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Artigo

Patogenic and indicator microorganisms in chicken cuts sold in the Recôncavo da Bahia area, Brazil

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Abstract: Hygiene-sanitary conditions, the presence of *Salmonella* spp. and indicator microorganisms of chicken cuts sold on the market in the Recôncavo da Bahia area, are analyzed and their co-relationship with conditions of commercialization is verified. Sixty samples were collected between May and August 2016 at informal selling sites (fairs, butcher shops, abattoirs) and in supermarkets of ten municipalities of the Recôncavo da Bahia region. Hygiene and sanitary conditions were assessed through a check list. Total coliforms, *Escherichia coli,* mesophylls, psychrotrophic organisms, molds and yeast, and *Salmonella* spp. were counted. Results revealed statistical difference for total coliforms and *Escherichia coli* only, featuring greater concentration rates in fairs, butcher shops and abattoirs. Further, 31.7% of samples indicated 31.7% *Salmonella* spp. and 16.6% of samples failed to comply with sanitary legislation on *Escherichia coli*. Observational analysis demonstrated that 61.83% and 38.1% of the samples respectively on informal sites and in supermarkets did not comply with legislation and indicated a co-relationship of some variables with the growth of total coliforms, *Escherichia coli* and mesophylls. Pathogenic and indicator microorganisms suggest flaws in hygiene and sanitary conditions in the commercialization of chicken cuts and health risks to consumers.

Indexing Terms: microbiological contamination, hygiene and sanitary conditions, *Salmonella*, chickens.

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Introduction

Chicken meat, highly relevant for human beings, is one of the most sold items in Brazil and worldwide, due to its low fat rate and low costs (OLIVEIRA; SALVADOR, 2011; PENTEADO; ESMERINO, 2011; SILVA; MENÃO, 2015). Data derived from Family Budget Research show that the predominance of chicken meat in Brazil reached 27% when compared to other food items. In the case of prevalence per region, it is mostly consumed in the north-eastern region of Brazil, at 29.7%, when compared to that in other regions (IBGE, 2011). In spite of high consumption rates, chicken meat is reported to be a vector of microorganisms indicating improper hygiene conditions and even pathogenic microorganisms, such as *Salmonella* spp. and *Escherichia coli*. In natura chicken meat ranks ninth among epidemic-causing foods in Brazil (BRASIL, 2016).

Resolution RDC N. 12 published on 2nd January 2001 on food microbiological standards in Brazil provides the maximum coliform limits (10^4 CFU/g) at 45ºC for *in natura* chicken

meat. However, other microorganisms may indicate flaws in the production or commercialization of this type of food.

The verification of the commercialization quality of chicken cuts is thus highly relevant to avoid health risks which this type of food may cause when contaminated by pathogenic microorganisms. Hygiene-sanitary conditions, the presence of *Salmonella* spp. and indicator microorganisms of chicken cuts sold on the market of the Recôncavo da Bahia area, are analyzed and counted, and the relationship between conditions of commercialization and microbiological results is verified.

Materials and Methods

Current study was undertaken in ten municipalities in the Recôncavo da Bahia area, comprising Santo Antônio de Jesus, Nazaré, Cachoeira, Cruz das Almas, Santo Amaro, São Felipe, Conceição do Almeida, Governador Mangabeira, Castro Alves and Sapeaçu, between May and August 2016. Three supermarket suppliers and three selling sites, such as fairs, butcher shops or abattoirs, were selected. All 60 samples consisted of chicken thigh and drumstick sold at each selling site. They were conditioned in thermal boxes and taken for analysis to the Laboratory of Microbiology of the Universidade Federal do Recôncavo da Bahia.

At the instance of sample collection, temperature was taken and samples were observed through a verification list based on Resolutions RDC n. 275/2002 and RDC n. 216/2004, featuring hygiene and sanitary conditions of chicken cuts, selling sites and handlers (BRASIL, 2002; BRASIL, 2004). Data obtained from verification identified sample percentages which complied or did not comply with the resolutions above. Correlation between results from observation and microbiological analyses was undertaken. Further, 25g of each sample were retrieved and added to 225 mL of peptone water 0.1% for the first dilution. Series dilutions were performed up to dilution 10^{-6} for counts of total coliforms, *Escherichia coli*, mesophylls, psychrotrophic organisms, molds and yeast (SILVA et al., 2007).

