Journal of Food Protection, Vol. 70, No. 4, 2007, Pages 930–936

An In Vitro System for the Comparison of Excision and Wet-Dry Swabbing for Microbiological Sampling of Beef Carcasses

B. T. CENCI-GOGA,[1]* D. MIRAGLIA,[1] D. RANUCCI,[1] R. BRANCIARI,[1] L. BUDELLI,[2] C. M. McCRINDLE,[3] A. CIOFFI,[1] AND R. MAMMOLI[1]

[1]*Dipartimento di Scienze Biopatologiche, Laboratorio di Ispezione degli Alimenti di Origine Animale, Facoltà di Medicina Veterinaria, Università degli Studi di Perugia, 06126 Perugia, Italy;* [2]*U.S.L. n. 2, Perugia, Dipartimento di Prevenzione, Servizio Veterinario di Igiene degli Alimenti di Origine Animale, 06100 Perugia, Italy; and* [3]*Department of Paraclinical Sciences, Faculty of Veterinary Science, University of Pretoria, Private Bag X04, Onderstepoort 0110, South Africa*

MS 06-465: Received 31 August 2006/Accepted 6 November 2006

ABSTRACT

An in vitro system for the comparison of wet-dry swabbing and surface tissue excision was developed to ascertain whether the commonly accepted statement of the advantage (in terms of bacterial recovery) of the tissue excision method is also legitimate when different kinds of bacteria are used. A total of 1,770 sections (2.5 by 10 cm) of bovine skin were individually inoculated on the subcutaneous fat side by spreading various suspensions of marker organisms (nalidixic acid–resistant *Escherichia coli*, vancomycin-resistant *Enterococcus faecalis*, and methicillin-resistant *Staphylococcus aureus*) at different concentrations and sampled by two standard methods: cotton wet-dry swabbing and excision. Most counts from cuts sampled by excision were significantly ($P < 0.05$) higher than the wet-dry swabs; however, no differences were observed between the control and the sampling method when sections were inoculated with bacterial solutions at a concentration of 10^3 CFU/ml and sampled by excision. For sections inoculated with bacterial solutions at a concentration of 10^3 CFU/ml, counts given as log CFU/25 cm^2 ranged from 1.97 (*S. aureus* sampled by wet-dry swab) to 3.06 (*S. aureus* sampled by excision). For sections inoculated at a concentration of 10^4, counts given as log CFU/25 cm^2 ranged from 2.15 (*E. faecalis* sampled by wet-dry swab) to 3.19 (*S. aureus* sampled by excision). For sections inoculated at 10^5, counts given as log CFU/25 cm^2 ranged from 2.94 (*E. faecalis,* wet-dry swab) to 3.98 (*S. aureus,* excision), and for sections inoculated at 10^6, counts given as log CFU/25 cm^2 ranged from 3.53 (*E. coli,* wet-dry swab) to 4.69 (*S. aureus,* excision). The proposed system, which enabled a considerable amount of samples to be analyzed under controlled experimental conditions and a large number of data to be generated in a short time, demonstrated among the tested microorganisms that whereas the excision method recovered the highest number of bacteria, control means were always (with the exception of an inoculum of 10^3/ml) significantly higher than means from either of the sampling methods. Our results indicate that particular attention should be paid to the diverse microflora that can contaminate carcasses in a given slaughterhouse and that it is not appropriate to generalize by saying that the destructive method is the reference technique for the bacteriological sampling of carcasses in slaughterhouses, especially when the contamination is higher than 10^3 CFU/25 cm^2.

The EC Decision 2001/471 of 8 June 2001 (hereinafter referred to as Decision 2001/471) and the following amendments and corrigenda *(1, 2, 4)* as well as, more recently, Commission Regulation 2073/2005 of 15 November 2005 (hereinafter referred to as Regulation 2073/2005) *(5)* set the achievement of microbial performances standards as a primary requirement for the European Union meat industry. After the publication of Decision 2001/471 and especially after the publication of Regulation 2073/2005, scientific debate on the best carcass sampling method has expanded, and the focal point of the matter has possibly become a detail of secondary importance. In fact, the focal point of article 2 of the cited Decision 2001/471 is the microbiological checks referred to in article 10 of Directive 64/433 *(3).* According to this directive, the operators of the establishment, the owners, or their agent must conduct regular checks on the general hygiene of conditions of production in their establishment, inter alia, by means of microbiological controls. Checks must include utensils, fittings, and machinery at all stages of production and, if necessary, products. The official veterinarian must regularly analyze the results of these checks. This individual may, on the basis of this analysis, conduct further microbiological examinations at all stages of production or on the products. It is clear that the focal point of this rule is the ''regular checks on the general hygiene of conditions of production, conducted, *inter alia,* by means of microbiological controls.'' In light of this rule, microbiological controls are tools for the verification of the conditions of production and should not be regarded as absolute. Regulation 2073/2005 has repealed Decision 2001/471, and ''microbiological checks'' have become ''microbiological criteria.'' This subtle difference hides a whole new approach because, according to Regulation 2073, a microbiological criterion means ''. . . a criterion defining the acceptability of a product, a batch of foodstuffs or a process, based on the absence, presence or number of micro-organisms, and/or on the quantity of their

