie, Legnaro, Padova, Italy

Contract title: Usefulness of *Escherichia coli* and *Enterobacteriaceae* as Process Hygiene Criteria in poultry

Contract number: CFT/EFSA/BIOHAZ/2012/03

INTRODUCTION AND OBJECTIVES

Routine examination of meat samples for potential pathogens is impractical mainly because of their low level; inconsistent distribution in meat samples and in some cases the need of laborious analytical methods (Schaffner and Smith, 2004). As indicator bacteria are found at much higher prevalences on foods of animal origin, they are frequently used as indicators of pathogen presence (Matias et al., 2010).

Ideally, an indicator bacterium should meet certain criteria (Jay et al., 2005); in particular, it should be:

- rapidly detectable and easily differentiable from other microorganisms present in the samples;

- strictly associated with the pathogen whose presence it should indicate (e.g. correlate counts, comparable growth rates).

However, controversy still remains over the degree to which the presence/amount of indicator bacteria can be indicative of the presence of pathogens (Schaffner and Smith, 2004). On the contrary, the recognition that indicator bacteria are an effective tool in process hygiene assessment has been well-documented (EFSA, 2012).

Several indicators can be useful to evaluate hygiene levels during meat slaughtering process. Aerobic colony count (ACC) is commonly used to evaluate the hygiene of the entire meat production process.

Enterobacteriaeceae and *E. coli* are more frequently used to assess enteric contamination (Ghafir et al., 2008). Psychrotrophic microorganisms, such as *Pseudomonas*, have great importance as indicators in products that are stored at low temperatures since they are responsible for the superficial alteration of these products (Gonzalez-Miret et al., 2006).

Enterobacteriaceae are defined as Gram-negative, glucose fermenting, oxidase negative, usually catalase-positive and nitrate reducing organisms. This family includes many bacteria associated with faeces, but also many non-faecal organisms (Schaffner and Smith, 2004). Faecal coliforms are defined as Gram-negative bacilli fermenting lactose within 48 h at 44.5 to 45.5 °C and this group includes several bacteria, such as *E. coli*, *Enterobacter* spp., *Klebsiella pneumoniae* and *Citrobacter fruendii* (Schaffner and Smith, 2004). Within this group, *E. coli* is the most relevant microorganism in relation to faecal contamination of foods, thereby it is the most widely used indicator of faecal contamination (Smooth and Pierson, 1997). *E. coli* counts are usually highly correlated with *Enterobacteriaceae* counts, which are commonly used in slaughterhouses as indicators of faecal as well as environmental contamination (Ghafir et al., 2008). The proliferation of *Enterobacteriaceae* on poultry carcasses has been routinely linked to inadequate or unhygienic processing or inappropriate handling or storage conditions (Whyte et al., 2003).

In the European Union, Regulation (EC) No 2073/2005[5] on microbiological criteria for food-stuffs has established the surveillance of ACC and *Enterobacteriaceae* as process hygiene criteria for carcasses of cattle, sheep, goats, horses and pigs. For broiler carcasses, as laid down by the same Regulation and its amendment (Regulation (EC) No 1086/2011[6]), the current process hygiene criterion is based on the evaluation of the presence of *Salmonella* on neck skin samples collected from carcasses after chilling.

In the United States, *E. coli* was identified as a useful indicator organism to verify the adequacy of the hazard analysis and critical control points (HACCP) plans in place in bovine, swine and poultry slaughterhouses (USDA, 1996). In 1996, the FSIS issued the Pathogen Reduction (PR)-HACCP System's Final Rule, prescribing that *E. coli* must be enumerated from 1/22000 and 1/3000 randomly collected broiler and turkey carcasses, respectively. The poultry carcasses will be selected after chilling and after the drip line, before packing/cut-up. A poultry establishment is considered to fulfil the *E. coli* process criteria if none out of the last 13 tests performed exceeds the upper limit of 1000 cfu/ml, and fewer than three samples are between 100 and 1000 cfu/ml for *E. coli* (Altekruse et al., 2009). These performance criteria for poultry allow microbial reduction during the slaughter processing to be monitored and interventions to reduce microbial numbers on poultry carcasses to be validated. Furthermore, poultry plants in the United States are required to meet the established *Salmonella* performance standard, consisting of a maximum of 12 *Salmonella*-positive samples in a complete set of 51 samples (Bilgili et al., 2010).

Obtaining poultry meat is a similar process in all the slaughterhouses, with some differences in specific stages. Basically, this process consists on a highly coordinated system of different operations aimed at slaughtering the birds, removing the inedible portions of the carcasses and preserving the edible portions for distribution to consumers (Sams and McKee 2010). The poultry slaughtering process involves the following phases: stunning and bleeding, scalding, defeathering, evisceration, washing and chilling. The whole process can be divided in two basic areas: the "dirty zone", including stunning, bleeding, scalding, defeathering and evisceration stages and the "clean zone" including washing and chilling (Escudero-Gilete et al., 2005).

[5] Commission Regulation (EC) No 2073/2005 of 15 November 2005 on microbiological criteria for foodstuffs. OJ L 338, 22.12.2005, p. 1-26.

[6] Commission Regulation (EC) No 1086/2011 of 27 October 2011 amending Annex II to Regulation (EC) No 2160/2003 of the European Parliament and of the Council and Annex I to Commission Regulation (EC) No 2073/2005 as regards salmonella in fresh poultry meat. OJ L 281, 28.10.2011, p. 7 - 11.

Different stages of the process influence and change bacterial types and loads on poultry carcasses. These variations are partly exacerbated by the differences in the processing practices routinely used for instance in Europe and the US (Hutchison et al., 2006). One of the main differences is related to the chilling process, which is recognized as one of the most critical step for inhibiting the growth of microorganisms. The majority of European slaughterhouses use air-based chilling (80% of the market), instead of water immersion chilling, which is the standard in the United States (Sanchez et al., 2002). Another main difference is the application of chemical decontaminants in poultry processing, which is permitted in the US (Whyte et al. 2001). Despite many chemical additives being allowed in the poultry industry in the United States, chlorine is the most widely used (Northcutt et al., 2005). The phases of the slaughter processing line where chemical antimicrobials are most commonly used include the rinse and/or spray washes that are applied at various places of the processing line (e.g. post-picking, post-evisceration, pre-chilling and post-chilling) and the immersion chilling (Stopforth et al., 2007). In the United States the Food Safety and Inspection Service (FSIS) allows for the addition of chlorine to the processing water at level up to 50 ppm during carcass washing and chilling (Russell and Axtell, 2005). Conversely, current European legislation states that only potable water can be used in poultry slaughterhouses.

A literature search considering all the main livestock species (poultry, pigs and ruminants) was conducted to obtain data on:

- the presence of the indicator organisms *E. coli* and *Enterobacteriaceae* and their counts on carcasses during different stages in the slaughter processing line;
- factors that could lead to the variability of the counts of these indicator organisms, such as the design of the slaughterhouses, the throughput of the slaughterhouse, the processing techniques, and any other batch specific information such as length of feed withdrawal, catching time and resting time before slaughtering;
- any potential relationship between the counts of indicator organisms and visual faecal contamination on carcasses.

The present report specifically considers data related to poultry. A second report (Barco et al., 2014) is available considering the data related to the other livestock species (pig and ruminant carcasses)

This review was conducted at the worldwide level. The majority of work on the use of *E. coli* and *Enterobacteriaceae* as PHI has been conducted in the US; in contrast, few scientific papers are available on the quantitative levels of these indicator bacteria on poultry in European slaughterhouses. Hence, despite the European situation not always being fully comparable with other countries (e.g. Canada and US), because of the different slaughter processing practices, all available information is reported and particular attention has been paid to critical discussion of the factors which could have influenced the variability evidenced among the different scenarios described.

1. Materials and Methods

The principles of "systematic review methodology" (EFSA, 2010) were applied to the present literature search. This involved the following steps:

- defining the review questions and developing the eligibility criteria for studies;
- searching for research studies;
- selecting studies for inclusion or exclusion in the review;
- collecting data from the included studies and creating evidence tables;
- assessing validity and quality of included studies;
- synthesising data from included studies;
- presenting data and results;
- interpreting results and drawing conclusions.

1.1. Defining the review questions and developing the eligibility criteria for studies

The first step of the literature review process consisted of the analysis of the three review questions in order to identify the key elements and to clarify their scopes.

Review question 1 is related to the presence of the indicator organisms, *E. coli* and *Enterobacteriaceae*, and their counts on carcasses during different stages of the slaughter processing line. The key elements of the question are:

- the population of interest, represented by the main livestock species;
- the outcome, represented by the presence and amounts of indicator organisms (*E. coli* and *Enterobacteriaceae*) on carcasses;
- the setting, represented by the slaughter processing line.

Review question 2 is related to the identification of the factors which could explain the differences in terms of amount of the indicator bacteria on carcasses. The key elements of the question are:

- the population of interest, represented by the main livestock species;
- the intervention strategies/scenarios that could influence the counts, which could be represented by structural/managerial characteristics of the slaughterhouse or pre-slaughter handling of animals (e.g. catching time and resting time before slaughter, diet and feed withdrawal period before slaughter);
- the comparators: represented by reference scenarios against which the interventions/strategies/scenarios have been compared (e.g. batches slaughtered in different ways, in slaughterhouses with different processing characteristics, animals handled in different ways before being slaughtered);

- the outcome, represented by the presence and amounts of indicator organisms (*E. coli* and *Enterobacteriaceae*) on carcasses;

- the setting, represented by the slaughter processing line.

Review question 3 is related to the potential relationship between the counts of indicator organisms and visual faecal contamination of carcasses. The key elements of the question are:

- the population of interest, represented by the main livestock species;

- the outcome, represented by the presence and amounts of indicator organisms (*E. coli* and *Enterobacteriaceae*) on carcasses in relation to their visual faecal contamination;

- the setting, represented by the slaughter processing line.

The three key elements (population, outcome and setting) identified for review question 1 remain unchanged also for review questions 2 and 3, although for these last two review questions additional aspects, such as variables influencing the outcome of interest and the relationship between the outcome of interest and other factors, have to be considered respectively. Since the review questions shared these main key elements it was decided to combine the three review questions in a unique literature search.

1.2. Searching for research studies

Electronic databases (search A) and Web-searching (search B) were used to retrieve pertinent studies.

1.2.1. Search A: electronic databases

For search A, the search terms used in relation to the specific key elements of the three review questions related to "outcome", "setting" and "intervention strategy /scenarios", are listed in Table 1.

Terms related to the "population" were omitted from the search string in order to get as many papers as possible and then to select the relevant ones in terms of species of interest in the following steps of the screening process.

Regarding the "intervention strategy /scenarios" related to review question 2 not all plausible terms were included in the search string. Conversely, for "outcome" and "setting" the terms used were selected in order to include as many synonymous terms as possible, since these two elements were considered the most important ones to retrieve relevant papers.

More specifically, the search string used was:

(E. coli) OR (Coliform*) OR (Escheric*) OR (Enterobacter*) OR (Indicator) OR (Hygien*) OR (Microbi*) AND (Slaughter) OR (Slaughterhouse*) OR (Abattoir*) OR (Carcas*) OR (HACCP) OR (Chill*) OR (Eviscerat*) OR (Defeathering) OR (De-feathering) OR (Post-harvest) OR (Post harvest) OR (Pre harvest) OR (Pre-harvest) OR (Holding pen*)

Before identifying the definitive search terms some other terms, such as "coli" and "process" were tested, but they were not included in the final search string since it was verified that they did not result in the retrieval of any additional relevant papers.

After having adjusted the search string for minor differences in syntax, it was applied to two electronic bibliographic databases: PubMed and Web of Science.

The search covered the period January 2000 - December 2012 (01.01.2000 - 19.12.2012).

The bibliographic software RefWorks was used to collect and manage the references downloaded from the electronic databases.

Table 1: List of the terms included in the search string in relation to the specific key elements of the three review questions

Outcome	Setting	Intervention strategy /scenarios
E. coli	Slaughter	HACCP
Escheric*	Slaughterhouse*	Chill*
Enterobacter*	Abattoir*	Eviscerat*
Coliform*	Carcas*	Defeathering
Indicator		De-feathering
Hygien*		Post-harvest
Microbi*		Post harvest
		Pre harvest
		Pre-harvest
		Holding pen*

1.2.2. Search B: Web-searching

Search B was conducted by using the Internet search engine Google Scholar.

Since Google Scholar looks for the search terms in the entire document the search process was conducted by using a few very specific terms, related to the "outcome" (*Escherichia coli* – *Enterobacteriaceae*) and the "setting" (slaughterhouse). Moreover, to limit the retrieval of non-pertinent articles the terms O157 and resistance were excluded from results.

The search string used was:

((Escherichia coli) OR Enterobacteriaceae) AND (slaughterhouse)) NOT (O157) NOT (resistance).

The titles of the first 500 returns were assessed in order to identify the pertinent documents.

1.3. Selecting studies for inclusion or exclusion in the review

For the purpose of the present review, primary research studies performed at the slaughterhouse and providing data on the presence/counts of *E. coli* and *Enterobacteriaceae* on carcasses of the livestock species of interest were considered.

Moreover, the relevant studies were defined as the ones:

- referring to slaughtering process from the point at which animals enter the slaughterhouse up to the conclusion of the chilling phase;
- providing data on the entire carcasses collected at the slaughterhouses. Studies providing data on parts of carcasses obtained after secondary processing, such as cutting, portioning and deboning of the entire carcass were not considered as pertinent.

Both for search A and B papers/documents in English, French, Spanish or Italian were considered. Geographical restrictions were not imposed.

The screening process was independently carried out by three veterinarians.

In the following paragraphs the eligibility criteria used to select relevant papers at abstract and full text level are described. Moreover, in Appendix A the checklists developed to identify relevant and irrelevant papers at the different steps of the screening process are reported. These checklists were beforehand validated by the three reviewers involved in the screening process using a subset of 50 retrieved papers.

1.3.1. Screening of the titles and abstracts for the relevance to the study questions

For Search A, the first level assessment was conducted considering the title and the abstract of the papers. For Search B, the first level assessment was conducted considering title and, if the document was pertinent, also abstract (when available), or in the case of evaluating directly, the full text.

The first level assessment consisted of two steps. Papers that did not fulfil one or more criteria considered in these steps were discarded and considered ineligible.

For the first step, it was defined that if the two initial criteria were not fulfilled (the papers were written in languages different from English, French, Spanish or Italian, or were review papers) it was not necessary to proceed with the screening and the article was considered ineligible.

This first step consisted of selecting papers that:

1) are written in English, French, Spanish or Italian;
2) describe data provided by primary researches (review articles were excluded);
3) provide data related to the main livestock species;
4) provide data on the presence and counts of generic *Escherichia coli* and/or *Enterobacteriaceae*;
5) do not have as their main purpose the investigation of antimicrobial resistance.

The second step consisted of selecting papers that:

1) provide data on more than one stage of the slaughter processing line or data on risk factors influencing the loads of indicator bacteria on carcasses;
2) provide data on carcasses (papers considering parts of the carcasses obtained after a secondary process were excluded);
3) provide data obtained in slaughterhouses that do not use chemical decontaminants other than chlorine.

When the abstract screening did not identify precise information concerning a specific eligibility criterion, the reviewers provided an inconclusive reply (e.g. unknown), that did not lead to the exclusion of the paper.

Each retrieved paper was individually evaluated by two independent reviewers (parallel review). In the case of disagreements between them, the paper was discussed to reach a consensus before proceeding to the next step of the screening process.

1.3.2. Examining full-text for the eligibility of studies

All retrieved articles (both from search A and B) related to poultry were submitted to the second level assessment, conducted examining the full-text of the papers.

At this stage some eligibility criteria already taken into account in the first level assessment were included since, in some cases, the abstract analysis did not allow precise information to be obtained, and a definite decision could not be made.

In particular, the following eligibility criteria were used to select the papers at second level assessment. Relevant papers:

1) describe data provided by primary research (review articles were excluded);
2) provide data obtained from entire carcasses (papers considering parts of the carcasses obtained after a secondary process were excluded);
3) provide data on the presence and counts of *E. coli* and/or *Enterobacteriaceae*;
4) do not provide data about counts of *E. coli* and/or *Enterobacteriaceae* from carcasses that have been artificially contaminated with these indicator bacteria;
5) report *E. coli* and/or *Enterobacteriaceae* counts at more than one stage of the slaughter processing line, or describe factors influencing the counts of *E. coli* and/or *Enterobacteriaceae*, or consider the relationship between visual faecal contamination and *E. coli* and/or *Enterobacteriaceae* counts.

Moreover, at this stage, further data (e.g. the country where the study was done, the setting of the study, at which stage of the slaughter processing line samples were collected, the procedure used to get samples, which type of risk factor was investigated) were gathered from the screened papers. The collection of these data was also useful to develop forms for the collection of pertinent data from the relevant papers (Appendix B).

The assessment of full-text papers was carried out by two reviewers. Data were extracted from each paper by one reviewer and then verified by a second person (sequential method). In case of disagreement, the paper was discussed between the two reviewers involved in the screening to reach a consensus.

1.4. Collecting data from the included studies and creating evidence tables

Three standardized forms were designed to extract pertinent data from the selected papers (Appendix B).

The first form was aimed at gathering general information. It considers the type of reference (e.g. article, technical report, meeting proceeding), the aim of the study, where and when the study was conducted, the type of study (observational or experimental), some characteristics of the animals sampled (species, age, weight, weight variability within the sampled batch), how animals were handled before arriving at the slaughterhouse (duration of feed withdrawal, transportation time from farm to the slaughterhouse) and sample size (number of samples collected, significance and power of the sample size).

The second form was aimed at collecting information concerning the characteristics of the slaughterhouses where the animals were slaughtered. It considers the capacity of the slaughterhouse (number of animals slaughtered), the slaughtering practices at the main steps of the slaughter

processing line, information concerning the use of chlorine and if appropriate, at which stage the decontamination treatment was used.

The last form was aimed at collecting pertinent analytical data for the scope of the review. It included information concerning the sampling and analytical methods used (type of sampling, single or pooled samples, indicator bacteria investigated, analytical procedure, unit of enumeration), data on prevalence and counts of indicator bacteria at each step of the processing line. Finally, it included an evaluation of the steps of the slaughter processing line where the counts decreased or increased, or the effect of the investigated factors on the counts of the indicator bacteria or the relationship between visual faecal contamination and the counts was reported.

Data related to multiple slaughterhouses or data from different visits at the same slaughterhouses or presenting different scenarios were considered separately, and counted as different studies within the same paper.

Data were extracted from each paper by one reviewer and then verified by a second person (sequential method). In cases where inconsistencies between the data reported in the paper and those included in the forms were observed, the reviewers again verified the paper, but together this time, and if appropriate, they modified data in the form accordingly.

In order to avoid double counting of the studies published more than once, the papers were compared for author names, geographic area where the study was conducted, sample size and data reported. The duplicates were discarded.

The extraction forms were used to minimize the transcription errors and to obtain a record of all the collected data. The management of references, the screening and the data extraction processes were done through the web-based software DistillerSR.

Since in different studies samples were collected at different stages of the slaughter processing line, some assumptions were made in order to make the data available more comparable. Post-scalding was considered to be the same sampling stage as pre-defeathering, post-defeathering the same as pre-evisceration, post-evisceration the same as pre-washing and post-washing the same as pre-chilling. These assumptions were plausible since the time between the two successive stages is usually very short, and there are generally no additional steps in between. The only exception could be between the post-defeathering and the pre-evisceration, since neck and feet are usually removed from the carcasses between these two stages (Löhren, 2012).

Decontaminant treatment of carcasses with chlorine was considered an import factor that likely affects the counts of indicator bacteria. Hence, studies were grouped according to whether they were conducted in a European or non-European country, and the use of chlorine as follows:

- Group 1: European studies where theoretically chlorine was not used;
- Group 2: studies performed in non-European countries conducted in slaughterhouses where chlorine was not used at any steps of the slaughter processing line;
- Group 3: studies performed in slaughterhouses where chlorine was used at least in one step of the slaughter processing line (mainly in non-European countries);
- Group 4: studies performed in non-European countries for which information concerning the use of chlorine was not available.

To ascertain the slaughterhouse setting in relation to the use of chlorine in cases where that detailed information was not provided in the papers in non-European studies (group 4 above), the authors of the studies were directly contacted by e-mail in order to collect further information concerning this aspect.

1.5. Assessing validity and quality of the included studies

As regards **review question 1**, the validity of the retrieved studies was appraised at different stages of the screening process and was related to the stages of the slaughter processing line where samples were collected.

Initially, the relevant studies for review question 1 had to provide data on *E. coli* and *Enterobacteriaceae* counts at more than one stage of the slaughter processing line, as stated in the eligibility criteria (reported in sections 1.3.1 and 1.3.2). Hence, data from studies describing counts at more than one steps of the slaughter chain were collected, whereas studies reporting counts at one single step were not included.

Then, evaluations in terms of changes of the level of the indicator bacteria along the slaughter processing line were limited to studies that sampled carcasses immediately before and after any of the major stages of the slaughtering process. This quality criterion was used in order to compare the data reported in different studies and to identify the steps of the chain where the counts increase or decrease.

Concerning **review question 2**, a quality assessment was not defined according to the criteria used for **review question 3** due to the fact that the choice of setting depended on the investigated risk factor and on the feasibility of studying the selected factor in a commercial slaughterhouse.

For **review question 3** the following criteria were used to categorize the studies in terms of the quality of the data provided.

- Medium value: studies conducted in a pilot slaughterhouse or considering carcasses artificially contaminated with faecal material

- High value: studies conducted in a commercial slaughterhouse or considering carcasses naturally contaminated with faecal material.

Data were extracted and presented for all papers, and then the above-mentioned criteria were taken into account when conclusions were drawn.

2. Results

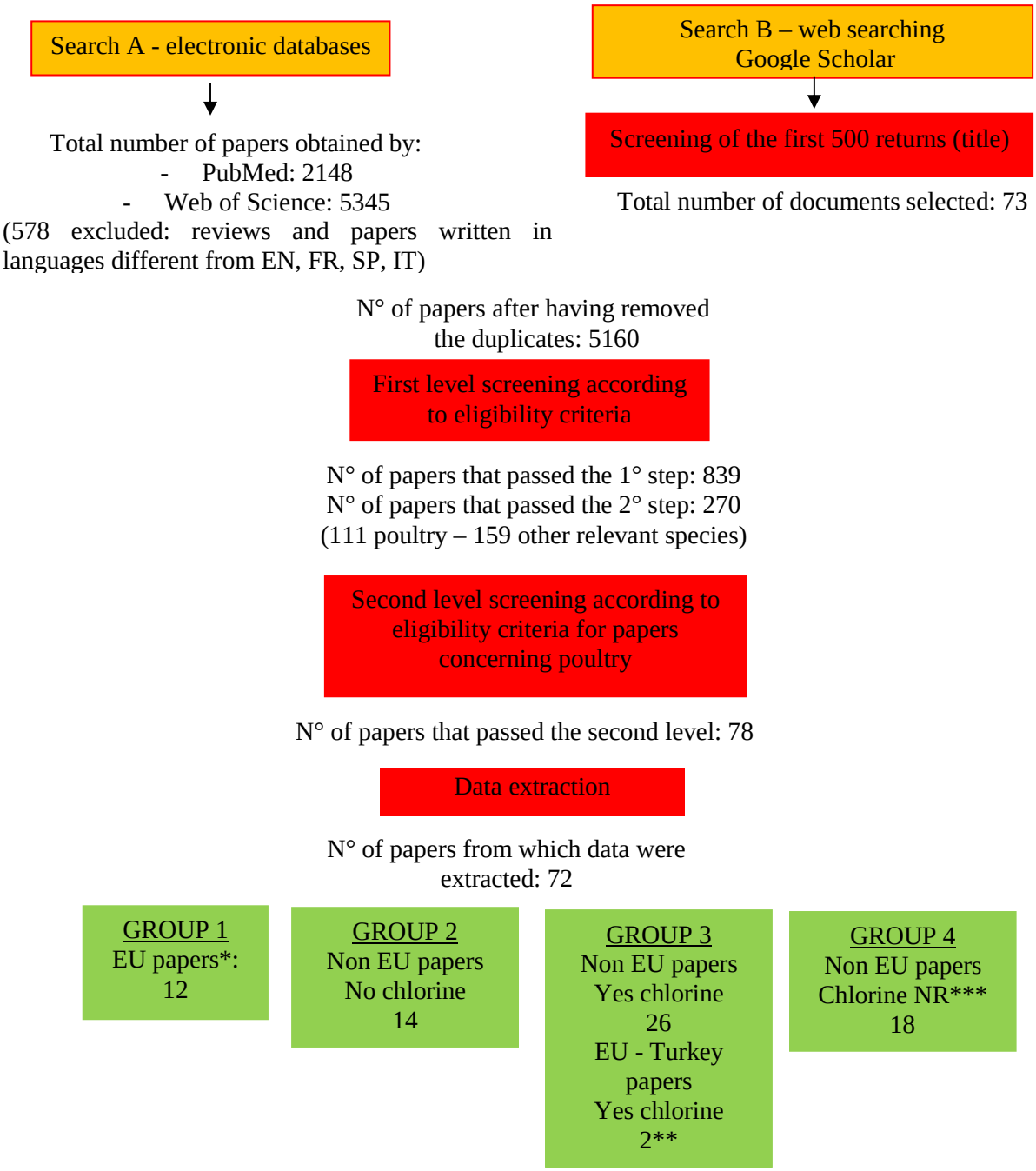
2.1. Literature search and relevance screening

Figure 1 shows the flow chart of the screening processes. Search A provided 2148 papers from PubMed and 5345 papers from Web of Science. A total of 578 papers were excluded before starting the screening since they were review papers and/or were written in languages different from English, French, Spanish or Italian. Google Scholar search (search B) resulted in 73 potentially pertinent documents out of the first 500 returns. After having eliminated the duplicates, a total of 5160 papers remained.

Of the 5160 retrieved papers, 4321 were excluded at step 1 (thus 839 remaining) and 569 at step 2 (thus 270 remaining) of the first level relevance screening assessment since they did not fulfil one or more eligibility criteria considered at that level.

Of the 270 papers that passed both steps of the first level assessment, 111 papers reported data on poultry. After having examined the full-text, 33 out of the 111 poultry-related papers were excluded at the second level assessment because one or more eligibility criteria were not fulfilled. After having concluded the screening, 78 poultry-related papers were selected since they provided appropriate data for the scope of the review and were assessed in detail in order to collect pertinent data. Six out of the 78 selected papers were eliminated at the data extraction stage for different reasons. In particular, some studies provided counts reported in a graphical way meaning it was not possible to easily extract precise quantitative data or the provided information was not compatible with the structure of the data extraction forms developed for the project, for one study, the setting was so specific and experimental that the conclusions drawn could not be generalized and applied to a commercial slaughterhouse and one study provided data concerning the amount of indicator bacteria on the respiratory tract of the birds instead of the entire carcass. As a result, 72 papers will move forward for data extraction**.**

Appendix C summarizes the number of papers excluded at each step of the process in relation to the eligibility criteria considered.



* EU Member States (MSs)+ Turkey

** Papers describing studies performed in EU MSs and Turkey, in slaughterhouses where chlorine was used

*** NR: not reported; papers not reporting information about chlorine use which authors didn't reply to reviewers' request of clarification.

Figure 1: Flow-chart summarizing the results of the literature search – the screening and the data extraction process

2.2. General information about the considered papers

The 72 selected papers considering poultry were classified into four different groups in relation to the origin of the study (European or non-European countries), and the use of chlorine along the slaughter processing line. In Figures 2 and 3, the number of the selected papers is reported according to categorisation of the papers into the four groups and the indicator bacteria investigated (Figure 2) or the classification into the four groups and the review question considered.

Twelve papers were included in Group 1 since they were done in a European country (including Turkey). Fourteen papers were included in Group 2 since they were done in a non-European country, but it was possible to ascertain that chlorine was not used at any stage of the slaughter processing line. Twenty-eight papers were included in Group 3 since they were done in a non-European country and the use of chlorine was reported in the paper or confirmed by the authors. One European and one Turkish paper were added to Group 3 since the use of chlorine was reported in the articles. Eighteen non-European papers were included in Group 4 because of the impossibility of ascertaining whether chlorine was used along the slaughter line.

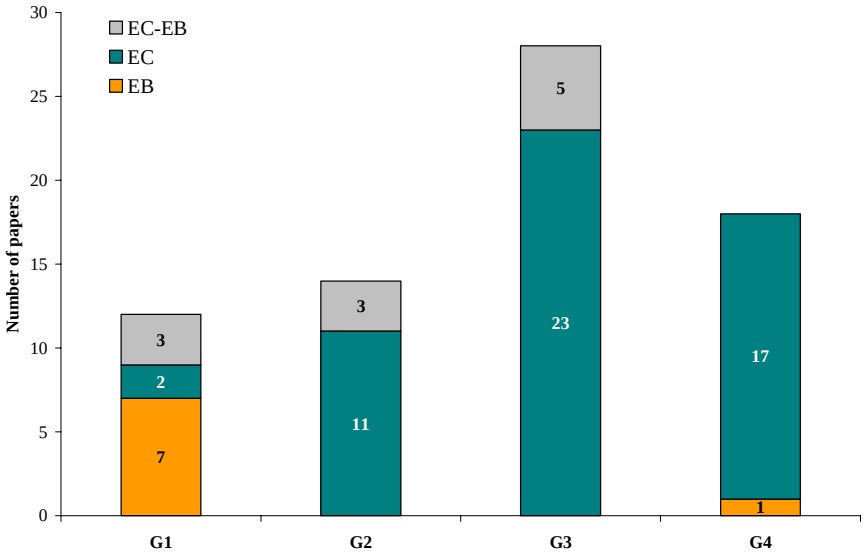


Figure 2: Number of selected papers grouped according to the country and use of chlorine and the indicator bacteria investigated (EB: *Enterobacteriaceae*; EC: *Escherichia coli*; EC-EB: *Escherichia coli-Enterobacteriaceae*). (G1: EU papers; G2: Non-EU papers, No chlorine; G3: Non-EU and EU papers, Yes chlorine; G4: Non-EU papers, Chlorine use not reported (NR))

The great majority of papers were carried out in the US (46) or Europe (14). The others were from Canada (4), South America (4), Asia (2), South Africa (1) or Australia (1).

All studies sampled broiler carcasses, and one of them considered also turkeys and quails.

Forty-two papers described observational studies and 30 papers experimental studies. The great majority of manuscripts (50) provided data obtained in commercial slaughterhouses, whereas the remaining papers (22) considered carcasses obtained from a slaughter process for which at least one phase was carried out in a pilot facility.

Twelve papers reported the weight of the slaughtered birds, which ranged between 1.3 and 6.1 kg. Moreover, 22 papers described the age of birds at the slaughterhouse, which was between 35 and 84 days.