Total coliforms and *Escherichia coli* were analyzed by pour plate technique by which 1 mL of diluted samples was transferred to petri plates with approximately 15 mL of culture medium HiCrome® Coliform Agar of HIMEDIA and homogenized. After solidification, the plates were inverted and incubated at 35°C during 24 hours (SILVA et al., 2007).

Total coliform and *Escherichia coli* colonies were counted: colonies ranging from dark blue to violet were *Escherichia coli* colonies, whilst colonies ranging from salmon pink to red were colonies of other coliforms, according to the manufacturer´s instructions.

Pour plate technique was also employed to count mesophyll and psychrotrophic microorganisms employing culture medium Plate Count Agar (PCA) from Merck®. Plates were incubated in a buffer at 35°C during 48 hours for the growth of mesophylls; in the case of psychrotrophic microorganisms they were kept in a refrigerator at 7ºC during seven days. Colony count was undertaken after these periods (SILVA et al., 2007).

Mold and yeast were counted by spread plate method where 0.1 mL of each diluted sample was transferred to petri plates with solidified culture medium Sabouraud Dextrose Agar from Merck® spread with sterile Drigalski spatula. Plates were then inverted and incubated at 20ºC in BOD for 5 days, after which colonies were counted (SILVA et al., 2007).

Further, 3M® Petrifilm *Salmonella* Express system was employed to detect *Salmonella* spp, where 25g of each sample were transferred to 225 mL enriched broth for *Salmonella* prepared with Enrichment Base 3M® *Salmonella* Express whilst supplement 3M® *Salmonella Express* was homogenized and incubated at 41.5º C for 18 hours. After incubation, 10 µL of the sample were striated with inoculation spatula on Petrifilm plates which had been previously hydrated with 2 mL of distilled water. Plates were again incubated at 41.5º C for 24 hours. Plates were then read by circling presumed *Salmonella* spp. colonies directly on the film characterized by red to brown color with a yellow zone or with the formation of associated gas or both.

Biochemical confirmation disc was placed on plates with colonies featuring these characteristics and newly incubated at 41.5º C for 4 hours. Changes from red-brown to green-bluish, dark blue or black confirmed the presence of *Salmonella* spp. with results given as positive/negative. Results of microorganism counts were given in log CFU/g and compared to Brazilian legislation, or rather, Resolution 12 published on the 2nd January 2001, providing microbiological standards for animal-derived food (BRASIL, 2001).

Statistical analysis was performed by SPSS 17.0 with descriptive analysis for microorganism count and proportion analysis for qualitative variables, or rather, research results on *Salmonella* spp. and checklist. Student´s *t* test compared the microbiological profile of chicken cuts according to commercialization sites and correlated concentrations of microorganisms with regard to variables of the verification list. Pearson´s correlation revealed correlation between temperature and growth of microorganisms in samples at 5% significance level ($p<0.05$).

Results and Discussion

Table 1 shows mean results of the microbiological analysis and minimum and maximum rates in samples, according to the selling site**.** Selling sites were statistically different for total coliforms and *Escherichia coli*, with higher concentrations rates in informal selling sites (fairs/butcher shops/abattoirs) at $p < 0.05$.

Table 1: Microbiological characterization of samples of chicken thighs and drumsticks in fairs/butcher shops/abattoirs and supermarkets in municipalities of the Recôncavo da Bahia area, Brazil, between May and August 2016.

