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# Sampling Strategies Used to Determine the Microbiological Recovery in Beef Carcass during Slaughter Operations: A Systematic Literature Review and Meta-Analysis

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**Abstract** **Original Research Article**

The use of microbiological sampling to test beef carcasses for ensuring food safety is a critical activity that food manufacturers need to prioritize. Differences in sampling strategy may affect the quality of the results being reported, possibly leading to misinformed action. Moreover, failure to use an appropriate sampling strategy directly impacts the validity of study results. A systematic literature, covering the period 1965-2020, was conducted to identify sampling strategies used to determine the microbiological quality of beef carcasses in slaughter operations in North America, South America, the European Union, and Australia. Six electronic bibliographic databases were searched for beef microbiological studies in English. Two independent trained reviewers analyzed the full text of articles to assess the quality of the study methods. A total of 30 articles were included for a full review. The number of carcass sites sampled ranged from 1 to 7. Brisket (23/27, 85.2%), flank (17/27, 63%), rump (13/27, 48.1%), and neck areas (8/27, 29.6%) were most often sampled. Most studies described sample characteristics, such as slaughter step to be sampled, carcass sites, and sampling tools used for sampling, sampling frequency, microbiological testing, and handling of sample. Seven had very small sample sizes (10, 18, and 25 beef carcasses). In 13 studies, samples were randomly collected. Only eight reported conducting a power analysis to determine sample size. The average of overall alignment score across all studies with government regulations (except Latin American studies) was 77 points (maximum point was 100). The average score was 62 points in the United States, 78 points in Canada, 90 points in Australia, and 77 points in European countries. Two main sampling tools (swabbing or excision or both) were used in 29/30 studies, with most (24) using swabbing. Microbiological analysis of carcass samples was mentioned in 28/30 studies, 18 used standard plate count, seven used 3M petrifilm, and four used membrane filtration method. Our analysis concluded that there were multiple flaws in the sampling strategies of many of the studies included in our sample, potentially impacting study quality hence limiting utility in the food industries.

**Keywords**: Sampling strategy, beef carcass, slaughter operations, microbial recovery.



## INTRODUCTION

According to the World Health Organization (WHO) in 2010, 600 million cases of foodborne disease were attributed to 31 etiologic agents, with a corresponding 230,000-420,000 deaths worldwide (Havelaar *et al*., 2015). Hoffmann and colleagues, (2017) reported that eating contaminated beef was attributed to approximately 15% of these cases; in the United States, beef accounted for 6.6% of cases of foodborne disease (Painter *et al*., 2013). Given this, there is a need to study food safety practices in beef slaughterhouses to protect public health and enhance consumer confidence (Lee *et al*., 2010) as microbial contamination can occur during animal slaughtering and processing (Kim and Yim, 2016).

Regulatory agencies, such as the USDA Food Safety and Inspection Service (FSIS), often require animal slaughtering and meat processing plants to implement food safety practices such as Hazard Analysis Critical Control Points (HACCP) to prevent and control pathogenic bacteria (USDA-FSIS, 1996). Compliance with regulations is commonly determined through sampling, testing, inspections, monitoring, and

surveillance to verify preventative controls are working. Microbiological sampling programs are particularly important as they provide the most objective data to inform food safety decisions (Institute of Medicine and National Research Council, 2003). However, to prevent the introduction of bias into results, a carefully chosen sampling strategy must be used (Charles, 1979; Corlett, 1974). Inappropriate sampling can lead to systematic bias and sampling error. Within the context of sampling in a meat slaughter operation, the sampling strategy (how samples will be selected) must address sampling method, slaughter stage of sampling, frequency of sampling, sampling tool, carcass sites to be swabbed, sample size (e.g., number of samples to be collected and how often), and microbiological testing approaches. Sampling strategies established by regulatory authorities across different countries and geographic regions are presented in Table 1. In general, one must determine which strategy applies to a situation before deciding how many samples are required to represent the target population.

**Table 1: Differences across Sampling Strategies for Beef Slaughter Established by Regulatory Authorities in Different Countries and Geographic Regions**

| **Sampling** | **Countries** | | | | |
|---|---|---|---|---|---|
| **Strategy Categories** | **United States** | **Canada** | **European Union** | **Australia** | **Latin America** |
| **Agency** | USDA[1] | CFIA[2] | EC[3, 4, 5] | AQIS[6, 7] | Sampled |
| **Slaughter Stage of Sampling** | Pre-evisceration and pre-chill | Pre-evisceration and pre-chill | After carcass dressing but before chilling | After a minimum of 12 hours chilling | according ISO[8], USDA, and European Commission (EC) |
| **Sampling Frequency** | One test per 300 beef carcasses every week for 13 tests | One test per 300 beef carcasses every week for 13 tests | Must take 5 samples at least once a week. | One sample per 300 carcasses | No specific |
| **Sampling Tool** | Nondestructive sponge swabbing 100 $cm^2$/site | Nondestructive sponge swabbing 100 $cm^2$/site | Destructive (excision) 20 $cm^2$/site or nondestructive sponge swabbing 100 $cm^2$/site for *Salmonella* | Nondestructive sponge swabbing 100 $cm^2$/site | regulations standard for sampling strategy |
| **Carcass Site** | Brisket, flank, and rump | Brisket, flank, and rump | Neck, brisket, flank, and rump | Brisket, flank, and rump | |
| **Microbiological Testing** | Generic *E. coli* | Generic *E. coli* | Aerobic colony counts (ACCs), Enterobacteriaceae or *Salmonella* | ACCs, *E. coli*, and *Salmonella* | |

**[1]**United States Department of Agriculture (USDA). Food Safety Inspection Services (FSIS). (1996). Pathogen Reduction: Hazard Analysis Critical Control Point (HACCP) systems.