* Author for correspondence. Tel: +39 075 585 7929; Fax: +39 075 585 7929; E-mail: cencigoga@unipg.it.

toxins/ metabolites, per unit(s) of mass, volume, area or batch.'' In other words, since test results are dependent on the method used, a given reference method should be associated with each microbiological criterion. Regulation 2073/2005, however, gives food business operators the possibility ''. . . to use analytical methods other than the reference methods . . . as long as the use of these alternative methods provides equivalent results.'' Excision is the preferred method of carcass sampling, and the annex to Decision 2001/471 and Regulation 2073/2005 establishes a performance standard relating to this technique. Swabbing, by the wet-dry technique, is also permitted if a correlation can be established with excision. There is no consensus in the literature on the most appropriate carcass sampling technique. In fact, although excision is generally considered the most effective bacterial carcass sampling method *(13, 14, 16, 22, 24, 25),* some authors believe that swabbing with abrasive materials may be a suitable alternative *(8, 12, 23).* Furthermore, the European Union meat industry is inclined toward swabbing *(7),* and this carcass surface-sampling method is the one most extensively used for both practical and economic reasons. Data available in the literature mainly derive from comparisons based on total aerobes and *Enterobacteriaceae* counts on groups of carcasses obtained by random selection at the end of commercial processes *(8, 12–14, 20, 22, 23, 25).* This approach, although generally accepted by the scientific community, has two unique flaws that make any statistical analysis imprecise: the nonuniform distribution of bacteria between near-consecutive carcasses and the low prevalence of *Enterobacteriaceae* on the carcass. In fact, it has been confirmed that there is a high degree of variation between the counts present on the surface of individual red meat carcasses closely positioned on commercial processing lines *(16)* and that a bacterial indicator can be used as potential process monitor only when at least 80% of the samples test positive for it (*14*). To overcome these flaws, several authors have intentionally contaminated skin cuts *(10),* meat cuts (*26*), or sides and carcasses *(24)* with marker microorganisms in order to perform unbiased comparisons of sampling methods for carcass contamination. All these studies have, to our knowledge, been conducted on a limited number of samples. This study proposes an in vitro system with marker microorganisms under controlled experimental conditions as a reliable alternative to comparative studies performed under commercial processing conditions. The system relies on the similarity of the subcutaneous fat attached to the skin of the tail region to the carcass surface after hide removal. The subcutaneous fat layer, the bottom or lowest layer of skin, consists of adipose cells, and its limited depth, together with the presence of abundant connective tissue at the tail region, render it a good in vitro system for studies of carcass contamination *(10).*

MATERIALS AND METHODS

Bacterial strains and culture media. The bacterial strains used as marker microorganisms for the inoculation were taken from the collection of the Laboratorio di Ispezione degli Alimenti di Origine Animale and are as follows: *Escherichia coli,* strain CSH26 K-12, resistant to 200 μg/ml of nalidixic acid; *Enterococcus faecalis,* strain NCTC 12201 *van*(A), resistant to 6 μg/ml of vancomycin; and *Staphylococcus aureus,* strain 27R *mec*(A), resistant to 2 μg/ml of methicillin. The microorganisms were grown aerobically in nutrient broth (NB; CM0001, Oxoid, Basingstoke, UK) at 37°C for 24 h. The total viable cell count on nutrient agar (NA; CM0003, incubated at 37°C on air for 24 h; Oxoid) at 24 h was approximately 1×10^9 CFU/ml. Decimal dilutions were performed to obtain the following concentrations in NB: 10^6 CFU/ml, 10^5 CFU/ml, 10^4 CFU/ml, and 10^3 CFU/ml. The total viable cell counts for all dilutions were recorded as controls on NA and on the following media. Violet red bile glucose agar (CM0485, Oxoid), to which a solution of nalidixic acid at a final concentration of 200 μg/ml was added, was used for counts of the *E. coli* nalidixic acid–resistant strain *(19).* Vancomycin-resistant *Enterococcus* agar (CM0984, Oxoid) with vancomycin supplement (SR0186, Oxoid) was used for the *E. faecalis* vancomycin-resistant strain *(15).* Oxacillin resistance screening agar base (ORSAB; CM1008, Oxoid) with ORSAB selective supplement (SR019, Oxoid) was used for the *S. aureus* methicillin-resistant strain *(18).*

Preparation of skin cuts. Bovine skin cuts were collected at the slaughterhouse from the tail region after hide removal, transported to the laboratory in refrigerated containers, carefully washed within 1 h in tap water at room temperature to remove all visible dirt, and stored at −80°C. Before storage, transversal sections of the skin were stained with hematoxylin and eosin and observed with a light microscope for the selection of cuts for the experiment and for comparison with nonfrozen samples. Samples were cut to size (1 by 0.5 by 0.5 cm) and either put in a plastic bag to be frozen at −80°C or immediately processed for histology as controls. Samples that underwent immediate processing for histology were turned in talcum powder, wrapped in aluminium foil, and fixed in liquid nitrogen. At the time of analysis, samples were collected and cut with a cryostat to obtain 7-μm-thick transversal sections of the skin that were then stained with hematoxylin and eosin.