	FAIRS/BUTCHER SHOPS/ ABATTOIRS log CFU/g				SUPERMARKETS log CFU/g			
MICROORGANISMS	MIN	MAX	AV	SD	MIN	MAX	AV	SD
Total coliforms	< 1.0	7.10	4.73*	1.23	2.00	6.00	4.13*	0.75
Escherichia coli	< 1.0	6.70	2.82*	1.84	< 1.0	5.20	1.56*	1.17
Mesophylls	< 1.0	8.30	5.57^{ns}	1.38	3.73	8.30	5.07^{ns}	0.75
Psychrotrophics	< 1.0	8.01	3.76^{ns}	2.30	< 1.0	9.10	3.88^{ns}	2.14
Molds and yeasts	2.90	8.48	5.18^{ns}	1.47	2.78	8.00	4.68^{ns}	1.67

MIN = minimum rate in samples; MAX = maximum rate in samples; AV = average rates in the municipalities; SD = standard deviation; * = significant at 5% probability ($p<0.05$); ^{ns} = not significant.

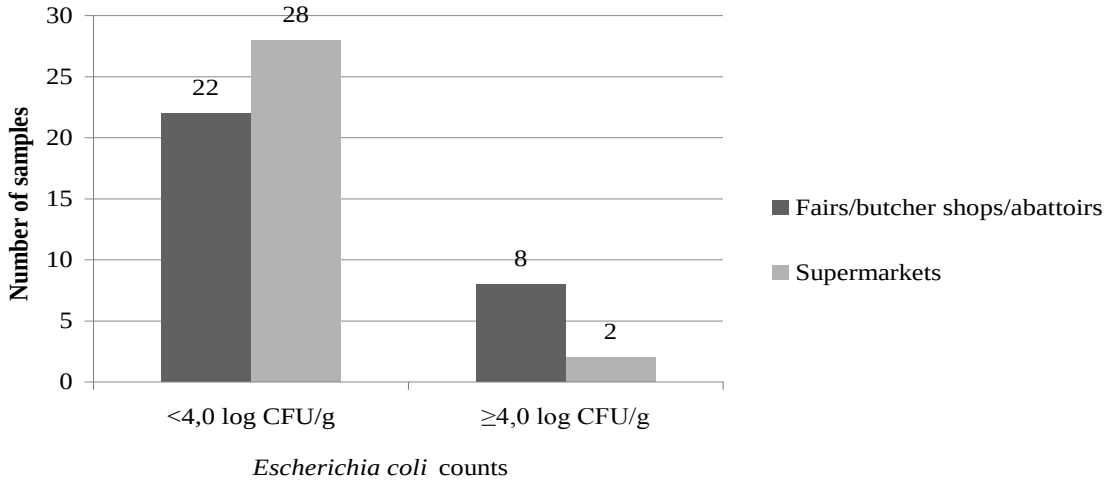
Similar averages (>2.7 log CFU/g) of thermotolerant coliforms in five chicken thighs and drumsticks sold at butcher shops in the city of São

Paulo, Brazil, were registered by Silva & Menão (2015), demonstrating serious flaws during processing. Carvalho et al. (2005) also assessed chicken thighs and drumsticks sold in the city of Jaboticabal, Brazil, and reported *Escherichia coli* counts ranging between 2.55 and 3.63 log CFU/g and between 3.17 and 3.63 log CFU/g for total coliforms.

In their research on the prevalence of microorganisms in chicken carcasses which had been supervised or not in the city of Viçosa, Brazil, Coussi et al. (2012) identified high rates of total coliforms. Mean total coliforms amounted to 2.99 log CFU/g for inspected samples and 2.54 log CFU/g for non-inspected ones, suggesting that high total coliform rates indicated improper hygiene and sanitary conditions in the production and commercialization of food.

Resolution RDC of the 2nd January 2001 establishes maximum number of coliforms at 45º or thermotolerant coliforms of 4.0 log CFU/g for *in natura* or refrigerated chicken cuts. Average *Escherichia coli* counts, the main representative of the coliform group, lie within the limits established by law but eight samples from fairs/butcher shops/abattoir and two samples from supermarkets had rates higher than those
legally recommended, or rather, 16.6% of total (**Figure 1**).

Figure 1: Number of samples of chicken thighs and drumsticks complying or not with Resolution RDC N. 12/2001 on *Escherichia coli* .



Species of *Escherichia coli* are one of the main pathogenic agents vectored by food, such as *Escherichia coli* O157:H7 (SHEKARFOROUSH et al., 2015).