**[2]**Canadian Food Inspection Agency (CFIA). (2013). Testing for *Escherichia coli* (*E. coli*) in slaughter establishments.

**[3]**Sampling, microbiological examinations, and analysis of results were performed in accordance with Decision 2001/471/EC.

**[4]**European Commission Regulation (EC). (2005). Sampling rules and frequencies for carcasses of cattle, pigs, sheep, goats, and horses set in Commission Regulation (EC) No 2073/2005, as amended.

**[5]**European Commission Regulation (EC). (2004). No. 853/2004 of the European Parliament and of the Council of 29 April 2004. laying down specific hygiene rules for on the hygiene of foodstuffs.

**[6]**Australian Quarantine and Inspection Service (AQIS). Meat Safety Enhancement Program (MSEP). (1998).

**[7]**European Food Safety Authority (EFSA). (2010). The assessment of the comparison of the Australian monitoring program for carcasses to requirements in Regulation (EC) No 2073/2005 on microbiological criteria on foodstuffs.

**[8]**International Organization for Standardization (ISO). (2015). Microbiology of the food chain -- carcass sampling for microbiological analysis.

Experts report that the most effective sampling method to recover bacteria from an animal carcass is the excision method (Dorsa *et al*., 1997; Ribas *et al*., 1993; Anderson *et al*., 1987). Even so, it is often stressed that excision is unacceptable or impractical in non-research settings because it results in visible evidence of sampling on the carcass, reducing the commercial value of the carcass (Korsak *et al*., 1998). Although swabbing

recovers only a proportion of the microbial load present on carcass surfaces, its performance is considered to be acceptable and reliable (Korsak *et al*., 1998). Moreover, swabbing enables sampling of a wider area of the carcass, which might improve the detection of different pathogens. Gill and Jones (2000) suggest that swabbing using more abrasive sponge materials may be a suitable alternative to excision. Sampling using the polyurethane sponge represents an equivalent alternative method as it is nondestructive and less labor intensive (Pearce and Bolton, 2005).

To our knowledge, no studies have been published to compare sampling strategies used in developed regions (North America, European Union, and Australia). Our systematic literature review aimed to analyze studies to identify sampling strategies used to determine the microbiological quality of beef carcasses in slaughter operations in North America, Latin America, the European Union, and Australia and how well they aligned with guidelines outlined in their respective governmental agency regulations.

## MATERIALS AND METHODS

The Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) was used to guide a transparent, valid review of studies conducted to evaluate the microbiological quality of beef slaughter operations (Figure 1) (Liberati *et al*., 2009). The search was performed using the following databases: Science Direct (1965-2014), Academic Search Complete (1965-2014), Academic OneFile (1965-2014), AgEco Search (1965-2014), Web of Science (1965-2014), and Google Scholar (1965-2014). Academic Search Complete is managed by EBSCO and allows for simultaneous searches through multiple databases, such as MEDLINE® and CINAHL®. The search terms to conduct our electronic search are shown in Table 2.

**Table 2: Literature Search Terms**

| **Microorganism** | | **Beef Meat** | | **Slaughtering** | | **Sampling** |
|---|---|---|---|---|---|---|
| Bacteria OR<br>Pathogens OR<br>Parasites OR<br>Viruses OR | AND | Cow OR Bulls OR<br>Heifers OR Steers OR<br>Bovine OR Veal OR<br>Cattle OR Calves OR | AND | Slaughterhouse OR<br>Abattoir OR<br>Butcher OR<br>Meat plant | AND | Swab OR<br>Excision OR |

To be included, studies had to: 1) pertain to red meat slaughter operations; 2) be conducted in North America, the European Union, Australia, or Latin America; 3) be peer-reviewed; 4) have used observational or experimental study designs; and 5) findings reported in English. After the initial search, duplicates were removed then titles and abstracts were screened to determine which articles met our eligibility criteria. A full-text article was retrieved if the title or abstract met all five of our eligibility criteria. In addition, we also hand searched the reference lists of all relevant articles to locate additional published studies.

Two trained reviewers analyzed the full text of articles to assess the quality of the study methods. No universal quality assessment checklist was available to evaluate the quality of microbiological studies, so we created a list of nine items assigned to four content domains: reporting (5 items), external validity (1 item), internal validity (2 items), and power (1 item). Two trained reviewers independently assessed the quality of all eligible studies using the checklist (Table 3). We initially evaluated studies using a binary response format (yes/no) then coded responses as a number (1/0). The two reviewers discussed disagreements in scoring and reached a consensus before mean quality scores were calculated.