Experimental contamination procedure. Before each trial, skin cuts were thawed at 8°C overnight and then left at 37°C until the temperature was similar to the carcass temperature after dressing and prior to refrigeration (30 to 35°C). Sections (8 by 12 cm) were removed with a surgical blade. To minimize variation, sections from the same cut were used for the same trial. All skin cuts used in this experiment had been previously tested for the absence of growth of the three marker organisms in the respective selective media. Skin sections were transferred into sterile aluminium trays and inoculated with the appropriate bacterial suspension by a sterile plastic hockey stick (Etaleur 5051, PBI International, Milano, Italy) to spread five 0.2-ml aliquots, which were distributed at different points on the sample surface to ensure an even spread of cells. The samples were then left at room temperature for 1 h to permit cell adherence and to simulate commercial processing conditions (temperature and moment in time of sampling after dressing). At the end of the entire experiment consisting of 295 trials, 1,770 sections of bovine skin were individually inoculated on the subcutaneous fat side by spreading the various suspensions of marker microorganisms. Each trial consisted of six skin sections, taken from the same skin cut, inoculated with the same bacterial suspension: three sections were sampled by wet-dry swabbing, and three were sampled by excision. For each trial, a negative control inoculated with sterile NB was set up. A direct contamination of the carcass surface was conducted with the highest marker concentration (10^6 CFU/ml) as a control of the pro-

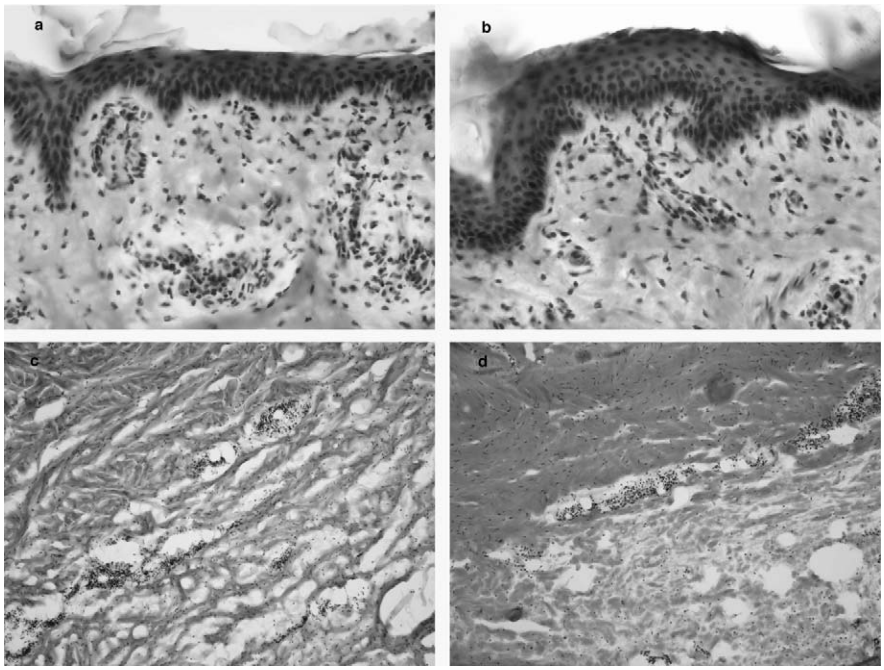

FIGURE 1. *Epidermis, dermis, and hypodermis stained with hematoxylin and eosin and observed with a light microscope. (a) Epidermis and dermis of skin frozen at −80°C, original magnification ×100; (b) epidermis and dermis of skin fixed in liquid nitrogen, original magnification ×100; (c) dermis and hypodermis of skin frozen at −80°C, original magnification ×40; (d) dermis and hypodermis of skin fixed in liquid nitrogen, original magnification ×40.*

posed system, for a total of 24 controls (four times for each marker microorganism).

