ENTEROBACTERIA ISOLATION IN BROILER CARCASSES FROM COMMERCIAL ESTABLISHMENTS IN FORTALEZA, CEARÁ STATE, BRAZIL

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ABSTRACT

Processing of poultry products requires a severe microbiological quality control, considering they are one of the main sources of foodborne infections. The objective of this research was to perform the isolation of enterobacteria in broiler carcasses from commercial establishments in the Metropolitan Region of Fortaleza in Ceará State, Brazil. Broiler carcasses were collected and selected as fresh (n = 14), refrigerated (n = 18) and frozen (n = 19). Carcasses were submitted to a rinsing method, followed by pre-enrichment and enrichment with Rappaport-Vassiliadis and Selenite-Cystine, streaked on plates with Brilliant Green, MacConkey and Salmonella-Shigella agars, and to a presumptive biochemical identification. It was verified that all broiler carcasses categories presented enterobacteria contamination, with the following frequency of isolation: *Proteus* sp., 66.7%; *Enterobacter* sp., 15.7%; *Citrobacter* sp., 2%; *Escherichia coli*, 47.1%; *Klebsiella* sp., 11.8%; *Shigella* sp., 5.9%, and *Salmonella* sp. 11.8%. It was observed that no combination of culture media was able to detect all enterobacteria contamination in the broiler carcasses. Thus, it may be necessary the use of several combinations of culture media to determine the real microbiological quality of broiler carcasses.

KEY WORDS: Enterobacteria, broiler carcass, culture media, microbiological quality, *Salmonella*, methodology.

RESUMO

ISOLAMENTO DE ENTEROBACTÉRIAS EM CARCAÇAS DE FRANGOS DE CORTE PROCE-DENTES DE PONTOS COMERCIAIS DE FORTALEZA, ESTADO DO CEARÁ, BRASIL. A carne de frango requer um rígido controle de qualidade microbiológica em seu processamento, uma vez que é uma das principais fontes de infecção alimentar para o homem. Desta forma, a presente pesquisa teve como objetivo realizar o isolamento de enterobactérias em carcaças de frango de corte obtidas em estabelecimentos comerciais na região metropolitana de Fortaleza no Estado do Ceará, Brasil. As carcaças foram coletadas e agrupadas em três categorias: frescas (n = 14), refrigeradas (n = 18) e congeladas (n = 19). As amostras foram submetidas a um método de enxaguadura, seguido por pré-eriquecimento e enriquecimento seletivo com os meios Rappaport-Vassiliadis e Selenito-Cistina, plaqueamento nos meios sólidos Verde Brilhante, MacConkey e Salmonella-Shigella e, posteriormente, a provas bioquímicas de identificação. Foram realizadas diversas combinações entre os meios para o processamento das amostras. Todas as categorias de carcaças apresentaram contaminação por enterobactérias, com as seguintes freqüências de isolamentos: *Proteus* sp., 66,7%; *Enterobacter* sp., 15,7%; *Citrobacter* sp., 2%; *Escherichia coli*, 47,1%; *Klebsiella* sp., 11,8%; *Shigella* sp., 5,9% e *Salmonella* sp., 11,8%. Nenhuma combinação dos meios de cultura empregados foi capaz de isolar todas as contaminações por enterobactérias nas carcaças. Desta forma, é necessário o uso de várias combinações de meios de cultura para determinação real da qualidade microbiológica de carcaças de frango.

PALAVRAS-CHAVE: Enterobactérias, carcaça de frango, meios de cultura, qualidade microbiológica, *Salmonella*, metodologia.

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INTRODUCTION

Microbial food safety is an increasing public health concern worldwide. It is estimated that each year in the United States there are approximately 76 million food-borne illnesses (MEAD et al., 1999).

Broiler meat consumption is known as one of the main sources of food-borne infections in humans (FITZGERALD et al., 2001). Processing of poultry products requires a severe microbiological quality control, considering that they are the most accessible protein sources.

The level of *Enterobactereaceae* in poultry carcasses can be routinely used as an indicator of improper hygiene methods during processing and incorrect storage conditions, which can lead to the proliferation of pathogens, such as *Salmonella*, and toxin production (ROBERTS et al., 1995). Most enterobacteria inhabit human and animal intestines as members of their normal microbiota or as infectious agents (TRABULSI; CAMPOS, 2002). Contamination control during slaughter and processing has been identified as an ultimate requirement in order to detect the prevalence of pathogenic microorganisms in poultry products. Microbiological status of broiler carcasses depends on several factors, such as: infection level of living birds, cross contamination, amount and variety of pathogens, among others (ABU-RUWAIDA et al., 1994).

Despite careful practices performed during processing, carcasses may become contaminated with feces. Approximately 0.8-5% of the broiler chickens processed in the USA and Canada must be reprocessed or trimmed due to contamination or disease (RUSSELL; WALKER, 1997).

Campylobacter, *Salmonella*, and pathogenic *E. coli* can colonize the gastrointestinal tracts of a wide range of wild and domestic animals, especially animals raised for human consumption (MENG; DOYLE, 1998).

BUZBY (2002) reported that the occurrence of *Salmonella* infection in Americans over 60 years old was 10.8 cases per 100,000 and 17.4 per 100,000 at all ages. NADVORNY et al. (2004) found that broiler meat was responsible for 11.4% of food-borne diseases outbreaks in 2000 in Rio Grande do Sul State. Since 1993, salmonellosis has been considered the major cause of illness and between 1987 and 2000, *Salmonella* was the cause of 34.1% of all outbreaks investigated in the same state.