We also calculated an alignment score comparing the sampling strategy to the required regulatory standard. A list of five categories was created: 1) slaughter stage to be sampled; 2) sampling tool; 3) carcass sites to be swabbed; 4) frequency of sampling and sample size, (e.g., number of samples to be collected and how often); and 5) microbiological testing. A weighted alignment score, expressed as a number (maximum = 100) was calculated based on the sum of all points earned for each of the five categories using the following equations:

$$\text{Category of alignment score} = \frac{100 \text{ points}}{5 \text{ (No.of categories)}} \text{ then,}$$

$$\text{Sampling plan score} = \frac{20 \text{ points of alignment in each category}}{\text{No.of sampling plan (s) in each category}} \text{ then,}$$

$$\text{Alignment score} = \text{sum all points of sampling plans.}$$

**Table 3: Sampling Categories and Plans According to Different Country Regulations**

| Categories | Country Regulations for Sampling Plan(s) | | | | Alignment Score (points) |
|---|---|---|---|---|---|
| | USA | Canada | EU | Australia | |
| **Slaughter Stage** | Two stages | Two stages | One stage | One stage | 20 |
| **Sampling Frequency** | Thirteen sampling time | Thirteen sampling time | Five sampling time | One sample per 300 carcasses | 20 |
| **Sampling Tool** | Swabbing | Swabbing | Swabbing or excision | Swabbing | 20 |
| **Carcass Site** | Three sites | Three sites | Four sites | Three sites | 20 |
| **Microbiological Testing** | One test | One test | Two tests | Three tests | 20 |
| **Total** | | | | | 100 points |

A non-aligned score for sampling strategy was chosen in case of total incompatibility between the sampling strategy used in a study with standards established by the regulatory authority in that country (alignment score = 0 point). The studies in Latin American were assigned an undetermined score, unable to compare the sampling strategies applied in these studies with standard legislation as a result of no regulations addressing sampling is available in these countries.

## RESULTS

### Search Strategy

A total of 972 records were identified within the electronic databases (Figure 1). After removing duplicates and screening titles and abstracts, 77 potentially eligible studies were included for full-text review.

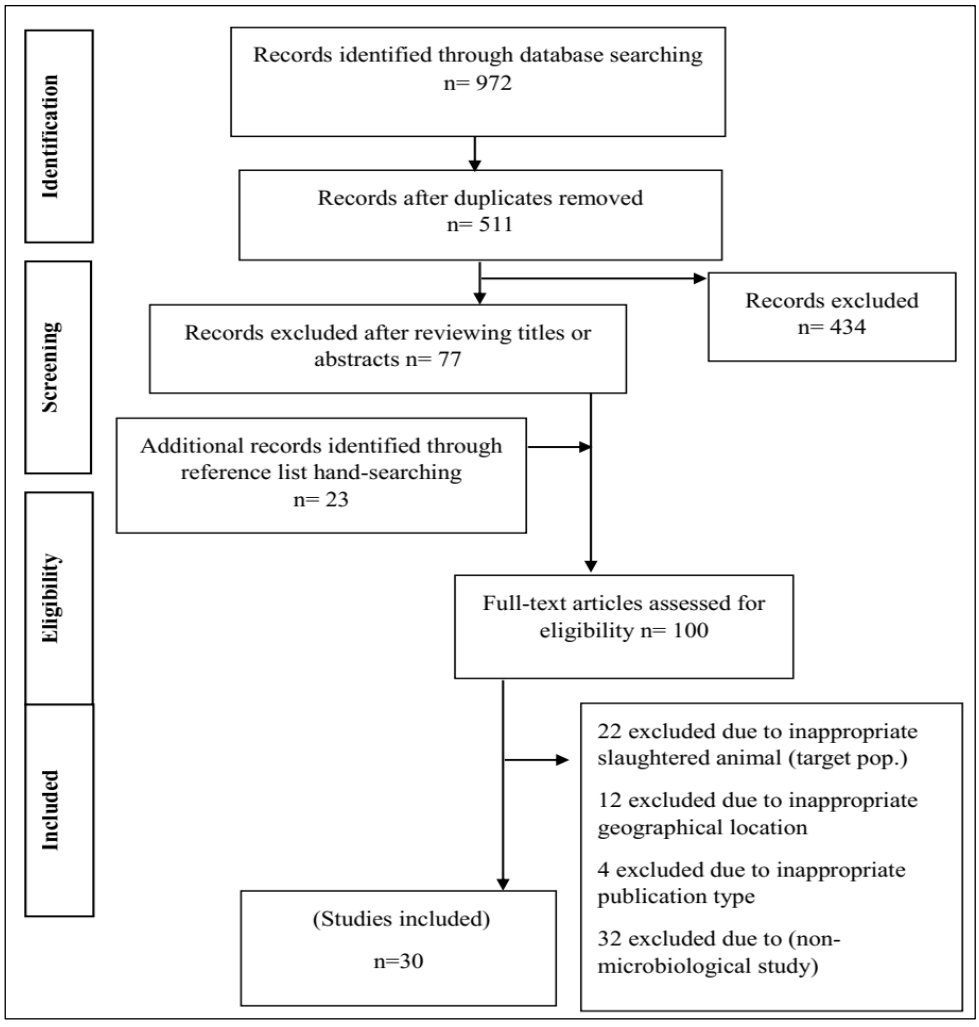


**Figure 1: PRISMA Flow Chart Describing the Search Procedure**

Hand searching the reference list of relevant articles resulted in 23 additional articles. After reviewing the full text, 70 articles were excluded because of the incorrect type of meat (22), wrong geographic location (12), inappropriate publication type (4), and non-microbiological study (32). A total of 30 articles were included in the analysis.