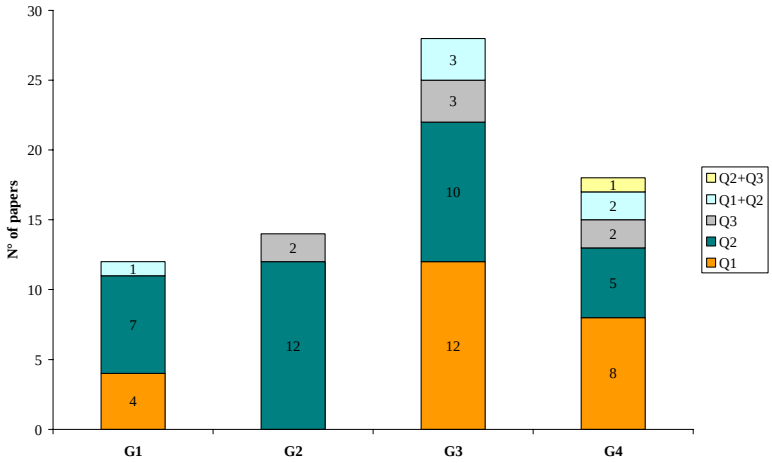


Figure 3: Number of selected papers grouped according to the country, the use of chlorine and the review question for which they provide data. Papers dealing with two review question are considered separately (G1: EU papers; G2: Non-EU papers, No chlorine; G3: Non-EU and EU papers, Yes chlorine; G4: Non-EU papers, Chlorine use not reported (NR))

Fifty-three papers provided quantitative data on *E. coli*, eight papers on *Enterobacteriaceae* and eleven papers considered both indicator bacteria. Figure 4 reports the number of the selected papers providing data for each of the three review questions.

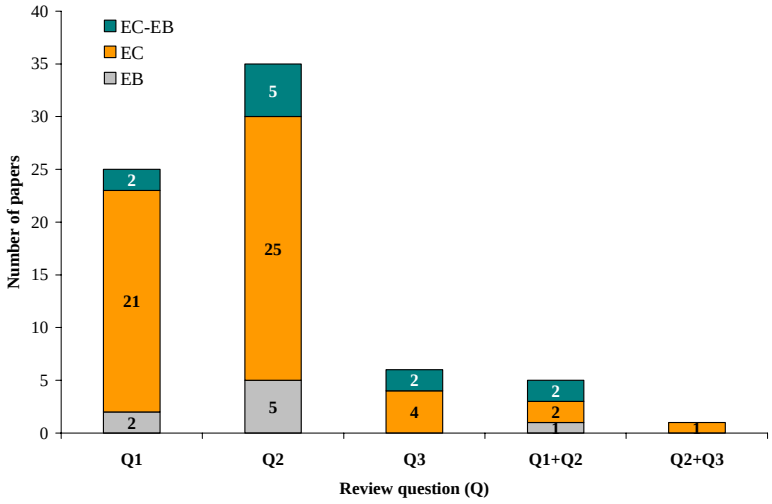


Figure 4: Selected papers grouped according to the review question addressed and the indicator bacteria considered (EB: *Enterobacteriaceae*; EC: *Escherichia coli*; EC-EB: *Escherichia coli-Enterobacteriaceae*)

Sixty-eight manuscripts describe studies that analysed single samples, three studies involved pooled samples and one study examined both single and pooled samples. The sampling methods used in the papers were: rinse, skin-meat excision, swab and drip. Figure 5 shows the number of selected papers for each type of sampling method. The most commonly used method was rinse (50 studies), most probably because this is the reference sampling method prescribed by the U.S. Regulation. Some differences were reported when using this method, in particular in relation to the rinse volumes used (ranging from 50 to 500 ml). Another commonly used method is skin excision, mainly of neck and breast regions, with sampling sizes ranging from 5 to 25 grams, reported in 17 papers. Analysis of swab and meat excision samples was described in three manuscripts.

Traditional colony count and in particular, the Petrifilm, was the analytical method most frequently used. Some differences were noted among studies in relation to the incubation temperatures used to quantify indictor bacteria. In particular for *E. coli*, the selective plates were usually incubated at 35 to 37°C, but six European studies reported an incubation temperature of 44°C. Heterogeneity among papers was noted also for the unit of enumeration selected to express the results obtained. For the majority of the papers, the units of enumeration used were log CFU/ml (42 papers), log CFU/cm^2 (seven papers), log CFU (six papers) and log CFU/g (eight papers).

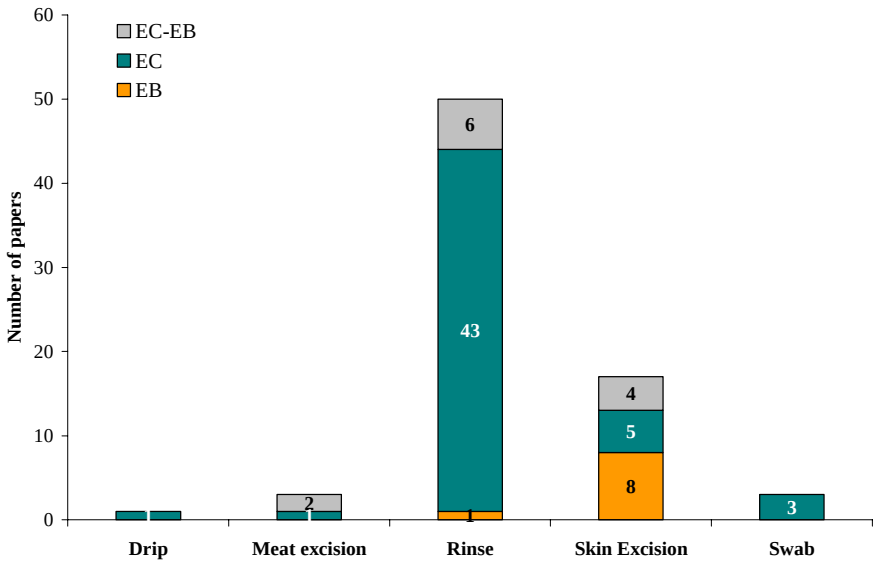


Figure 5: Selected papers grouped according to the type of sample collected and the indicator bacteria considered. Two papers were counted twice because they collected two different type of sample. (EB: *Enterobacteriaceae*; EC: *Escherichia coli*; EC-EB: *Escherichia coli-Enterobacteriaceae*).

Appendix D reports some considerations concerning the comparability of data obtained by using different sampling methods and units of enumeration.

Appendix E, Tables 4a, 4b, 4c and 4d, report general information concerning the 72 selected papers and the review questions (1, 2, 3) for which each paper provided pertinent data.

2.3. Review question 1

2.3.1. General information about the relevant studies providing data for review question 1

As regards the presence and amount of *E. coli* and *Enterobacteriaceae* on carcasses during different stages of the slaughter processing line, among the 30 eligible papers, 23 papers provided information on *E. coli*, three papers on *Enterobacteriaceae* and four papers on *E. coli* and *Enterobacteriaceae.* All the papers except one provided data obtained through observational studies and all the papers described results obtained in commercial slaughterhouses.

As regards the country of origin, half of the papers were from the US; the remainders were from Canada, Turkey, Brazil, India, South Africa, Taiwan and some European countries (Austria, Spain, Czech Republic and Ireland). All the papers developed studies providing data on broiler carcasses. Five papers reported the weight of the sampled animals (ranging from 1.3 to 3.2 kg) and five papers recorded the age of the animals at the time of slaughter (ranging from 35 to 56 days).

As regards the type of sample, 17 out of the 30 papers collected rinse samples. The other types of tested samples were skin excision (eight papers), swab (three), meat excision (one) and drip (one). In 17 papers, the whole carcass was sampled while six papers provided data obtained from different parts of the carcass, and in six papers, neck samples were analysed. In one paper this information was not reported.

The enumeration unit used differs according to the type of sample. The most common ways to express the counts were log cfu/ml (fifteen papers) and log cfu/cm^2 (five papers). Other types of enumeration units were: cfu/cm^2, cfu/ml, cfu/g, log cfu, logcfu/g, logMPN/ml, MPN/cm^2.

As regards the analytical method**,** 25 papers used the traditional colony count (TCC). In particular, *E. coli* quantification was carried out mainly by using EC/coliform Petrifilm (nine papers) or Petri dishes (nine papers) incubated at different conditions of time and temperatures (35, 37 or 44 °C for 24 or 48 hours); furthermore in seven papers, EC Petrifilm was used, while for the remaining four papers, the analytical procedure followed was not detailed. As regards the detection of *Enterobacteriaceae*, all papers used traditional Petri dishes incubated mostly at 37 °C for 24 hours.

Fifteen papers reported the use of chlorine along the slaughter processing line. For 10 papers information about the use of chlorine was not available and in five papers, chlorine was not used or it was used at a concentration comparable to that of the tap water (chlorine level from 0.5 mg/L to 2.0 mg/L). Among the group of papers describing the use of chlorine, 11 papers reported the chlorination of water at the chilling step and nine at the washing step.

2.3.2. General information about the features of the slaughterhouses described in the relevant studies providing data for review question 1

Fourteen out of the 30 papers reported the number of slaughtered animals expressed as birds per day or per hour.

The type of stunning was described in only one case, while information about the scalding step was available for 12 papers as time-temperature (seven papers), temperature (four papers) or length of the process (one paper). Scalding temperatures ranged from 51 to 58°C and the length of the scalding step was between 90 and 207 seconds.

Information regarding the defeathering and evisceration processes was rarely available and was reported only in five and six papers, respectively. Fourteen papers reported details about the washing procedure used, which mainly consisted of the inside-outside washing. As regards the chilling step, 13

papers reported the use of water immersion chilling, while in nine papers, air chilling was applied. Air chilling was used mostly in Europe (four out of nine papers), followed by Canada and Turkey (four out of nine papers) and the US (one out of nine papers). Water immersion chilling was the method mostly used in the US (nine out of 13 papers), followed by other countries such as Canada, Brazil, South Africa and Taiwan.

The temperature of the carcasses at the end of the chilling process was available only in three papers and it ranged from <4 °C to 9 °C.

Figure 6 reports the number of papers according to the slaughterhouse features and the use of chlorine. Looking at the figure, it is clear that the greatest amount of information is available for the number of slaughtered animals, the scalding process, the washing and the chilling steps, while information regarding the stunning step, the defeathering method, the type of evisceration and the temperature of the carcass at the end of the chilling process is rarely available.

Moreover, in Appendix F, Table 5 reports the details about the features of the slaughterhouses described in studies taken into account for review question 1.

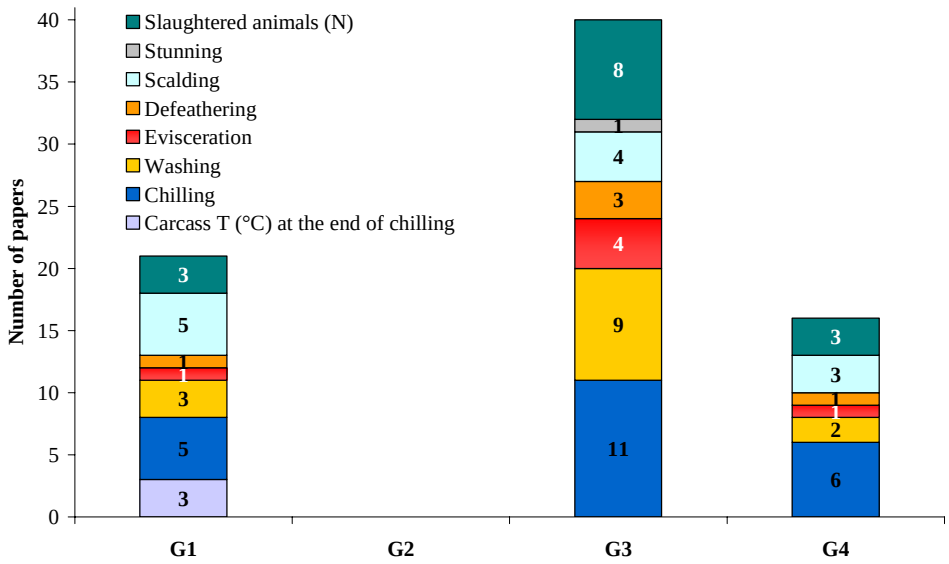


Figure 6: Number of papers reporting technical details of the slaughterhouse features (slaughtered animals (N); type of stunning, scalding and defeathering, type of evisceration, washing, chilling, carcass temperature at the end of the chilling), divided according to the origin of the paper and the use of chlorine. (G1: EU papers; G2: Non-EU papers, No chlorine; G3: Non-EU and EU papers, Yes chlorine; G4: Non-EU papers, Chlorine not reported (NR))

2.3.3. Counts of the indicator bacteria on carcasses collected at the different stages of the slaughter processing line

Among the 30 papers, 40 studies provided data regarding counts on *E. coli* and nine studies on *Enterobacteriaceae*. As regards *E. coli*, 12 out of the 40 studies collected samples at only two steps of the slaughter processing line that were distant to each other; in particular five studies collected samples after evisceration and after chilling, six studies collected samples before evisceration and after chilling and one study investigated the points before evisceration and after washing. Conversely, 28 studies reported data of carcasses sampled immediately before and after a specific stage of the process (e.g. scalding, defeathering, evisceration, washing, chilling).

In particular, Figure 7 shows the number of studies providing counts of *E. coli* at the different stages of the slaughter processing line. The step "before stunning and bleeding" was covered by one study, six studies took into account the "after stunning and bleeding - before scalding" step, seven studies considered the "after scalding - before defeathering" stage and 22 studies provided data of the "after defeathering - before evisceration" stage. Moreover, 23 studies collected information at the "after evisceration - before washing" step and 24 studies gathered the counts at the "after washing - before chilling" stage, while 29 studies considered the "after chilling" step; three studies included counts from "other stages".

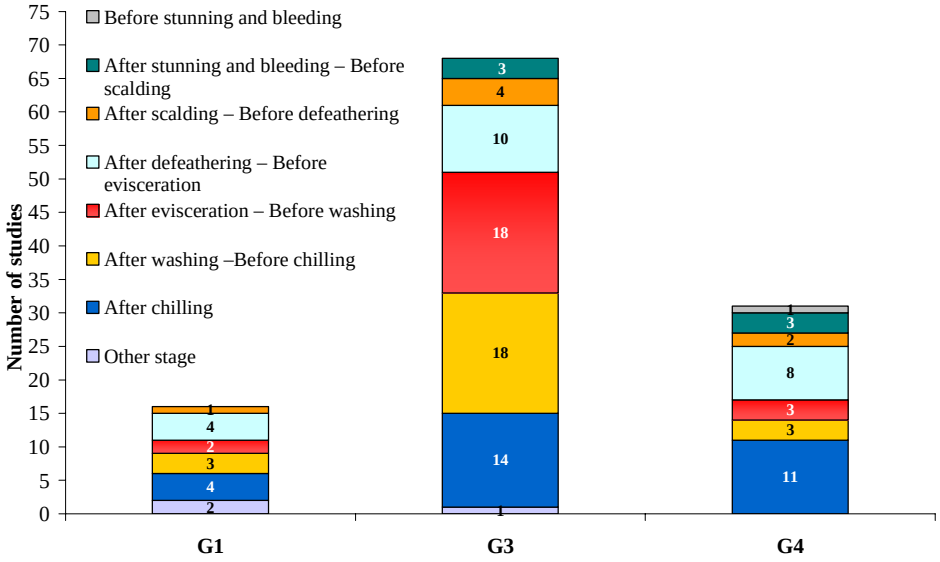


Figure 7: Number of studies providing counts of *E. coli* before and after a specific stage of the slaughter processing line. (G1: EU papers; G3: Non-EU and EU papers, Yes chlorine; G4: Non-EU papers, Chlorine not reported (NR))

Figure 8 brings into focus the number of studies describing counts of *Enterobacteriaceae*. Nine studies gathered samples along the slaughtering line and seven out of nine studies provided data on the counts before and after a specific step along the process. Two studies provided data on counts obtained "before stunning and bleeding", three studies "after stunning and bleeding - before scalding", six studies "after scalding - before defeathering" and seven studies "after defeathering - before evisceration". The steps "after evisceration - before washing", "after washing - before chilling" and "after chilling" were covered by six, nine and two studies, respectively.

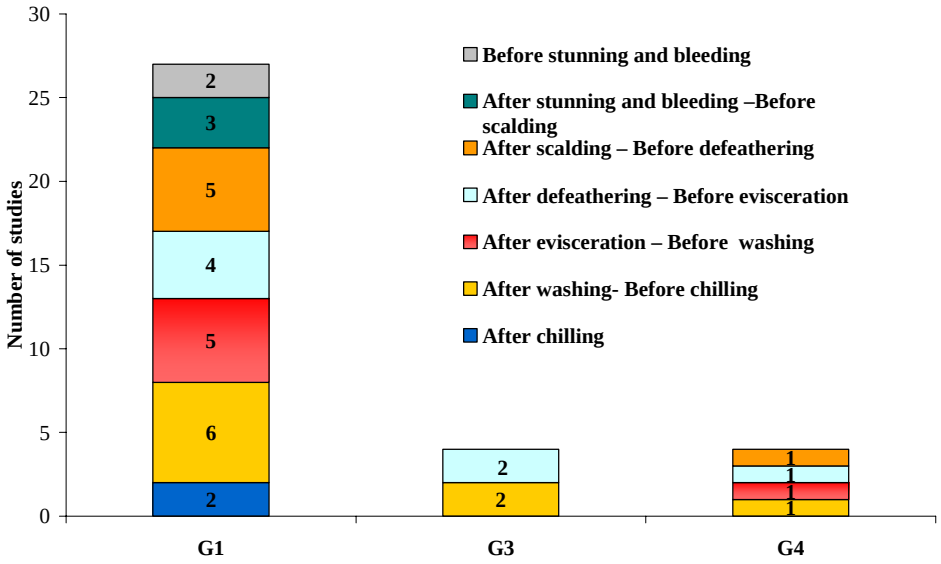


Figure 8: Number of studies providing counts of *Enterobacteriaceae* before and after a specific stage of the slaughter processing line. (G1: EU papers; G3: Non-EU and EU papers, Yes chlorine; G4: Non-EU papers, Chlorine not reported (NR))

In Appendix G, the counts of *E. coli* on poultry carcasses described in the selected papers at the different stages of the slaughter process are reported in Table 6a, while the counts of *Enterobacteriaceae* are presented in Table 6b. In these tables, the number of samples analysed at each step, the mean counts and the standard deviation are reported when these were available; information about the use of chlorine is also included.

2.3.4. Evaluation of the effect of the different stages of the slaughter processing line on the counts of the indicator bacteria

The retrieved papers provided data that are not always comparable. In particular the units of enumeration and analytical methods used vary considerably. Moreover, the slaughter processes described are rather heterogeneous. For these reasons, it is not appropriate to provide a direct and meaningful comparison among the different studies. Rather, it seems more informative to analyse the trend of the counts at the different stages of the slaughter processing line within each of the studies.

Evaluations in terms of changes in counts of the indicator bacteria along the slaughter processing line was limited to studies that sampled carcasses immediately before and after any of the major stages of the slaughtering process. This criterion was used in order to identify the steps of the chain where the counts increase or decrease.

In Figure 9 the change in counts of *E. coli* on poultry carcasses before and after a specific stage along the slaughter processing line is reported considering only studies providing data expressed by using the two most commonly used units of enumeration (log cfu/ml and log cfu/cm^2); according to these criteria, totally 24 out of 28 studies were considered. Moreover, the studies not reported in Figure 11 will be briefly described in this paragraph. In Figure 10, the change in counts of *Enterobacteriaceae* before and after a specific stage of the slaughtering line is shown; all the seven available studies using as units of enumeration log cfu/ml and log cfu/g are reported.

At each stage of the slaughter processing line, the change between the two sampling points (before and after) was calculated as the difference between the mean values of the counts at the latter chronological sampling point and the counts at the previous sampling stage. The use of terms increase or decrease simply refers to the change in counts between the two sampling points at each stage of the slaughter processing line. In the reading of these figures and of this paragraph it should be taken into account that some studies provide the counts of indicator bacteria at more than one point along the slaughter line.

As regards ***E. coli***, a decrease in the counts of more than one log unit immediately after *scalding* was observed in four out of the five considered studies. In two of these four studies, three counterflow scald tanks were used, in one study a single scald tank was used and in the other study, no info was provided. Moreover, two out of the five studies used chlorine at this step. In the study giving increased counts (> 1.5 log units), the collected samples were represented by respiratory tract rinses of carcasses. According to this study (Berrang et al., 2003 (2)), it appears that bacteria are added to the respiratory tract during the immersion scalding process and it's unclear how this happened. The authors hypothesized that bacteria could enter the respiratory tract due to the muscular activity of the carcass during the slaughtering process or that the highly contaminated air of the scalding area could be pulled in the respiratory tract, due to the presence of a negative pressure in the airsacs.

The results of the three studies that investigated the *defeathering* step show inconsistencies: two studies showed a decrease after defeathering of between 0.5-0.7 log unit, while one study reported an increase of around 0.7 log unit after this stage. The reasons for this inconsistency could not be clarified as information about the technical characteristics of the defeathering process was provided by only one study (stating it is an automatic process). Moreover, chlorine was used in two out of three studies for this particular step.

Among the four studies collecting samples at the *evisceration* step, two studies recorded an increase after evisceration above about 0.5 log unit, one study (Barbut et al., 2009) reported an increase of 0.07 log unit, while another study (Gill et al., 2006) recorded an decrease 0.05 log unit. Moreover, a study of Hecer et al. (2007), not shown in Figure 9, reported a decrease of 2.2 $*10^3$ CFU/g at this stage. Also in this case limited information has been provided on the process (one study mentions automatic process).

Twelve studies sampled both before and after the *washing* step; eleven out of these twelve studies showed an overall decrease of the counts after this step ranging from 0.1 to 1.1 log unit increase, while one study (Stopforth et al., 2007 (2)) recorded a very small increase of the count (0.2 log unit) after this step. Eleven out of twelve studies specified the type of washing as inside-outside washing, and in one of these (Berrang and Bailey, 2009), five washing intervention steps were applied along the chain. Seven out of twelve studies used chlorine at this step. Moreover other two studies not reported in Figure 9, described a decrease of the counts at this step. Matias et al. (2010) collected samples before and after this stage in two slaughterhouses with distinct slaughter capacity (Sh1 and Sh2) and recorded a decrease of 0.53 MPN/cm^2 in Sh1 and of 0.18 MPN/cm^2 in Sh2. Chlorine was not used at this step. Finally, Hecer et al. (2007) indicated a decrease of 0.5 * 10^3 CFU/g using chlorinated water at this level.

Among the nine studies reporting the counts before and after the *chilling* step, five studies used water immersion chilling, three studies used air chilling and one study did not report the chilling method. Regardless of the chilling method, eight out of the nine studies showed a decrease in the number of *E. coli* on the carcasses, while in one study no change in the counts was recorded. Six out of nine studies applied chlorine at this step; among these six studies, five used water immersion chilling while one adopted the air chilling system. Moreover in two studies information on the use of chlorine was not reported and in one study no chlorine was added at the chilling step. Finally two studies not showed in

Figure 9, reported a decrease at this step. Matias et al. (2010) described during the immersion chilling stage a reduction of 0.72 MPN/cm^2 in slaughterhouse 1 (Sh1: high capacity) and of 0.6 MPN/cm^2 in slaughterhouse 2 (Sh2: low capacity). According to the authors the passage through the chiller was important in order to promote a significant reduction of bacterial load. Furthermore Hecer et al. (2007) observed a reduction of 0.1 * 10 ^{3} CFU/g at this step: the type of chilling and the use of chlorine at this stage are not specified.

As regards ***Enterobacteriaceae*** (Figure 10), two studies reported a decrease of 0.32 and 0.99 log CFU/g respectively after *scalding*. Three studies concerning the *defeathering* step, described a decrease of 0.7 and 0.67 log CFU/g and 0.59 log CFU/ml. The scalding step consisted in both studies of a single scald tank with a water temperature between 52 and 54 °C and a process time ranging from 150 to 180 seconds. Two out of three studies specified that defeathering step was an automatic process. The *evisceration* step was investigated by two studies, Geornaras and von Holy (2000) reported an increase in *Enterobacteriaceae* counts of 0.2 log CFU/g while Smulders et al. (2011) recorded an decrease of 0.15 log CFU/ml. Three out of four studies demonstrated that *washing* resulted in an overall decrease in the numbers of *Enterobacteriaceae* (ranging from 0.23 to 0.78 log CFU/g), while one study showed this step had no effect. Washing (as reported by three studies) consisted of inside-outside washing in one study and of spray washing in the two other studies. There was a discrepancy among the six studies including the *chilling* step: two studies showed an decrease of 0.13 and 0.4 log CFU/g respectively and four studies reported an overall increase (ranging from 0.04 to 0.42 log CFU/g) of the counts after this process. As regards the chilling method, five out of the six studies used the air chilling system with an air temperature between -6 and 2 °C and a processing time ranging from 45 to 100 mins; one study used the water immersion chilling with a processing time of 20 mins. Finally, all the studies providing data on the counts of *Enterobacteriaceae* immediately before and after specific stages of the processing belong to the group of studies in which chlorine was not used or for which the use of chlorine was not reported.

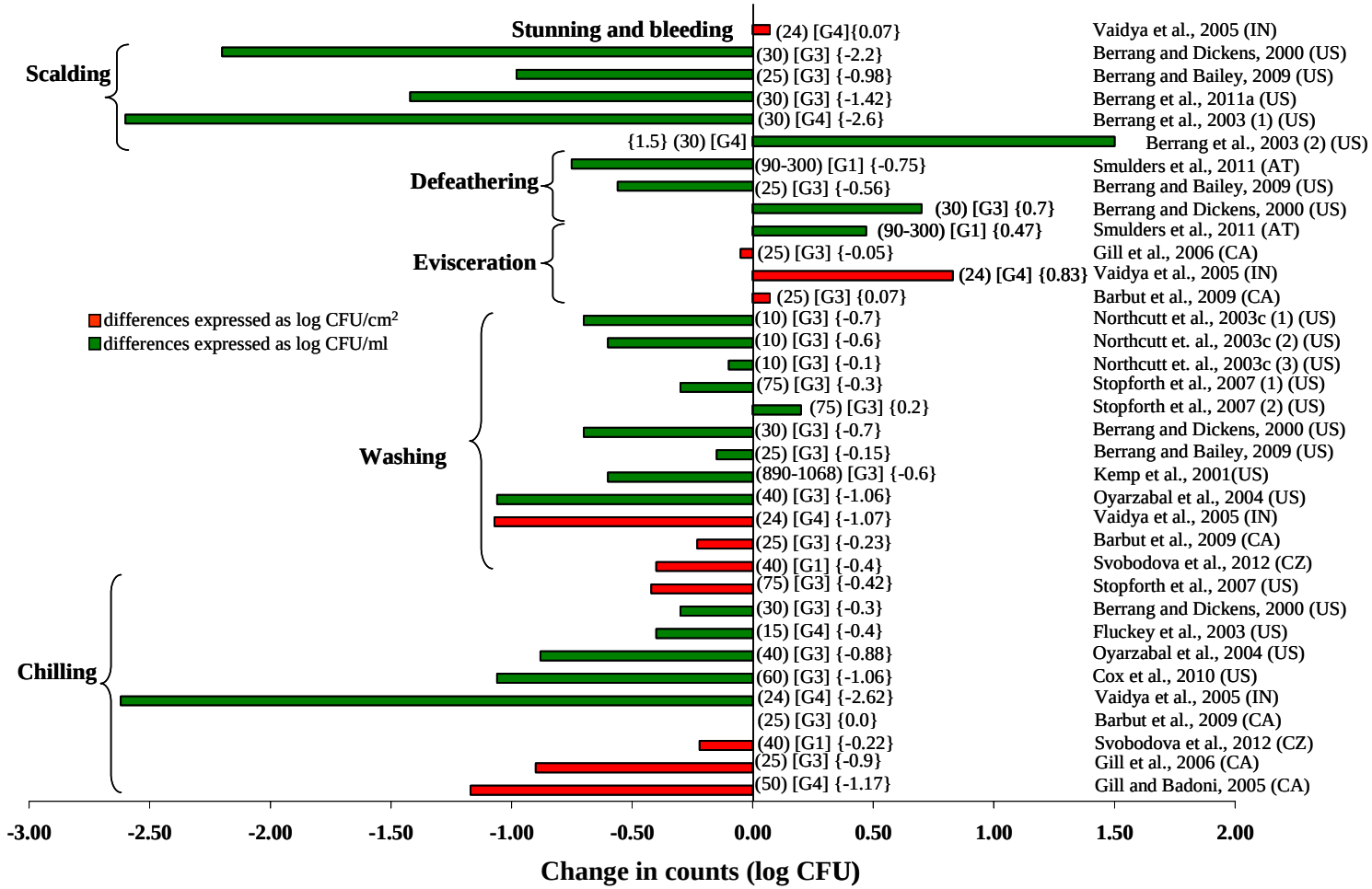


Figure 9: Change in counts of *E. coli* on poultry carcasses before and after a specific stage along the slaughter processing line reported in 24 studies. Number of samples is shown in the round brackets, chlorine groups are shown in the square brackets, change in count is reported in the brace brackets

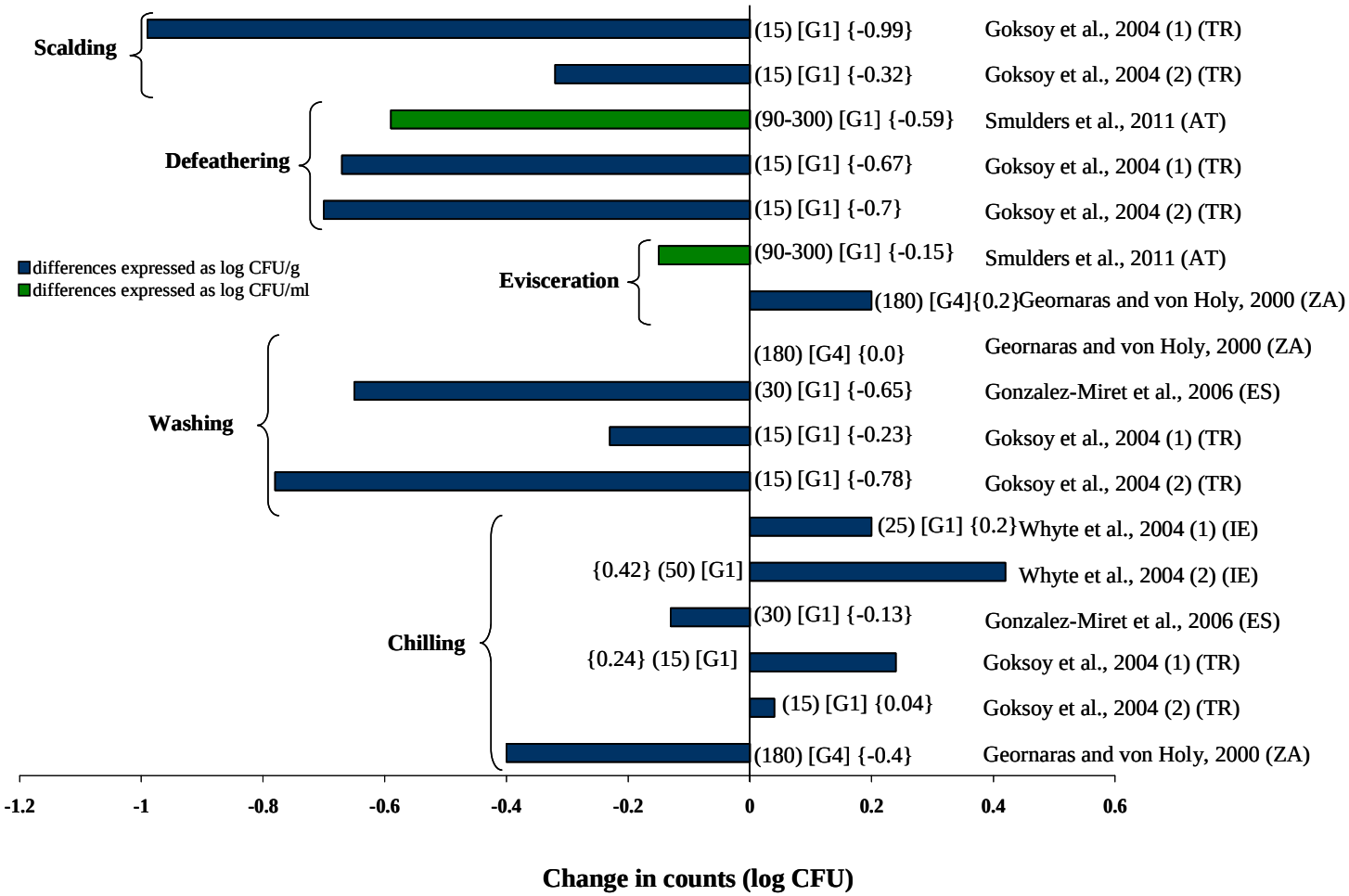


Figure 10: Change in counts of *Enterobacteriaceae* on poultry carcasses before and after a specific stage along the slaughter processing line reported in seven studies. Number of samples is shown in the round brackets, chlorine groups are shown in the square brackets, change in count is reported in the brace brackets

2.3.5. Discussion

According to the majority of the retrieved papers, as far as *E. coli* is concerned, the steps of the slaughter processing line where a decrease of the counts was more consistent were scalding, washing and chilling. For the defeathering step contrasting results have been provided by the studies. As regards the washing and chilling stages it should be remarked that the great majority of studies reporting a decrease of the *E. coli* counts used chlorine as decontaminant. Conversely, at the evisceration step an increase of the counts was reported for the majority of the studies.