Sampling procedure. The wet-dry sampling method involved the use of jumbo-head cotton swabs (PBI International). Samples were collected within an area of 25 cm^2 (2.5 by 10 cm). The sampling area was delimited by an autoclaved aluminum foil frame (2.5 by 10 cm). For each sampling area, a swab was moistened in an isotonic diluent (peptone bacteriological, 1.0 g/liter [LP0037, Oxoid], and sodium chloride, 8.5 g/liter) and then rubbed firmly across the sampling area with five strokes. This procedure was then repeated twice, with an angle of approximately 60° each time to ensure an even recovery of bacteria. The procedure was immediately repeated on the same area with a dry swab. Each pair of wet and dry swabs was combined into a single sample in a sterile universal test tube containing 10 ml of isotonic diluent and placed in the refrigerator until microbiological analyses were carried out. Excision-based sampling involved removing a sliver of tissue (2.5 by 10 cm, 3 mm thick) from each skin section. An autoclaved aluminum foil frame (2.5 by 10 cm) was placed over the section, and an initial cut to a depth of approximately 4 mm was made with a sterile surgical blade. The same blade was then used to cut free the tissue sliver from the skin section. Each sample was stored in a single, sealed, polyethylene stomacher bag (PBI International) and placed in the refrigerator for later microbiological analyses. The same procedure was applied for all direct control contaminations of carcasses. All analyses were conducted within 30 min after sampling.

Microbiological analysis. Wet-dry swabs were decimally diluted directly in buffered peptone water (CM1049, Oxoid) before plating. One hundred milliliters of buffered peptone water was added to the stomacher bags containing excised samples, homogenized for 2 min with a stomacher (PBI International), and decimally diluted before plating. Counts of marker bacteria were performed by plating 0.1-ml aliquots of appropriate decimal dilution onto violet red bile glucose agar, to which nalidixic acid, vancomycin-resistant *Enterococcus* agar, and ORSAB were added, respectively, for *E. coli, E. faecalis,* and *S. aureus,* and then these aliquots were incubated in air at 37°C for 24 to 48 h. Uninoculated control samples were treated by the same procedures and plated both on NA and on the three selective media.

Analysis of results. For each trial, the log of the arithmetic means for the three skin sections was calculated, following which all log values were analyzed with GraphPad InStat, version 3.0b, for Mac OS X for the analysis of variance with the Tukey-Kramer multiple comparisons test and the Dunnett multiple comparisons test *(21).*

RESULTS

Light microscope observation of transversal skin sections showed a similar pattern for the epidermis from skin frozen at −80°C and from skin immediately fixed in liquid nitrogen. The dermis and hypodermis, on the other hand, showed the formation of ice crystals and a slender alteration of the structure of the tissue (Fig. 1). *E. coli* K-12, *E. faecalis tet*(M), and *S. aureus mec*(A) were cultured in NB and inoculated on the skin sections. Following the plating of each dilution on NA and specific selective media (violet red bile glucose agar, to which nalidixic acid, vancomycin-resistant *Enterococcus* agar, and ORSAB were added, respectively, for *E. coli, E. faecalis,* and *S. aureus*), no differences were recorded when plating on NA or selective media. The numbers of CFU per milliliter were hence used as controls by calculating the number of microorganisms inoculated in 25 cm^2. No *E. coli* K-12, *E. faecalis tet*(M), or *S. aureus mec*(A) strains were detectable in control skin cuts (skin cuts inoculated with sterile NB) given the absence of growth in violet red bile glucose agar to which nalidixic acid, vancomycin-resistant *Enterococcus* agar base, and ORSAB were added, respectively. Mean values for background microflora in NA were 2.85 CFU/25 cm^2 (skin cuts inoculated with sterile NB and sampled by wet-dry swab) and 3.60 CFU/25 cm^2 (skin cuts inoculated with sterile NB and sampled by excision). The results from the 295 trials are presented in Table 1, which shows the counts

TABLE 1. *Results after inoculum of skin sections with bacterial suspensions at different concentrations*[a]

Inoculum	Control[b]			Wet-dry swabbing			Excision		
	Mean	SE	*n*	Mean	SE	*n*	Mean	SE	*n*
E. coli $\simeq 10^3$	3.23 a[c]	0.08	7	2.21 bc	0.24	7	2.56 ac	0.31	7
E. coli $\simeq 10^4$	3.79 A	0.07	29	2.39 B	0.14	29	3.17 C	0.12	29
E. coli $\simeq 10^5$	4.79 A	0.06	32	3.12 B	0.16	32	3.88 C	0.14	32
E. coli $\simeq 10^6$	5.67 A	0.05	25	3.53 B	0.18	25	4.39 C	0.15	25
E. faecalis $\simeq 10^3$	3.21 a	0.13	7	2.36 bc	0.22	7	2.91 ac	0.26	7
E. faecalis $\simeq 10^4$	3.79 A	0.07	34	2.15 B	0.11	34	3.03 C	0.11	34
E. faecalis $\simeq 10^5$	4.79 A	0.07	34	2.94 B	0.11	34	3.93 C	0.11	34
E. faecalis $\simeq 10^6$	5.68 A	0.06	27	3.73 b	0.11	27	4.36 c	0.13	27
S. aureus $\simeq 10^3$	3.06 A	0.08	8	1.97 bB	0.11	8	2.06 A	0.11	8
S. aureus $\simeq 10^4$	3.73 A	0.05	33	2.34 B	0.09	33	3.19 C	0.09	33
S. aureus $\simeq 10^5$	4.74 A	0.05	34	3.19 B	0.09	34	3.98 C	0.09	34
S. aureus $\simeq 10^6$	5.62 A	0.05	25	3.80 B	0.09	25	4.69 C	0.09	25

[a] Values are expressed as log CFU/25 cm^2.