**Study Characteristics**

Of the 30 eligible studies, published between 1992 and 2014, most were conducted in the European Union (14), followed by North America (8), Latin American (6), and Australia (2). The total number of samples/studies ranged from 10 to 5965 beef carcasses collected in 1-110 slaughterhouses. The number of carcass sites sampled ranged from 1 to= 7. Brisket (23/27, 85.2%), flank (17/27, 63%), rump (13/27, 48.1%), and neck areas (8/27, 29.6%) were most often sampled (Figure 2). The whole carcass was swabbed in only one study. Three of the 30 studies did not report carcass site sampling (3/30, 10%).

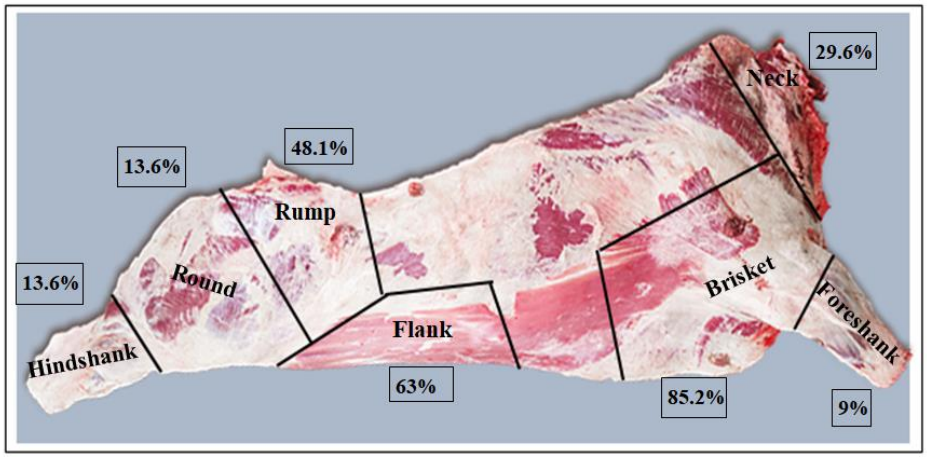


**Figure 2: Percentage of Studies (N=27) Used Multiple Carcass Sites Sample for Microbiological Analysis**

NOTE: Three studies did not report the site on the carcass for sampling

**Quality Assessment**

The median quality assessment score was 7 (range 5 to 9), with 9 being the highest possible score. Most studies described sample characteristics, such as slaughter step to be sampled, carcass sites, and sampling tools used for sampling, sampling frequency, microbiological testing, and handling of sample. Seven had very small sample sizes (10, 18, and 25 beef carcasses). Every study clearly described the main outcomes measured. In 13 studies, samples were randomly collected. Only eight reported conducting a power analysis to determine sample size (Table 4).

**Table 4: Results of Quality Assessment Review (N=30)**

| Questions | Yes % (N) | No % (N) |
|---|---|---|
| **REPORTING** | | |
| Q1: Is the hypothesis/aim/objective the study clearly described? | 96.6 (29) | 3.4 (1) |
| Q2: Are the main outcomes to be measured clearly described in the introduction or methods section? | 100.0 (30) | 0.0 (0) |
| Q3: Are the characteristics of the samples included in the study clearly described? | 90.0 (27) | 10.0 (3) |
| Q4: Are the main findings of the study clearly described? | 96.6 (29) | 3.4 (1) |
| Q5: Have actual probability values been reported for the main outcomes? | 33.3 (10) | 66.7 (20) |
| **EXTERNAL VALIDITY** | | |
| Q6: Were samples representative? | 76.7 (23) | 23.3 (7) |
| **INTERNAL VALIDITY** | | |
| Q7: Were the statistical tests used to assess the main outcomes appropriate? | 86.7 (26) | 13.3 (4) |
| Q8: Were samples randomly collected? | 43.3 (13) | 56.7 (17) |
| **POWER** | | |
| Q9: Did the study have sufficient power to detect an effect? | 26.7 (8) | 73.3 (22) |

**Key Findings**

Thirty (30) articles provided pertinent data related to the sampling strategies of beef carcasses in slaughterhouses were identified. The general characteristics of each study are reported in Table 5.