According to the selected studies providing data on *Enterobacteriaceae* counts, scalding, defeathering and washing were the stages that contributed to reducing the bacterial loads on carcasses. Conversely, for the chilling step both an increase and decrease of the counts was observed.

Hence, for both indicator bacteria, a reduction of the bacterial loads were obtained after scalding and washing, whereas for the defeathering and chilling steps diverging results were obtained for the two indicator bacteria.

It should be remarked that the number of the available studies considering *Enterobacteriaceae* counts was very limited compared to the ones considering *E. coli* counts. In addition, all studies, describing the trends of *Enterobacteriaceae* along the processing line were conducted in slaughterhouses where chlorine was not used or information concerning the use of chlorine was not available. On the contrary, the great majority of studies providing data on *E. coli* were conducted in plants where chlorine was used.

Despite the fact that *scalding* has been shown to facilitate cross contamination even when the bacteria of interest are present in low numbers (Berrang et al., 2011), the data obtained from the retrieved studies demonstrated that this stage can also lead to a decrease of the bacteria loads on carcasses. The control of microbial levels could be explained by the water usage and its temperature. Due to the use of hot water, which is commonly in a state of constant agitation, many organisms can be removed from the carcass surface and the subsequent reduction of the bacterial load depends on the water temperature (Mead, 2004) and the replacing of the water in the scalding tank with fresh water (Göksoy et al., 2004). Scalding is a process that not only helps to loose feathers, facilitating defeathering, but also lowers the numbers of bacteria on the external surface of broiler carcasses.

The next step of the slaughter processing line is the *defeathering*, which involves high-speed rotation of multiple metal discs bearing rubber fingers, and causes considerable scattering of bacteria from carcass surfaces, so that cross contamination is an obvious risk (Mead, 2004). Another possibility of contamination is due to the contact between the picker fingers and the abdomen of the carcass, which could cause the release of gut content still present in the bowel (Berrang and Dickens, 2000). Despite defeathering being identified as a major source of cross contamination among carcasses, the great majority of the retrieved studies reported that defeathering tends to reduce bacterial numbers on broiler carcasses.

Evisceration is another step that, if carried out badly, can cause a significant increase of the microbial levels on carcasses. A certain level of contamination is unavoidable because of the natural variation in bird-size that is responsible for some degree of breakage of intestines and also because of the spillage of intestinal content that can occur during evisceration. In practice, according to the retrieved papers the variations of the bacterial loads at this stage were quite limited (Mead et al., 2004). Only two out of the six studies considering variation of indicator bacteria counts at this stage reported an increase after this stage higher than 0.5 log.

Once evisceration is concluded the carcasses are commonly cleaned before being transferred to the chilling process. The *washing* step is essential to remove organic debris and it is commonly carried out by spray washing. This practice causes a reduction of the microbial contamination of carcasses, but the level of reduction depends upon several aspects, such as the washer design, the water pressure, the degree of carcass washing during previous steps of the slaughtering process, and the use of decontaminants, such as chlorine (Mead et al., 2004). The importance of the washing step for reducing indicator bacteria loads was demonstrated by the data collected from the retrieved studies. Despite the level of reduction varying among studies, mainly due to the different practices described, almost all retrieved studies reporting bacterial counts at this stage demonstrated that washing was effective in reducing the *E. coli* and *Enterobacteriaceae* counts on carcasses.

Chilling is the last step of the process and it is aimed at lowering the temperature of the carcasses in order to control microbial growth. The two most commonly used practices are water immersion chilling and air chilling, with and without incorporation of water sprays to maintain carcass yield and enhance cooling by evaporation (Mead et al., 2004). Data obtained from the retrieved studies demonstrated the efficacy of this step in reducing *E coli* counts on carcasses. Different studies described several practices and reported a certain variability in terms of *E. coli* counts reduction. For *Enterobacteriaceae* the data obtained were quite conflicting, leading to both limited increases and decreases of the counts at the end of the chilling process.

2.4. Review question 2

2.4.1. General information about the relevant studies proving data for review question 2

The aim of this section is to describe results of papers investigating the effects of poultry farm management and slaughter characteristics, as well as other factors, on *E. coli* and *Enterobacteriaceae* loads on poultry carcasses.

The great variability among the selected papers is due to the multitude of aspects considered in the different studies and to the complexity of the slaughter processing line. Moreover, other variables like the indicator organism considered, sampling and analytical method used, the use of chlorine, the setting where the studies were carried out and the specific steps of the slaughter processing line investigated, render the available data barely comparable.

In particular, according to the defined search process and the established eligibility criteria, a total of **41 papers** dealing with risk factors were obtained. Eight papers were included in Group 1, 12 papers Group 2, 13 papers in Group 3, and eight papers in Group 4. Since some papers provided data on both types of indicator bacteria, or considered more than one factor influencing bacterial counts, it was more convenient to consider the individual studies (trials), instead of the papers.

Altogether, **63 studies** related to factors which could potentially influence indicator bacteria counts on poultry carcasses were described in the retrieved papers

Studies were divided according to the indicator bacteria considered and the factor investigated (Figure 11) resulting in a total of 46 studies dealing with *E. coli*. Of these, ten focused on batch information, one on transport conditions and 35 on slaughtering techniques or sampling time (e.g. time during the working day or annual season). Studies dealing with *Enterobacteriaceae* (17 in total) included one study on farming conditions and 16 on slaughtering techniques.

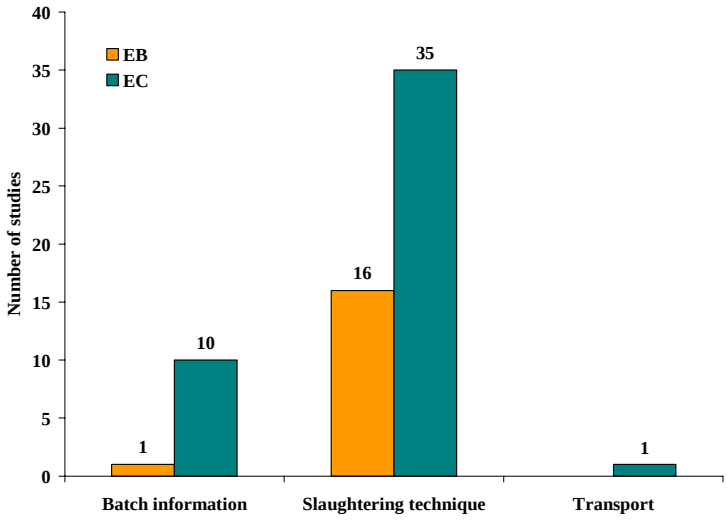


Figure 11: Studies providing data for review question 2 grouped according to the risk factors considered and the indicator bacteria investigated

Tables 7a and 7b (Appendix H) list the papers and studies that provided data on factors influencing *E. coli* and *Enterobacteriaceae* counts.

As already mentioned for review question 1, the comparability of data provided by different studies was hampered by different aspects, such as the sampling method and the unit of enumeration, which were not consistent across studies. Hence, different studies considering the same factor were compared in terms of conclusions drawn and not in terms of counts reported. Moreover, in order to assess the burden of different characteristics not directly addressed in studies on the indicator bacteria counts, we also collected general information about farming conditions and slaughter characteristics from all studies, as reported in Appendix E.

2.4.2. Factors related to batch characteristics

This section reports results of the different studies considering risk factors related to batch characteristics and influencing *E. coli* and *Enterobacteriaceae* counts on poultry carcasses. For this purpose, a risk factor was defined as a particular condition which could increase, in a significant way, the counts of these indicator bacteria on carcasses.

Tables 8a, 8b and 8c (Appendix I) report the *E. coli* and *Enterobacteriaceae* counts at different stages of the slaughter processing line for all pertinent studies considered. Figure 12 reports an overview of retrieved studies. They are grouped according to both batch characteristics which could impact on the indicator bacteria counts, and observed study impacts on those counts.

Among the retrieved papers, only one study dealt with *Enterobacteriaceae* counts on carcasses and farming conditions. In particular, this study, investigating different feeding practices, concluded that introduction of essential oil or organic acids in broiler diets did not lead to a reduction of counts on carcasses (Aksit et al., 2006). Diet was also investigated in relation to *E. coli* counts on carcasses, but again, correlations were not observed (Acikgoz et al., 2011, Northcutt et al., 2003a). In particular Acikgoz et al. (2011) evaluated the effect of formic acid administration to drinking water, while Northcutt et al. (2003a) evaluated the effect of the use of a finisher feed.

Two studies (Northcutt et al., 2003b; Thanissery et al., 2012) considered the age of birds as a potential factor influencing *E. coli* counts on carcasses and both of them reported a significant effect of age of birds on these counts, but drew opposite conclusions. In the first case *E. coli* counts increased with the age of the slaughtered animals (aged 42, 49 and 56 days) (Northcutt et al., 2003b). The other study reported that older subjects had lower bacterial loads (Thanissery et al., 2012). However, this last finding must be evaluated not only in relation to the age of birds, but also to their growth conditions, because the main aim of this study was to evaluate bacterial loads on carcasses between fast growing subjects and medium growing ones.

Two studies, investigating the effect of the length of the feed withdrawal period (0, 4, 8 and 12h) before slaughtering on the *E. coli* counts on carcasses, found no evidence of any correlation between these two factors (Northcutt et al., 2003a; 2003b). However, in one of these studies, cotton plugs were inserted into the cloaca of each sampled carcass during bleeding in order to limit intestinal leakage and this practice could have led to a bias in the amount of indicator bacteria retrieved.

Another study evaluated the effect of health status of animals on *E. coli* counts. In particular, birds from batches with and without a history of airsacculitis were compared. No evident differences were reported for the *E. coli* counts on broilers carcasses between the two groups (Russell, 2003).

Moreover, the effect of the presence of feathers on *E. coli* counts on the carcasses was studied. In this case, genetically featherless birds were compared with feathered subjects. No protective effect due to the absence of feathers emerged from samples collected after plucking (Buhr et al., 2003; Cason et al., 2004b) or after chilling (Buhr et al., 2005). However, when the birds sampled before defeathering had plugged vents, the absence of feathers was correlated with lower *E. coli* counts (Buhr et al., 2003). However, this experiment was performed on a limited number of animals and, as reported before, the practice of cloacal plugging could bias the impact the lack of feathers had on the amount of indicator bacteria detected on carcasses.

A study investigating the effect of the transport conditions on the *E. coli* counts observed that the type of flooring could influence the bacterial load on samples taken immediately after bleeding, with higher *E. coli* counts on carcasses when animals were held on solid flooring compared to these held on wire floors. In contrast, samples collected after plucking did not show any differences in terms of bacterial loads (Buhr et al., 2000).

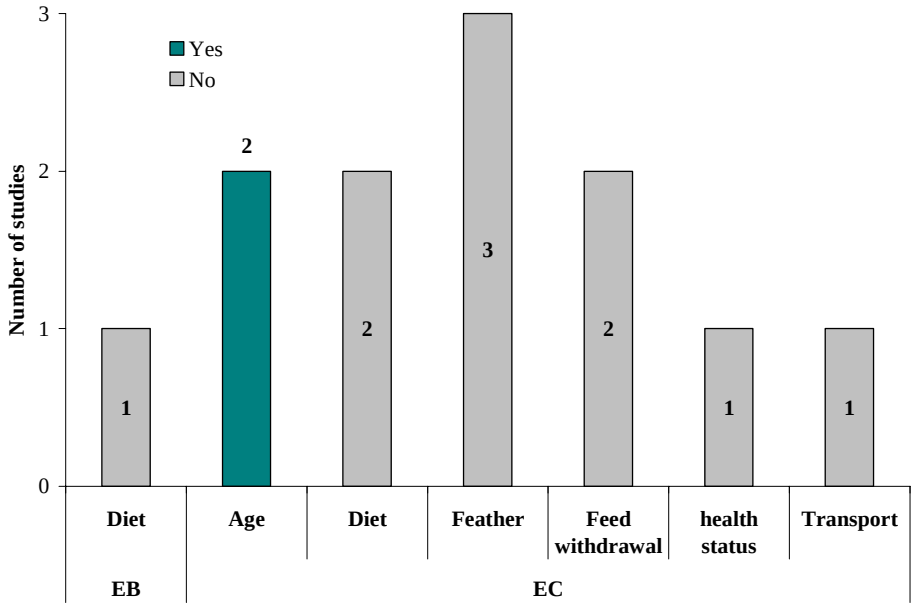


Figure 12: Studies dealing with batch risk factors classified according to the ability of the risk factor considered in explaining counts variations.

2.4.3. Factors related to slaughterhouse

2.4.3.1. General

When assessing the effect of the slaughterhouse processing line on the indicator bacteria load of poultry carcasses, the setting should be considered, and in particular, the use of chlorine should be evaluated since this treatment could directly influence the counts. As previously described, to interpret results properly and to avoid potential biases, the pertinent papers were classified in four groups in relation to the country where the study was conducted and the use of chlorine at the slaughterhouse.

An Australian paper dealing with the dimensions of the slaughterhouses stated that the lowest *E. coli* counts were reported in broiler slaughterhouses with the extreme conditions: in the biggest plant with the highest level of mechanization, but also in the smallest one with a limited level of mechanization. Concerning other poultry species in the same paper, the lowest counts were observed in small slaughterhouses for turkeys (only three plants considered), while for quails no correlations were found between the counts on the carcasses and the dimension of the plant (Sumner et al., 2004). Another paper aimed at obtaining a baseline for bacterial presence in poultry slaughterhouses in Alberta, Canada ended with opposite conclusions, depending on the unit of enumeration used. The authors observed that if counts were expressed as CFU/ml of rinse, low volume abattoirs (<100,000 birds killed/year) showed higher *E. coli* counts, while if results were converted to CFU/cm^2, the situation was reversed with higher counts in higher volume abattoirs (Bohaychuk et al., 2009).

Correlations were not observed between the time when the samples were collected and *Enterobacteriaceae* counts (Lindblad et al., 2006; Geornaras and van Holy, 2000, Whyte et al., 2004) or *E. coli* counts (Lindblad et al., 2006; Whyte et al., 2004) on poultry/broiler carcasses. More specifically, different sampling times were evaluated in order to observe potential variations in bacterial loads of carcasses during the working day, with the exception of Hutchison et al. (2006), who

evaluated a narrow time interval (30, 60, 120, 180 minutes), thus addressing an intrabatch potential variation. It was described that a weak relationships between levels of indicator bacteria (*Enterobacteriaceae*) and duration of the process was observed, although the cleanliness of the environment diminished visibly during processing time. In some cases, samples were collected at different points of the slaughterline. Whyte and others (2004) did not find a positive correlation between counts and the time of the day (AM and PM) for either *E. coli* or *Enterobacteriaceae*.

As far as the *season* is concerned, diverging results were obtained. Lindblad et al. (2006) did not find any correlations for either *E. coli* or *Enterobacteriaceae*, while Hutchison et al. (2006) did find a seasonal influence, with higher *Enterobacteriaceae* counts reported during the summer period in the UK.

To conclude, as summarized in Figure 13, the use of *chlorine* was reported as a significant tool to reduce bacterial contamination (Sumner et al., 2004), as was the *sanitation* programme used by the slaughterhouse, and which, according to Potter et al. (2012), could lead to lower counts if scheduled in a performance-based way instead of in the traditional one.

Internal *inspection* model, HACCP versus HIMP (HACCP-Inspection Models Project) did not result in any differences in terms of indicator bacteria counts (Berrang et al., 2008b). The same finding was obtained when the slaughter line speed was investigated (Northcutt et al., 2008d), but in this case, we considered only results reported in a proceeding abstract level.

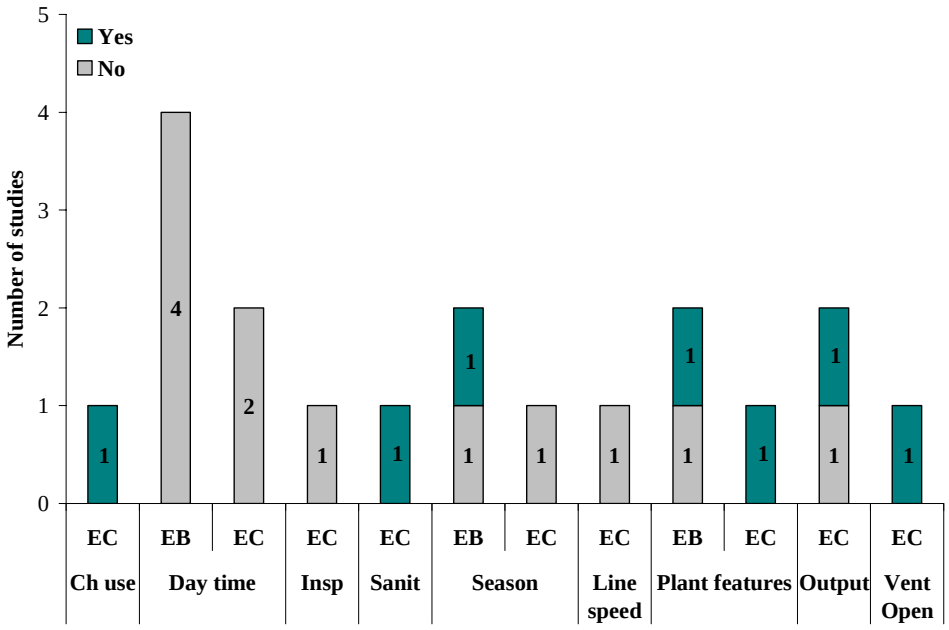


Figure 13: Effect of the slaughterhouse (general characteristics)-related risk factors considered in the retrieved studies. Ch use: chlorine use – Day time: sampling time – Insp: inspection model – Sanit: sanitation programme – Line speed: slaughterline speed– Output: animals slaughtered/time (day/year).

2.4.3.2. Different stages

The aim of the majority of papers included was to evaluate the influence of specific practices applied at one or more stages of the processing line on indicator bacteria counts on carcasses. In this section, the results reported in different studies are presented following the normal order of the slaughter

processing operations. Details about the use of chlorine are specified only when studies provided controversial results.

After stunning and bleeding, carcasses pass through the *scalding* process. This procedure was investigated by Cason et al. (2001), who evaluated the effect of lowering the temperature of the first of three tanks where the carcasses are scalded from 57 °C to 24 °C, in order to achieve a reduction in costs of the process. They demonstrated that despite the lower temperature used in the first tank, significant alterations (i.e. increases) of *E. coli* counts were not observed. Also, the number of scalding baths (one or three) did not influence *E. coli* counts (Buhr et al., 2005). Moreover, rescalding after defeathering, in order to minimize the effect of this operation on bacterial loads of carcasses was investigated without evidencing any significant differences (Berrang et al., 2000).

Studies also evaluated the duration of defeathering (Cason et al., 2004b) and the use of chlorine as an antimicrobial agent to reduce cross contamination at the defeathering stage (Berrang et al, 2011b). The first study (Cason et al., 2004b) reported that the duration of plucking (30 seconds versus 60 seconds) did not influence *E. coli* counts on the carcass. Conversely, the second study (Berrang et al, 2011b) demonstrated that spraying a solution containing 50 ppm chlorine during defeathering was effective in lowering *E. coli* counts on carcasses and, according to the authors, this was probably due to the reduction of cross contamination. This last claim has also been confirmed by Buhr and others (2003), who plugged the vents of the carcasses before plucking to avoid intestinal leakage and subsequent carcass contamination, thus obtaining better results in terms of *E. coli* counts. However, since the experimental size of this study was small, these results can be considered only as preliminary. Authors evaluated also the difference between skinned and unskinned broiler without drawing final conclusion about this practice (Berrang et al., 2001; 2002).

Washing has been investigated as a potential risk factor influencing indicator bacteria loads on carcasses in five studies focusing on the use of chlorine or other alternative decontamination treatments. Papers describing the use of chemical decontamination other than chlorine were excluded during the screening process and only studies providing data generated by using physical decontamination treatments or chlorine were considered.

Results about chlorine efficacy at the carcass washing step are rather inconsistent. Whyte et al. (2001) reported that treatment with 25 ppm chlorine effectively reduced both *E. coli* and *Enterobacteriaceae* counts on carcasses. This result was confirmed by Northcutt et al. (2007). On the contrary, others found that using either 20 ppm (Buhr et al., 2005) or 50 ppm (Northcutt et al., 2005) chlorine did not significantly impact/reduce *E. coli* counts on carcasses.

As far as physical treatments are concerned, the temperature of water used at the washing step has been investigated. Northcutt et al. (2005), did not find any effect of applying water temperatures, within a 21 to 55°C range, on *E. coli* on carcasses. On the contrary, in a pilot study, a hot water immersion treatment was effective against *Enterobacteriaceae* when applied at 75 °C for 30 sec, but leads to detrimental effects on the carcass skin; the same treatment, at 70 °C for 40 sec, significantly reduced the counts in one out of two trials (Purnell et al., 2004). Positive results were obtained also after processing carcasses with a steam pasteurization unit commonly used in a bovine slaughter plant. This treatment was effective at 90 °C for 24 sec, but showed less efficacy when applied for 12 sec (Whyte et al., 2003). A high pressure washing treatment was also effective according to an observational study conducted in Spain by Escudero-Gilete et al. (2005).

Finally, the impact of the *chilling* stage on indicator bacteria counts was assessed, as that is a crucial phase to achieve a quick decrease of carcass temperature and consequently to prevent microbial proliferation. In slaughterhouses, according to the papers evaluated, two practices are commonly used: air chilling and water immersion chilling. Some of the studies aimed at comparing these two chilling

treatments in relation to the bacterial reduction, while other studies focused on the effectiveness of different immersion chilling practices. It is important to take into account the use of chlorine.

Results disagree in relation to *E. coli* counts variations between air and water chilling without the addition of chlorine because in one study water chilling was more effective (Berrang et al., 2008a), while in another study no differences were found (Huezo et al., 2007). Interestingly, equivalence between treatments in terms of *E. coli* counts was confirmed also by two studies performed in slaughterhouses where chlorine was used in chilling immersion tank (Sanchez et al., 2002; Barbut et al., 2009). The only significant difference was observed in one of these studies against *Enterobacteriaceae* counts that resulted lower if chilling was performed in an immersion tank (Barbut et al., 2009). In relation to the efficacy of the use of chlorine during immersion chilling, no differences were observed on *E. coli* counts between water with 20 ppm chlorine and tap water (Russell and Axtell, 2005).

In Brazil when immersion chilling is used, water has to be renewed every 8 h according to the current legislation. Souza et al. (2012) evaluated the effect of widening this interval to 16 hours without addition of chlorine, and concluded that the *E. coli* and *Enterobacteriaceae* counts on broiler carcasses remained the same. These results are confirmed, for both indicators, by Cavani et al. (2010) using water renewal times of 8, 16 and 24 hours in immersion chilling tank. Also the reuse of chilling water (with addition of chlorine) was tested and it was demonstrated as a valid alternative because no significant effects on *E. coli* counts were observed (Northcutt et al., 2008b). Another option to reduce costs related to water usage was the reduction in the ratio of water per kg of carcass. In this case no differences in *E. coli* and *Enterobacteriaceae* counts were observed between 3.3 L of distilled water /Kg and 6.7 L of distilled water /kg (Northcutt et al., 2008c), while differences were identified between 16.8 L of water /Kg and 2.1 L of water/Kg. In the latter case, results were considered not significant from a microbiological point of view (Northcutt et al., 2006).

In Figures 14, 15 and 16 the effect of risk factors associated with slaughterhouse practices are summarized. In Tables 2a and 2b, the effects of the slaughter and batch related factors on *E. coli* and *Enterobacteriaceae* counts are reported for the considered studies.

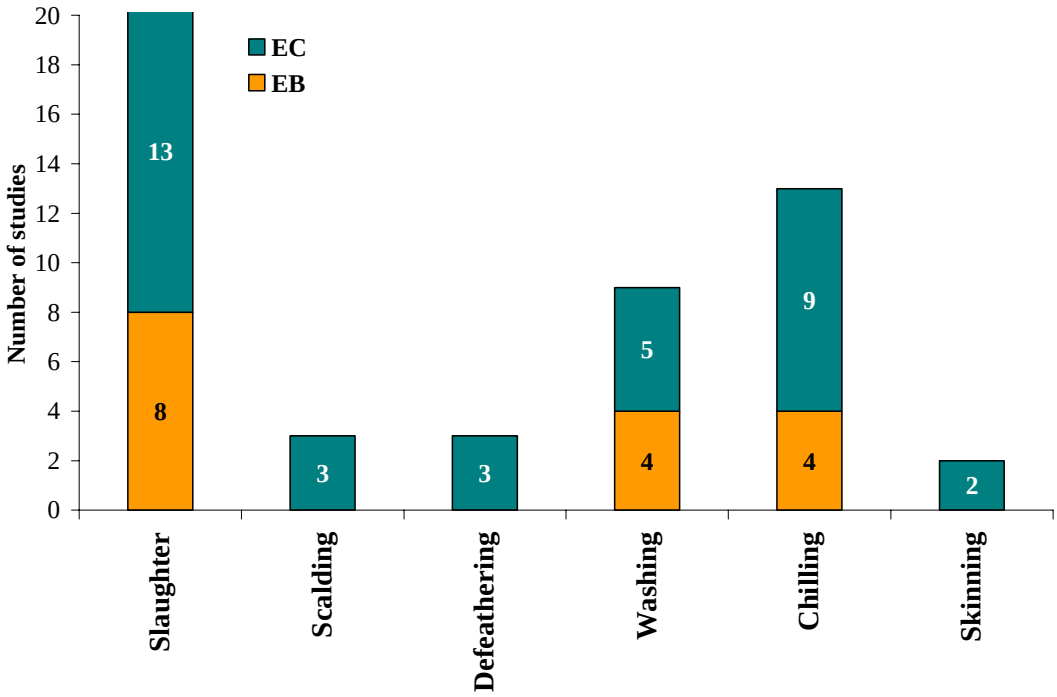


Figure 14: Studies considering risk factors related to the slaughterhouse practices grouped according to the step of slaughter line and the indicator bacteria investigated.

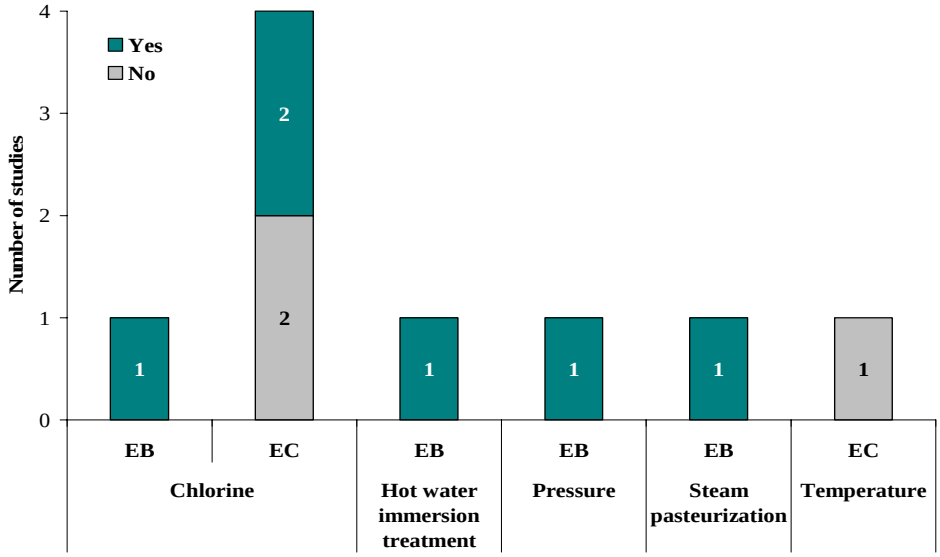


Figure 15: Effect of the specific risk factors considered by the selected studies on the indicator bacteria counts at washing phase.

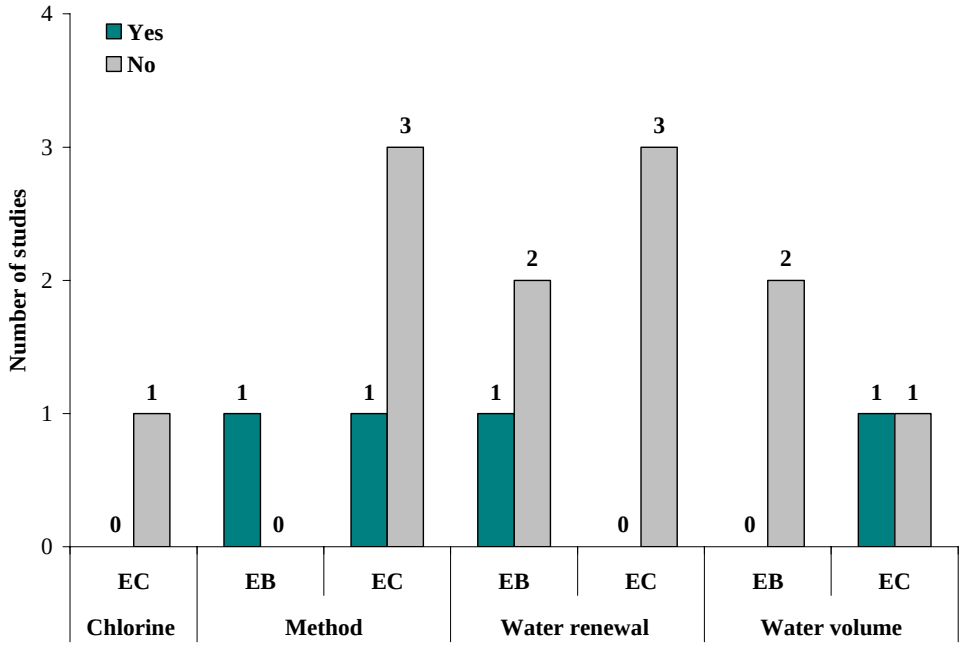


Figure 16: Effect of the specific risk factors considered by the selected studies on the indicator bacteria counts at chilling phase. The total number of studies is twelve because one study failed to include a control group. One study which effect is classified as ND in Table 2a is not reported in this figure.