Although Brazilian law does not prescribe a limit for mesophylls in chicken cuts, high rates of the microorganism have been reported, corroborating with results by Coussi et al. (2012) featuring mean 5.45 log CFU/g in chicken samples commercialized in Viçosa, MG, Brazil.

Mesophylls are a health risk for consumers since most pathogenic microorganisms are mesophylls (FRANCO; LANDGRAF, 2008). Further, high counts of these microorganisms in chicken meat are

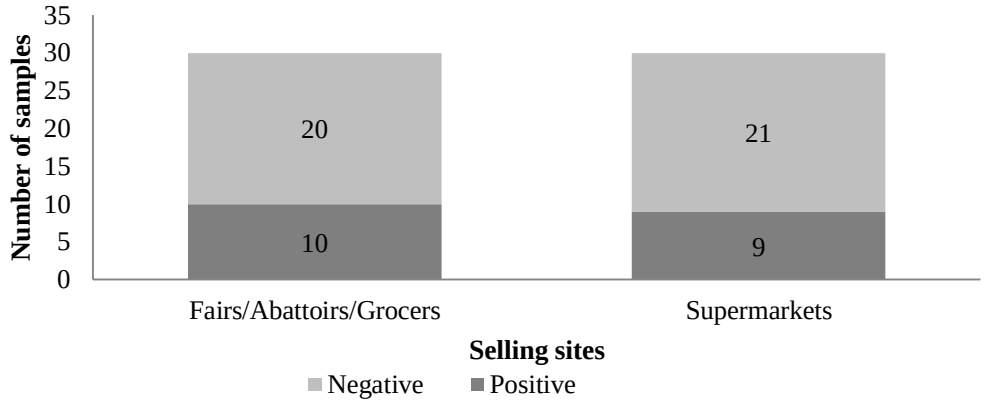
associated with poor hygiene and sanitary conditions during slaughter or carcass processing (OLIVEIRA et al., 2009).

Similar data on psychrotrophic organisms were reported by Galarz, Fonseca and Prentice-Hernández (2010) who obtained mean 3.66 log CFU/g in samples from a chicken meat-processing industry in the state of Santa Catarina, Brazil. A similar average was obtained in chicken meat samples from abattoirs in the northern region of the state of Paraná, Brazil (LOPES et al., 2007). Although sanitary legislation lacks reference limits for psychrotrophic microorganisms, high rates may indicate the product´s contamination, conservation flaws, an estimate of product life and degree of deterioration (JAY et al., 2005; GALARZ; FONSECA; PRENTICE-HERNÁNDEZ, 2010).

Sanitary legislation for chicken meat fails to give a standard in mold and yeast counts. However, their presence in food makes them the main deterioration agents (PENTEADO; ESMERINO, 2011). Oliveira et al. (2009) also reported high rates of mold and yeast in chicken samples in abattoirs inspected by sanitary officials. Contamination reached 4.1 log CFU/g.

Figure 2 demonstrates the number of samples contaminated by *Salmonella* spp. according to selling sites. Nineteen (31.7%) out of sixty samples were contaminated by *Salmonella* spp, with 10 positive samples (33.3%) from informal selling sites (fairs, butcher shops and abattoirs). Nine samples (30%) with *Salmonella* spp were extant in supermarkets.

Figure 2. *Salmonella* sp. detected in chicken meat samples according to selling sites.



Guran, Mann and Alali (2017) also registered similar tallies for *Salmonella* spp. in chicken thighs commercialized in Atlanta GA USA, with 31.9% testing positive for *Salmonella* spp. Zhu et al. (2017) evaluated chicken meat contamination commercialized in several towns in China and detected 41.5% of samples with *Salmonella* spp. High prevalence of *Salmonella* spp. was reported in 43% of chicken samples analyzed by Yamatogi et al. (2011) on the retail market in Botucatu SP Brazil.

Table 2 shows compliance or non-compliance percentages of samples according to indicators in the check list. In the case of characteristics (color, aspect and smell) of chicken thighs and

drumsticks, all samples were compliant to legislation and showed typical features. However, 83.3% of samples in fairs, butcher shops and abattoirs were sold in improper packaging, such as in bags manufactured for any type of article, without any identification or reference to sanitary inspection stamp and expiring date. Only 6.7% of samples were inadequate in supermarkets.