**Table 5: Descriptive Characteristics of Studies Investigating Indicator and Pathogenic Bacteria on Beef Carcasses during Slaughter Operations**

| Author(s)/Year | Origin | Number of Plants | Plant Size | Samples Number (carcasses) | Carcass Sample Sites | Type of Sample | Target Bacteria | Maximum Quality Score=9* |
|---|---|---|---|---|---|---|---|---|
| Cossi *et al.*, (2014) | BR[10] | 3 | VLV[20] | 209 | Shoulder and brisket | Swab | *Salmonella* spp. | 8 |
| Silva *et al.*, (2014) | BR | 1 | VLV | 120 | Brisket | Swab | ECCs and *Salmonella* spp. | 8 |
| Zweifel *et al.*, (2014) | CH[12] | 2 | M[22] | 500 | Brisket, flank, rump, and neck | Swab | APCs[1] and ECs[3] | 6 |
| Prata *et al.*, (2013) | BR | 1 | NM[24] | 10 | Brisket, flank, rump, and neck | Swab | TVCs[2], ECCs[5], TCCs[4], *E. coli O157:H7* | 7 |
| Carranza *et al.*, (2013) | MX[15] | 1 | NM | 150 | Brisket and flank | Swab | APCs, TCCs, fecal coliform | 9 |
| Paszkiewicz and Pyz-lukasik (2012) | PL[16] | 1 | VLV | 72 | Brisket, flank, leg, and shoulder | Swab and excision | ECs, *Enterococci* spp., and *Salmonella* spp. | 8 |
| Bass *et al.*, (2011) | AU[8] | 12 | NM | 100 | Brisket and flank | Swab | APCs and ECCs | 5 |
| Calicioglu *et al.*, (2010) | US[19] | 3 | NM | 135 | Brisket, flank, and round | Swab | APCs | 5 |
| Martinez *et al.*, (2010) | ES[17] | 1 | NM | 55 | brisket, flank, rump, and neck | Swab and Excision | ECs and TVCs | 8 |
| Ghafir *et al.*, (2008) | BE[9] | 110 | VLV | 5965 | Brisket, flank, thigh, and forelimb | Swab | APCs, ECs, and ECCs | 7 |
| Ghafir *et al.*, (2007) | BE | 110 | M | 1210 carcasses and meat cutting | - | Swab | *Campylobacter* spp. | 7 |
| Barros *et al.*, (2007) | BR | 1 | NM | 151 carcasses and meat cutting | - | Swab | APCs, ECCs, TCCs, mold, and yeast | 5 |
| Guy *et al.*, (2006) | CA[11] | 1 | S[21] | 45 | Brisket, flank, and rump | Swab | *E. coli* O157:H7, *L. monocytogenes*, *Salmonella* spp., and TCCs | 5 |
| Tergney and Bolton (2006) | IE[14] | 1 | M | 180 | Brisket, flank, rump, anus, and hock | Swab | ECCs, TCCs, TECs[6], and TVCs | 7 |
| Hutchison *et al.*, (2005) | GB[13] | 8 | NM | 1352 | Brisket, flank, rump, and neck | Swab and excision | ECs and TVCs | 7 |
| Zweifel *et al.*, (2005) | CH | 5 | M | 800 | Brisket, flank, rump, and neck | Swab | ECs and TVCs | 7 |
| Gill and Landers (2004) | CA | 4 | M | 100 | Brisket, foreleg, and rump | Swab | APCs and ECCs | 7 |

| Author(s)/Year | Origin | Number of Plants | Plant Size | Samples Number (carcasses) | Carcass Sample Sites | Type of Sample | Target Bacteria | Maximum Quality Score=9* |
|---|---|---|---|---|---|---|---|---|
| McEvoy *et al*., (2004) | IE | 1 | M | 36 | Brisket, hock, cranial back, bung, inside round, and outside round | Swab | ECs, ECCs, TCCs, and TVCs | 5 |
| Sumner *et al*., (2003) | AU | 17 | VLV | 159 | Brisket and flank | Swab | TVCs and ECCs | 6 |
| Rose *et al*., (2002) | US | 70 | VLV, S, L[23] | 5783 | Brisket, flank, and rump | Swab | *Salmonella* spp. | 6 |
| Chapman *et al*., (2001) | GB | 1 | NM | 1500 | Neck | Excision | *E. coli* O157:H7 | 6 |
| Hansson (2001) | SE[18] | 8 | VLV, S | 200 | Loin (flank) and sternum | Swab | APCs, ECCs, TCCs, and *Staphylococcus* spp. | 6 |
| Madden *et al*., (2001) | GB | 10 | NM | 780 | Neck | Excision | *E. coli* O157:H7 *Listeria* spp., *Salmonella* spp., and *Campylobacter* spp. | 6 |
| Byrne *et al*., (2000) | GB | 1 | NM | 30 | Triangle of hind-quarters and rectangle of fore-quarters | Swab | *E. coli* O157:H7 | 5 |
| Gill and Jones (1999) | CA | 1 | M | 25 | Brisket and rump | Swab | APCs, ECCs, and TCCs | 5 |
| Little *et al*., (1999) | GB | - | NM | | - | - | *Campylobacter* spp., *Salmonella* spp., and *E. coli* O157:H7 | 5 |
| Sofos *et al*., (1999) | US | 7 | M | 1260 | Brisket, flank, and rump | Excision | APCs, ECCs, TCCs, and *Salmonella* spp. | 6 |
| Gill *et al*., (1998) | CA | 3 | VLV, M | 75 | Brisket, flank, and rump | Swab | APCs, ECCs, and TCCs | 8 |
| Gill *et al*., (1996) | CA | 1 | NM | 18 | Brisket, rump, and neck | Swab | APCs and ECCs | 7 |
| Lasta *et al*., (1992) | AR[7] | 6 | NM | 523 | Whole carcass | Swab | TVCs, TCCs, *Enterobacteria*, fecal coliforms and *Staphylococcus aureus* | 8 |