Table 2a: Effect of the slaughter related factors on the *E. coli* (EC) and *Enterobacteriaceae* (EB) counts. IB indicator bacteria; NS: not specified; ND: not defined; B<A; * result considered not significant from a microbiological point of view.

	IB	Factor	Specifications	Reference (country)	Batch	Study	Counts	Effect
Group 1	EB	Slaughter	Sampling time	Hutchison et al., 2006 (GB)	NS	O	-	No
				Lindblad et al., 2006 (SE)	NS	O	-	No
				Whyte et al., 2004 (IE)	After 7-8 h working	O	HIGH	No
					Morning	O	HIGH	
			Season	Hutchison et al., 2006 (GB)	NS	O	-	Yes
				Lindblad et al., 2006 (SE)	NS	O	-	No
			Slaughterhouse characteristics	Lindblad et al., 2006 (SE)	NS	O	-	Yes
		Washing	Hot water immersion treatment	Purnell et al., 2004 (GB)	70°C 40s	E	LOW	Yes
					70°C 40s		HIGH	
					75°C 30s		LOW	
			Pressure	Escudero-Gilete et al., 2005 (ES)	High pressure	O	LOW	Yes
					Low pressure	O	HIGH	
					Medium pressure	O	HIGH	
			Steam pasteurization	Whyte et al., 2003 (IE)	12s 90°C	E	HIGH	Yes
					24s 90°C	E	LOW	
					Control	E	HIGH	
	EC	Slaughter	Sampling time	Lindblad et al., 2006 (SE)	NS	O	-	No
				Whyte et al., 2004 (IE)	After 7-8 h working	O	HIGH	No
					Morning	O	HIGH	
			Season	Lindblad et al., 2006 (SE)	NS	O	-	No
			Slaughterhouse characteristics	Lindblad et al., 2006 (SE)	NS	O	-	Yes
Group 2	EB	Chilling	Water renewal	Souza et al., 2012 (BR)	16h	E	HIGH	No
					8h	E	HIGH	
				Cavani et al., 2010 (BR)	8h	O	HIGH	No
					16h	O	HIGH	
					24h	O	HIGH	
			Water volume	Northcutt et al., 2006 (US)	16.8 L/Kg	E	LOW	Yes*
					2.1 L/Kg	E	HIGH	
	EC	Chilling	Method (immersion - air)	Berrang et al., 2008a (US)	Air	E	HIGH	Yes
					Immersion	E	LOW	
				Huezo et al., 2007 (US)	Air	E	HIGH	No
					Immersion	E	HIGH	
			Water renewal	Souza et al., 2012 (BR)	16h	E	HIGH	No
					8h	E	HIGH	
				Cavani et al., 2010 (BR)	8h	O	HIGH	No
					16h	O	HIGH	
					24h	O	HIGH	
			Water volume	Northcutt et al., 2006 (US)	16.8 L/Kg	E	LOW	Yes
					2.1 L/Kg	E	HIGH	
		Scalding	First bath temperature	Cason et al., 2001 (US)	24-57-57°C	E	HIGH	No
					57-57-57°C	E	HIGH	
		Skinning	Skin elimination	Berrang et al., 2002 (US)	Skin off	E	-	ND
					Skin on	E	-	
		Slaughter	Slaughtering output	Bohaychuk et al., 2009 (CA)	High volume	O	HIGH/LOW	Yes/No
					Low volume	O	LOW/HIGH	
			Vent open or close	Buhr et al., 2003 (US)	Close	E	LOW	Yes
					Open	E	HIGH	

Table 2a (Continued): Effect of the slaughter related factors on the *E. coli* (EC) and *Enterobacteriaceae* (EB) counts. IB indicator bacteria; ND: not defined.

	IB	Factor	Specifications	Reference (country)	Batch	Study	Counts	Effect
Group 3	EB	Chilling	Method (immersion vs air)	Barbut et al., 2009 (CA)	Air	O	HIGH	Yes
					Immersion	O	LOW	
			Water volume	Northcutt et al., 2008c (US)	3.3 L/kg	E	HIGH	No
					6.7 L/kg	E	HIGH	
		Washing	Chlorine	Whyte et al., 2001 (IE)	1-2 ppm	E	HIGH	Yes
					25 ppm	E	LOW	
	EC	Chilling	Chlorine	Russell and Axtell, 2005 (US)	50 ppm	E	HIGH	No
					No chlorine	E	HIGH	
			Method (immersion vs air)	Barbut et al., 2009 (CA)	Air	O	HIGH	No
					Immersion	O	HIGH	
				Sanchez et al., 2002 (US)	Air	O	HIGH	No
					Immersion (40 ppm)	O	HIGH	
			Recycled water with chlorine	Northcutt et al., 2008b (US)	Recycled	O	-	ND
			Water volume	Northcutt et al., 2008c (US)	3.3 L/kg	E	HIGH	No
					6.7 L/kg	E	HIGH	
		Defeathering	Chlorine during defeathering	Berrang et al., 2011b (US)	50 ppm	E	LOW	Yes
					No	E	HIGH	
		Scalding	Number of baths	Buhr et al., 2005 (US)	Single	E	HIGH	No
					Triple	E	HIGH	
		Slaughter	Use of chlorine	Sumner et al., 2004 (AU)	No	O	HIGH	Yes
					Yes	O	LOW	
			Dimension	Sumner et al., 2004 (AU)	Dimension	O	-	No
			Slaughterhouse characteristics	Matias et al., 2010 (BR)	Large-high mecanization	O	-	Yes
					Small-manual procedures			
				Sumner et al., 2004 (AU)	Mechanization	O	-	No
		Washing	Chlorine	Buhr et al., 2005 (US)	20 ppm	E	HIGH	No
					No	E	HIGH	
				Northcutt et al., 2005 (US)	50 ppm	E	HIGH	No
					No	E	HIGH	
				Northcutt et al., 2007 (US)	50 ppm	E	LOW	Yes
					No	E	HIGH	
				Whyte et al., 2001 (IE)	1-2 ppm	E	HIGH	Yes
					25 ppm	E	LOW	
			Temperature	Northcutt et al., 2005 (US)	21.1 °C	E	HIGH	No
					43.3 °C	E	HIGH	
					54.4 °C	E	HIGH	
Group 4	EB	Slaughter	Day time	Geornaras and von Holy., 2000 (ZA)	1h	O	HIGH	No
					1h before day end	O	HIGH	
					Tea break	O	HIGH	
	EC	Defeathering	Duration	Cason et al., 2004b (US)	30s	E	HIGH	No
					60s	E	HIGH	
		Scalding	Rescalding after defeathering	Berrang et al., 2000 (US)	30 min after	E	HIGH	No
					Immediately	E	HIGH	
					Immersion 28s-60°C	E	HIGH	
					Spray 20s-73°C	E	HIGH	
		Skinning	Skin elimination	Berrang et al., 2001 (US)	Skin off	O	-	ND
					Skin on	O	-	
		Slaughter	Method of inspection	Berrang et al., 2008 (US)	HACCP	O	HIGH	No
					HIMP	O	HIGH	
			Sanitation	Potter et al., 2012 (US)	PBS (performance)	O	LOW	Yes
					TS (traditional)	O	HIGH	
			Shackle line speed	Northcutt et al., 2008d (US)	NS	E	-	No

Table 2b: Effect of the batch related factors on the *E. coli* (EC) and *Enterobacteriaceae* (EB) counts. IB indicator bacteria; *Effect only if vents are plugged; **Effect only before plucking

	IB	Factor	Specifications	Reference (country)	Batch	Study	Counts	Effect
Group 1	EB	Diet	Feed	Aksit et al., 2006 (TR)	Control	E	HIGH	No
					Essential oils	E	HIGH	
					Organic acids	E	HIGH	
	EC	Diet	Feed	Acikgoz et al., 2011 (TR)	Control	E	HIGH	No
					Formic acid 8h before slaughtering	E	HIGH	
Group 2	EC	Age	Slaughter age	Thanissery et al., 2012 (US)	Fast growing species 64-71 d	O	HIGH	Yes
					Medium growing species 73 d	O	LOW	
		Diet	Finisher or control feed	Northcutt et al., 2003a (US)	Control	E	HIGH	No
					Finisher	E	HIGH	
		Feather	Presence of feathers	Buhr et al., 2003 (US)	Absence	E	HIGHB	Yes/No*
					Presence	E	HIGH/HIGH	
		Feed withdrawal	Time	Northcutt et al., 2003a (US)	0h	E	HIGH	No
					12h	E	HIGH	
					4h	E	HIGH	
					8h	E	HIGH	
		Transport	Transport flooring	Buhr et al., 2000 (US)	Solid	E	HIGH/HIGH	Yes/No**
					Wire	E	HIGH/LOW	
Group 3	EC	Age	Slaughter age	Northcutt et al., 2003b (US)	42 d	E	LOW	Yes
					49 d	E	HIGH	
					56 d	E	HIGH	
		Feather	Presence of feathers	Buhr et al., 2005 (US)	Absence	E	HIGH	No
					Presence	E	HIGH	
		Feed withdrawal	Time	Northcutt et al., 2003b (US)	0h	E	HIGH	No
					12h	E	HIGH	
Group 4	EC	Feather	Feather presence	Cason et al., 2004b (US)	Absence	E	HIGH	No
					Presence	E	HIGH	
		Health status	History of airsacculitis	Russell 2003 (US)	Negative	E	HIGH	No
					Positive	E	HIGH	

Discussion

2.4.3.3. Batch related risk factors

Since a bacterial process control indicator must respond to changes in process hygiene, the possibility of correlating a specific factor with the counts of the indicator bacteria on carcasses is of interest. Although it has been very difficult to consolidate results of the retrieved studies and draw conclusions because of the scattered nature of results collected, results collected have been discussed and data gaps underlined. The greatest challenge has been evaluating the effects of farming characteristics/practices on carcass counts. In this case, according to the eligibility criteria used to select pertinent studies, it was mandatory that such studies investigated the effect of the farming techniques on carcass counts and not on gut content. This was because although intestinal content is an important source of carcass contamination depending on rupture and leakage of the gastrointestinal tract, such events do not always occur.

According to these last considerations, the length of the feed withdrawal period has frequently been considered as an important factor influencing indicator bacteria counts on poultry carcasses. Ideally, the length of feed withdrawal before processing should be the shortest amount of time required for the birds' digestive tracts to become empty. The recommended length of time off feed for broilers before processing is between 8 to 12 hours. During this time period, most of the birds in the flock evacuate their gastrointestinal tracts, preventing intestinal ruptures due to excessive repletion and subsequent cross contamination among carcasses during the slaughter process. However, the feed withdrawal time should not be too long to avoid excessive loss of carcass yield (Casey et al., 2010). The two studies retrieved, investigating the effect of length of feed withdrawal period on indicator bacteria loads on carcasses, failed to demonstrate any link between feed withdrawal time and *E. coli* counts on carcasses (Northcutt et al, 2003a; 2003b). However, in one of these studies, the authors plugged the anus of broilers before defeathering and evisceration in order to avoid intestinal leakage and this treatment could have influenced the microbial loads of the carcasses.

The same consideration about the risk of intestinal ruptures drove other authors to investigate the effect of the health status of slaughtered animals that can be associated with cachectic conditions and within-batch heterogeneity in terms of size of animals. Russell et al. (2003) compared the indicator bacteria loads of two batches of broilers with and without an anamnesis of airsacculitis and did not find any correlations between health status and indicator bacteria loads. The same authors remarked that this finding could be explained by the fact that, irrespective of the previous health status of the two batches, all the carcasses sampled in the study passed ante-mortem inspection.

Feed regimen was never described as a significant risk factor in any relevant study as it did not impact on indicator bacteria loads (Aksit et al., 2006; Acikgoz et al., 2011; Northcutt et al., 2003a).

Age of animals at slaughter was considered as a potential risk factor which could affect indicator bacteria loads, but different studies provided contrasting results (Thanissery et al., 2012; Nortcutt et al., 2003b). The inconsistency among the retrieved studies could be a consequence of the different study designs. Northcutt et al. (2003b) tested the effect of age on bacterial load of carcasses considering three age groups, while Thanissery et al. (2012) investigated the differences between fast and medium growing broiler lines, rather than between two ages.

Moreover, studies evaluating the role of the presence of feathers on carcass contamination during slaughtering operations were considered (Buhr et al., 2003, Buhr et al., 2005; Cason et al., 2004b). The presence of feathers increased the indicator bacteria counts when samples were collected after defeathering in one out of three studies retrieved. However, this last result was obtained analysing carcasses with plugged vents (Buhr et al., 2003). This preliminary result suggests that during

defeathering, counts are likely to be homogenized because of squeezing and cross contamination. However, this result seems to not be of particular interest since, to our knowledge, genetically featherless broiler lines are raised only for experimental reasons, but are not used in commercial farming practices.

Between the farm and slaughter, transport is an important step that could influence indicator bacteria loads on carcasses. Bacterial shedding potentially occurs because of close contact among animals, stressful conditions and crate characteristics. As far as this topic was concerned, only one study fitted our eligibility criteria. This study evaluated the effect of crate flooring type on bacterial loads of carcasses during transport and holding of broilers prior to slaughter. Solid floor was compared to a wired floor and a significant correlation with *E. coli* counts on carcasses was observed in samples taken immediately after bleeding. *E. coli* counts were significantly higher on carcasses of animals transported on solid flooring. This outcome was not observed when *E. coli* counts were measured after picking (Buhr et al., 2000). This result confirmed the importance of ante-mortem practices on carcass indicator bacterial load, but raised some concerns about the persistence of these differences throughout the slaughter line. Moreover, the effect of sampling point on interpretation of results should be carefully considered before concluding that a practice is a risk factor, and indeed contributes to higher loads of indicator bacteria on carcasses.

2.4.3.4. Slaughter related risk factors

E. coli and *Enterobacteriaceae* counts on poultry carcasses depend on the plant where the slaughtering process took place. The effect of the size of the slaughterhouse on the indicator bacteria counts was investigated in several papers (Sumner et al., 2004; Lindblad et al., 2006, Bohaychuk et al., 2009). Summer et al. (2004) provided a description of slaughterhouses according to their main technical features, whereas in the latter two papers, specific descriptions of the slaughtering practices were not provided. It was concluded that slaughterhouses play an important role in the indicator bacteria counts on carcasses; however, it is not possible to understand what effect the practices applied at the different stages of the slaughter processing line have on indicator bacteria counts. Bohaychuk et al. (2009), dealing with this point, concluded that the high volume abattoir had lower counts if the results of rinse analyses were expressed as CFU/ml, but had higher counts if the results were transformed into CFU/cm^2. This finding should be carefully considered when evaluating results obtained with the rinse sampling technique. Sumner et al. (2004) concluded that the dimension of the slaughterhouse had no effect on indicator bacteria counts (expressed on a cm^2 basis), because the lowest counts were found both in the largest and the smallest abattoirs. No effect was observed also in relation to the level of mechanization.

In conclusion, slaughterhouses could induce different effects on the bacterial counts of carcasses, but neither the dimension of the slaughterhouse nor the level of mechanization are clearly correlated to the counts of *E. coli* and *Enterobacteriaceae*. Moreover, attention should be paid to the unit of enumeration used to express the results.

The time of the day when samples are collected should be considered as the possibilities of contamination theoretically increase during the working day and also because the flocks that are processed later usually spend more time in cages before being slaughtered. This issue, however, has never been supported with data, according to data reported in the studies retrieved. Available studies dealt with this factor without observing an impact (Hutchison et al., 2006; Lindblad et al., 2006; Geornaras and van Holy, 2000; Whyte et al., 2004). However, the results show that data are barely comparable. Five papers were evaluated, three belonging to Group 1 (no chlorine), one to Group 2 (chlorine, but the level in this case was low, around 2 ppm) and one to Group 4 (no information available regarding chlorine). Moreover, the studies took place in different periods of the year and considered samples collected at different times during the working day. According to this other

authors focused on a shorter working time, and finally Hutchison et al. (2006) studied the difference along slaughter line in a 180 minute period.

Sampling season was investigated in two papers, which provided contrasting results, thus making it impossible to draw definitive conclusions (Lindblad et al., 2006; Hutchison et al., 2006). Therefore, neither season nor time of slaughtering during the day when samples are collected seemed to have any definitive impact on indicator bacteria levels on carcasses.

With regards to other general slaughter features, the use of chlorine as well as the sanitation programme used were reported as significant factors (Sumner et al., 2004; Potter et al., 2012) influencing the indicator bacteria counts on carcasses. In this last paper, a sanitation system based on microbiological monitoring showed better results in terms of bacterial loads on carcasses. In contrast, internal inspection model, HACCP versus HIMP (HACCP-Inspection Models Project) and slaughterline speed did not contribute towards indicator bacteria loads, and are not considered risk factors (Berrang et al., 2008b; Northcutt et al., 2008d).

The effect of each single step of the slaughter processing line on the bacterial loads of carcasses has been frequently investigated, in particular, the washing and chilling steps. In this case, it should be noted that the changes of bacterial counts observed after one slaughtering operation will not necessarily affect the counts at the end of the entire process (after chilling).

Scalding (Berrang et al., 2000, Cason et al., 2001, Buhr et al., 2005) practices were never identified as a risk factor, as *E. coli* counts on carcasses was not affected. In particular, concerning scalding different practices such as lowering the temperature in the first bath (Cason et al., 2001), the number of scalding baths (Buhr et al., 2005), and a rescalding step after defeathering (Berrang et al., 2000) were evaluated without evidencing any effects on the indicator bacteria counts.

Plucking is theoretically considered challenging for carcass hygiene because of the potential for cross-contamination between carcasses. However, as reported for review question 1, evidence concerning the effect of this stage on indicator bacteria counts is lacking and results are conflicting. The evaluation of the three studies dealing with this step revealed inconsistent results. Defeathering duration was not associated with higher counts on carcasses (Cason et al., 2004b); in contrast, the use of 50 ppm chlorine significantly reduced *E. coli* counts, probably by limiting the cross-contamination among carcasses (Berrang et al., 2011b). Finally, a pilot study also evaluated the effect of plugging the anus to avoid squeezing and obtained positive results, since plugged carcasses sampled at the defeathering stage showed lower counts (Buhr et al., 2003). However, this result was obtained on a limited number of subjects and considers a process which would be barely applicable in a commercial setting.

During washing, the use of chlorine in water was considered an effective factor, reducing indicator bacteria loads on carcasses in two out of four papers considered (White et al., 2001; Northcutt et al., 2007, Buhr et al., 2005; Northcutt et al., 2005). However, this finding is of limited interest because according to current EU legislation, chlorine is not permitted during slaughter operations.

The use of physical treatments could be a valid alternative to produce a reduction of carcass bacterial counts. In particular carcass treatment with steam or hot water has been tested. Their potential application, however, should be further assessed and carefully considered in relation to cost effectiveness and carcass skin damages. Also a high pressure washing treatment was effective in reducing *Enterobacteriaceae* counts. The use of water immersion chilling has the advantage of lowering the temperatures of carcasses more quickly, but has also some disadvantages mainly related to the risk of cross-contamination among carcasses and the high costs. Accordingly, in the US, the practice of adding chlorine to the chilling bath is very common. It is not, however, effective in directly

reducing the bacteria load on the carcass surfaces, but rather, it is used to maintain the microbiological quality of the water for longer periods, thus helping to avoid cross contamination linked with bacteria that are released from carcasses into water. The problem of water quality is addressed in EU legislation prescribing a daily renewal of water. Several studies considered the effect of the chilling stage on the indicator bacteria counts on carcasses; unfortunately, none of these studies took place in Europe. Air chilling resulted in higher counts of *Enterobacteriaceae* on carcasses in one study (Barbut et al., 2009) and of *E. coli* in another one (Berrang et al., 2008a), but in contrast, no effects of chilling technique on *E. coli* levels on carcasses were observed in studies with chlorination (Sanchez et al., 2002; Barbut et al 2009) and without chlorination (Huezo et al., 2007). Chlorine was also judged as ineffective against indicator bacteria counts on carcasses compared to untreated water in immersion chilling (Russell and Axtell, 2005). However, these results are of limited utility in an EU perspective, because air chilling is widely applied, while the immersion technique is quite unusual. For these reasons, studies considering different renewal times for water used during immersion (Souza et al., 2012) are of limited value as are studies considering different ratios of water/Kg for processing (Northcutt et al., 2006; 2008c).

2.5. Review question 3

2.5.1. General information about the relevant studies proving data for review question 3

Seven papers provided information on the relationship between faecal contamination of carcasses and their *E. coli* and/or *Enterobacteriaceae* counts; six out of the seven papers included studies conducted in US and one in Argentina. Five out of seven papers described experimental studies, four papers provided data obtained in pilot establishments and three papers gathered information from a commercial slaughterhouse.

All papers reported data on broiler carcasses; three papers provided information about the weight of sampled animals (ranging between 1.3 and 6.1 kg) and two papers gave information about the age of birds (ranging from 42 to 70 days).

All studies collected single samples and used the rinse sampling procedure. The sampled area was, in most of the cases, the whole carcass. All papers provided data on *E. coli* and two papers also provided data on *Enterobacteriaceae*, in addition to *E. coli*.

Petrifilm was the most common method used for quantifying *E. coli*, while traditional Petri dishes incubated at 35°C for 24 hours were used for quantification of *Enterobacteriaceae*. Six out of seven papers expressed the counts as logCFU/ml.

Two papers described studies conducted in slaughterhouses where chlorine was used at the chilling step.

Finally, four papers described results obtained on carcasses artificially contaminated with faecal material. The remaining three papers considered naturally contaminated carcasses which were classified for visible faecal contamination at several points in the slaughtering line, such as after evisceration, after inside-outside washing or either before or after immersion chilling.

2.5.2. Data concerning the counts of indicator bacteria in relation to the level of visual faecal contamination

Table 3 summarizes the counts of *E. coli* and *Enterobacteriaceae* on broiler carcasses at different stages of the slaughtering processing line in relation to the level of visual faecal contamination.

The papers retrieved examined the relationship between visual faecal contamination and bacterial counts at different stages of the slaughter processing line. Considering that, according to the eligibility criteria defined for literature review, the papers providing information on this aspect were only seven, a further selection of these papers could result in a loss of the available observations and data.

Nevertheless, a different weight has been given to the papers that provided information collected in a commercial slaughterhouse instead of a pilot plant or that provided data on natural contaminated instead of artificially contaminated carcasses. Thus, the two following quality criteria have been considered: study setting (commercial slaughterhouse: high value or pilot slaughterhouse: medium value) and type of contamination (natural: high value or artificial: medium value).

Among the seven retrieved papers, four papers reported studies considering artificially contaminated carcasses collected in pilot slaughterhouses, while three papers provided data on naturally contaminated carcasses obtained in commercial slaughterhouses.

2.5.2.1. Studies conducted in pilot slaughterhouses – data on artificially contaminated carcasses

Cason et al. (2004a) contaminated carcasses with 0.1 g of faecal material, then washed the carcasses with tap water, and collected samples before and after water immersion chilling (without addition of chlorine). *Enterobacteriaceae* counts were higher on artificially contaminated carcasses collected before chilling than on control carcasses (no artificial contamination), while *E. coli* counts did not differ. Conversely, after water immersion chilling, neither type of indicator bacteria counts differed between artificially contaminated and control carcasses. Hence, the authors concluded that indicator bacteria counts of carcasses collected after chilling do not reveal if faecal contamination occurred before chilling. The same conclusion was drawn by Smith et al. (2005). These authors demonstrated that immersion chilling (without chlorine) equilibrated indicator bacterial numbers between carcasses which had been either directly contaminated with faecal material or cross-contaminated.

In addition, Northcutt et al. (2008a) assessed indicator bacteria counts on featherless broilers, after carcasses passed through a machine designed to induce defecation (squeezed carcasses), and after washing these to remove faecal material (squeezed and washed carcasses). The comparison of indicator bacteria counts between the group of carcasses that was only squeezed, the group that was only washed and the group that was both squeezed and washed demonstrated that *E. coli* counts did not vary with the treatment used.

Smith et al. (2007) provided the results of a study considering three groups of eviscerated carcasses. The first group of carcasses were artificially contaminated with 1 g of caecal material on the internal cavity of carcasses, the second group were carcasses artificially contaminated on the breast region, whereas the last group were control carcasses. After washing these carcasses using an inside-outside bird washer (IOBW), the highest *E. coli* load was registered on the externally-contaminated carcasses, whereas the internal contamination resulted in significantly lower counts and, finally, the lowest counts were reported for the control group. These data suggest that the IOBW procedure was not able to reduce *E. coli* counts on visibly contaminated carcasses to a level comparable to non-artificially contaminated carcasses. Moreover, it demonstrated that the bacterial load differed between carcasses contaminated with faecal material on the internal cavity or on the external surface for various reasons. Faecal contamination may adhere less readily to the internal cavity than to the external surface; in addition the IOBW could be more effective in removing internal than external contamination and finally, the whole carcass rinse sampling method, may not provide a reliable estimation of the *E. coli* load on the internal cavity of carcasses.

In conclusion, the studies conducted on artificially contaminated carcasses suggest that the *Enterobacteriaceae* and *E. coli* counts in samples taken at different stages along the slaughter

processing line are generally not influenced by the level of faecal contamination of carcasses, especially when samples were collected at the end of the slaughter processing line. A distinction should be done as regards external vs internal contamination: external contamination recorded higher *E. coli* counts compared to the internal contamination counts.

However, these data were obtained on artificially contaminated carcasses, which may not always faithfully mimic natural contamination since bacteria may adhere less persistently on artificially contaminated carcasses than on naturally contaminated ones.

2.5.2.2. Studies conducted in commercial slaughterhouses – data on naturally contaminated carcasses

Jimenez et al. (2003) compared *E. coli* and *Enterobacteriaceae* counts on carcasses with and without visual faecal contamination at different steps of the slaughter processing line (after evisceration, after inside-outside washing, and after chilling).

After evisceration, the counts of visually contaminated carcasses were significantly higher only for *E. coli*. After the IOBW, there were significant differences between carcasses with and without visible faecal contamination for both indicator bacteria. In contrast, after chilling (with 25 ppm of chlorinated water) those differences were no longer significant for either of the indicator bacteria. These data confirmed that up to the last step of the slaughter process a difference in terms of indicator bacteria counts was present between carcasses with and without visible faecal contamination. The immersion chilling, when chlorine was used, however, resulted in a considerable reduction of the level of contamination for all the analysed samples.

A similar investigation, conducted in seven processing plants in the US, compared the *E. coli* counts on broiler carcasses with and without visible ingesta contamination at the pre- and post-immersion chilling steps in which chlorine was applied at different concentration. No statistically significant differences in *E. coli* loads between these two groups were detected either before or after chilling. These finding suggested the lack of direct correlation between the presence of faecal material and *E. coli* contamination on carcasses (Bilgili et al., 2002).

Finally Russell (2003) investigated the percentage of carcasses with visual faecal contamination before the IOBW comparing a batch of broilers with a history of airsaccultis with a healthy group. The first group presented a higher level of faecal contamination and this finding was likely due to the fact that the airsaccultis-positive group presented more variability in terms of weight, which resulted in an increased number of processing errors along the slaughtering line. *E. coli* counts were higher for the airsacculitis group for only two out of the five samples, but the opposite was found when repeating this experiment. So, this study did not find evidence of a direct correlation between the level of faecal contamination of carcasses and *E. coli* load on those carcasses.

In conclusion, the results of the studies conducted on carcasses naturally contaminated with faecal material confirmed that the presence of visible faecal contamination has no predictive value for estimating the microbial quality of carcasses especially at the end of the slaughter processing line. However, for two out of three retrieved studies chlorine was used and for the third study no information was available about the use of chlorine.

Despite the retrieved studies providing relevant data for review question 3 were quite limited, both the data obtained on artificially and naturally contaminated carcasses suggested that there is no correlation between the bacteria loads of carcasses and their faecal contamination during the slaughtering process.

Table 3: Relationship between visual faecal contamination (VFC) and *E. coli* (EC), *Enterobacteriaceae* (EB) counts on broiler carcasses at different stages: natural contamination. AE: After evisceration; AW: after washing; BC: Before chilling; BW: Before washing; EU: unit of enumeration; IB: indicator bacteria; NR: not reported; N*: number of samples; M*: mean; SD*: standard deviation.