[b] Theoretical value obtained by calculating the number of microorganisms inoculated in 25 cm^2 of skin.

[c] Different letters within the same row indicate significantly different means (lowercase: $P < 0.05$; underlined lowercase: $P < 0.01$; capital: $P < 0.001$).

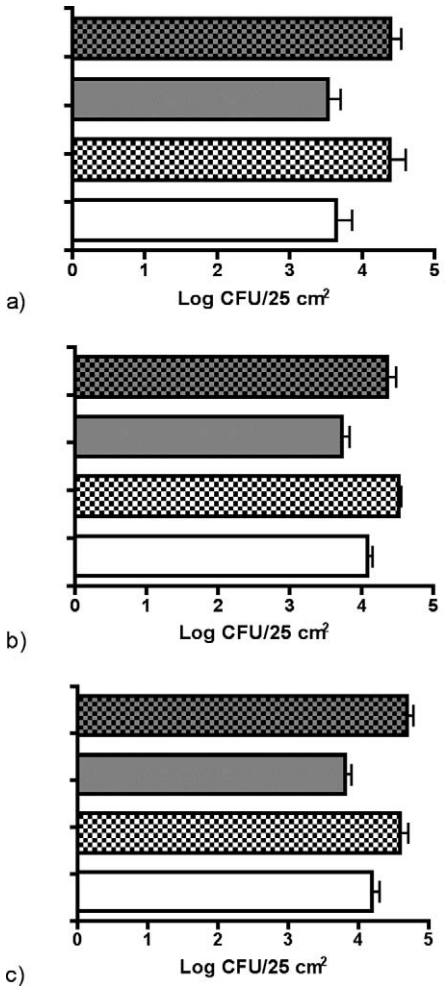

FIGURE 2. *Recovery of marker microorganisms after inoculum of carcasses and skin sections with bacterial suspension at 10^6 CFU/ml. (a)* E. coli, *(b)* E. faecalis, *(c)* S. aureus. □, *Carcass sampled by wet-dry swabbing (*n = *4);* ▨, *carcass sampled by excision (*n = *4);* ▤, *skin sampled by wet-dry swabbing (*n = *25,* n = *27 for* E. faecalis*);* ▦, *skin sampled by excision (*n = *25,* n = *27 for* E. faecalis*).*

from the three marker microorganisms. *E. coli* counts, given as log CFU/25 cm^2, ranged from 2.21 (wet-dry swab) to 2.56 (excision) for skin sections inoculated with bacterial suspension at the concentration of 10^3 CFU/ml, from 2.39 (swab) to 3.17 (excision) for skin sections inoculated with bacterial suspension at 10^4 CFU/ml, from 3.12 (swab) to 3.88 (excision) for skin sections inoculated with bacterial suspension at 10^5 CFU/ml, and from 3.53 (swab) to 4.39 (excision) for the highest inoculum (10^6 CFU/ml). *E. faecalis* counts ranged from 2.36 (swab) to 2.91 (excision) for skin sections inoculated with bacterial suspension at 10^3 CFU/ml, from 2.15 (swab) to 3.03 (excision) for skin sections inoculated with bacterial suspension at 10^4 CFU/ml, from 2.94 (swab) to 3.93 (excision) for skin sections inoculated with bacterial suspension at 10^5 CFU/ml, and from 3.73 (swab) to 4.36 (excision) for skin sections inoculated with bacterial suspension at 10^6 CFU/ml. *S. aureus* counts ranged from 1.97 (swab) to 3.06 (excision) for skin sections inoculated with bacterial suspension at 10^3 CFU/ml, from 2.34 (swab) to 3.19 (excision) for skin sections inoculated with bacterial suspension at 10^4 CFU/ml, from 3.19 (swab) to 3.98 (excision) for skin sections inoculated with bacterial suspension at 10^5 CFU/ml, and from 3.80 (swab) to 4.69 (excision) for skin sections inoculated with bacterial suspension at 10^6 CFU/ml. Control direct contaminations of carcasses (inoculum, 10^6 CFU/ml) yielded similar results. Mean values, expressed as CFU/25 cm^2, for *E. coli* were 3.64 (carcass sampled by wet-dry swab) and 4.38 (carcass sampled by excision); for *E. faecalis,* these mean values were 4.01 and 4.52; and for *S. aureus,* these mean values were 4.18 and 4.56 (Fig. 2). Statistical analysis of the data indicated that for all but two tested inoculum concentrations (*E. coli* and *E. faecalis* at 10^3 CFU/ml), the excision method recovered significantly higher ($P < 0.05$) counts. Statistical analysis also indicated that the extent of marker recovery was generally low and that differences between controls and the two sampling methods considered were statis-