*: Maximum quality score for quality assessment is 9, [1]APCs: Aerobic plate counts, [2]TVCs: Total viable counts, [3]ECs: Enterobacteriaceae counts, [4]TCCs: Total coliforms counts, [5]ECCs: *Escherichia coli* counts, [6]TECs: Total enteric counts, [7]AR: Argentine, [8]AU: Australia, [9]BE: Belgium, [10]BR: Brazil, [11]CA: Canada, [12]CH: Switzerland, [13]GB: United Kingdom, [14]IE: Ireland, [15]MX: Mexico, [16]PL: Poland, [17]ES: Spain, [18]SE: Sweden, [19]US: United States, [20]VLV: Very low volume > 6000 slaughter animals annually, [21]S: Small 10000- 99999, [22]M: Medium 100000- 999999, [23]L: large over 1000000 per year. [24]NM: Not mentioned. [20, 21, 22, 23]Source: FSIS Nationwide Beef and Veal Carcass Microbiological Baseline Survey, 2013

Alignment scores presented in Table 6 showed that the average overall alignment score across all studies with government regulations (except Latin American studies) was 77 points. The average score was 62 points in the United States, 78 points in Canada, 90 points in

Australia, and 77 points in European countries. One study was non-aligned (0-point score) in the United Kingdom. Latin American studies were undetermined scores as no standard legislations addressing sampling were available in the countries included in this region.

**Table 6: Sampling Strategies Aligned with Regulatory Legislations**

| Author(s)/Year | Country | Alignment Score (points) | Standard Regulations for Sampling Strategy/Country (align requirement) |
|---|---|---|---|
| Calicioglu *et al*., (2010) | United States | 51.5 | USDA-FSIS. Pathogen Reduction; HACCP Systems; Final Rule. Federal Register. 1996; 61: 38806-38989. |
| Rose *et al*., (2002) | United States | 70.0 | |
| Sofos *et al*., (1999) | United States | 63.1 | |
| Guy *et al*., (2006) | Canada | 80.0 | Canadian Food Inspection Agency (CFIA). HACCP Generic Model: Beef Slaughter. 1994. Ottawa, ON, Canada. |
| Gill and Landers (2004) | Canada | 90.0 | |
| Gill and Jones (1999) | Canada | 74.8 | |
| Gill *et al*., (1998) | Canada | 71.2 | |
| Gill *et al*., (1996) | Canada | 74.8 | |
| Bass *et al*., (2011) | Australia | 86.6 | Australian Standard for Hygienic Production of Meat (AS 4461:1997) and (AS 4696:2002). |
| Sumner *et al*., (2003) | Australia | 93.3 | |
| Cossi *et al*., (2014) | Brazil | Undetermined[1] | No specific sampling regulations. |
| Silva *et al*., (2014) | Brazil | Undetermined | |
| Prata *et al*., (2013) | Brazil | Undetermined | |
| Carranza *et al*., (2013) | Mexico | Undetermined | |
| Barros *et al*., (2007) | Brazil | Undetermined | |
| Lasta *et al*., (1992) | Argentina | Undetermined | |
| Hutchison *et al*., (2005) | United Kingdom | 80 | Sampling, microbiological examinations, and analysis of results were performed in accordance with Decision 2001/471/EC or PN-ISO 17604:2005 or ISO 21528-2:2004 or 1994/65/EC. |
| Chapman *et al*., (2001) | United Kingdom | 65 | |
| Madden *et al*., (2001) | United Kingdom | 55 | |
| Byrne *et al*., (2000) | United Kingdom | 44 | |
| Little *et al*., (1999) | United Kingdom | Non-aligned[2] | |
| Tergney and Bolton (2006) | Ireland | 95 | |
| McEvoy *et al*., (2004) | Ireland | 95 | |
| Zweifel *et al*., (2014) EU | Switzerland | 100 | |
| Zweifel *et al*., (2005) | Switzerland | 80 | |
| Ghafir *et al*., (2008) | Belgium | 95 | |
| Ghafir *et al*., (2007) | Belgium | 80 | |
| Paszkiewicz and Pyz-lukasik (2012) | Poland | 65 | |
| Martinez *et al*., (2010) | Spain | 80 | |
| Hansson (2001) | Sweden | 72 | |

[1] Undetermined: Means unable to compare the sampling strategies applied in these studies with standard legislation as a result of no regulations addressing sampling is available in these countries

[2] Non-aligned: Means total incompatibility between the sampling strategy used in a study with standard legislation established by regulatory authorities in that country (alignment score = 0-point)

Two main sampling tools (swabbing or excision or both) were used in 29/30 studies (one study did not report the sampling tool), with most (24) using swabbing. Of the 24 studies, nine used only sterile cotton swabs, six polyurethane sponges, seven sterile cellulose sponges, and four sterile gauze (Table 7). Excision was used in five studies, with most (3) conducted in the United Kingdom. One study did not mention the sampling tool.