	Reference (country)	Study type	Contamination	Batch ID	Point where VFC was assessed	IB	EU	After defeathering - Before evisceration			After evisceration – Before washing			After washing – Before chilling			After chilling		
								N*	M*	SD*	N*	M*	SD*	N*	M*	SD*	N*	M*	SD*
Group 3	Jimenez et al., 2003 (AR)	Commercial slaughterhouse	Natural	Faecal	AE; AW	EC	log CFU/ml	-	-	-	-	3.54	-	-	3.44	-	NR	2.88	-
				Clean				-	-	-	-	2.72	-	-	2.49	-	NR	1.60	-
				Faecal		EB		-	-	-	-	3.83	-	-	3.72	-	NR	2.70	-
				Clean				-	-	-	-	3.65	-	-	3.29	-	NR	2.93	-
	Bilgili et al., 2002 (US)	Commercial slaughterhouse	Natural	1- visible ingesta	BC	EC	log CFU/ml	-	-	-	-	-	-	280	2.36	-	280	1.22	-
				2- no visible ingesta				-	-	-	-	-	-	280	2.35	-	280	1.22	-
Group 4	Russell, 2003 (US)	Commercial slaughterhouse	Natural	Airsac + replicate 1	BW	EC	log CFU/ml	-	-	-	2/100[+]	2.88[++]	-	-	-	-	-	-	-
				Airsac - replicate 1				-	-	-	1/100[+]	2.38[++]	-	-	-	-	-	-	-
				Airsac + replicate 2				-	-	-	9/100[+]	2.02[++]	-	-	-	-	-	-	-
				Airsac + replicate 2				-	-	-	0/100[+]	1.85[++]	-	-	-	-	-	-	-
				Airsac + replicate 3				-	-	-	13/100[+]	2.08[++]	-	-	-	-	-	-	-
				Airsac - replicate 3				-	-	-	2/100[+]	1.59[++]	-	-	-	-	-	-	-
				Airsac + replicate 4				-	-	-	9/100[+]	1.89[++]	-	-	-	-	-	-	-
				Airsac - replicate 4				-	-	-	0/100[+]	1.91[++]	-	-	-	-	-	-	-
				Airsac + replicate 5				-	-	-	5/100[+]	1.98[++]	-	-	-	-	-	-	-
				Airsac - replicate 5				-	-	-	0/100[+]	2.30[++]	-	-	-	-	-	-	-

[+] N° of carcasses with visible faecal contamination/N° of sampled carcasses; [++] Counts obtained from 20 carcasses for each batch

Table 3 (Continued): Relationship between visual faecal contamination (VFC) and *E. coli* (EC), *Enterobacteriaceae* (EB) counts on broiler carcasses at different stages: artificial contamination. AE: Evisceration: After evisceration; BW: Before Washing; CC Cross-contamination; DC: Direct Contamination; FC: Faecal Contamination; IB: indicator bacteria; NR: Not Reported; S: Squeezing; SW: Squeezing-Washing; W: Washing**;** N*: number of samples; M*: mean: SD*: standard deviation

	Reference (country)	Study type	Contamination	Batch ID	Point where VFC was assessed	IB	EU	After defeathering - Before evisceration			After evisceration – Before washing			After washing – Before chilling			After chilling		
								N*	M*	SD*	N*	M*	SD*	N*	M*	SD*	N*	M*	SD*
Group 2	Cason et al., 2004a (US)	Pilot slaughterhouse	Artificial	Control	BW	EB	log CFU	-	-	-	-	-	-	18	5.9	0.4	18	5.6	0.5
				FC#				-	-	-	-	-	-	18	6.3	0.5	18	5.6	0.3
				Control		EC		-	-	-	-	-	-	18	6.3	0.4	18	5.4	0.5
				FC#				-	-	-	-	-	-	18	6.4	0.5	18	5.5	0.4
	Smith et al., 2005 (US)	Pilot slaughterhouse	Artificial	Artificial contamination	NR	EC	log CFU/ml	-	-	-	-	-	-	24/24	6.2	-	-	-	-
				1- DC#/contaminated chiller				-	-	-	-	-	-	-	-	-	24/24	2.7	0.1
				2- DC#/control chiller				-	-	-	-	-	-	-	-	-	24/24	2.6	0.1
				3- CC°/contaminated chiller				-	-	-	-	-	-	-	-	-	24/24	2.6	0.1
				4- CC°/control chiller				-	-	-	-	-	-	-	-	-	24/24	2.6	0.1
Group 4	Northcutt et al., 2008a (US)	Pilot slaughterhouse	Artificial	S (faecal)	NR	EC	log CFU/ml	50	5.4	0.1	-	-	-	-	-	-	-	-	-
				W (clean)				50	5.3	0.1	-	-	-	-	-	-	-	-	-
				SW (clean)				50	5.4	0.1	-	-	-	-	-	-	-	-	-
	Smith et al., 2007 (US)	Pilot slaughterhouse	Artificial	External contamination ##	AE	EC	log CFU/ml	-	-	-	-	-	-	11/12	4.9	0.2	-	-	-
				Internal contamination ##				-	-	-	-	-	-	12/12	4.2	0.2	-	-	-
				Control (clean)				-	-	-	-	-	-	12/12	3.6	0.2	-	-	-

carcasses artificially contaminated with 0.1 g of faeces; ## carcasses artificially contaminated with 1 g of faeces; ° carcasses in the same chiller with the artificially contaminated halves but not directly contaminated with caecal content

3. Conclusions and Remarks

- The studies described in the selected papers used a wide range of experimental designs and thus differ in relation to settings, laboratory methods, sample types and unit of enumeration. All these parameters impacted the results of the counts of the considered indicator bacteria.

- The great variability of bacterial loads on carcasses in the different studies makes it very difficult to compare the data produced by the different studies in a quantitative way and to assess the overall reduction of indicator bacteria loads at the different stages along the slaughter processing line and in relation to the investigated risk factors.

REVIEW QUESTION 1. Presence of the indicator organisms *E. coli* and *Enterobacteriaceae* and their counts on carcasses during different stages in the slaughter processing line.

- According to the majority of the retrieved papers, the steps of the slaughter processing line where a decrease of the *E. coli* counts on poultry/broiler carcasses was reported were scalding, washing and chilling.

- Considering *Enterobacteriaceae* counts, scalding and washing were the stages that contributed to reduce the bacterial loads on broiler carcasses; the effect of the chilling step is controversial.

REVIEW QUESTION 2. Information that could explain the variability of the counts of the indicator organisms.

- As far as batch-related risk factors are concerned, the studies evaluated failed to demonstrate any relationship between farming practices and the counts of indicator bacteria broiler carcasses, with the exception of one study that found an increase in *E. coli* counts according to the age of the animals.

- Considering the effect of risk factors on bacterial counts, the use of physical treatments at the washing step (steam pasteurization, hot water, high pressure washing treatment) could be a valid solution to reduce the carcass bacterial counts. However, the studies generally refer to pilot scenarios and report that even small variations in such treatments are able to produce different results and to cause damage to the broiler carcasses.

- In relation to the chilling step, the water immersion chilling practices, like widening water renewal time, reusing of chilling water, reducing the ratio of litres of water per Kg of carcass did not influence the indicator bacteria counts on broiler carcasses significantly.

- Chlorine use represents an important factor of variability among the evaluated studies; however, its use was not always linked in a significant way with differences observed during the slaughter operations. In fact, some studies failed to classify the absence of chlorine use as a risk factor.

- The trend of bacterial counts depends on slaughterhouse characteristics, but it is difficult to assess which factors most impact the counts. Moreover, neither the slaughterhouse dimension nor the level of mechanization were found to be correlated to the counts of *E. coli* or *Enterobacteriaceae* on poultry carcasses.

REVIEW QUESTION 3. The potential relationship between the counts of indicator organisms and visual faecal contamination on carcasses.

- Among the seven retrieved papers, four studies considered artificially contaminated broiler carcasses collected in pilot slaughterhouses, and three studies provided data on naturally contaminated carcasses obtained in commercial slaughterhouses.

- Both the data obtained on artificially and naturally contaminated poultry/broiler carcasses suggest that there is no correlation between the bacteria loads of carcasses (*E.coli* and *Enterobacteriaceae*) and faecal contamination.

Remarks

- Further studies are necessary and should strive to provide more details in order to allow comparison of studies and to quantitatively summarize their data.

- Results of papers included in this review are strongly influenced by several fundamental aspects, such as type of sample (rinse, skin excision, swab) and unit of enumeration. Consequently, it would be important to standardize the experimental design and the expression of results in order to allow proper scientific comparison between studies.

REFERENCES

Acikgoz Z, Bayraktar H, Altan O, 2011. Effects of formic acid administration in the drinking water on performance, intestinal microflora and carcass contamination in male broilers under high ambient temperature. Asian Australas J Anim Sci 24(1):96-102.

Aksit M, Goksoy E, Kok F, Ozdemir D, Ozdogan M, 2006. The impacts of organic acid and essential oil supplementations to diets on the microbiological quality of chicken carcasses. Archiv Fur Geflugelkunde 70(4):168-73.

Altekruse SF, Berrang ME, Marks H, Patel B, Shaw WK,Jr, Saini P, Bennett PA, Bailey JS, 2009. Enumeration of *Escherichia coli* cells on chicken carcasses as a potential measure of microbial process control in a random selection of slaughter establishments in the United States. Appl Environ Microbiol 75(11):3522-7.

Barbut S, Moza LF, Nattress F, Dilts B, Gill CO, 2009. The microbiological conditions of air- or water-chilled carcasses produced at the same poultry packing plant. J Appl Poultry Res 18(3):501-7.

Barco L, Belluco S, Roccato A and Ricci A, 2014. *Escherichia* coli and *Enterobacteriaceae* counts on pig and ruminant carcasses along the slaughterline, factors influencing the counts and relationship between visual faecal contamination of carcasses and counts: a review. EFSA supporting publication 2014:EN-634, 111 pp.

Berrang M and Dickens J, 2000. Presence and level of *Campylobacter* spp. on broiler carcasses throughout the processing plant. Journal of Appl Poultry Res 9(1):43-7.

Berrang ME, Dickens JA, Musgrove MT, 2000. Effects of hot water application after defeathering on the levels of campylobacter, Coliform bacteria, and *Escherichia coli* on broiler carcasses. Poult Sci 79(11):1689-93.

Berrang ME, Ladely SR, Buhr RJ, 2001. Presence and level of *Campylobacter*, coliforms, *Escherichia coli*, and total aerobic bacteria recovered from broiler parts with and without skin. J Food Prot 64(2):184-8.

Berrang ME, Buhr RJ, Cason JA, Dickens JA, 2002. Microbiological consequences of skin removal prior to evisceration of broiler carcasses. Poult Sci 81(1):134-8.

Berrang ME, Meinersmann RJ, Buhr RJ, Reimer NA, Philips RW, Harrison MA, 2003. Presence of campylobacter inthe respiratory tract of broiler carcasses before and after commercial scalding. Poult Sci 82(12):1995-9.

Berrang ME and Bailey JS. 2009. On-line brush and spray washers to lower numbers of *Campylobacter* and *Escherichia coli* and presence of salmonella on broiler carcasses during processing. J Appl Poultry Res 18(1):74-8.

Berrang ME, Windham WR, Meinersmann RJ, 2011a. *Campylobacter*, *Salmonella*, and *Escherichia coli* on broiler carcasses subjected to a high pH scald and low pH postpick chlorine dip. Poult Sci 90(4):896-900.

Berrang ME, Meinersmann RJ, Cox NA, Fedorka-Cray PJ, 2011b. Application of chlorine dioxide to lessen bacterial contamination during broiler defeathering. J Appl Poultry Res 20(1):33-9

Berrang ME and Bailey JS, 2008. *Campylobacter*, *Salmonella* and *E. coli* on broiler carcasses from commercial plants under HIMP inspection. Poult Sci 87:134-.

Berrang ME, Meinersmann RJ, Smith DP, Zhuang H, 2008a. The effect of chilling in cold air or ice water on the microbiological quality of broiler carcasses and the population of campylobacter. Poult Sci 87(5):992-8.

Berrang ME, Bailey JS, Altekruse SF, Shaw WK, Jr, 2008b. Presence and numbers of *Campylobacter*, *Escherichia coli*, and *Salmonella* determined in broiler carcass rinses from United States processing plants in the hazard analysis and critical control point-based inspection models project. J Appl Poultry Res 17(3):354-60.

Bilgili S, Waldroup A, Zelenka D, Marion J, 2002. Visible ingesta on prechill carcasses does not affect the microbiological quality of broiler carcasses after immersion chilling. J Appl Poultry Res 11(3):233-8.

Bohaychuk VM, Checkley SL, Gensler GE, Barrios PR, 2009. Microbiological baseline study of poultry slaughtered in provincially inspected abattoirs in Alberta, Canada. Can Vet J 50(2):173-8.

Buhr RJ, Cason JA, Dickens JA, Hinton A, Jr, Ingram KD, 2000. Influence of flooring type during transport and holding on bacteria recovery from broiler carcass rinses before and after defeathering. Poult Sci 79(3):436-41.

Buhr RJ, Berrang ME, Cason JA, 2003. Bacterial recovery from breast skin of genetically feathered and featherless broiler carcasses immediately following scalding and picking. Poult Sci 82(10):1641-7.

Buhr RJ, Bourassa DV, Northcutt JK, Hinton A, Jr, Ingram KD, Cason JA, 2005. Bacteria recovery from genetically feathered and featherless broiler carcasses after immersion chilling. Poult Sci 84(9):1499-504.

Cason JA, Buhr RJ, Hinton A, Jr, 2001. Unheated water in the first tank of a three-tank broiler scalder. Poult Sci 80(11):1643-6.

Cason JA, Berrang ME, Buhr RJ, Cox NA, 2004a. Effect of prechill fecal contamination on numbers of bacteria recovered from broiler chicken carcasses before and after immersion chilling. J Food Prot 67(9):1829-33.

Cason JA, Hinton A, Jr, Buhr RJ, 2004b. Impact of feathers and feather follicles on broiler carcass bacteria. Poult Sci 83(8):1452-5.

Cavani R, Schocken-Iturrino RP, Fernandes Lopes Garcia TC, de Oliveira AC, 2010. Comparison of microbial load in immersion chilling water and poultry carcasses after 8, 16 and 24 working hours. Ciencia Rural 40(7):1603-9.

Cibin V, Mancin M, Pedersen K, Barrucci F, Belluco S, Roccato A, Cocola F, Ferrarini S, Sandri A, Lau Baggesen D, Ricci A, 2014. Usefulness of *Escherichia coli* and *Enterobacteriaceae* as Process Hygiene Criteria in poultry: experimental study. EFSA supporting publication 2014:EN-635, 121 pp.

Cox NA, Richardson LJ, Cason JA, Buhr RJ, Vizzier-Thaxton Y, Smith DP, Fedorka-Cray PJ, Romanenghi CP, Pereira LV, Doyle MP, 2010. Comparison of neck skin excision and whole carcass rinse sampling methods for microbiological evaluation of broiler carcasses before and after immersion chilling. J Food Prot 73(5):976-80.

Escudero-Gilete M, Gonzalez-Miret M, Heredia F, 2005. Multivariate study of the decontamination process as function of time, pressure and quantity of water used in washing stage after evisceration in poultry meat production. J Food Eng 69(2):245-51.

Fluckey WM, Sanchez MX, McKee SR, Smith D, Pendleton E, Brashears MM, 2003. Establishment of a microbiological profile for an air-chilling poultry operation in the United States. J Food Prot 66(2):272-9.

Geornaras I and von Holy A, 2000. Bacterial counts associated with poultry processing at different sampling times. J Basic Microbiol 40(5-6):343-9.

Gill C and Badoni M, 2005. Recovery of bacteria from poultry carcasses by rinsing, swabbing or excision of skin. Food Microbiol 22(1):101-7.

Gill CO, Moza LF, Badoni M, Barbut S, 2006. The effects on the microbiological condition of product of carcass dressing, cooling, and portioning processes at a poultry packing plant. Int J Food Microbiol 110(2):187-93.

Goksoy EO, Kirkan S, Kok F, 2004. Microbiological quality of broiler carcasses during processing in two slaughterhouses in turkey. Poult Sci 83(8):1427-32.

Gonzalez-Miret ML, Escudero-Gilete ML, Heredia FJ, 2006. The establishment of critical control points at the washing and air chilling stages in poultry meat production using multivariate statistics. Food Control 17(12):935-41.

Hannah JF, Fletcher DL, Cox NA, Smith DP, Cason JA, Northcutt JK, Richardson LJ, Buhr RJ, 2009. Impact of added sand on the recovery of *Salmonella, Sampylobacter, Escherichia coli,* and coliforms from prechill and postchill commercial broiler carcass halves. J Appl Poultry Res 18(2):252-8.

Hecer C, Balci F, Udum CD, 2007. The effects of ozone and chlorine applications on microbiological quality of chickens during processing. J Biol Environ Sci 1:131-8.

Ho CP, Huang NY, Chen BJ, 2004. A survey of microbial contamination of food contact surfaces at broiler slaughter plants in Taiwan. J Food Prot 67(12):2809-11.

Huezo R, Northcutt JK, Smith DP, Fletcher DL, Ingram KD, 2007. Effect of dry air or immersion chilling on recovery of bacteria from broiler carcasses. J Food Prot 70(8):1829-34.

Hutchison ML, Walters LD, Mead GC, Howell M, Allen VM, 2006. An assessment of sampling methods and microbiological hygiene indicators for process verification in poultry slaughterhouses. J Food Prot 69(1):145-53.

Jimenez SM, Tiburzi MC, Salsi MS, Pirovani ME, Moguilevsky MA, 2003. The role of visible faecal material as a vehicle for generic *Escherichia coli*, coliform, and other Enterobacteria contaminating poultry carcasses during slaughtering. J Appl Microbiol 95(3):451-6.

Kemp GK, Aldrich ML, Guerra ML, Schneider KR, 2001. Continuous online processing of fecal- and ingesta-contaminated poultry carcasses using an acidified sodium chlorite antimicrobial intervention. J Food Prot 64(6):807-12.

Lindblad M, Lindmark H, Lambertz ST, Lindqvist R, 2006. Microbiological baseline study of broiler chickens at Swedish slaughterhouses. J Food Prot 69(12):2875-82.

Line JE, Oakley BB, Stern NJ, 2013. Comparison of cumulative drip sampling with whole carcass rinses for estimation of *Campylobacter* species and quality indicator organisms associated with processed broiler chickens. Poult Sci 92(1):218-24.

Line JE, Stern NJ, Oakley BB, Seal BS, 2011. Comparison of an automated most-probable-number technique with traditional plating methods for estimating populations of total aerobes, Coliforms, and *Escherichia coli* associated with freshly processed broiler chickens. J Food Prot 74(9):1558-63.

Matias BG, Pinto PS, Cossi MV, Nero LA, 2010. *Salmonella* spp. and hygiene indicator microorganisms in chicken carcasses obtained at different processing stages in two slaughterhouses. Foodborne Pathog Dis 7(3):313-8.

Northcutt JK, Buhr RJ, Berrang ME, Fletcher DL, 2003a. Effects of replacement finisher feed and length of feed withdrawal on broiler carcass yield and bacteria recovery. Poult Sci 82(11):1820-4.

Northcutt JK, Berrang ME, Dickens JA, Fletcher DL, Cox NA, 2003b. Effect of broiler age, feed withdrawal, and transportation on levels of coliforms, Campylobacter, *Escherichia coli* and *Salmonella* on carcasses before and after immersion chilling. Poult Sci 82(1):169-73.

Northcutt J, Berrang M, Smith D, Jones D, 2003c. Effect of commercial bird washers on broiler carcass microbiological characteristics. J Appl Poultry Res 12(4):435-8.

Northcutt JK, Smith DP, Musgrove MT, Ingram KD, Hinton A, Jr, 2005. Microbiological impact of spray washing broiler carcasses using different chlorine concentrations and water temperatures. Poult Sci 84(10):1648-52.

Northcutt JK, Cason JA, Smith DP, Buhr RJ, Fletcher DL, 2006. Broiler carcass bacterial counts after immersion chilling using either a low or high volume of water. Poult Sci 85(10):1802-6.

Northcutt J, Smith D, Ingram KD, Hinton A, Jr, Musgrove M, 2007. Recovery of bacteria from broiler carcasses after spray washing with acidified electrolyzed water or sodium hypochlorite solutions. Poult Sci 86(10):2239-44.

Northcutt JK, McNeal WD, Ingram KD, Buhr RJ, Fletcher DL, 2008a. Microbial recovery from genetically featherless broiler carcasses after forced cloacal fecal expulsion. Poult Sci 87(11):2377-81.

Northcutt JK, Smith D, Huezo RI, Ingram KD, 2008b. Microbiology of broiler carcasses and chemistry of chiller water as affected by water reuse. Poult Sci 87(7):1458-63.

Northcutt JK, Cason JA, Ingram KD, Smith DP, Buhr RJ, Fletcher DL, 2008c. Recovery of bacteria from broiler carcasses after immersion chilling in different volumes of water, part 2. Poult Sci 87(3):573-6.

Northcutt JK, McNeal WD, Ingram KD, 2008d. Effects of shackle line speed on broiler carcass microbiology and presentation scores. Poult Sci 87:122-.

Oyarzabal OA, Hawk C, Bilgili SF, Warf CC, Kemp GK, 2004. Effects of postchill application of acidified sodium chlorite to control *Campylobacter* spp. and *Escherichia coli* on commercial broiler carcasses. J Food Prot 67(10):2288-91.

Potter BD, Marcy JA, Owens CM, Slavik MF, Goodwin HL, Apple JK, 2012. Impact of performance-based sanitation systems on microbiological characteristics of poultry processing equipment and carcasses as compared with traditional sanitation systems. J Appl Poultry Res 21(3):669-78.

Purnell G, Mattick K, Humphrey T, 2004. The use of 'hot wash' treatments to reduce the number of pathogenic and spoilage bacteria on raw retail poultry. J Food Eng 62(1):29-36.

Russell SM, 2003. The effect of airsacculitis on bird weights, uniformity, fecal contamination, processing errors, and populations of *Campylobacter* spp. and *Escherichia coli*. Poult Sci 82(8):1326-31.

Russell SM and Axtell SP, 2005. Monochloramine versus sodium hypochlorite as antimicrobial agents for reducing populations of bacteria on broiler chicken carcasses. J Food Prot 68(4):758-63.

Sanchez MX, Fluckey WM, Brashears MM, McKee SR, 2002. Microbial profile and antibiotic susceptibility of *Campylobacter* spp. and *Salmonella* spp. in broilers processed in air-chilled and immersion-chilled environments. J Food Prot 65(6):948-56.

Smith DP, Cason JA, Berrang ME, 2005. Effect of fecal contamination and cross-contamination on numbers of coliform, *Escherichia coli*, *Campylobacter*, and *Salmonella* on immersion-chilled broiler carcasses. J Food Prot 68(7):1340-5.

Smith D, Altekruse SF, Bailey JS, 2007a. Assessment of post-hurricane Katrina recovery in poultry slaughter establishments. J Food Prot 70(6):1498-501.

Smith DP, Northcutt JK, Cason JA, Hinton A,Jr, Buhr RJ, Ingram KD, 2007b. Effect of external or internal fecal contamination on numbers of bacteria on prechilled broiler carcasses. Poult Sci 86(6):1241-4.

Smulders FJM, Gleisz B, Sofka D, Sacher A, Omurtag I, Paulsen P, Hilbert F, 2011. Microbial ecology on poultry carcasses along the production line. Archiv Fur Lebensmittelhygiene 62(5):170-4.

Souza LC, Jr, Pereira JG, Spina TL, Izidoro TB, Oliveira AC, Pinto JP, 2012. Microbiological evaluation of chicken carcasses in an immersion chilling system with water renewal at 8 and 16 hours. J Food Prot 75(5):973-5.

Stopforth JD, O'Connor R, Lopes M, Kottapalli B, Hill WE, Samadpour M, 2007. Validation of individual and multiple-sequential interventions for reduction of microbial populations during processing of poultry carcasses and parts. J Food Prot 70(6):1393-401.

Sumner J, Raven G, Dean P, Dowsett P, Petrenas E, Weiring R, West G, Lillie M, Holds G, Pointon A, 2004. A microbiological profile of poultry processed in south Australia. Food Aust 56(7):335-40.

Svobodova I, Borilova G, Hulankova R, Steinhauserova I, 2012. Microbiological quality of broiler carcasses during slaughter processing. Acta Veterinaria Brno 81(1):37-42.

Thanissery R, Kathariou S, Siletzky RM, Smith DP, 2012. Microbiology of prechill carcasses from medium- and fast-growing pastured broiler chicken strains. J Appl Poultry Res 21(3):623-9.

Vaidya V, Paturkar A, Waskar V, Zende R, Rawool D, 2005. Detection of indicator organisms on poultry carcass sites in an organized slaughterhouse. Journal of Muscle Foods 16(4):289-97.

Whyte P, Collins JD, McGill K, Monahan C, O'Mahony H, 2001. Quantitative investigation of the effects of chemical decontamination procedures on the microbiological status of broiler carcasses during processing. J Food Prot 64(2):179-83.

Whyte P, McGill K, Collins J, 2003. An assessment of steam pasteurization and hot water immersion treatments for the microbiological decontamination of broiler carcasses. Food Microbiol 20(1):111-7.

Whyte P, McGill K, Monahan C, Collins J, 2004. The effect of sampling time on the levels of micro-organisms recovered from broiler carcasses in a commercial slaughter plant. Food Microbiol 21(1):59-65.

OTHER REFERENCES CITED

Bilgili SF, 2010. Poultry meat inspection and grading, pp. 67-97. In Poultry meat processing, Second Edition, CRC Press.

EFSA (European Food Safety Authority), 2012. Application of systematic review methodology for food and feed safety assessments to support decision making. EFSA Journal 2012;8(6):1637, 90 pp. doi:10.2903/j.efsa.2010.1637

EFSA (European Food Safety Authority), 2012. Scientific Opinion of the EFSA Panels on Biological Hazards (BIOHAZ), on Contaminants in the Food Chain (CONTAM), and on Animal Health and Welfare (AHAW) on the public health hazards to be covered by inspection of meat (poultry). EFSA Journal 2012;10(6):2741, 179 pp. doi:10.2903/j.efsa.2012.2741

Ghefir Y, China B, Dierick K., De Zutter, Daube G, 2008. Hygiene indicator microorganisms for selected pathogens on beef, pork, and poultry meats in Belgium. J Food Prot, 71: 35-45.

Guerin MT, Sir C, Sargeant JM, Waddell L, O'Connor AM, Wills RW, Bailey RH, Byrd JA, 2010. The change in prevalence of *Campylobacter* on chicken carcasses during processing: a systematic review. Poult Sci 89: 1070-1084.

Jay JM, Loessner MJ and Golden DA, 2005. Indicators of food microbial quality and safety pp. 473-495. In Modern food microbiology, Seventh Edition, Food Science Text Series, Springer.

Löhren U, 2012. Overview on current practices of poultry slaughtering and poultry meat inspection. Supporting publication 298, pp. 1-58.

Mead GC, 2004. Microbial hazards in producing and processing, pp. 232-251. In poultry meat processing and quality, CRC Press, Boca Raton Boston New York Washington, DC.

Sams AR and McKee SR, 2010. First processing – Slaughter through chilling, pp 25-49. In Poultry meat processing, Second Edition, CRC Press

Schaffner DW and Smith S, 2004. Indicator organisms, pp. 773-773. In Encyclopaedia of Meat Sciences, Elsevier, Academic Press, Amsterdam.

Smooth LM and Pierson MD, 1997. Indicator microorganisms and microbiological criteria, pp. 66-80. In Food Microbiology fundamentals and frontiers, ASM Press, Washington D.C.

USDA, 1996. Pathogen reduction: hazard analysis and critical control point (HACCP) systems, final rule, 9 CFR Part 304. Food Safety and Inspection Service, U.S. Department of Agriculture, Washington D.C.

APPENDICES

Appendix A. Relevance Screening

The checklists used for the assessment of the relevance of the retrieved papers are reported below. Neutral means that the answer was only informative and didn't lead to paper exclusion.

First level assessment – step 1

Questions	Answers	Inclusion/Exclusion
Language of the paper	English	Inclusion
	French	Inclusion
	Italian	Inclusion
	Spanish	Inclusion
	German	Exclusion
	Other	Exclusion
Is the paper a primary research paper?	Yes	Inclusion
	No	Exclusion
	Unknown	Inclusion
Does the paper consider main livestock species?	Yes	Inclusion
	No	Exclusion
	Unknown	Inclusion
Does the paper provides data on presence/counts of (non pathogenic) *Escherichia coli* or *Enterobacteriaceae*?	Yes	Inclusion
	No	Exclusion
	Unknown	Inclusion
Is the main aim of the paper the investigation of antimicrobial resistance and does the paper provide no pertinent data for the scope of the review?	Yes	Exclusion
	No	Inclusion
	Unknown	Inclusion

First level assessment – step 2

Questions	Answers	Inclusion/Exclusion
Which animal species are considered in the paper ?	Ruminants	Neutral
	Swine	Neutral
	Poultry	Neutral
	Horses	Neutral
	Others	Neutral

Does the paper provide data on more than one stage of the slaughter processing line or data on risk factors influencing the loads of indicator bacteria on carcasses?	Yes	Inclusion
	No	Exclusion
	Unknown	Inclusion
Does the paper provide data on carcasses?	Yes	Inclusion
	No	Exclusion
	Unknown	Inclusion
Does the paper provide data obtained in slaughterhouses that do not use chemical decontaminants other than chlorine	Yes	Inclusion
	No	Exclusion
	Unknown	Inclusion
Does the paper consider a real setting?	Yes	Neutral
	No	Neutral
	Unknown	Neutral

Second level assessment

Questions	Answers	Inclusion/Exclusion
Where was the paper conducted?	Europe	Neutral
	Canada and US	Neutral
	Central and South America	Neutral
	Africa	Neutral
	Asia	Neutral
	Oceania	Neutral
Does the paper provide original data?	Yes	Inclusion
	No	Exclusion
Does the paper provide data on the carcasses?	Yes	Inclusion
	No	Exclusion
Which indicator organism/s is/are considered in the paper?	*Eschericha coli*	Inclusion
	Enterobacteriaceae	Inclusion
	None of them	Exclusion
	Total coliforms	Neutral

	Faecal coliforms	Neutral
Which kind of scenario is considered?	Real setting	Inclusion
	Pilot slaughterhouse	Inclusion
	Artificial contamination with *E. coli* and/or *Enterobacteriaceae* strains	Exclusion
Which kind of sample is collected?	Rinse	Neutral
	Excision	Neutral
	Drip	Neutral
	Swab	Neutral
	Others	Neutral
What is the study aim?	Counts at different stages	Inclusion
	Factors influencing the counts	Inclusion
	Relation between visual faecal contamination and counts of indicator bacteria	Inclusion
	None	Exclusion
At which stage/s of the processing line were the samples collected?	Killing	Neutral
	Before Scalding	Neutral
	After scalding	Neutral
	Before defeathering	Neutral
	After defeathering	Neutral
	Before evisceration	Neutral
	After evisceration	Neutral
	Before washing	Neutral
	After washing	Neutral
	Before chilling	Neutral

	After chilling	Neutral
	Others	Neutral
If a factor influencing the counts was considered, is that factor related to:	Slaughterhouse characteristics	Neutral
	Batch information (e.g. feed withdrawal)	Neutral
	Analytical Method	Neutral

Appendix B. Data Collection

The forms used for the collection of data from the selected papers related to poultry are reported below

General information

Questions	Answers
Reference ID	
Type of reference	Article
	Technical report
	Proceeding/meeting abstract
	Other
Study aim	Counts at different stages
	Factors influencing the counts
	Relationship between visual faecal contamination and counts
Start year of the study	(YYYY)
End year of the study	(YYYY)
Country where the study was conducted	
Type of study	Experimental
	Observational
Species considered in the study	Broilers
	Laying hens
	Turkeys
	Ducks
	Others
Weight (Kg)	
Age (days)	
Feed withdrawal before slaughtering (hours)	
Transport time to the slaughterhouse (hours)	
Weight variability within the sampled group	
If it is an experimental study it can be classified as	Randomized Control Trial
	Challenge Trial (no control group)
If it is an observational study it can be classified as	Cohort
	Case-control

	Cross-sectional
Sample size (number)	
Significance (alfa) e.g. 0.05	
Power (1-beta) e.g. 0.80	
Effect size (delta)	

Slaughterhouse features

Questions	Answers
Study ID	
Slaughter ID	
Number of animals slaughtered/year	
Number of animals slaughtered/day	
Number of animals slaughtered/hour	
Type of stunning	Electrical
	Gas
	Other
	NR
Scalding temperature (°C)	
Scalding time (seconds)	
Type of scalding	Single bath scalding tank
	Single bath with counterflow
	Multi bath scalding tanks
	Multi bath scalding tanks with counterflow
	NR
Type of defeathering	Vertical disk
	Counter rotating
	NR
Type of evisceration	Automatic
	Manual
	NR
Type of washing	

Type of chilling	Air
	Immersion
	Spray
	NR
Chilling time (minutes)	
Chilling temperature (°C)	
Temperature of the carcasses at the end of chilling (°C)	
Was chlorine used as decontaminant?	Yes
	No
	NR
At which stage/s was chlorine used	Scalding
	Washing
	Chilling
	NR

Data collection

Questions	Answers
Study aim	Counts at different stages
	Factors influencing the counts
	Relationship between visual fecal contamination and counts
Slaughterhouse identification	
Group identification	
The factor influencing the counts considered in the paper is related to the	Batch
	Slaughter technique – management
	Faecal contamination
	None
Factor related to the batch	Age
	Feed withdrawal
	Diet
	Other

Specify the factor related to slaughter technique – management investigated in the study		
How was the level faecal contamination of carcasses categorized?		
When was the level of faecal contamination evaluated?	Ante-mortem	
	Post- mortem	
Sample type 1	Single	
	Pool	
Sample type 2	Rinse	(ml)
	Skin Excision	(g)
	Meat Excision	(g)
	Drip	
	Swab	(cm^2)
	Other	
Sampled region of the carcass	Neck	
	Breast	
	Whole carcass	
	Other	
	NR	
Analytical method	Traditional colony count	
	Petrifilm	
	MPN	
	Membrane filtration	
	Molecular method	
	Others	
Unit of enumeration	CFU/ml	
	CFU/g	
	CFU/cm^2	
	logCFU/ml	
	logCFU/g	
	$logCFU/cm^2$	
	MPN/ml	

	MPN/g	
	MPN/cm^2	
	Other	
Indicator bacteria considered	*Escherichia coli*	
	Enterobacteriaceae	
Counts before killing	Number of samples	
	Mean	
	SD	
	Min	
	Max	
	5 percentile	
	95 percentile	
	Prevalence (%)	
	Other	
Counts after killing - before scalding	Number of samples	
	Mean	
	SD	
	Min	
	Max	
	5 percentile	
	95 percentile	
	Prevalence (%)	
	Other	
Counts after scalding - before defeathering	Number of samples	
	Mean	
	SD	
	Min	
	Max	
	5 percentile	
	95 percentile	
	Prevalence (%)	

	Other	
Counts after defeathering - before evisceration	Number of samples	
	Mean	
	SD	
	Min	
	Max	
	5 percentile	
	95 percentile	
	Prevalence (%)	
	Other	
Counts after evisceration - before washing	Number of samples	
	Mean	
	SD	
	Min	
	Max	
	5 percentile	
	95 percentile	
	Prevalence (%)	
	Other	
Counts after washing - before chilling	Number of samples	
	Mean	
	SD	
	Min	
	Max	
	5 percentile	
	95 percentile	
	Prevalence (%)	
	Other	
Counts after chilling	Number of samples	
	Mean	
	SD	
	Min	

Counts after chilling	Max	
	5 percentile	
	95 percentile	
	Prevalence (%)	
	Other	
Counts at other stages	Number of samples	
	Mean	
	SD	
	Min	
	Max	
	5 percentile	
	95 percentile	
	Prevalence (%)	
	Other	
Stage/s where counts decrease	Killing	
	Scalding	
	Defeathering	
	Evisceration	
	Washing	
	Chilling	
	None	
Stage/s where counts increase	Killing	
	Scalding	
	Defeathering	
	Evisceration	
	Washing	
	Chilling	
	None	
Effect of the factor considered on the carcasses counts	Increase	
	Decrease	
	None	
Effect of the faecal contamination on carcass counts	Increase	

	Decrease
	None
Final statements	

Appendix C. Papers excluded at each stage of the screening process

The number of papers excluded at each step of the screening process in relation to the eligibility criteria are summarized in the tables below.