Non-compliance for shelf display occurred only in fairs, butcher shops and abattoirs since 80% of the items were sold lacking hygiene and refrigeration. They were even handled by consumers. Further, chicken cuts at all selling sites were sold close to other products.

Although all samples retrieved from supermarkets were refrigerated, the temperature of several products was rather high: refrigeration temperature should range between 0 and 7ºC and freezing should be below 0ºC (ABERC, 2003). High temperatures favor microbial growth and chicken cuts should be cooled or frozen to avoid health risks to consumers (BRASIL, 2001).

Expiring date and sanitary inspection stamp on packages occurred in 86.7% of samples from supermarkets, although the occurrence in the other selling sites was low (16.7%) since most had neither specific packaging nor any identification. Vectors and pests were reported at 13.3% of informal selling sites, especially where chicken cuts were exposed without any protection or refrigeration.

The use of uniform by handlers was low at all selling sites, namely, 3.3% in informal selling sites and 13.3% in supermarkets. All selling sites showed somewhat lack of personal hygiene in handlers, such as dirt on finger nails, untrimmed beards and the use of trinkets. Percentage was high at fairs/butcher shops/abattoirs with 70% of handlers simultaneously dealing with money and meat cutting. Tavakoli et al. (2017) reported personal hygiene as one of the main factors in bacteria growth due to handlers´ direct contact with food.

Table 2: Compliance and non-compliance percentages of chicken meat samples in fairs/butcher shops/abattoirs in the municipalities of the Recôncavo da Bahia area between May and August 2016.

INDICATOR	FAIRS/ BUTCHER SHOPS/ ABATTOIRS		SUPERMARKETS	
	%C	%NC	%C	%NC
Color	100	0	100	0
Physical aspect	100	0	100	0
Smell	100	0	100	0
Packaging	16.7	83.3	93.3	6.7
Shelf	20.0	80.0	100	0
Refrigeration	26.7	73.3	100	0
Closeness to other products	0	100	0	100
Expiring date	16.7	83.3	86.7	13.3
Sanitary inspection stamp	16.7	83.3	86.7	13.3
Presence of vectors and pests	86.7	13.3	100	0
Use of uniform by handlers	3.3	96.7	13.3	86.7
Personal hygiene	0	100	0	100
Simultaneous money and food handling	30	70	100	0
Total (%)	38.17	61.83	61.90	38.10

C = Compliant; N = Non-compliant.

Table 3 shows variables on the check list correlated to microbial growth. There was statistical difference ($p<0.05$) between *Escherichia coli* and the variables Refrigeration, Package, Shelf display, Expiring date, Sanitary inspection stamp and simultaneous money-food handling, with high concentration of microorganisms in the non-compliant samples.

Table 3: Correlation between observation analysis and microbiological results of chicken meat samples retrieved from fairs/butcher shops/abattoirs and supermarkets in the municipalities of the Recôncavo da Bahia area between May and August 2016.

Variables on checklist	TC		*Escherichia coli*		Mesophylls	
	C	NC	C	NC	C	NC
Refrigeration	4,23	4,78*	1,52	3,35*	5,13	5,66 ^{ns}
Package	4,32	4,57 ^{ns}	1,58	2,93*	5,26	5,40 ^{ns}
Shelf	4,27	4,66 ^{ns}	1,57	3,12*	5,17	5,54 ^{ns}
Expiring date	4,31	4,56 ^{ns}	1,63	2,79*	5,22	5,43 ^{ns}
Sanitary inspection stamp	4,31	4,56 ^{ns}	1,63	2,79*	5,22	5,43 ^{ns}
Use of uniform by handlers	6,44	4,39 ^{ns}	5,30	2,14 ^{ns}	8,20	5,27*
Simultaneous Money and Food handling	4,20	4,75*	1,74	2,80*	5,03	5,54 ^{ns}

C=Compliant; NC=Non-compliant; * = Significant at 5% probability ($p<0.05$); ^{ns} = non-significant.