**Table 7: Sampling Instruments Used for Collecting Samples (N=30)**

| Sampling Tool(s) | Sampling Instrument | Number of Studies | Geographical Location of Studies |
|---|---|---|---|
| Swabbing | Polyurethane sponge | 5 (30) | USA, Ireland, Australia |
| Swabbing | Sterile cellulose sponge | 5 (30) | Brazil, Canada, Argentine |
| Swabbing | Sterile cotton swabs, sterile cellulose sponge | 1 (30) | United Kingdom |
| Swabbing | Sterile cotton swab | 9 (30) | Sweden, Switzerland, Poland, Ireland, Belgium, Mexico, Brazil |
| Swabbing | Sterile gauze | 4 (30) | Canada |
| Swabbing and excision | Cellulose sponge, polyurethane sponge, sterile gauze, aseptic excision | 1 (30) | Spain |
| Swabbing and excision | Cotton swabs, aseptic excision | 1 (30) | United Kingdom |
| Excision only | Aseptic excision | 3 (30) | USA, United Kingdom |
| Not mentioned | - | 1 (30) | United Kingdom |

The sampling frequency and study duration varied widely (1-49 times), (1-7 years), respectively. Random samples were mentioned in 13/30 studies, and more than half (17/30) did not report sample selection methods. Microbiological analysis of carcass samples was mentioned in 28/30 studies, 18 used standard plate count, seven used 3M petrifilm, and four used membrane filtration method (Table 8).

**Table 8: Summary of Sampling Frequency, Slaughter Stage, Carcass Selection, and Analytical Method of Detection, (N= 30)**

| Author(s)/year | Carcass Selection | Slaughter Stage | Sampling Frequency and/or Study Duration | Analytical Method |
|---|---|---|---|---|
| Cossi *et al*., (2014) | NM[2] | After bleeding, after skinning, after evisceration, and after end washing | 10 times during two-years | PCR[1] |
| Silva *et al*., (2014) | NM | After bleeding and before evisceration | 13 times during nine-months | 3M petrifilm and standard plate count |
| Carranza *et al*., (2013) | Random | Before washing and 4 treatment wash | 4 day's | Standard plate count |
| Prata *et al*., (2013) | Random | NM | 4 months | 3M petrifilms and standard plate count |
| Calicioglu *et al*., (2010) | Random | Pre-evisceration (skin on carcass) | One time | Standard plate count |
| Barros *et al*., (2007) | NM | NM | One time | 3M petrifilm |
| Rose *et al*., (2002) | Random | > 12 hrs after slaughter | 13 times/set for 3 years | Standard plate count |
| Sofos *et al*., (1999) | NM | Pre-evisceration, post-final carcass washing, and 24 hrs carcass chilling | Twice time (one on the wet season and one in dry season | Standard plate count and 3M petrifilm |
| Guy *et al*., (2006) | NM | > 12 hrs after slaughter | One year | 3M petrifilm and PCR |
| Gill and Landers (2004) | Random | Before trimming, after trimming, and after dressing | Every day/5 days | Membrane filtration Method |
| Gill and Jones (1999) | Random | 16 breaking carcass operations | Every day/5 day's | Membrane filtration method |
| Gill *et al*., (1998) | Random | Skinning carcass hindquarters | Every day/5 day's | Membrane filtration method |
| Gill *et al*., (1996) | Random | Skinning, carcass splitting, trimming, and washing | Every day/4 day's | Membrane filtration method |
| Bass *et al*., (2011) | NM | 4-24 hrs chilling | Three times | 3M petrifilm |
| Sumner *et al*., (2003) | NM | 8-48 hrs chilling | 1-week | 3M petrifilm |
| Hutchison *et al*., (2005) | Random | NM | 49 times | Standard plate count |
| Chapman *et al*., (2001) | NM | After slaughter pre-chilling | 1 year/every month (12 times) | Standard plate count |
| Madden *et al*., (2001) | NM | Less than 48 hrs chilling | 13 times | Standard plate count |
| Byrne *et al*., (2000) | NM | End of slaughter after washing | One time | Standard plate count |
| Little *et al*., (1999) | NM | NM | NM | NM |
| Tergney and Bolton (2006) | NM | Final inspection | 18 visits/6 months | Standard plate count |
| McEvoy *et al*., (2004) | NM | 8 slaughter stages | 12 months | Standard plate count |
| Zweifel *et al*., (2014) | NM | Skinning, evisceration, trimming, washing, and blast chilling | Seven months | Standard plate count |
| Zweifel *et al*., (2005) | NM | NM | Eight months/ every week | Standard plate count |
| Ghafir *et al*., (2008) | Random | 2-4 hrs chilling | 3 years | Standard plate count |
| Ghafir *et al*., (2007) | Random | 2-4 hrs after slaughtering | 7 years | Standard plate count |
| Paszkiewicz and Pyz-lukasik (2012) | Random | 5 slaughter stages (stages NM.) | NM | NM |
| Martinez *et al*., (2010) | Random | End of slaughter before chilling | NM | Standard plate count |
| Lasta *et al*., (1992) | NM | After washing | Four years | Most probable number |
| Hansson (2001) | NM | End of slaughter | 3 times | Standard plate count |

[1]PCR: Polymerase Chain Reaction, [2]Not mentioned (NM).