TABLE 2. *Dunnett multiple comparison test between controls and different sampling methods for the three markers at various inocula*

	Control vs wet-dry swabbing		Control vs excision	
10^3	Control vs *E. coli* wet-dry swabbing	$P < 0.05$	Control vs *E. coli* excision	NS[a]
10^4	Control vs *E. coli* wet-dry swabbing	$P < 0.01$	Control vs *E. coli* excision	$P < 0.01$
10^5	Control vs *E. coli* wet-dry swabbing	$P < 0.01$	Control vs *E. coli* excision	$P < 0.01$
10^6	Control vs *E. coli* wet-dry swabbing	$P < 0.01$	Control vs *E. coli* excision	$P < 0.01$
10^3	Control vs *E. faecalis* wet-dry swabbing	$P < 0.01$	Control vs *E. faecalis* excision	NS
10^4	Control vs *E. faecalis* wet-dry swabbing	$P < 0.01$	Control vs *E. faecalis* excision	$P < 0.01$
10^5	Control vs *E. faecalis* wet-dry swabbing	$P < 0.01$	Control vs *E. faecalis* excision	$P < 0.01$
10^6	Control vs *E. faecalis* wet-dry swabbing	$P < 0.01$	Control vs *E. faecalis* excision	$P < 0.01$
10^3	Control vs *S. aureus* wet-dry swabbing	$P < 0.01$	Control vs *S. aureus* excision	NS
10^4	Control vs *S. aureus* wet-dry swabbing	$P < 0.01$	Control vs *S. aureus* excision	$P < 0.01$
10^5	Control vs *S. aureus* wet-dry swabbing	$P < 0.01$	Control vs *S. aureus* excision	$P < 0.01$
10^6	Control vs *S. aureus* wet-dry swabbing	$P < 0.01$	Control vs *S. aureus* excision	$P < 0.01$

[a] NS, not significant.

tically significant (Table 2), with the exception of sections inoculated with bacterial solutions at the concentration of 10^3 CFU/ml and sampled by excision, i.e., for inoculum higher than 10^3 CFU/ml, control counts were significantly higher than counts obtained either after wet-dry swabbing or excision.

DISCUSSION

A total of 22 of 24 comparisons produced significantly different means when controls were compared to sampling methods. This means that none of the sampling methods recovered an adequate amount of bacteria. In fact, the bacterial counts between controls and sampling were different, given that statistically significant differences were always recorded, with the exception of when sections were inoculated with bacterial solutions at a concentration of 10^3 CFU/ml and sampled by excision. For the other comparisons, control counts were always significantly higher than counts from sampled skin sections. In other words, wet-dry swabbing counts were always significantly lower than the controls, and even excision yielded counts significantly lower than the controls. On the other hand, the general observation that excision recovered the highest number of marker microorganisms from inoculated skin sections, compared with swabbing, is the same as practically all existing information, as reported by recent reviews and research articles *(7–9),* and was also a predictable result. The range of inoculum used in this experiment (from 10^3 to 10^6 CFU/25 cm^3) yielded contamination levels usually found in real commercial processing conditions *(16).* In light of the results obtained, some considerations are required. First, the attachment of bacteria to meat surfaces and carcasses needs to be considered. Researchers have studied this phenomenon extensively; however, the exact mechanisms involved are still not fully understood. Attachment is generally considered a two-stage process. The first stage is regulated by physicochemical forces; in the second stage, following a consolidation of the interaction by the secretion of extracellular polysaccharides, growth leading to the formation of complex communities of attached cells may take place *(6, 26).* The stronger binding of bacteria to carcasses (and, in our case, to subcutaneous fat and connective tissue) can be facilitated by exopolysaccharide (glycocalyx) surrounding the cell. Bacterial attachment as a whole is indeed influenced by cell surface charge, hydrophobicity, and structures such as extracellular polysaccharides and flagella *(11).* Our results indicate that particular attention should be paid to the diverse microflora that can contaminate carcasses in a given slaughterhouse and that it may not be appropriate to generalize that the destructive method is the reference technique for the bacteriological sampling of carcasses in slaughterhouses, especially for contaminations higher than 10^3 CFU/25 cm^2. Our data, in fact, demonstrate that, although excision recovers more bacteria than wet-dry swabbing, counts obtained with both methods for contaminations higher than 10^3 CFU/25 cm^2 were significantly lower than the controls. The second consideration refers to alternative, indicator bacteria. Several authors suggest that alternative, indicator bacteria, such as enterococci, are a viable choice as a possible alternative indicator group for use in detecting the fecal contamination of beef carcasses *(17).* Our findings demonstrate that the choice of sampling method for a given indicator should be carefully selected after a thorough search in the available literature or possibly after sampling validation trials. In fact, at lower contamination levels (10^3 CFU/25 cm^3), no significant differences were observed between counts from wet-dry swabbing and excision for *E. coli* and *E. faecalis.* Has the scientific community overstressed the importance of the best sampling method? This is the third consideration. In fact, article 10 of Directive 64/433 *(3)* states that "... the operator of the establishment, the owner or his agent must conduct regular checks on the general hygiene of conditions of production in its establishment, inter alia, by means of microbiological controls." In other words, microbiological controls can demonstrate the general hygienic conditions in a given establishment—but not necessarily. Our results demonstrate that the publication of absolute limits, along with reference sampling methods, such as those introduced by Decision 2001/471 and its amendments *(1, 2, 4)* and by Regulation 2073/2005