First level assessment – step 1

4321 out of 5160 papers were excluded at this stage

Eligibility criteria (EC)	Total n° of papers excluded for each EC	Total n° of papers that have not already been excluded for another EC reported above
Language (No: EN, FR, SP, IT)	34	34
No: The paper was a primary research paper	242	241
No: The paper provides data on the main livestock species	1814	1812
No: The paper provides data on the presence/counts of *Escherichia coli* and/or *Enterobacteriaceae*	3550	2146
Yes: The main scope of the study is antimicrobial resistance and no pertinent data are provided for the aims of the review	112	88

First level assessment – step 2

569 out of 839 papers were excluded at this stage

Eligibility criteria (EC)	Total n° of papers excluded for each EC	Total n° of papers that have not already been excluded for another EC reported above
No: The paper provides data on more than one stage of the slaughter processing line or data on risk factors influencing the loads of indicator bacteria on carcasses	464	464
No: The paper provides data on carcasses	334	59
No: The paper provides data obtained in slaughterhouses that do not use chemical decontaminants (chlorine was not considered)	86	46

Second level assessment

111 out of the 270 papers that arrived at the second level assessment provided data on poultry

33 out of the 111 papers related to poultry were excluded at this stage

Eligibility criteria (EC)	**Total n° of papers excluded for each EC**	**Total n° of papers that have not already been excluded for another EC reported above**
No: The paper was a primary research paper	6	6
No: The paper provides data on the presence/counts of *Escherichia coli* and/or *Enterobacteriaceae* . Coliforms were excluded at this stage	14	14
Yes: Animals/carcasses were artificially contaminated with *E. coli* and/or *Enterobacteriaceae*	6	6
No: The paper provides data on counts of *E. coli* and/or *Enterobacteriaceae* at more than one stage of the slaughter processing line or data on risk factors influencing the loads of *E. coli* and/or *Enterobacteriaceae* on carcasses or relationship between visual faecal contamination and *E. coli* and/or *Enterobacteriaceae* counts	7	7

Appendix D. Biases on indicator bacteria counts due to the sampling methods

Appendix D is aimed at discussing some of the variables related to the sampling and analytical methods that could lead to variability in the recovery of indicator bacteria from poultry carcasses, in order to address the interpretation and the discussion of the results presented in the review.

Since the topic of this Appendix was out of the scope of the present systematic review, it should be remarked that the data reported below do not represent an exhaustive presentation of all available relevant literature related to these issues.

The type of sampling technique used to collect samples from poultry carcass is a crucial factor affecting the accuracy of bacterial counts (Cossi et al., 2012). Differences in sample types and methodology used to collect them can hamper the comparability of data across different studies (Cox et al., 2010).

For poultry carcasses, different sampling techniques have been described, and they can be broadly categorized as destructive and non-destructive methods.

Among the non-destructive techniques, the most commonly used method is the carcass rinse. Briefly, a rinse sample consists of placing a carcass in a bag with a known volume (usually 100, 250, 300or 400 ml) of a suitable diluent. The carcass is either shaken or massaged within the bag for 60 to 120 s and then a portion of the diluent is used for analysis (Williams et al., 2010).

Swab sampling is included among the non-destructive methods, whereas the destructive methods include mainly tissue and skin excision.

The swabbing method can be used on small or large areas of the carcasses and different devices can be applied for this purpose. The collected swabs are shaken or stomached in the diluent, which is then analysed (McEvoy et al., 2005).

Excision is applied to relatively small areas of the carcass, and the collected tissue and/or skin samples are homogenized in diluent by stomaching or blending before being analysed (McEvoy et al., 2005).

Food industries tend to prefer non-destructive methods which maintain the integrity and quality of the carcass. However, the destructive methods can be adopted in such a way to preserve the characteristics of marketable parts of the carcasses. For instance, the neck is not generally a commercially viable cut, so it is a part of the carcass that can feasibly be used for excision sampling.

Currently, the U.S. Department of Agriculture Food Safety and Inspection Service (FSIS) prescribes the rinsing of individual broiler carcass with 400 ml of diluents for *Salmonella* detection and *E. coli*/coliform counts. The European Commission requires that a composite sample of 25g of neck skin of broiler carcasses is analysed for *Salmonella* detection (Cox et al., 2010).

Several studies have been conducted to clarify the comparability among these sampling methods.

Cossi et al. (2012) compared skin and tissue excision, rinse and skin swab methods and evidenced that, although tissue excision (both skin and meat) provided the highest counts of indicator microorganisms, the rinsing method is fully comparable with tissue excision. Also, Gill et al. (2005) confirmed that, in general, excision and rinsing provided comparable results. However, the authors demonstrated that when bacteria such as *E. coli* are present at low levels on poultry carcass portions, rinsing is the most effective technique. In the case of a target organism either found infrequently or heterogeneously distributed on the carcasses surface, the sample size influences the accuracy of the bacterial population estimations. For such cases, swabbing and rinsing are likely most effective, since these methods sample larger areas of the carcass. On the contrary, for bacteria that are homogeneously distributed on the carcasses, methods investigating relatively small areas, such as excision, can provide reliable data (McEvoy et al., 2005). No differences in accuracy between rinsing and tissue excision were reported by Cox et al. (2010).

As far as swabbing is concerned, it is generally accepted that for poultry carcasses, this method is less effective in recovering indicator bacteria than excision or rinsing (Gill et al., 2005; Zhang et al., 2011; Cossi et al., 2012). The lower recovery level obtained by using swabbing method could be due to the topography of the poultry skin or to the attachment of bacteria to the skin (Zhang et al., 2011).

Because, some bacteria are firmly attached to the skin of poultry, some authors have suggested that skin excisions might be better than the rinse method for determining the incidence of microorganisms. However, it was also demonstrated that the rinse method provide a picture that is more representative of the entire carcass than other sampling methods (Sanchez et al., 2002).

Collection of relatively large numbers of whole carcasses rinse samples, especially in the case of large sized birds, would be impractical and economically demanding (Gill et al., 2006). Swabbing and excision offer practical advantages over carcass rinse, because of the ease and rapidity of sample collection and the smaller amount of enrichment medium required (Zhang et al., 2011). Moreover, excision, in particular of neck samples, is easier to perform because carcasses can be sampled without being removed from the processing line (Hutchison et al., 2006).

Poultry carcasses sampling methods, and in particular the aspects that could influence the recovery of bacteria, have been well-studied during recent years.

One of the reasons that could explain the variability across the different sampling methods is the homogenization step. This phase of sample preparation should be well-standardized, whereas for some sampling protocols, such as rinsing, the homogenization is carried out manually because the use of mechanical equipment is impractical. Hence, the sample manipulation by the analysts could hamper the method standardization and lead to a high level of variability in the recovery of indicator bacteria (Cossi et al., 2012).

This hypothesis was confirmed also by Hutchison et al. (2006), who demonstrated that the coefficients of variation calculated for several indicator bacteria were significantly lower for neck skin excision than whole carcass rinsing, and thus, neck excision seems to be a more reproducible method. The authors suggested that this result was likely due to the manual shaking procedure used to detach bacteria into the diluents for the rinsing method, whereas for excision, a mechanical stomaching machine is used for the same purpose, reducing the variability.

The volume of diluents used for the rinse could also have some influence on the recovery of bacteria, as demonstrated by Buhr et al. (2005). These authors attributed the contrasting results obtained in two experiments to the differences in the volume used for the rinse (100 or 400 ml). The same conclusion was drawn by Williams et al. (2010), who demonstrated that approximately 11 times as many bacteria were removed from the carcass when using a 400 ml rinse sample than with a 100 ml rinse sample. According to the authors, carcass weight could be an important variable to take into account and the appropriate rinse volume should also be defined.

Also, the unit of enumeration used to express results can have some influence on the counts. For instance, Bohaychuk et al. (2009) estimated the *E. coli* counts on carcasses collected from 65 low and high volume abattoirs in Canada. The rinse method was used, and enumeration data were initially recorded as CFU/ml and then converted to CFU/cm^2 using a formula considering the rinse volume and carcass weight. When CFU/ml data were considered, significantly higher counts were obtained in low-volume abattoirs compared with the high-volume ones. Conversely, when data were converted to CFU/cm^2, the opposite results were obtained and high volume abattoirs had significantly higher log mean counts than low-volume abattoirs. The discrepancies were attributed to the differences in the weight of the carcasses slaughtered in low and high volume abattoirs.

For red meat carcasses, it is well documented that some parts of the carcass surface are generally more heavily contaminated than others. On broiler carcasses, the situation is rather different. Although evisceration could lead to the contamination of specific parts of the carcass, before and after this step,

broiler carcasses are subjected to repeated washing steps and further procedures that may homogeneously distribute bacteria over the entire carcass surface. Hence, at the end of the process, all parts of the broiler carcass may be similarly contaminated (Gill and Badoni, 2005).

However, when Smith (2010) compared the *E. coli* counts from dorsal and ventral parts of broiler carcasses, he concluded that not only the sampling method, but also the area of the carcass sampled could influence the recovery of bacteria.

REFERENCES

Bohaychuk VM, Checkley SL, Gensler GE, Barrios PR, 2009. Microbiological baseline study of poultry slaughtered in provincially inspected abattoirs in Alberta, Canada. Can Vet J 50(2):173-8.

Buhr RJ, Bourassa DV, Northcutt JK, Hinton A, Ingram KD, Cason JA, 2005. Bacteria recovery from genetically feathered and featherless broiler carcasses after immersion chilling. Poult Sci 84: 1499-1504.

Cox NA, Richardson LJ, Cason JA, Buhr RJ, Vizzier-Thaxton Y, Smith DP, Fedorka-Cray PJ, Romanenghi CP, Pereira LBV, Doyle MP, 2010 . Comparison of neck skin excision and whole carcass rinse sampling methods for microbiological evaluation of broiler carcasses before and after immersion chilling. J Food Prot 73: 976-980.

Cossi MVC, de Almeida MV, Dias MR, de Arruda Pinto PS, Nero LA, 2012. Comparison of destructive and non destructive sampling tecniques of retail chicken carcasses for enumeration of hygiene indicator organisms. J Food Prot 75: 29-33.

Gill CO, Badoni M, Moza LF, Barbut S, Griffith MW, 2005. Microbiological sampling of poultry carcasses portions by excision, rinsing or swabbing. J Food Prot 68: 2718-2720.

Gill CO, Moza FL, Badoni M and Barbut S., 2006. The effects on the microbiological condition of product of carcass dressing, cooling, and portioning processed at poultry packing plant. Int J Food Micro 110: 187-193.

Hutchison ML, Walters LD, Mead GC, Howell M, Allen VM, 2006. An assessment of sampling methods and microbiological hygiene indicators for process verification in poultry slaughterhouses. J Food Prot 69: 145-153.

McEvoy JM, Nde CW, Sherwood JS, Logue S, 2005. An evaluation of sampling methods for the detection of Escherichia coli and Salmonella on turkey carcasses. J Food Prot 68: 34-39.

Sanchez MX, Fluckey WM, Brashears M, McKee S, 2002. Microbial profile and antibiotic susceptibility of *Campylobacter* spp. and *Salmonell*a spp. In broilers processed air-chilled and immersion-chilled environments. J Food Prot 65, 948-956

Smith DP, 2010. Sampling method and location affect recovery of coliforms and *Escherichia coli* from broiler carcasses. Poult Sci 89: 169-172.

Williams MS, Ebel ED, Golden NJ, Berrang ME, Bailey JS, Hartnett, 2010. Estimating removal rates of bacteria from poultry carcasses using two whole-carcass rinse volumes. Int J Food Micro 139: 140-146.

Zhang QQ, Ye KP, Xu XL, Zhou GH and Cao JX, 2011. Comparison of excision, swabbing and rinsing sampling methods to determine the microbiological quality of broiler carcasses. J Food Safety 32: 134-139.

Appendix E. General characteristics of the selected papers

Table 4a: General characteristics of the 12 studies within the Group 1 (EU – no chlorine) *E: experimental, O: observational; **CS: commercial slaughterhouse, P: pilot setting; ***S: single, P: pool; ****EC: *Escherichia coli*, EB: *Enterobacteriaceae*; *****TCC: traditional colony count, MF: membrane filtration.

	Reference (country)	Study *	Setting **	Animals			Sample characteristics		Indicator bacteria ****	Method characteristics			Review question
				Weight (kg)	Age (days)	S/P ***	Type of sample	Region sampled		Method used *****	Analytical procedure	Unit of enumeration	
Group 1	Whyte et al., 2003 (IE)	E	CS	--	-	S	Skin excision (10 g)	Breast	EB	TCC	-	logCFU/g	2
	Goksoy et al., 2004 (TR)	O	CS	-	-	S	Skin excision (10 g)	Neck	EB	TCC	37°C 24h	logCFU/g	1
	Purnell et al., 2004 (GB)	E	P	-	-	S	Skin excision (25 g) + Rinse (225 ml)	Neck - Whole carcass	EB	TCC	37°C 24 h	logCFU/ml	2
	Whyte et al., 2004 (IE)	O	CS	-	35-54	S	Skin excision (25 g)	Neck	EC, EB	MF	EC: 44°C -EB: 37°C 24h	logCFU/g	1,2
	Escudero-Gilete et al., 2005 (ES)	O	CS	-	-	S	Skin excision (10 g)	Breast	EB	TCC	37°C 24 h	logCFU	2
	Aksit et al., 2006 (TR)	E	P	-	42	S	Skin excision (10 g)	Neck	EB	TCC	37°C 24 h	logCFU/g	2
	Gonzalez-Miret et al., 2006 (ES)	O	CS	-	-	S	Skin excision (10 g)	Breast	EB	TCC	37°C 24 h	logCFU	1
	Hutchison et al., 2006 (GB)	O	CS	-	-	S	Skin excision (10 g)	Neck	EB	TCC	EB: 37°C 24h	CFU/g	2
	Lindblad et al., 2006 (SE)	O	CS	-	-	S	Rinse (400 ml)	Whole carcass	EC, EB	TCC	EC: 44°C 24h - EB: 37°C 24h	logCFU/cm2	2
	Acikgoz et al., 2011 (TR)	E	CS	-	43	S	Skin excision	-	EC	TCC	44°C	logCFU/g	2
	Smulders et al., 2011 (AT)	O	CS	-	-	S	Rinse (300 ml)	Whole carcass	EC, EB	TCC	37°C 24h	logCFU/ml	1
	Svobodova et al., 2012 (CZ)	O	CS	2.1	36	S	Rinse (400 ml)	Whole carcass	EC	TCC	44°C 24 h	logCFU/cm2	1

Table 4b: General characteristics of the 14 studies within Group 2 (non-EU – no chlorine)*E: experimental, O: observational; **CS: commercial slaughterhouse, P: pilot setting; ***S: single, P: pool; ****EC: *Escherichia coli*, EB: *Enterobacteriaceae*; *****TCC: traditional colony count, MF: membrane filtration. @: an e-mail was sent to authors in order to obtain information on the use of chlorine.

	Reference (country)	Study *	Setting **	Animals		Sample characteristics			Indicator bacteria ****	Method characteristics			Review question
				Weight (kg)	Age (days)	S/P ***	Type of sample	Region sampled		Method used *****	Analytical procedure	Unit of enumeration	
Group 2	Buhr et al., 2000 (US) @	E	P	-	49	S	Rinse (200-400 ml)	Whole carcass	EC	TCC	EC/coliform Petri (37°C 24h)	logCFU/ml	2
	Cason et al., 2001 (US)	E	P	-	42	S	Rinse (200 ml)	Whole carcass	EC	TCC	EC/coliform Petri (35°C 24h)	logCFU/ml	2
	Berrang et al., 2002 (US)	E	P	-	-	S	Rinse (100 ml)	Whole carcass	EC	TCC	EC Petri (37°C 18-24h)	logCFU/ sample	2
	Buhr et al., 2003 (US) @	E	P	1.39 - 1.58	56 - 63	S	Skin Excision (NR)	Breast	EC	TCC	EC Petri (35°C 18-24h)	logCFU/ml	2
	Northcutt et al., 2003a (US) @	E	P	2.5	42	S	Rinse (100 ml)	Whole carcass	EC	TCC	EC Petri (37°C 18-24h)	logCFU/ml	2
	Cason et al., 2004a (US) @	E	P	-	42	S	Rinse (400 ml)	Half carcass	EC, EB	TCC	EC/coliform Petri (35°C 18 - 24h); 35°C 18-24h	logCFU/ml	3
	Smith et al., 2005 (US)	E	P	-	-	S	Rinse (100 ml)	half carcass	EC	TCC	EC/coliform Petri (35°C 18-24h)	logCFU/ml	3
	Northcutt et al., 2006 (US) @	E	P	-	35	S	Rinse (100 ml)	Whole carcass - half carcass	EC	TCC	EC Petri (35°C-24 to 48h)	logCFU/ml	2
	Huezo et al., 2007 (US)	E	P	-	44-45	S	Rinse (100 ml)	Whole carcass	EC	TCC	EC Petri (35°C-24h)	logCFU/ml	2

Table 4b (Continued): General characteristics of the 14 studies within Group 2 (non-EU – no chlorine)*E: experimental, O: observational; **CS: commercial slaughterhouse, P: pilot setting; ***S: single, P: pool; ****EC: *Escherichia coli*, EB: *Enterobacteriaceae*; *****TCC: traditional colony count, MF: membrane filtration. NR: not reported. @: an e-mail was sent to authors in order to obtain information on the use of chlorine.

	Reference (country)	Study *	Setting **	Animals		Sample characteristics			Indicator bacteria ****	Method characteristics			Review question
				Weight (kg)	Age (days)	S/P ***	Type of sample	Region sampled		Method used *****	Analytical procedure	Unit of enumeration	
Group 2	Berrang et al., 2008a (US)	E	P	-	42 - 56	S	Rinse (100 ml)	Whole carcass	EC	TCC	EC/coliform Petri (35°C-24h)	logCFU/ml	2
	Bohaychuk et al., 2009 (CA) @	O	CS	-	-	S	Rinse (400 ml)	Whole carcass	EC	TCC	35°C 24h	logCFU/ml	2
	Cavani et al., 2010 (BR)	O	CS	-	-	P (25g)	Meat excision (5 g/carcass)	-	EC, EB	TCC	EC/coliform - EB Petri (35°C 24h)	logCFU	2
	Souza et al., 2012 (BR)	E	CS	-	-	P (5 samples)	Skin and Meat Excision (25 g)	Pericloacal area, thighs, wings, neck	EC, EB	TCC	EC Petrifilm (NR); EB Petri (35°C 24h)	CFU/g	2
	Thanissery et al., 2012 (US) @	O	P	3	64 - 81	S	Rinse (400 ml)	Whole carcass	EC	TCC	EC/coliform Petri (35°C 24h)	logCFU/ml	2

Table 4c: General characteristics of the 28 studies within Group 3 (26 non-EU + 2 (EU+ Turkey) – yes chlorine) *E: experimental, O: observational; **CS: commercial slaughterhouse, P: pilot setting; ***S: single, P: pool; ****EC: *Escherichia coli*, EB: *Enterobacteriaceae*; *****TCC: traditional colony count, MF: membrane filtration; ******S: scalding, W: washing, C: chilling. @: an e-mail was sent to authors in order to obtain information on the use of chlorine.

	Reference (country)	Study *	Setting **	Animals			Sample characteristics		Indicator bacteria ****	Method characteristics			Review question	Chlorine ******
				Weight (kg)	Age (days)	S/P ***	Type of sample	Region sampled		Method used *****	Analytical procedure	Unit		
Group 3	Berrang and Dickens, 2000 (US)	O	CS	-	-	S	Rinse (100 - 300 ml)	Whole carcass	EC	TCC	EC Petri (37°C 18-24 h)	logCFU/ml	1	S, W, C
	Kemp et al., 2001 (US)	O	CS	1.6-3.2	42-56	S	Rinse (400 ml)	Whole carcass	EC	TCC	EC Petri	logCFU/ml	1	W, C
	Whyte et al., 2001 (IE)	E	CS	-	-	S	Skin excision (20g)	Neck	EC, EB	MF TCC	EC: 44°C EB: 37° 24h	logCFU/g	2	W
	Bilgili et al., 2002 (US)	O	CS	-	-	S	Rinse (400 ml)	Whole carcass	EC	-	-	logCFU/ml	3	C
	Sanchez et al., 2002 (US)	O	CS	-	42	S	Rinse (400 ml)	Whole carcass	EC	TCC	37°C 24h	logCFU/ml	2	Immersion C
	Northcutt et al., 2003c (US) @	O	CS	-	-	S	Rinse (100 ml)	Whole carcass	EC	TCC	EC Petri (37°C 48h)	logCFU	1	W
	Jimenez et al., 2003 (AR)	O	CS	2.2-2.5	42-56	S	Rinse (400 ml)	Whole carcass	EC; EB	TCC	EC Petri (35°C 24-48h); (35°C 24)	logCFU/ml	3	C
	Northcutt et al., 2003b (US) @	E	P	-	42-49-56	S	Rinse (100 ml)	Whole carcass	EC	TCC	EC Petri (37°C 18-24h)	log counts	2	C
	Sumner et al., 2004 (AU)	O	CS	-	-	S	Rinse (500 ml)	Whole carcass	EC	TCC	EC Petri (37°C 48h)	logCFU/cm2	2	NR
	Ho et al., 2004 (TW) @	O	CS	1.8-2.2	-	S	Swab (4 cm^2)	Thigh	EC	TCC	EC/coliform Petri (35°C 48h)	logCFU/cm2	1	C
	Oyarzabal et al., 2004 (US) @	O	CS	-	-	S	Rinse (400 ml)	Whole carcass	EC	TCC	EC/coliform Petri	logCFU/ml	1	W,C
	Buhr et al., 2005 (US) @	E	P	2-3.5	56-63	S	Rinse (100 - 400 ml)	Whole carcass	EC	TCC	EC/coliform Petri (35°C 24h)	logCFU/100 ml	2	C

Table 4c (Continued): General characteristics of the 28 studies within Group 3 (26 non-EU + 2 (EU+ Turkey) – yes chlorine) *E: experimental, O: observational; **CS: commercial slaughterhouse, P: pilot setting; ***S: single, P: pool; ****EC: *Escherichia coli*, EB: *Enterobacteriaceae*; *****TCC: traditional colony count, MF: membrane filtration; ******S: scalding, W: washing, C: chilling. @: an e-mail was sent to authors in order to obtain information on the use of chlorine.

	Reference (country)	Study *	Setting **	Animals			Sample characteristics		Indicator bacteria ****	Method characteristics			Review question	Chlorine ******
				Weight (kg)	Age (days)	S/P ***	Type of sample	Region sampled		Method used *****	Analytical procedure	Unit of enumeration		
Group 3	Northcutt et al., 2005 (US) @	E	P	-	-	S	Rinse (100 ml)	Whole carcass	EC	TCC	EC Petri (35°C-24 to 48h)	logCFU/ml	2	W
	Russell and Axtell, 2005 (US)	E	P	-	-	S	Rinse (100 ml)	Whole carcass	EC	TCC	35°C 24h	logCFU/ml	2	C
	Gill et al., 2006 (CA)	O	CS	1.3-1.6	-	S	Skin excision	Different parts of the carcass (5x2 cm)	EC	MF	-	logCFU/cm^2	1	C
	Smith et al., 2007a (US) @	O	CS	-	-	S	Rinse (100 ml)	Whole carcass	EC	TCC	EC Petri (35°C-24h)	logCFU/ml	1	W, C
	Stopforth. 2007 (US)	E	CS	-	-	S	Rinse (400 ml)	Whole carcass	EC	TCC	EC/coliform Petri (37°C 24h)	logCFU/ml	1	W,C
	Northcutt et al., 2007 (US) @	E	P	-	-	S	Rinse (100 ml)	Whole carcass	EC	TCC	EC Petri (35°C-24h)	logCFU/ml	2	W
	Hecer et al., 2007 (TR)	O	CS	-	-	S	Meat excision (25g)	-	EC	TCC	44°C-24h	CFU/g	1	W
	Northcutt et al., 2008b (US) @	O	CS	-	-	S	Rinse (100 ml)	Whole carcass	EC	TCC	EC/coliform Petri (35°C-24h)	logCFU/ml	2	C
	Northcutt et al., 2008c (US) @	E	CS	-	-	S	Rinse (100 ml)	Half carcass	EC, EB	TCC	EC Petri (35°C-24-48h) EB (35°C-24-48h)	logCFU/ml	2	W
	Berrang and Bailey, 2009 (US)	O	CS	-	-	S	Rinse (100-500 ml)	Whole carcass	EC	TCC	EC Petri (35°C 24h)	logCFU/ml	1	W

Table 4c (Continued): General characteristics of the 28 studies within Group 3 (26 non-EU + 2 (EU-Turkey) – yes chlorine) *E: experimental, O: observational; **CS: commercial slaughterhouse, P: pilot setting; ***S: single, P: pool; ****EC: *Escherichia coli*, EB: *Enterobacteriaceae*; *****TCC: traditional colony count, MF: membrane filtration; ******S: scalding, W: washing, C: chilling. @: an e-mail was sent to authors in order to obtain information on the use of chlorine.

	Reference (country)	Study *	Setting **	Animals			Sample characteristics		Indicator bacteria ****	Method characteristics			Review question	Chlorine ******
				Weight (kg)	Age (days)	S/P ***	Type of sample	Region sampled		Method used *****	Analytical procedure	Unit of enumeration		
Group 3	Barbut et al., 2009 (CA) @	O	CS	-	-	S	Skin excision (5x2 cm)	Different parts of the caracass	EC, EB	MF	35°C 3 h	logCFU/cm^2	1, 2	C
	Cox et al., 2010 (US) @	O	CS	-	37-40	S	Skin Excision (8.3 g)	Neck	EC	TCC	EC/coliform Petri (37°c 24h)	logCFU/ml	1	C
	Matias et al., 2010 (BR) @	O	CS	-	-	S	Swab (50 cm^2)	Chest near the neck and dorsal region near the cloacae	EC	TCC	35°C 24h	MPN/cm^2	1,2	C
	Berrang et al., 2011a (US)	O	CS	-	-	S	Rinse (400 ml)	Whole carcass	EC	TCC	EC/coliform Petri (35°C 24h)	logCFU/ml	1	S, W
	Berrang et al., 2011b (US)	E	CS	-	-	S	Rinse (100 - 500 ml)	Whole carcass	EC	TCC	EC/coliform Petri (35°C 24h)	logCFU/ml	2	-
	Line et al., 2013 (US)	O	CS	-	-	S	Rinse (100 ml)	Whole carcass	EC, EB	MPN	35°C 48h	logMPN/ml	1	C

Table 4d: General characteristics of the 18 studies within Group 4 (non-EU – no information about use of chlorine) *E: experimental, O: observational; **CS: commercial slaughterhouse, P: pilot setting; ***S: single, P: pool; ****EC: *Escherichia coli*, EB: *Enterobacteriaceae*; *****TCC: traditional colony count, MF: membrane filtration. @: an e-mail was sent to authors in order to obtain information on the use of chlorine.