## DISCUSSION

In order to develop preventive systems in food plants, microbiological data are needed to identify microbial hazards. The sampling strategy is an essential part of this preventive approach (FDA, 2019). Therefore, we aimed to identify sampling strategies used to determine the microbiological quality of beef carcasses in slaughter operations in North and South America, the European Union, and Australia and to determine how

well these sampling strategies aligned with the respective governmental agency's regulations.

**Quality Assessment**

Our review revealed two common flaws in many of the studies, based on the quality assessment checklist we developed. First of all, samples were not randomly selected (17/30, 57%), so results are not representative. Moreover, randomization can eliminate possible bias that may arise in the study. It is important to note that randomization might have occurred but was not reported. Secondly, most (22/30, 73%) did not power their sample size. Insufficient sample size may affect the reliability of the study results as it leads to higher variability and bias. Sample sizes were small (7/30, 23%), which reduces the statistical power. Also, a small sample size leads to a lack of representation of the target population, which affects the generalizability of the study results (greater representativeness = greater generalizability). However, small sample sizes are often used because of cost of sampling equipment, difficulty in collecting data (practicality), and using the prior information of similar study to reduce sample sizes (use mean, and variance estimates of previous studies to reduce sample sizes) (Stephanie, 2017). Poor to fair quality studies are impacting our knowledge about beef slaughterhouses. Moreover, food safety regulations might be informed by less rigorously designed studies.

**Sampling Strategies Alignment**

The mean alignment score between the sampling strategies used in the 23 research studies with corresponding standard legislative regulations related to sampling was 77/100 points. The six Latin American studies were excluded as we could not compare the study sampling strategies to standard legislation due to the absence of regulations addressing sampling. To begin with, it is important to note that the absence of an item does not necessarily mean that it did not occur during the execution of the study. Rather it was a problem with reporting, the reporting of sampling strategy provides information needed to ensure a study can be understood by a reader, replicated by a researcher, and used for developing industries.

Governmental agencies develop sampling strategies to support regulations. Official sampling standards provide guidance on how to create a sampling strategy to collect reliable and valid microbiological data. However, using unofficial methods, which might not be reliable and valid, may lead to biased results. Although compliance with official microbiological sampling standards requires a lot of resources, they are presumably the most beneficial to identifying food safety issues for the food industry.

Variation in applying sampling standards was recorded in the EU studies. Two possible reasons for this include the large number of countries in the EU (28 members), and some EU countries have their own standards for beef sampling that differ from EU regulations. The highest alignment score was in Australia (90 points) presumably because only two studies were included in our sample. The lowest score of alignment was in the United States, presumably because the U.S. has the most detailed sampling standards [e.g., two slaughter stages, 13 times of sampling (sampling frequency), and three carcass sites] compared with other country regulations.

Lastly, there are other plausible reasons study authors did not align their methods with regulatory standards. These include: (1) nature of the study (e.g., potential interferences, including environmental conditions, and weather impacts) might have required deviation from set standards; (2) the aim of the study (project goals and objectives), such as determining specific target microorganisms or sampling different slaughter stages, required deviation; (3) limitation in the study design (e.g., difficulty getting participants, sample locations, and frequencies); and (4) cost of sampling may affect the sampling duration, choosing a sampling tool, and an analytic method (laboratory capabilities). Also, some countries have no standard guidelines (e.g., Latin America, Asian, and Africa – the latter two were not reviewed as part of this study) which may lead to using other standard methods (e.g., ISO, USDA, and European Commission) suitable for the study design.

**Limitations**

In our review, we observed several limitations. The primary constraint in our analysis of the studies included in our sample was all journals do not require completion of a reporting checklist. As a result, essential elements needed to review the study methods might have occurred but was not reported. In addition, the difficulties faced in comparing sampling strategies (five sampling categories) was difficult because of the variability among the various studies such as different regulatory authorities in North America, South America, European Union, and Australia. Lastly, we only included studies published in English, whereas, contrasting negative results may be published in non-English journals.

## CONCLUSIONS

Regardless of the purpose of sampling, reliable, and accurate sampling strategies are needed to ensure the validity of the data collected. Our analysis concluded that there were multiple flaws in the sampling strategies of many of the studies included in our sample, potentially impacting study quality hence limiting utility in the food industries. Approved sampling strategies by the country authority or official validated methods may reduce confounding bias in the results. Consequently, it has a positive contribution to public health by improving and developing food safety practices in the meat industry. Further research is needed to study the weaknesses of

microbiological sampling standards in different countries.

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