(5), can be misleading. In fact, our results clearly demonstrate that both methods, generally considered the worst (wet-dry swabbing) and the best (excision) in terms of bacterial recovery *(7, 8, 12, 14, 16, 23–25),* fail when challenged with known contamination levels higher than 10^3 CFU/25 cm^2. Consequently, neither of these two methods could generate ''absolute'' data nor fulfilment of ''criteria''; they could be used only for relative data or trend analysis. We believe, and our data confirm this belief, that figures obtained from a given slaughterhouse with a specific sampling protocol should be regarded as relative to that establishment. Finally, since conventional comparisons of methods under commercial processing conditions are cumbersome and expensive, require lengthy trials, and are sometimes inaccurate because of the nonuniform distribution of bacteria between different carcasses *(16),* the method described in the present study has the potential for providing a rapid comparison and generating a large amount of data in a short time. The direct contamination of the carcass surface, conducted with the highest marker concentration (10^6 CFU/ml), as a control of the proposed system, yielded similar results when compared to the average recovery obtained throughout the experiment. The light microscope observation of transversal skin sections demonstrates that the epidermis, probably because of its low water content, was unaffected by the slow freezing procedure. The dermis and hypodermis, on the other hand, show the consequences of the formation of ice crystals that have only faintly altered the structure of the tissue. Nonetheless, according also to microbiological results, such a negligible alteration has not influenced bacterial adhesion. These two important observations are a guarantee of the appropriateness of the model proposed. The entire experiment described in this article (1,872 microbial analyses) was completed in only 8 weeks (2 weeks per man). The proposed system may prove useful for the rapid assessment and validation of sampling methods, thus increasing our knowledge regarding the behavior of microorganisms on carcasses and meat surfaces.

ACKNOWLEDGMENTS

This research was supported by grants from the Ministero degli Affari Esteri, Direzione Generale per la Promozione e Cooperazione Culturale, Rome, Italy, executive program of scientific and technological cooperation between the Italian Republic and the Republic of South Africa 2005–2007, significant bilateral project between the Facoltà di Medicina Veterinaria, Università degli Studi di Perugia, Italy, and the Faculty of Veterinary Medicine, University of Pretoria, South Africa. The authors thank the Cooperativa Macellatori, Perugia, for support and interest and Drs. A. Codega de Oliveira and E. Bartocci for preparing the sampling material and express sincere appreciation to members of Polyglot, Perugia, for their careful reading and comments on the manuscript.