	Reference (country)	Study *	Setting **	Animals		Sample characteristics			Indicator bacteria ****	Method characteristics			Review question
				Weight (kg)	Age (days)	S/P ***	Type of sample	Region sampled		Method used *****	Analytical procedure	Unit	
Group 4	Berrang et al., 2000 (US) @	E	P	-	-	S	Rinse (100-300 ml)	Whole carcass	EC	TCC	EC Petri (35°C 18-24h)	logCFU/ml	2
	Georgnaras and von Holy, 2000 (ZA) @	O	CS	-	-	P (20g)	Skin Excision (5 g/carcass)	Neck	EB	TCC	30°C 24h	logCFU/g	1,2
	Berrang et al., 2001 (US) @	O	CS	-	-	S	Rinse (50 ml)	Breast , thigh, drum	EC	TCC	EC/coliform Petri (35°C 18-24h)	logCFU/g, logCFU/part	2
	Berrang et al., 2003 (US) @	O	CS	-	-	S	Rinse (500 ml 60 ml)	Whole carcass; respiratory tract	EC	TCC	EC/coliform Petri (35°C 18-24h)	logCFU/ml	1
	Fluckey et al., 2003 (US) @	O	CS	-	42	S	Rinse (400 ml)	Whole carcass	EC	TCC	EC/coliform Petri (37°C 48h)	logCFU/ml	1
	Russell 2003 (US) @	E	CS	1.3-1.5	-	S	Rinse (400 ml)	Whole carcass	EC	TCC	E. coli Petri	logCFU/ml	2, 3
	Cason et al., 2004b (US)	E	P	-	84	S	Rinse (200 ml)	Whole carcass	EC	TCC	EC Petri	logCFU/ml	2
	Gill and Badoni, 2005 (CA)	O	CS	1.9 - 2.4	-	S	Skin excision (10 cm^2)	Neck	EC	MF	-	logCFU/cm^2	1
	Vaidya et al., 2005 (IN)	O	CS	-	-	S	Swab (100 cm^2)	Neck, breast, wing, leg	EC	TCC	37°C 24-48 h	logCFU/cm^2	1

Table 4d (Continued): General characteristics of the 18 studies within Group 4 (non-EU – no information about use of chlorine) *E: experimental, O: observational; **CS: commercial slaughterhouse, P: pilot setting; ***S: single, P: pool; ****EC: *Escherichia coli*, EB: *Enterobacteriaceae*; *****TCC: traditional colony count, MF: membrane filtration. @: an e-mail was sent to authors in order to obtain information on the use of chlorine.

	Reference (country)	Study	Setting	Animals		Sample characteristics			Indicator bacteria	Method characteristics			Review question
				Weight (kg)	Age (days)	S/P	Type of sample	Region sampled		Method used	Analytical procedure	Unit	
Group 4	Smith et al., 2007b (US) @	E	P	-	-	S	Rinse (200 ml)	Whole carcass	EC	TCC	EC/coliform Petri (35°C 24h)	logCFU/ml	3
	Berrang et al., 2008b (US) @	O	CS	-	-	S	Rinse (100 ml)	Whole carcass	EC	TCC	EC/coliform Petri (35°C 24h)	logCFU/ml	1,2
	Berrang and Bailey, 2008 (US) @	O	CS	-	-	S	Rinse (NR)	Whole carcass	EC	TCC	-	logCFU/ml	1
	Northcutt et al., 2008d (US) @	E	CS	-	-	S	Rinse (400 ml)	Whole carcass	EC	-	-	logCFU/ml	2
	Northcutt et al., 2008a (US) @	E	P	1.8-6.1	56-70	S	Rinse (100 ml)	Whole carcass	EC	TCC	EC/coliform Petri (35°C-24h)	logCFU/ml	3
	Altekruse et al., 2009 (US) @	O	CS	-	-	S	Rinse (100 ml)	Whole carcass	EC	TCC	EC Petri (35°C-24h)	logCFU/ml	1
	Hannah et al., 2009 (US) @	O	CS	-	-	S	Rinse (200 ml)	Half carcass	EC	TCC	EC/coliform Petri (35°C 24h)	logCFU/ml	1
	Line et al., 2011 (US) @	O	CS	-	-	P	Drip	Whole carcass	EC	TCC	EC Petri (35°C 48h)	CFU/ml	1
	Potter et al., 2012 (US) @	O	CS	-	-	S	Rinse (400 ml)	Whole carcass	EC	TCC	-	logcounts	2

Appendix F. Features of the slaughterhouses

Table 5: General information about the features of the slaughterhouses described in papers providing data for review question 1. Sl: Slaughterhouse; E: evisceration; C: chilling.

	Reference (country)	Slaughter ID	Slaughtered animals (N)	Stunning	Scalding	Defeathering	Evisceration	Washing	Chilling	Carcass T (°C) at the end of chilling
Group 1	Goksoy et al., 2004 (TR)	1	8,000 (hour)	-	1 scald tank (150s, 52 to 53.5°C)	-	-	Spray	Air	-
		2	12,000 (hour)	-	1 scald tank (180s, 52 to 54°C)	-				
	Whyte et al., 2004 (IE)	1	60,000 (day)	-	1 scald tank (52°C)	-	-	-	Air (45 to 60 min)	4 °C
	Gonzalez-Miret et al.,2006 (E)	1	-	-	52°C	Automatic	Automatic	Inside - outside*	Air (100 min, -6 to 2 °C)	4 to 9 °C
	Smulders et al., 2011 (AT)	Overall	-	-	51°C	-	-	-	Air	-
	Svobodova et al., 2012 (CZ)	1	8,500 (hour)	-	180s, 54°C	-	-	Inside-outside	Air (70 min, 0 °C)	< 4 °C
Group 3	Berrang and Dickens, 2000 (US)	1	-	Electrical	3 counterflow scald tanks (150s, 55.3°C)	Automatic	Automatic	Inside - outside	Immersion (50 to 55min, 2 to 4°C)	-
	Kemp et al., 2001 (US)	1	-	-	-	-	-	Inside - outside	Immersion	-
	Northcutt et al., 2003c (US)	1, 2, 3	-	-	-	-	-	Inside - outside	-	-
	Ho et al., 2004 (TW)	Overall (3 Sl)	160,000 80,000 40,000 (day)	-	-	-	Automatic	-	Immersion	-
	Oyarzabal et al., 2004 (US)	1	-	-	-	-	-	Inside - outside	Immersion	-
	Gill et al., 2006 (CA)	1	250,000 (day)	-	90s, 58°C	-	Automatic	4 washing steps: bef. E - during E - after E - bef. C	Immersion	-

* Mean temperature at the washing step: 12 to 14°C; carcasses enter the washing stage at 39°C and leave at 37°C

Table 5 (Continued): General information about the features of the slaughterhouses described in papers providing data for review question 1. Sl: Slaughterhouse; W: washing; D: defeathering; E: evisceration; IOBW: inside-outside bird washing; CC: Chlorine chilling.

	Reference (country)	Slaughter ID	Slaughtered animals (N)	Stunning	Scalding	Defeathering	Evisceration	Washing	Chilling	Carcass T (°C) at the end of chilling
Group 3	Smith et al., 2007a (US)	Overall (11 Sl)	-	-	-	-	-	-	-	-
		Sl 6	-	-	-	-	-	-	-	-
		Sl 7	-	-	-	-	-	-	Immersion	-
	Stopforth et al.,2007 (US)	Sl A - W after D	8,400 (hour)	-	-	-	-	Inside – outside	Immersion	-
		Sl B - W after E	16,500 (hour)							
		Sl C - IOBW 1	5,400 (hour)							
		Sl D – CC								
	Hecer et al., 2007 (TR)	1	-	-	-	-	-	-	(120 min; -1 to -3 °C)	-
	Berrang and Bailey, 2009 (US)	1	8,400 (hour)	-	-	-	-	Inside - outside**	-	-
	Barbut et al., 2009 (CA)	1	250,000 (day)	-	90s, 58 °C	-	-	Inside – outside	Air (360 min, -0.5 to 0.5 °C)	-
	Cox et al.,2010 (US)	Plant B	4,200 (hours)	-	-	-	-	Inside - outside	Immersion	-
	Matias et al., 2010 (BR)	Sl 2	4,000 (day)	-	-	Automatic	Manual	-	Immersion	-
		Sl 1	165,000 (day)	-	-	Automatic	Automatic	-	Immersion	-
	Berrang et al., 2011a (US)	1	8,400 (hours)	-	1 scald tank (207s, 52.7 °C)	Automatic	-	-	-	-
	Line et al., 2013 (US)	1	-	-	-	-	-	-	-	-

** Five washing steps along the chain

Table 5 (Continued): General information about the features of the slaughterhouses described in papers providing data for review question 1. Sl: Slaughterhouse.

	References (country)	Slaughter ID	Slaughtered animals (N)	Stunning	Scalding	Defeathering	Evisceration	Washing	Chilling	Carcass T (°C) at the end of chilling
Group 4	Georgnaras and von Holy, 2000 (ZA)	1	10,000 (day)	-	50.8 to 53.5 °C	2 machines in series	Automatic/Manual	Spray	Immersion (20 min)	-
	Berrang et al., 2003 (US)	1	-	-	3 counterflow scald tanks (110s; 57s; 45s)	-	-	-	-	-
	Fluckey et al., 2003 (US)	1	-	-	-	-	-	-	Air	-
	Gill and Badoni, 2005 (CA)	1	9,000 (hour)	-	150s, 52 °C	-	-	-	Air (85 min)	-
	Vaidya et al., 2005 (IN)	1	-	-	-	-	-	-	-	-
	Berrang et al., 2008b (US)	Overall (20 Sl)	4,200 to 9,600 (hours)	-	-	-	-	-	Immersion	-
	Berrang and Bailey, 2008 (US)	Overall (20 Sl)	-	-	-	-	-	-	-	-
	Altekruse et al., 2009 (US)	Overall (20 Sl)	-	-	-	-	-	-	-	-
	Hannah et al., 2009 (US)	1	-	-	-	-	-	Inside - outside	Immersion	-
	Line et al., 2011 (US)	1	-	-	-	-	-	-	Immersion	-

Appendix G. Counts at different stages along the slaughter line

Table 6a: Counts of *E. coli* reported at different stages of the slaughter processing line (before stunning and bleeding - before evisceration). S: Scalding; W: Washing C: Chilling; Sl: slaughterhouse; NS: Neck skin; D: Defeathering; E: Evisceration; IOBW: Inside Outside Bird Washing; CC: Chorine Chiller; N: number of samples; M: mean; SD: standard deviation.

	Reference (country)	Stages where chlorine was used	Slaughter ID	Batch ID	Unit of enumeration	Before stunning and bleeding			After stunning and bleeding – Before scalding			After scalding – Before defeathering			After defeathering – Before evisceration		
						N	M	SD	N	M	SD	N	M	SD	N	M	SD
Group 1	Whyte et al., 2004 (IE)	-	1	beginning of the day	logCFU/g	-	-	-	-	-	-	-	-	-	50	3.01	0.56
				after 7 to 8 hours		-	-	-	-	-	-	-	-	-	25	2.90	0.21
	Smulders et al., 2011 (AT)	-	Overall	-	log CFU/ml	-	-	-	-	-	-	90-300	5.91	0.83	90-300	5.16	0.45
	Svobodova et al., 2012 (CZ)	-	1	-	logCFU/cm^2	-	-	-	-	-	-	-	-	-	40	3.5	0.7
Group 3	Berrang and Dickens, 2000 (US)	S - W – C	1	-	logCFU/ml	-	-	-	28/30	4.3	0.2	30/30	2.1	0.3	26/30	2.8	0.2
	Kemp et al., 2001 (US)	W – C	1	-	logCFU/ml	-	-	-	-	-	-	-	-	-	-	-	-
	Northcutt et al., 2003c (US)	W	1	-	logCFU/ml	-	-	-	-	-	-	-	-	-	-	-	-
			2	-													
			3	-													
	Ho et al., 2004 (TW)	C	Overall	-	logCFU/cm^2	-	-	-	-	-	-	30	3.0	-	30	3.5	-
	Oyarzabal et al., 2004 (US)	W – C	1	Experiment 1	logCFU/ml	-	-	-	-	-	-	-	-	-	-	-	-
				Experiment 2		-	-	-	-	-	-	-	-	-	-	-	-
	Gill et al., 2006 (CA)	C	1	-	logCFU/cm^2	-	-	-	-	-	-	-	-	-	25	1.61	0.74
	Hecer et al., 2007 (TR)	W	1	-	CFU/g	-	-	-	-	-	-	-	-	-	10	3.8x10^3	-
	Smith et al., 2007a (US)	C	Sl 7	-	log CFU/ml	-	-	-	-	-	-	-	-	-	5	3.1	-
	Stopforth et al., 2007 (US)	W – C	Overall	Sl A - W after D	logCFU/ml	-	-	-	-	-	-	-	-	-	75	3.1	0.8
				Sl B - W after E		-	-	-	-	-	-	-	-	-	-	-	-
				Sl C - IOBW 1		-	-	-	-	-	-	-	-	-	-	-	-
				Sl D – CC		-	-	-	-	-	-	-	-	-	-	-	-

Table 6a (Continued): Counts of *E. coli* reported at different stages of the slaughter processing line (before stunning and bleeding - before evisceration). S: Scalding; W: Washing C: Chilling; Sl: slaughterhouse; NS: Neck skin; CR: Carcass Rinse; CD: Cumulative Drip; RTW: Respiratory Tract Washes; *carcasses pre-treated with chlorine; N: number of samples; M: mean; SD: standard deviation.

	Reference (country)	Stages where chlorine was used	Slaughter ID	Batch ID	Unit of enumeration	Before stunning and bleeding			After stunning and bleeding – Before scalding			After scalding – Before defeathering			After defeathering – Before evisceration		
						N	M	SD	N	M	SD	N	M	SD	N	M	SD
Group 3	Barbut et al., 2009 (CA)	C	1	1 (air chilling)	logCFU/cm^2	-	-	-	-	-	-	-	-	-	25	1.64	0.41
	Berrang and Bailey, 2009 (US)#	W	1	-	logCFU/ml	-	-	-	25	4.60	0.18	25	3.62	0.33	25	3.06	0.27
	Cox et al., 2010 (US)	C	Plant B	NS	logCFU/ml	-	-	-	-	-	-	-	-	-	-	-	-
	Matias et al., 2010 (BR)	C	Sl 1	-	MPN/cm^2	-	-	-	-	-	-	-	-	-	27	1.71	0.76
			Sl 2	-		-	-	-	-	-	-	-	-	-	30	3.35	0.50
	Berrang et al., 2011a (US)	S – W	1	1*	logCFU/ml	-	-	-	30	3.93	0.09	30	2.51	0.11	-	-	-
	Line et al., 2013 (US)	C	1	CR	log MPN/ml	-	-	-	-	-	-	-	-	-	-	-	-
				CD		-	-	-	-	-	-	-	-	-	-	-	-
Group 4	Berrang et al., 2003 (US)	-	1	CR	logCFU/ml	-	-	-	30	4.6	0.1	30	2.0	0.3	-	-	-
				RTW		-	-	-	30	1.2	0.9	30	2.7	0.5	-	-	-
	Fluckey et al., 2003 (US)	-	1	-	logCFU/ml	-	-	-	-	-	-	-	-	-	15	3.74	-
	Gill and Badoni, 2005 (CA)	-	1	NS	logCFU/cm^2	-	-	-	-	-	-	-	-	-	50	3.85	0.84
	Vaidya et al., 2005 (IN)	-	1	-	logCFU/cm^2	24	3.10	0.11	24	3.17	0.19	-	-	-	24	2.24	0.08
	Smith et al., 2007a (US)	-	A_ Overall	-	logCFU/ml	-	-	-	-	-	-	-	-	-	55	2.8	-
		-	Sl 6	-		-	-	-	-	-	-	-	-	-	5	2.4	-
	Berrang and Bailey, 2008 (US)	-	Overall	-	logCFU/ml	-	-	-	-	-	-	-	-	-	100	2.88	-
	Berrang et al., 2008b (US)	-	20 Sl	Overall 2006	logCFU/ml	-	-	-	-	-	-	-	-	-	100	2.88	0.13
	Altekruse et al., 2009 (US)	-	20 Sl	-	logCFU/ml	-	-	-	-	-	-	-	-	-	800	3.3	-
	Hannah et al., 2009 (US)	-	1	-	logCFU/ml	-	-	-	-	-	-	-	-	-	-	-	-
	Line et al., 2011 (US)	-	1	CR	CFU/ml	-	-	-	-	-	-	-	-	-	-	-	-
				CD		-	-	-	-	-	-	-	-	-	-	-	-

Five washing steps along the chain

Table 6a (Continued): Counts of *E. coli* reported at different stages of the slaughter processing line (after evisceration – after chilling). S: Scalding; W: Washing C: Chilling; Sl: slaughterhouse; D: Defeathering; E: Evisceration; IOBW: Inside Outside Bird Washing; CC: Chorine Chiller; N: number of samples; M: mean; SD: standard deviation; ** freezing tunnel; ***-0.5 log cfu/sample was assigned for samples for which bacteria were not recovered.

	Reference (country)	Stages where chlorine was used	Slaughter ID	Batch ID	Unit of enumeration	After evisceration – Before washing			After washing – Before chilling			After chilling			Other stage		
						N	M	SD	N	M	SD	N	M	SD	N	M	SD
Group 1	Whyte et al., 2004 (IE)	-	1	beginning of the day	logCFU/g	-	-	-	50	3.26	0.43	50	3.35	0.48	50**	3.28	0.63
				after 7 - 8 hours		-	-	-	25	2.95	0.35	25	3.11	0.23	25**	3.20	0.31
	Smulders et al., 2011 (AT)	-	Overall	-	log CFU/ml	90-300	5.63	0.62	-	-	-	90-300	3.83	0.41	-	-	-
	Svobodova et al., 2012 (CZ)	-	1	-	logCFU/cm^2	40	3.1	0.7	40	2.7	0.6	40	1.8	0.8	-	-	-
Group 3	Berrang and Dickens, 2000 (US)	S - W - C	1	-	logCFU/ml	30/30	2.2	0.2	30/30	1.5	0.2	30/30	1.1	0.4	-	-	-
	Kemp et al., 2001 (US)	W - C	1	-	logCFU/ml	890	2.87		1068	2.27	-	-	-	-	1070	2.37	-
	Northcutt et al., 2003c (US)	W	1	-	logCFU/ml	10	2.2	0.4	10	2.1	0.5	-	-	-	-	-	-
			2	-		10	3.3	0.7	10	2.7	0.6	-	-	-	-	-	-
			3	-		10	3.1	0.1	10	2.4	0.3	-	-	-	-	-	-
	Ho et al., 2004 (TW)	C	Overall	-	logCFU/cm^2	30	3.5	-	-	-	-	30	0.5	-	-	-	-
	Oyarzabal et al., 2004 (US)	W - C	1	Experiment 1	logCFU/ml	40	2.51	-	40	1.45	-	40	1.22	-	-	-	-
				Experiment 2		40	2.76	-	40	2.80	-	40	1.74	-	-	-	-
	Gill et al., 2006 (CA)	C	1	-	logCFU/cm^2	25	1.56	0.83	25	1.09	0.80	25	-0.08	0.79 ***	-	-	-
	Hecer et al., 2007 (TR)	W	1	-	CFU/g	10	1.6x10	-	10	1.1x10	-	10	1x10^3	-	-	-	-
	Smith et al., 2007a (US)	C	Sl 7	1	log CFU/ml	-	-	-	-	-	-	5	0	-	-	-	-
	Stopforth et al., 2007 (US)	W - C	Overall	Sl A - W after D	logCFU/ml	-	-	-	75	2.9	0.8	-	-	-	-	-	-
				Sl B - W after E		75	2.6	0.8	75	2.8	1.0	-	-	-	-	-	-
				Sl C - IOBW 1		75	2.5	0.7	75	2.2	0.9	-	-	-	-	-	-
				Sl D – CC		-	-	-	75	1.3	0.6	75	1.0	0.6	-	-	-

Table 6a (Continued): Counts of *E. coli* reported at different stages of the slaughter processing line (after evisceration – after chilling). S: Scalding; W: Washing; C: Chilling; Sl: slaughterhouse; NS: Neck skin; CR: Carcass Rinse; CD: Cumulative Drip; RTW: Respiratory Tract Washes;*carcasses pre-treated with chlorine; N: number of samples; M: mean; SD: standard deviation.

	Reference (country)	Stages where chlorine was used	Slaughter ID	Batch ID	Unit of enumeration	After evisceration – Before washing			After washing – Before chilling			After chilling		
						N	M	SD	N	M	SD	N	M	SD
Group 3	Barbut et al., 2009 (CA)	C	1	1 (air chilling)	logCFU/cm^2	25	1.71	0.75	25	1.48	0.36	25	1.26	0.51
	Berrang and Bailey, 2009 (US)#	W	1	-	logCFU/ml	25	2.84	0.35	25	2.69	0.30	-	-	-
	Cox et al.,2010 (US)	C	Plant B	NS	logCFU/ml	-	-	-	60	2.76	0.14	60	0.14	0.04
	Matias et al., 2010 (BR)	C	Sl 1 Sl 2	1 2	MPN/cm^2	27 30	2.10 3.19	1.11 0.62	27 30	1.57 3.01	0.45 0.58	27 30	0.85 2.41	0.47 0.49
	Berrang et al., 2011a (US)	S - W	1	1 *	logCFU/ml	-	-	-	-	-	-	-	-	-
	Line et al., 2013 (US)	C	1	CR CD	log MPN/ml	140 140	3.5 3.6	- -	- -	- -	- -	140 140	1.2 0.3	- -
Group 4	Berrang et al., 2003 (US)	-	1	CR RTW	logCFU/ml	- -	- -	- -	- -	- -	- -	- -	- -	- -
	Fluckey et al., 2003 (US)	-	1	-	logCFU/ml	-	-	-	15	3.08	-	15	2.20	-
	Gill and Badoni, 2005 (CA)	-	1	NS	logCFU/cm^2	-	-	-	50	1.95	0.79	50	1.53	0.84
	Vaidya et al., 2005 (IN)	-	1	-	logCFU/cm^2	24	3.07	0.18	24	2.00	0.10	24	2.00	0.24
	Smith et al., 2007a (US)	-	A_ Overall Sl 6	- -	logCFU/ml	- -	- -	- -	- -	- -	- -	55 5	0.5 1.5	- -
	Berrang and Bailey, 2008 (US)	-	Overall	-	logCFU/ml	-	-	-	-	-	-	100	0.49	-
	Berrang et al., 2008b (US)	-	20 Sl	Overall 2006	logCFU/ml	-	-	-	-	-	-	100	0.48	0.12
	Altekruse et al., 2009 (US)	-	20 Sl	-	logCFU/ml	-	-	-	-	-	-	798	0.8	-
	Hannah et al., 2009 (US)	-	1	-	logCFU/ml	24	2.4	0.16	-	-	-	24	0.5	0.16
	Line et al., 2011 (US)	-	1	CR CD	CFU/ml	- 36	- 148.7	- 1.51	- -	- -	- -	120 40	10.9 0.3	0.95 0.40

Five washing steps along the chain

Table 6b: Counts of *Enterobacteriaceae* reported at different stages of the slaughter processing line (before stunning and bleeding – after chilling). C: Chilling; Ch: Chlorine; Carcass Rinse; CD: Cumulative Drip; * freezing tunnel.

	Reference (country)	Ch	Slaughter ID	Batch ID	Unit of enumeration	Before stunning and bleeding			After stunning and bleeding – Before scalding			After scalding – Before defeathering			After defeathering – Before evisceration			After evisceration – Before washing			After washing - Before chilling			After chilling		
						N	M	SD	N	M	SD	N	M	SD	N	M	SD	N	M	SD	N	M	SD	N	M	SD
Group 1	Goksoy et al., 2004 (TR)	No	1	-	log CFU/g	15	5.36	0.14	15	5.04	0.08	15	4.34	0.11	15	4.55	0.13	15	3.77	0.13	15	3.81	0.77	-	-	-
			2	-		15	5.75	0.09	15	4.76	0.03	15	4.09	0.13	15	3.90	0.18	15	3.67	0.14	15	3.91	0.28	-	-	-
	Gonzalez-Miret et al.,2006 (ES)	No	1	-	log CFU/g	-	-	-	-	-	-	-	-	-	30	4.18	0.31	30	3.53	0.31	30	3.40	0.21	-	-	-
	Smulders et al., 2011 (AT)	No	Overall	Overall	log CFU/ml	-	-	-	90-300	5.86	0.71	90-300	5.27	0.40	90-300	5.12	0.52	-	-	-	90-300	4.01	0.41	-	-	-
	Whyte et al., 2004 (IE)	No	1	beginning of the day after 7-8 hours	log CFU/g	-	-	-	-	-	-	50	3.28	0.39	-	-	-	50	3.37	0.44	50	3.79	0.30	50*	3.50	0.39
						-	-	-	-	-	-	25	3.25	0.28	-	-	-	25	3.17	0.19	25	3.37	0.13	25*	3.33	0.15
Group 3	Line et al., 2013 (US)	Yes C	1	CR	log MPN/ml	-	-	-	-	-	-	-	-	-	140	3.5	-	-	-	-	140	1	-	-	-	-
				CD		-	-	-	-	-	-	-	-	-	140	3.7	-	-	-	-	140	0.2	-	-	-	-
Group 4	Geornaras and von Holy, 2000 (ZA)	No	1	Overall	log CFU/g	-	-	-	-	-	-	180	4.4	0.5	180	4.6	0.3	180	4.6	0.6	180	4.2	0.3	-	-	-

Appendix H. Risk factors: detailed results

Table 7a: List of papers describing factors related to the batch and slaughterhouse (sampling time and slaughter technique) influencing *Enterobacteriaceae* (EB) counts. *E: experimental, O: observational; Gr: group.

IB	Setting	Risk Factor	Specifications	D	Gr	Reference (country)	Total
EB	Batch information	Diet	Feed	E	1	Aksit et al., 2006 (TR)	1
		Diet Totale					1
	Batch information Total						**1**
	Slaughtering technique	Chilling	Method (immersion vs air)	O	2	Barbut et al., 2009 (CA)	1
			Water renewal	E	2	Souza et al., 2012 (BR)	1
				O	2	Cavani et al., 2010 (BR)	1
			Water volume	E	2	Northcutt et al., 2006 (US)	1
					3	Northcutt et al., 2008c (US)	1
		Chilling Total					5
		Slaughter	Day time	O	1	Hutchison et al., 2006 (GB)	1
						Lindblad et al., 2006 (SE)	1
						Whyte et al., 2004 (IE)	1
					4	Geornaras and von Holy., 2000 (ZA)	1
			Slaughterhouse characteristics	O	1	Lindblad et al., 2006 (SE)	1
			Season	O	1	Hutchison et al., 2006 (GB)	1
						Lindblad et al., 2006 (SE)	1
		Slaughter Total					7
		Washing	Chlorine	E	3	Whyte et al., 2001 (IE)	1
			Hot water immersion treatment	E	1	Purnell et al., 2004 (GB)	1
			Pressure	O	1	Escudero-Gilete et al., 2005 (ES)	1
			Steam pasteurization	E	1	Whyte et al., 2003 (IE)	1
		Washing Total					4
	Slaughtering technique Total						**16**
EB							**17**

Table 7b: List of papers describing factors related to the batch influencing *E. coli* (EC) counts. *E: experimental, O: observational; Gr: group.

IB	Setting	Risk Factor	Specifications	D	Gr	Reference (country)	Total
EC	Batch information	Age	Slaughter age	E	3	Northcutt et al., 2003b (US)	1
				O	2	Thanissery et al., 2012 (US)	1
		Age Total					2
		Diet	Feed	E	1	Acikgoz et al., 2011 (TR)	1
			Finisher or control feed	E	2	Northcutt et al., 2003a (US)	1
		Diet Total					2
		Feather	Presence or absence	E	2	Buhr et al., 2003 (US)	1
					3	Buhr et al., 2005 (US)	1
					4	Cason et al., 2004b (US)	1
		Feather Total					3
		Feed withdrawal	Time	E	2	Northcutt et al., 2003a, 2003b (US)	2
		Feed withdrawal Total					2
		Health status	History of aerosacculites	E	4	Russell et al., 2003 (US)	1
		Health status Total					1
		Transport	Transport flooring	E	2	Buhr et al., 2000 (US)	1
		Transport Total					1
	Batch information Total						**11**

Table 7b (Continued) List of papers describing factors related to the slaughterhouse influencing *E. coli* (EC) counts. *E: experimental, O: observational; Gr: group.

IB	Setting	Risk Factor	Specifications	D	Gr	Reference (country)	Total
EC	Slaughtering technique	Chilling	Chlorine	E	3	Russell and Axtell, 2005 (US)	1
			Method (immersion vs air)	E	2	Berrang et al., 2008a (US)	1
						Huezo et al., 2007 (US)	1
				O	2	Barbut et al., 2009 (CA)	1
					3	Sanchez et al., 2002 (US)	1
			Recycled water	O	3	Northcutt et al., 2008b (US)	1
			Water renewal	E	2	Souza et al., 2012 (BR)	1
				O	2	Cavani et al., 2010 (BR)	1
			Water volume	E	2	Northcutt et al., 2006 (US)	1
					3	Northcutt et al., 2008c (US)	1
		Chilling Total					10
		Defeathering	Chlorine during defeathering	E	3	Berrang et al., 2011b (US)	1
			Duration	E	4	Cason et al., 2004b (US)	1
		Defeathering Total					2
		Scalding	Bath number	E	3	Buhr et al., 2005 (US)	1
			Firts bath temperature	E	2	Cason et al., 2001 (US)	1
			Rescalding after defeathering	E	4	Berrang et al., 2000 (US)	1
		Scalding Total					3
		Skinning	Skin elimination	E	2	Berrang et al., 2002 (US)	1
				O	4	Berrang et al., 2001 (US)	1
		Skinning Total					2

Table 7b (Continued) List of papers describing factors related to the slaughterhouse influencing *E. coli* (EC) counts. *E: experimental, O: observational; Gr: group.

IB	Setting	Risk Factor	Specifications	D	Gr	Reference (country)	Total
EC	Slaughtering technique	Slaughter	Chlorine	O	3	Sumner et al., 2004 (AU)	1
			Day time	O	1	Lindblad et al., 2006 (SE)	1
						Whyte et al., 2004 (IE)	1
			Sanitation	O	4	Potter et al., 2012 (US)	1
			Shackle line speed	E	4	Northcutt et al., 2008d (US)	1
			Slaughterhouse characteristics	O	1	Lindblad et al., 2006 (SE)	1
					3	Matias et al., 2010 (BR)	1
						Sumner et al., 2004 (AU)	1
			Slaughtering output	O	2	Bohaychuk et al., 2009 (CA)	1
					3	Sumner et al., 2004 (AU)	1
			Season	O	1	Lindblad et al., 2006 (SE)	1
			Inspection	O	4	Berrang et al., 2008 (US)	1
		Slaughter Total					12
		Vent	Open or closed	E	2	Buhr et al., 2003 (US)	1
		Vent Total					1
		Washing	Chlorine	E	3	Buhr et al., 2005 (US)	1
						Northcutt et al., 2005 (US)	1
						Whyte et al., 2001 (IE)	1
						Northcutt et al., 2007 (US)	1
			Temperature	E	3	Northcutt et al., 2005 (US)	1
		Washing Total					5
	Slaughtering technique Total						**35**
EC Total							**46**

Appendix I. Data collected for review question 2

Table 8a: Counts at different stages of the slaughter processing line reported on papers describing factors related to batch characteristics influencing indicator bacteria counts. GR: group; IB: indicator bacteria – EC: *Escherichia coli*, EB: *Enterobacteriaceae*; N: number of sample, M: mean, SD: standard deviation; NR: not reported.