Total coliforms had positive and significant correlation with the variables Refrigeration and Simultaneous Money-Food handling, featuring higher rates in the non-compliant samples. There was a statistical difference between the variable Use of Uniform and mesophylls, with great concentration rates in the non-compliant groups. **Figure 3** shows mean, minimum and maximum temperatures of samples. Mean temperature of samples in fairs/butcher shops/abattoir was 18.6ºC, with maximum temperature at 36.5ºC. Temperature control is a basic factor that directly affects the food´s microbiological quality (TAVAKOLI et al., 2017).

Current research shows slight but significant correlation between *Escherichia coli* counts and temperature of samples during selling, or rather, the higher the temperature, the greater was the concentration of *Escherichia coli*.

There was also a significant correlation in psychrotrophic microorganisms, or rather, the higher the temperature, the lower is the concentration of the microorganisms (**Table 4**).

No statistical difference ($p<0.05$) occurred between temperature and variables on checklist with regard to *Salmonella* spp. in the samples.

Figure 3. Temperature rates of chicken thighs and drumsticks sold in the municipalities in the Recôncavo da Bahia area between May and August 2016.

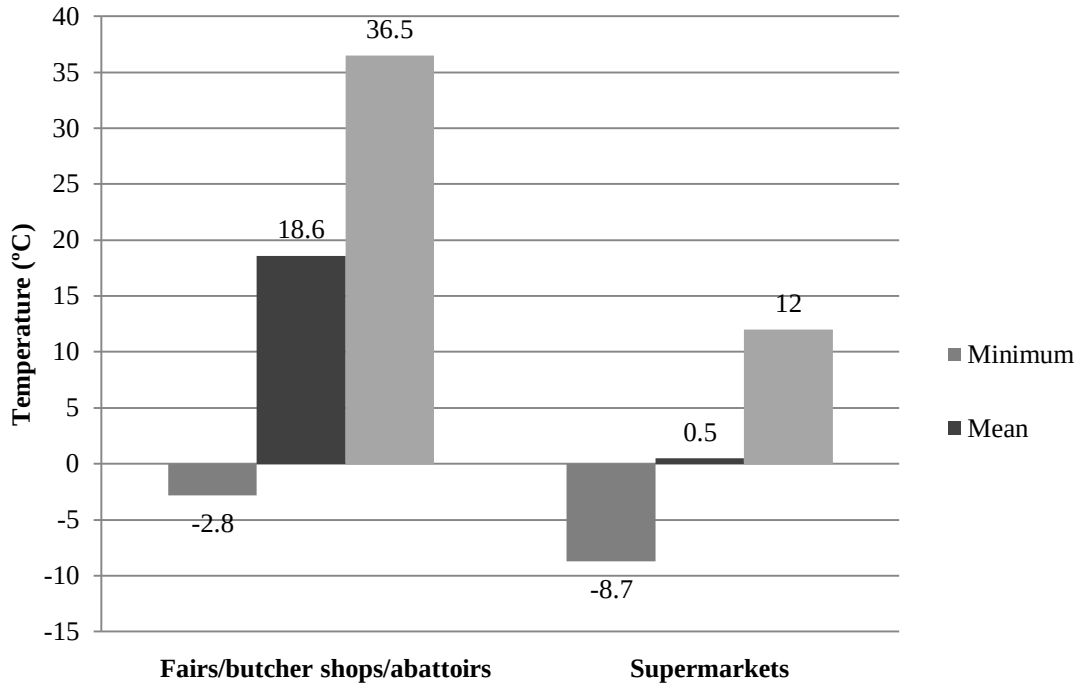


Table 4: Correlation between temperature during selling and the number of microorganisms in chicken meat samples from fairs/butcher shops/abattoirs and supermarkets in the municipalities of the Recôncavo da Bahia area between May and August 2016.

Microorganisms	Pearson´s correlation	Significance
Total coliforms	0.23 ^{ns}	0.68
Escherichia coli	0.46*	0.00
Mesophylls	0.10^{ns}	0.44
Psychrotrophic microorganisms	-0.20*	0.01
Molds and yeasts	0.20 ^{ns}	0.12

* = Significant at 5% probability ($p<0.05$); ^{ns} = non-significant

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