REFERENCES

1. Anonymous. 2001. Commission Decision of 8 June 2001 laying down rules for the regular checks on the general hygiene carried out by the operators in establishments according to Directive 64/433/EEC on health conditions for the production and marketing of fresh meat and Directive 71/118/EEC on health problems affecting the production and placing on the market of fresh poultry meat. Commission Decision 2001/471/EC. *Off. J. Eur. Communities* L 165:48–53.
2. Anonymous. 2004. Commission Decision of 26 April 2004 amending Decision 2001/471/EC as regards bacteriological tests in certain meat establishments. Commission Decision 2004/379/EC. *Off. J. Eur. Communities* L 144:1–4.
3. Anonymous. 2004. Consolidated text—CONSLEG: 1964L0433, p. 1–40 Vol. 64/433/EEC. Office for Official Publications of the European Communities.
4. Anonymous. 2004. Corrigendum to Commission Decision 2004/379/EC of 26 April 2004 amending Decision 2001/471/EC as regards bacteriological tests in certain establishments. Commission Decision 2004/379/EC. *Off. J. Eur. Communities* L 199:1–2.
5. Anonymous. 2005. Commission Regulation (EC) no. 2073/2005 of 15 November 2005 on microbiological criteria for foodstuffs. Commission Regulation 2073/2005/EC. *Off. J. Eur. Communities* L 338: 1–26.
6. Benito, Y., C. Pin, M. L. Marin, M. L. Garcia, M. D. Selgas, and C. Casas. 1997. Cell surface hydrophobicity and attachment of pathogenic and spoilage bacteria to meat surfaces. *Meat Sci.* 45:419–425.
7. Bolton, D. J. 2003. The EC decision of the 8th June 2001 (EC/471/2001): excision versus swabbing. *Food Control* 14:207–209.
8. Byrne, B., G. Dunne, J. Lyng, and D. J. Bolton. 2005. Microbiological carcass sampling methods to achieve compliance with 2001/471/EC and new hygiene regulations. *Res. Microbiol.* 156:104–106.
9. Capita, R., M. Prieto, and C. Alonso-Calleja. 2004. Sampling methods for microbiological analysis of red meat and poultry carcasses. *J. Food Prot.* 67:1303–1308.
10. Cenci-Goga, B. T., L. Budelli, R. Branciari, D. Ranucci, D. Miraglia, and R. Ortenzi. 2005. Comparison of two microbiological sampling techniques using marker microorganisms in an in-vitro model. Atti SISVet LIX, Viareggio, Italy 2005. LIX:377–378. Available at: http://www.sisvet.it/convegni/LIX/issn.htm, ISSN 1825-4454. Accessed September 2006.
11. Dickson, J. S., and M. Koohmaraie. 1989. Cell surface charge characteristics and their relationship to bacterial attachment to meat surfaces. *Appl. Environ. Microbiol.* 55:832–836.
12. Dorsa, W. J., G. R. Siragusa, C. N. Cutter, E. D. Berry, and M. Koohmaraie. 1997. Efficacy of using a sponge sampling method to recover low levels of *Escherichia coli* O157:H7, *Salmonella* typhimurium, and aerobic bacteria from beef carcass surface tissue. *Food Microbiol.* 14:63–69.
13. Gill, C. O., and T. Jones. 1998. Comparison of methods for sampling and enumerating *Escherichia coli* on pig carcasses. *Food Microbiol.* 15:617–623.
14. Gill, C. O., and T. Jones. 2000. Microbiological sampling of carcasses by excision or swabbing. *J. Food Prot.* 63:167–173.
15. Gold, H. S., and R. C. Moellering, Jr. 1996. Antimicrobial-drug resistance. *N. Engl. J. Med.* 335:1445–1453.
16. Hutchison, M. L., L. D. Walters, S. M. Avery, C. A. Reid, D. Wilson, M. Howell, A. M. Johnston, and S. Buncic. 2005. A comparison of wet-dry swabbing and excision sampling methods for microbiological testing of bovine, porcine, and ovine carcasses at red meat slaughterhouses. *J. Food Prot.* 68:2155–2162.
17. Ingham, S. C., and D. J. Schmidt. 2000. Alternative indicator bacteria analyses for evaluating the sanitary condition of beef carcasses. *J. Food Prot.* 63:51–55.
18. Kluytmans, J., A. V. Griethuysen, W. Piet, and P. V. Keulen. 2002. Performance of CHROMagar selective medium and oxacillin resistance screening agar base for identifying *Staphylococcus aureus* and detecting methicillin resistance. *J. Clin. Microbiol.* 40:2480–2482.
19. Mead, G. C., W. R. Hudson, and M. Hinton. 1994. Use of a marker organism in poultry processing to identify sites of cross-contamination and evaluate possible control measures. *Br. Poult. Sci.* 35: 345–354.
20. Miraglia, D., D. Ranucci, V. D'Ovidio, R. Branciari, and M. Severini. 2005. Comparison between carcass microbial load recovered by swabbing surfaces of different size and using the reference excision method. *Vet. Res. Commun.* 29:339–341.
21. Motulsky, H. 2003. The InStat guide to choosing and interpreting statistical tests. GraphPad Software, Inc., San Diego, Calif.

22. Palumbo, S. A., P. Klein, J. Capra, S. Eblen, and A. J. Miller. 1999. Comparison of excision and swabbing sampling methods to determine the microbiological quality of swine carcass surfaces. *Food Microbiol.* 16:459–464.
23. Pearce, R. A., and D. J. Bolton. 2005. Excision vs sponge swabbing—a comparison of methods for the microbiological sampling of beef, pork and lamb carcasses. *J. Appl. Microbiol.* 98:896–900.
24. Pepperell, R., C. A. Reid, S. N. Solano, M. L. Hutchison, L. D. Walters, A. M. Johnston, and S. Buncic. 2005. Experimental comparison of excision and swabbing microbiological sampling methods for carcasses. *J. Food Prot.* 68:2163–2168.
25. Ransom, J. R., K. E. Belk, R. T. Bacon, J. N. Sofos, J. A. Scanga, and G. C. Smith. 2002. Comparison of sampling methods for microbiological testing of beef animal rectal/colonal feces, hides, and carcasses. *J. Food Prot.* 65:621–626.
26. Warriner, K., K. Eveleigh, J. Goodman, G. Betts, M. Gonzales, and W. M. Waites. 2001. Attachment of bacteria to beef from steam-pasteurized carcasses. *J. Food Prot.* 64:493–497.