Reference (country)	GR	Factor	Batch ID	Unit of enumeration	IB	Stages of the processing line where samples were collected											
						After defeathering			After evisceration			After washing			Post chilling		
						N	M	SD	N	M	SD	N	M	SD	N	M	SD
Northcutt et al., 2003a (US)	2	Feed withdrawal - diet	0 h - Control	logCFU/ml	EC	8	3.1	0.7	8	2.5	0.6	-	-	-	-	-	-
			0 h - Finisher feed	logCFU/ml	EC	8	3.1	0.4	8	3.1	0.9	-	-	-	-	-	-
			4 h - Control	logCFU/ml	EC	8	2.7	0.6	8	2.0	0.6	-	-	-	-	-	-
			4h - Finisher feed	logCFU/ml	EC	8	3.0	0.7	8	3.0	0.8	-	-	-	-	-	-
			8 h - Control	logCFU/ml	EC	8	2.1	0.4	8	3.0	0.8	-	-	-	-	-	-
			8h - Finisher feed	logCFU/ml	EC	8	2.4	0.9	8	3.2	0.7	-	-	-	-	-	-
			12 h - Control	logCFU/ml	EC	8	3.1	0.6	8	3.6	0.7	-	-	-	-	-	-
			12h - Finisher feed	logCFU/ml	EC	8	2.90	0.80	8	3.3	0.9	-	-	-	-	-	-
Buhr et al., 2003 (US)	2	Presence of feathers	Featherless	logCFU/ml	EC	16	1.6	-	-	-	-	-	-	-	-	-	-
			Featherless	logCFU/ml	EC	8	0.7	-	-	-	-	-	-	-	-	-	-
			Featherless	logCFU/ml	EC	8	2.8	-	-	-	-	-	-	-	-	-	-
			Feathered	logCFU/ml	EC	16	1.5	-	-	-	-	-	-	-	-	-	-
			Feathered	logCFU/ml	EC	8	1.4	-	-	-	-	-	-	-	-	-	-
			Feathered	logCFU/ml	EC	8	2.6	-	-	-	-	-	-	-	-	-	-
Russell, 2003 (US)	4	Health status	Airsacculitis +	logCFU/ml	EC	-	-	-	20	2.88	-	-	-	-	-	-	-
			Airsacculitis -	logCFU/ml	EC	-	-	-	20	2.38	-	-	-	-	-	-	-
			Airsacculitis +	logCFU/ml	EC	-	-	-	20	2.02	-	-	-	-	-	-	-
			Airsacculitis -	logCFU/ml	EC	-	-	-	20	1.85	-	-	-	-	-	-	-
			Airsacculitis +	logCFU/ml	EC	-	-	-	20	2.08	-	-	-	-	-	-	-
			Airsacculitis -	logCFU/ml	EC	-	-	-	20	1.59	-	-	-	-	-	-	-
			Airsacculitis +	logCFU/ml	EC	-	-	-	20	1.89	-	-	-	-	-	-	-
			Airsacculitis -	logCFU/ml	EC	-	-	-	20	1.91	-	-	-	-	-	-	-
			Airsacculitis +	logCFU/ml	EC	-	-	-	20	1.98	-	-	-	-	-	-	-
			Airsacculitis -	logCFU/ml	EC	-	-	-	20	2.30	-	-	-	-	-	-	-
Northcutt et al., 2003b (US)	3	Age	42 gg	log10	EC	-	-	-	-	-	-	-	-	-	48	2.2	-
			49 gg	log10	EC	-	-	-	-	-	-	-	-	-	48	2.5	-
			56 gg	log10	EC	-	-	-	-	-	-	-	-	-	48	2.8	-
		Feed withdrawal	0 h	log10	EC	-	-	-	-	-	-	-	-	-	72	2.4	-
			12 h	log10	EC	-	-	-	-	-	-	-	-	-	72	2.6	-
Cason et al., 2004b (US)	4	Presence of feathers	Featherless	logCFU/ml	EC	42	2.8	0.9	-	-	-	-	-	-	-	-	-
			Feathered	logCFU/ml	EC	42	3	0.8	-	-	-	-	-	-	-	-	-
Buhr et al., 2005 (US)	3	Presence of feathers	Feathered	other	EC	-	-	-	-	-	-	-	-	-	18+9	4.13/4.66	-
			Feathered	other	EC	-	-	-	-	-	-	-	-	-	18+9	3.42/4.80	-
			Feathered	other	EC	-	-	-	-	-	-	-	-	-	18+9	3.98/5.03	-
			Feathered	other	EC	-	-	-	-	-	-	-	-	-	18+9	3.62/4.78	-
			Featherless	other	EC	-	-	-	-	-	-	-	-	-	18+9	4.29/5.53	-
			Featherless	other	EC	-	-	-	-	-	-	-	-	-	18+9	3.78/5.16	-
			Featherless	other	EC	-	-	-	-	-	-	-	-	-	18+9	4.47/5.01	-
			Featherless	other	EC	-	-	-	-	-	-	-	-	-	18+9	4.07/4.48	-

Table 8a (Continued): Counts at different stages of the slaughter processing line reported on papers describing factors related to batch characteristics influencing indicator bacteria counts. GR: group; IB: indicator bacteria – EC: *Escherichia coli*, EB: *Enterobacteriaceae*; N: number of sample, M: mean, SD: standard deviation.

Reference (country)	GR	Factor	Batch ID	Unit of enumeration	IB	Stages of the processing line where samples were collected											
						After defeathering			After evisceration			After washing			After chilling		
						N	M	SD	N	M	SD	N	M	SD	N	M	SD
Acikgoz et al., 2011 (TR)	1	Diet	Control	logCFU/g	EC	-	-	-	6	2.85	0.28	-	-	-	-	-	-
			Formic acid + water 8 h before slaughtering	logCFU/g	EC	-	-	-	6	2.2	0.35	-	-	-	-	-	-
Thanissery et al., 2012 (US)	2	Race / Age	CX / 64 - 71gg	logCFU/ml	EC	-	-	-	-	-	-	40	3.7	0.1	-	-	-
			FR / 83 gg	logCFU/ml	EC	-	-	-	-	-	-	40	3.4	0.1	-	-	-
Aksit et al., 2006 (TR)	1	Diet	control diet	logCFU/g	EB	-	-	-	-	-	-	-	-	-	20	4.07	0.18
			organic acids	logCFU/g	EB	-	-	-	-	-	-	-	-	-	20	3.58	0.12
			essential oil	logCFU/g	EB	-	-	-	-	-	-	-	-	-	20	3.71	0.16
			essential oil + organic acids	logCFU/g	EB	-	-	-	-	-	-	-	-	-	20	3.77	0.19

Table 8b: Counts at different stages of the slaughter processing line reported in papers describing factors related to the slaughterhouse influencing *E. coli* counts. GR: group; UE: enumeration unit; N: number of sample, M: mean, SD: standard deviation. +Mean square error; ++ standard error.

Reference (country)	GR*	Factor		Batch ID	UE*	Stages of the slaughter processing line where samples were collected																				
						After stunning and bleeding			After scalding			After defeathering			After evisceration			After washing			After chilling			Other stage		
						N	M	SD	N	M	SD	N	M	SD	N	M	SD	N	M	SD	N	M	SD	N	M	SD
Buhr et al., 2000 (US)	2	Transport floor		Solid floor	logCFU/ml	32	5.9	0.2^{+}				32	3	0.23^{+}	-	-	-	-	-	-	-	-	-	-	-	-
				Wire floor		32	5.4	0.2^{+}				32	2.8	0.23^{+}	-	-	-	-	-	-	-	-	-	-	-	-
Berrang et al., 2000 (US)	4	Rescalding (different conditions) at different times after defeathering		30 mins (28 s_60°C)	logCFU/ml	-	-	-	8	2.3	0.15^{++}	8	2.7	0.16^{++}	-	-	-	-	-	-	-	-	-	24	2.2	0.22^{++}
				(28 s_60°C)		-	-	-	8	1.8	0.13^{++}	8	2.4	0.25^{++}	-	-	-	-	-	-	-	-	-	24	1.9	0.22^{++}
				30 mins (20s_73°C)		-	-	-	8	3.3	0.08^{++}	8	3	0.13^{++}	-	-	-	-	-	-	-	-	-	24	2.9	0.19^{++}
				(20 s_70°C)		-	-	-	8	2.1	0.13^{++}	8	2.9	0.21^{++}	-	-	-	-	-	-	-	-	-	24	2.2	0.26^{++}
Cason et al., 2001 (US)	2	T° first tank - 3 tanks scalding		Low scald (24-57-57)	logCFU/ml	-	-	-	24	2.9	0.8	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
				Control (57-57-57)		-	-	-	24	3	0.9	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Berrang et al., 2001 (US)	4	Tissue type and carcass region	Non-eviscerated carcasses	Breast skin	logCFU/g	-	-	-	-	-	-	10/10	1.9	0.4	-	-	-	-	-	-	-	-	-	-	-	-
				Breast meat		-	-	-	-	-	-	0/10	-	-	-	-	-	-	-	-	-	-	-	-	-	-
				Thigh skin		-	-	-	-	-	-	10/10	2.3	0.4	-	-	-	-	-	-	-	-	-	-	-	-
				Thigh meat		-	-	-	-	-	-	1/10	0.7	-	-	-	-	-	-	-	-	-	-	-	-	-
				Drum skin		-	-	-	-	-	-	10/10	2.1	0.3	-	-	-	-	-	-	-	-	-	-	-	-
				Drum meat		-	-	-	-	-	-	1/10	0.3	-	-	-	-	-	-	-	-	-	-	-	-	-
			Prechilling carcasses	Breast skin + meat	logCFU/part	-	-	-	-	-	-	-	-	-	-	-	-	10/10	2.8	0.4	-	-	-	-	-	-
				Breast skin		-	-	-	-	-	-	-	-	-	-	-	-	10/10	2.7	0.4	-	-	-	-	-	-
				Breast meat		-	-	-	-	-	-	-	-	-	-	-	-	5/10	1.6	0.2	-	-	-	-	-	-
				Thigh skin + meat		-	-	-	-	-	-	-	-	-	-	-	-	10/10	2.6	0.4	-	-	-	-	-	-
				Thigh skin		-	-	-	-	-	-	-	-	-	-	-	-	10/10	2.7	0.4	-	-	-	-	-	-
				Thigh meat		-	-	-	-	-	-	-	-	-	-	-	-	3/10	1.8	1	-	-	-	-	-	-
				Drum skin + meat		-	-	-	-	-	-	-	-	-	-	-	-	9/10	2.3	0.5	-	-	-	-	-	-
				Drum skin		-	-	-	-	-	-	-	-	-	-	-	-	9/10	2.3	0.2	-	-	-	-	-	-
				Drum meat		-	-	-	-	-	-	-	-	-	-	-	-	9/10	2.1	0.4	-	-	-	-	-	-

Table 8b (Continued): Counts at different stages of the slaughter processing line reported in papers describing factors related to the slaughterhouse influencing *E. coli* counts. GR: group; EU: enumeration unit; N: number of sample, M: mean, SD: standard deviation +++ IOBW: inside outside bird washing.

Reference (country)	GR	Factor	Batch ID	UE	Stages of the slaughter processing line where samples were collected																				
					After stunning and bleeding			After scalding			After defeathering			After evisceration			After washing			After chilling			Other stage		
					N	M	SD	N	M	SD	N	M	SD	N	M	SD	N	M	SD	N	M	SD	N	M	SD
Whyte et al., 2001 (IE)	3	Chlorine concentration	1-2 ppm	logCFU/g	-	-	-	-	-	-	25	3.30	0.13	-	-	-	25	3.48	0.38	25	3.27	0.35	-	-	-
			25 ppm		-	-	-	-	-	-	25	3.32	0.46	-	-	-	25	3.2	0.28	25	3.22	0.29	-	-	-
Sanchez et al., 2002 (US)	3	Chilling method	Water	logCFU/ml	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	150	1.17	0.54	-	-	-
			Air		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	150	1.43	0.54	-	-	-
Berrang et al., 2002 (US)	2	Type of sampling - Skin presence	Rinse-skin on	logCFU/ sample	-	-	-	-	-	-	-	-	-	15	4.4	-	-	-	-	-	-	-	-	-	-
			Rinse - skin off		-	-	-	-	-	-	-	-	-	15	3.9	-	-	-	-	-	-	-	-	-	-
			External sponge- skin on		-	-	-	-	-	-	-	-	-	15/15	3.7	-	-	-	-	-	-	-	-	-	-
			External sponge - skin off		-	-	-	-	-	-	-	-	-	10/15	2.3	-	-	-	-	-	-	-	-	-	-
			Internal sponge skin on		-	-	-	-	-	-	-	-	-	14/15	3.4	-	-	-	-	-	-	-	-	-	-
			Internal sponge-skin off		-	-	-	-	-	-	-	-	-	14/15	3.4	-	-	-	-	-	-	-	-	-	-
			Rinse-IOBW^{+++}-skin on		-	-	-	-	-	-	-	-	-	-	-	-	15	3.3	-	-	-	-	-	-	-
			Rinse-IOBW^{+++}-skin off		-	-	-	-	-	-	-	-	-	-	-	-	15	3.8	-	-	-	-	-	-	-
			IOBW^{+++}-external sponge-skin on		-	-	-	-	-	-	-	-	-	-	-	-	14/15	2.2	-	-	-	-	-	-	-
			IOBW^{+++}-external sponge-skin off		-	-	-	-	-	-	-	-	-	-	-	-	9/15	1.8	-	-	-	-	-	-	-
			IOBW^{+++}-internal sponge-skin on		-	-	-	-	-	-	-	-	-	-	-	-	10/15	2.7	-	-	-	-	-	-	-
			IOBW^{+++}-internal sponge-skin off		-	-	-	-	-	-	-	-	-	-	-	-	13/15	3.2	-	-	-	-	-	-	-
Buhr et al., 2003 (US)	2	Feathered / Featherless birds	Featherless	logCFU/ml	-	-	-	-	-	-	16/16	1.6	NR	-	-	-	-	-	-	-	-	-	-	-	-
			Feathered		-	-	-	-	-	-	16/16	1.5	NR	-	-	-	-	-	-	-	-	-	-	-	-
		Vents open/close plugged and sutured	Featherless		-	-	-	-	-	-	8/8	0.7	NR	-	-	-	-	-	-	-	-	-	-	-	-
			Feathered		-	-	-	-	-	-	8/8	1.4	NR	-	-	-	-	-	-	-	-	-	-	-	-
		Vents opened	Featherless		-	-	-	-	-	-	8/8	2.8	NR	-	-	-	-	-	-	-	-	-	-	-	-
			Feathered		-	-	-	-	-	-	8/8	2.6	NR	-	-	-	-	-	-	-	-	-	-	-	-

Table 8b (Continued): Counts at different stages of the slaughter processing line reported in papers describing factors related to the slaughterhouse influencing *E. coli* counts. GR: group; UE: enumeration unit – EC: *Escherichia coli*, EB: *Enterobacteriaceae*; N: number of sample, M: mean, SD: standard deviation.

Reference (country)	GR	Factor		Batch ID	UE	Stages of the slaughter processing line where samples were collected																
						After stunning and bleeding			After scalding			After defeathering			After evisceration			After washing			After chilling		
						N	M	SD	N	M	SD	N	M	SD	N	M	SD	N	M	SD	N	M	SD
Cason et al., 2004b (US)	4	Feathered / Featherless birds Time post-defeathering		Featherless 30s	logCFU/ml	-	-	-	-	-	-	21	2.9	1	-	-	-	-	-	-	-	-	-
				Feathered 30s		-	-	-	-	-	-	21	3	0.8	-	-	-	-	-	-	-	-	-
				Featherless 60s		-	-	-	-	-	-	21	2.8	0.8	-	-	-	-	-	-	-	-	-
				Feathered 60s		-	-	-	-	-	-	21	3	0.8	-	-	-	-	-	-	-	-	-
Sumner et al., 2004 (AU)	3	Slaughter characteristics (dimension-evisceration - chilling) / species	A-L-Mec-2 st. wash-Spin	Broiler	logCFU/cm2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	80	0.07	1.12
			B-L-Mec-2 st. wash-Spin	Broiler		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	40	1.09	0.9
			C-L-M-Spin chiller	Broiler		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	40	0.19	1.13
			D-M-Mec-Spin	Broiler		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	20	0.97	0.47
			E-L-Mec-Spin	Broiler		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	20	1.2	0.68
			F-S-Man-Tub	Broiler		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	20	0.92	0.27
			G-M-Man-Spin	Broiler		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	40	0.88	0.51
			H-M-Mec-Spin	Turkey		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	39	1	0.5
			I-Sl-Man-Immersion	Turkey		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	10	0	0.2
			J-S-Man-Tub	Turkey		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	10	0.4	0.5
			K-S-Man-Tub	Quail		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	10	0.73	0.4
			L-M-Man-Air Immersion	Quail		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	10	0.74	0.4
Whyte et al., 2004 (IE)	1	Sampling time	AM		log CFU/g	-	-	-	-	-	-	50	3.01	0.56	-	-	-	50	3.26	0.43	50	3.35	0.48
			PM			-	-	-	-	-	-	25	2.9	0.21	-	-	-	25	2.95	0.35	25	3.11	0.23

Table 8b (Continued): Counts at different stages of the slaughter processing line reported in papers describing factors related to the slaughterhouse influencing *E. coli* counts. GR: group; UE: enumeration unit; N: number of sample, M: mean, SD: standard deviation.

Reference (country)	GR	Factor		Batch ID	UE	Stages of the slaughter processing line where samples were collected																	
						After stunning and bleeding			After scalding			After defeathering			After evisceration			After washing			After chilling		
						N	M	SD	N	M	SD	N	M	SD	N	M	SD	N	M	SD	N	M	SD
Northcutt et al., 2005 (US)	3	Washing: chlorine and water temperature		No Cl - 21.1°C	logCFU/ml	-	-	-	-	-	-	-	-	-	-	-	-	16	3.8	0.1	-	-	-
				No Cl - 43.3°C		-	-	-	-	-	-	-	-	-	-	-	-	16	4.1	0.1	-	-	-
				No Cl - 54.4°C		-	-	-	-	-	-	-	-	-	-	-	-	16	4	0.2	-	-	-
				50 ppm Cl - 21.1°C		-	-	-	-	-	-	-	-	-	-	-	-	16	3.8	0.1	-	-	-
				50 ppm Cl - 43.3°C		-	-	-	-	-	-	-	-	-	-	-	-	16	4	0.2	-	-	-
				50 ppm Cl - 54.4°C		-	-	-	-	-	-	-	-	-	-	-	-	16	3.9	0.2	-	-	-
Russell and Axtell, 2005 (US)	3	Chilling		Tap (control)	logCFU/ml	-	-	-	-	-	-	-	-	-	-	-	-	30	7.6	-	30	7.5	-
				Sodium hypochlorite		-	-	-	-	-	-	-	-	-	-	-	-	30	7.6	-	30	7.7	-
Buhr et al., 2005 (US)	3	Scalding	Single bath	Feathered	logCFU/100ml	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	18+9	4.13/4.66	-
				Featherless		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	18+9	4.29/5.53	-
				Feathered + Chlorine		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	18+9	3.42/4.80	-
				Featherless+Chlorine		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	18+9	3.78/5.16	-
			Multi baths	Feathered	logCFU/100ml	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	18+9	3.98/5.03	-
				Featherless		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	18+9	4.47/5.01	-
				Feathered+Chlorine		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	18+9	3.62/4.78	-
				Featherless+Chlorine		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	18+9	4.07/4.48	-
Lindblad et al., 2006 (SE)	1	Sampling time		10 slaughterhouses	logCFU/cm^2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	634	2.8	0.6

Table 8b (Continued): Counts at different stages of the slaughter processing line reported in papers describing factors related to the slaughterhouse influencing *E. coli* counts. GR: group; UE: enumeration unit; N: number of sample, M: mean, SD: standard deviation ++ standard error.

Reference (country)	GR	Factor		Batch ID	UE	Stages of the slaughter processing line where samples were collected																	
						After stunning and bleeding			After scalding			After defeathering			After evisceration			After washing			After chilling		
						N	M	SD	N	M	SD	N	M	SD	N	M	SD	N	M	SD	N	M	SD
Northcutt et al., 2006 (US)	2	Chilling water volume		2,1 L/kg	logCFU/ml	-	-	-	-	-	-	-	-	-	-	-	-	8	4.5	0.6++	24	2.5	0.2++
				16,8 L/kg		-	-	-	-	-	-	-	-	-	-	-	-	8	4.5	0.6++	24	1.7	0.2++
Northcutt et al., 2007 (US)	3	Washing with chlorine (HOCl)	Pilot	Control (faecal contaminated)	logCFU/ml	-	-	-	-	-	-	-	-	-	-	-	-	30	5.9	0.2	-	-	-
				Treated (faecal contaminated)		-	-	-	-	-	-	-	-	-	-	-	-	30	4.4	0.4	-	-	-
Huezo et al., 2007 (US)	2	Chilling method	Pilot: Air chilling	Rinse	logCFU/ml	-	-	-	-	-	-	-	-	-	-	-	-	32	3.4	0.1	32	2.4	0.1
						-	-	-	-	-	-	-	-	-	-	-	-				8	2.5	0.2
				Skin	logCFU/g	-	-	-	-	-	-	-	-	-	-	-	-				8	3	0.3
			Pilot: Water chilling	Rinse	logCFU/ml	-	-	-	-	-	-	-	-	-	-	-	-	32	3.5	0.1	32	2.6	0.1
						-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	8	2.8	0.2
				Skin	logCFU/g	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	8	2.7	0.2
Northcutt et al., 2008d (US)	4	Line speed		105 BPM	logCFU/ml	-	-	-	-	-	-	-	-	-	-	-	-	12	1.6	-	-	-	-
				140 BPM		-	-	-	-	-	-	-	-	-	-	-	-	12	1.6	-	-	-	-
Northcutt et al., 2008b (US)	3	Recycled water with chlorine			logCFU/ml	-	-	-	-	-	-	-	-	-	-	-	-	40	2.6	0.1	40	1.1	0.1
Berrang et al., 2008a (US)	2	Chilling method		Water	logCFU/ml	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	80	1.86	0.12
				Air		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	80	2.42	0.13

Table 8b (Continued): Counts at different stages of the slaughter processing line reported in papers describing factors related to the slaughterhouse influencing *E. coli* counts. GR: group; UE: enumeration unit; N: number of sample, M: mean, SD: standard deviation.

Reference (country)	GR	Factor		Batch ID	UE	After stunning and bleeding			After scalding			After defeathering			After evisceration			After washing			After chilling		
						N	M	SD	N	M	SD	N	M	SD	N	M	SD	N	M	SD	N	M	SD
Northcutt et al., 2008c (US)	3	Chilling water volume		EC 3.3L/Kg	logCFU/ml	-	-	-	-	-	-	-	-	-	-	-	-	12	3.9	0.3	18	3.3	0.5
				EC 6.7L/Kg		-	-	-	-	-	-	-	-	-	-	-	-	12	3.9	0.3	18	3.5	0.5
Berrang et al., 2008b (US)	4	Inspection method - season	Random U.S. slaughterhouses	HACCP	logCFU/ml	-	-	-	-	-	-	320	3.3	0.06	-	-	-	-	-	-	320	0.89	0.05
				HIMP		-	-	-	-	-	-	80	3.4	0.13	-	-	-	-	-	-	80	0.98	0.11
Barbut et al., 2009 (CA)	3	Chilling method		Water	logCFU/cm^2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	NR	NR	NR
				Air		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	NR	NR	NR
Bohaychuk et al., 2009 (CA)	2	Dimension of the slaughterhouse		Low-volume	logCFU/ml	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1285/1296	2.49(2.45 -2.54)	-
				High volume		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1285/1296	2.36(2.32 -2.40)	-
				Low-volume	logCFU/cm^2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1285/1296	1.64(1.60 -1.69)	-
				High volume		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1285/1296	1.68(1.64 -1.72)	-
Matias et al., 2010 (BR)	3	Plant characteristics	Sh1	Automatic	MPN/cm^2	-	-	-	-	-	-	27	1.71	0.76	27	2.1	1.11	27	1.57	0.45	27	0.85	0.47
			Sh2	Manual		-	-	-	-	-	-	30	3.35	0.5	30	3.19	0.62	30	3.01	0.58	30	2.41	0.49

Table 8b (Continued): Counts at different stages of the slaughter processing line reported in papers describing factors related to the slaughterhouse influencing *E. coli* counts. GR: group; UE: enumeration unit; N: number of sample, M: mean, SD: standard deviation.

Reference	GR	Factor	Batch ID	UE	Stages of the slaughter processing line where samples were collected																	
					After stunning and bleeding			After scalding			After defeathering			After evisceration			After washing			After chilling		
					N	M	SD	N	M	SD	N	M	SD	N	M	SD	N	M	SD	N	M	SD
Cavani et al., 2010 (BR)	2	Chilling water renewal	8h	CFU/g	-	-	-	-	-	-	-	-	-	-	-	-	23 (115)	5.33		23 (115)	3.86	-
			16h		-	-	-	-	-	-	-	-	-	-	-	-	23(115)	3.60		23 (115)	2.77	-
			24h		-	-	-	-	-	-	-	-	-	-	-	-	23(115)	5.34		23 (115)	3.42	-
Berrang et al., 2011b (US)	3	Chlorine	Control	logCFU/ml	-	-	-	30	3.3	0.13	30	3.6	0.14	-	-	-	-	-	-	-	-	-
			Chlorine 50ppm		-	-	-	30	2.8	0.16	30	2.8	0.36	-	-	-	-	-	-	-	-	-
Souza et al., 2012 (BR)	2	Chilling water renewal	8h	CFU/g	-	-	-	-	-	-	-	-	-	-	-	-	90	3.9	1.3	90	2.65	0.59
			16h		-	-	-	-	-	-	-	-	-	-	-	-	90	3.3	0.89	90	1.96	1.2
Potter et al., 2012 (US)	4	Sanitation system	TS (Traditional)	logCFU/ml	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	360	0.13	0.02
			PBS (Performance based)		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	360	0.07	0.01

Table 8c: Counts at different stages of the slaughter processing line reported in papers describing factors related to the slaughterhouse influencing *Enterobacteriaceae* counts. GR: group; UE: enumeration unit; N: number of sample, M: mean, SD: standard deviation. NR: not reported.

References (country)	GR	Factor	Batch ID	UE	Stages of the slaughter processing line where samples were collected											
					After defeathering			After evisceration			After washing			After chilling		
					N	M	SD	N	M	SD	N	M	SD	N	M	SD
Georgnaras and von Holy., 2000 (ZA)	4	Sampling time	1 h after start-up	logCFU/g	-	-	-	-	-	-	-	-	-	-	-	-
			10 min after break		-	-	-	-	-	-	-	-	-	-	-	-
			1 h before shut-down		-	-		-	-	-	-	-	-	-	-	-
Whyte et al., 2001 (IE)	3	Chlorine concentration	1-2 ppm	logCFU/g	25	3.44	0.12	-	-	-	25	3.53	0.2	25	3.48	0.21
			25 ppm		25	3.37	0.31	-	-	-	25	3.16	0.16	25	3.44	0.45
Whyte et al., 2003 (IE)	1	Steam pasteurization	control	logCFU/g	-	-	-	-	-	-	10	3.65	0.23	-	-	-
			12s 90°C		-	-	-	-	-	-	10	3.04	0.32	-	-	-
			24s 90°C		-	-	-	-	-	-	10	2.96	0.27	-	-	-
Purnell et al., 2004 (GB)	1	Hot water immersion treatment	1 (75°C/30s)+10 sec*	logCFU/ml	-	-	-	5	3.84	NR	5	<2.78	NR	-	-	-
			2 (70°C/40s)+13sec*		-	-	-	5	1.98	NR	5	<1.82	NR	-	-	-
			3 (70°C/40s)+13 sec*		-	-	-	5	4.67	NR	5	3.06	NR	-	-	-
Whyte et al., 2004 (IE)	1	Sampling time	AM	logCFU/g	50	3.28	0.39	-	-	-	50	3.37	0.44	50	3.79	0.30
			PM		25	3.25	0.28	-	-	-	25	3.17	0.19	25	3.37	0.13
Escudero-Gilete et al., 2005 (ES)	1	Washing pressure	1 A (all samples)	logCFU/g	-	-	-	70	4.08	NR	70	3.44	NR	-	-	-
			1- two stopcocks open		-	-	-	37	4.09	NR	37	3.19	NR	-	-	-
			1B(one stopcock is open - blocks 1-2)		-	-	-	17	4.01	NR	17	3.63	NR	-	-	-
			1C(one stopcock open - blocks 3-4)		-	-	-	16	4.13	NR	16	3.58	NR	-	-	-

Table 8c (Continued): Counts at different stages of the slaughter processing line reported in papers describing factors related to the slaughterhouse influencing *Enterobacteriaceae* counts. GR: group; UE: enumeration unit; N: number of sample, M: mean, SD: standard deviation NR: not reported.

References (country)	GR	Factor	Batch ID	UE	Stages of the slaughter processing line where samples were collected											
					After defeathering			After evisceration			After washing			After chilling		
					N	M	SD	N	M	SD	N	M	SD	N	M	SD
Lindblad et al., 2006 (SE)	1	Slaughter		logCFU/cm^2	-	-	-	-	-	-	-	-	-	636	2,5	0,6
Hutchison et al., 2006 (GB)	1	Season (18 slaugterhouses)	summer and winter	CFU/g	-	-	-	-	-	-	NR	NR	NR	NR	NR	NR
		Sampling time (3 slaughterhouses)	0, 30m, 60m, 120m, 180m after start up		-	-	-	-	-	-	NR	NR	NR	NR	NR	NR
Northcutt et al., 2006 (US)	2	Chilling water volume	2,1 L/kg	logCFU/ml	-	-	-	-	-	-	8	3,8	0,8	24	2,6	0,1
			16,8 L/kg		-	-	-	-	-	-	8	3,8	0,8	24	1,6	0,2
Northcutt et al., 2008c (US)	3	Chilling water volume	3.3 L/Kg	logCFU/ml	-	-	-	-	-	-	12	4,3	0,3	18	3,6	0,1
			6.7 L/Kg		-	-	-	-	-	-	12	4,3	0,3	18	3,8	0,6
Barbut et al., 2009 (CA)	3	Chilling method	Air	logCFU/cm^2	-	-	-	-	-	-	-	-	-	20/25	1,6	0,6
			Water		-	-	-	-	-	-	-	-	-	6/25	NR	NR
Cavani et al., 2010 (BR)	2	Chilling water renewal	8h	CFU/g	-	-	-	-	-	-	23	5,79	NR	23	3,4	NR
			16h		-	-	-	-	-	-	23	5,01	NR	23	3,49	NR
			24h		-	-	-	-	-	-	23	5,93	NR	23	3,7	NR
Souza et al., 2012 (BR)	2	Chilling water renewal	8h	CFU/g	-	-	-	-	-	-	90	4,54	1,12	90	3,32	0,34
			16h		-	-	-	-	-	-	90	4,61	0,68	90	3,25	0,58

ABBREVIATIONS

ACC: Aerobic colony count

AT: Austria

AU: Australia

BR: Brazil

CA: Canada

CZ: Czech Republic

ES: Spain

GB: United Kingdom

IE: Ireland

PHC: Process Hygiene Criteria

PHI: Process Hygiene Indicators

SE: Sweden

TR: Turkey

TW: Taiwan

US: United States

ZA: South Africa