The objective of this research was to evaluate the isolation of enterobacteria in broiler carcasses from commercial establishments in the Metropolitan Region of Fortaleza in Ceara State, Brazil.

MATERIAL AND METHODS

Broiler carcasses were collected from sales points and processing plants, and were selected into three categories: fresh carcasses (14 samples), collected in two processing plants with no federal sanitary inspection (7 carcasses in each plant); refrigerated carcasses (18 samples), ten in one supermarket and eight in another; and frozen carcasses (19 samples), ten samples collected in one supermarket, and nine from another.

The microbiological procedure for enterobacteria presumptive identification started with the samples submission to a rinsing method, reported by Cox et al. (1978), performed with the addition of 300 mL of buffered peptone water to each sample. These solutions were then placed in Erlenmeyer and incubated at 37° C for 24 hours. Aliquots from pre-enrichment were inoculated into selective enrichment liquid media at a ratio of 1/100 in Rappaport-Vassiliadis broth and at 1/10 in Selenite-Cysteine broth. A loopful of each broth was streaked on plates of Brilliant Green agar, MacConkey agar, and *Salmonella-Shigella* agar. The temperature and the period of incubation were standardized at 37° C for 24 hours. Two to three suspected colonies of *Salmonella* from each plate were collected for presumptive biochemical identification with Triple Sugar Iron (TSI); Lysine-Iron agar (LIA); Sulfur Indol Motility agar (SIM), and Citrate agar, incubated at the same time and temperature.

Companies and categories of carcasses were compared using the Chi-square (x^2) non-parametric test for qualitative analysis at a probability of 5% ($p < 0.05$).

RESULTS

The results showed the presence of enterobacteria in all broiler carcasses categories commercialized in Metropolitan Region of Fortaleza. As for the identified bacteria, there were qualitative variations, as we can see on Table 1.

The biochemical presumptive identification showed the following enterobacteria isolation in broiler carcasses: *Proteus* sp. (66.7%), *Enterobacter* sp. (15.7%), *Citrobacter* sp. (2%), *Escherichia coli* (47.1%), *Klebsiella* sp. (11.8%), *Shigella* sp. (5.9%) and *Salmonella* sp. (11.8%).

Fresh carcasses showed higher contamination levels compared to refrigerated and frozen ones. This difference was significant in cases of *Proteus* contamination. Regarding *E. coli*, there was no significant difference between the refrigerated and fresh carcasses, differing from the frozen ones ($p < 0.05$), classified as the lowest contamination level (Table 1).

Regarding the *Salmonella* isolation, a greater presence of this genus was verified in refrigerated carcasses. Frozen carcasses presented the lowest contamination levels for *Salmonella*. Genus *Citrobacter* was isolated only from frozen carcasses.

Table 1 - Enterobacteria species isolated, according to their origin, from broiler carcasses commercialized in the Metropolitan Region of Fortaleza, Ceara, Brazil.

Samples	Fresh n=14		Refrigerated n=18		Frozen n=19		Total n=51	
Bacteria		(%)		(%)		(%)		(%)
Proteus sp.	13[a]	(92.8)	11[b]	(61.1)	10[b]	(52.6)	34/51	(66.7)
Enterobacter sp.	4[a]	(28.6)	2[a]	(11.1)	2[a]	(10.5)	8/51	(15.7)
Citrobacter sp.	0[a]	(0)	0[a]	(0)	1[a]	(5.3)	1/51	(2)
Escherichia coli.	10[b]	(71.4)	9[ab]	(50)	5[a]	(26.3)	24/51	(47.1)
Klebsiella sp.	0[a]	(0)	3[a]	(16.7)	3[a]	(15.8)	6/51	(11.8)
Shigella sp.	2[a]	(14.3)	1[a]	(5.5)	0[a]	(0)	3/51	(5.9)
Salmonella sp.	2[a]	(14.3)	3[a]	(16.7)	1[a]	(5.3)	6/51	(11.8)

a, b – the different letters mean the existence of significant difference among the values ($p < 0.05$).
n- number of isolated samples

Table 2 - Number of enterobacteria isolated in different culture media combinations, from broiler carcasses commercialized in the Metropolitan Region of Fortaleza, Ceara, Brazil.

Bacteria	Culture media						Positive Carcass Samples
	RB	SB	RM	SM	RS	SS	
Proteus sp.	3[a]	6[ab]	13[bc]	28[d]	11[b]	20[c]	34
Enterobacter sp.	0[a]	0[a]	5[b]	5[b]	0[a]	2[ab]	8
Citrobacter sp.	0[a]	0[a]	1[a]	1[a]	1[a]	0[a]	1
Escherichia coli	6[a]	10[ab]	19[c]	9[a]	16[bc]	5[a]	24
Klebsiella sp.	0[a]	0[a]	3[b]	0[a]	2[ab]	3[b]	6
Shigella sp.	0[a]	0[a]	0[a]	1[ab]	2[ab]	3[b]	3
Salmonella sp.	3[b]	3[b]	1[ab]	0[a]	0[a]	1[ab]	6
Total	9[a]	16[a]	41[bc]	44[c]	32[b]	31[b]	82

a,b,c,d – The different letters mean the existence of significant difference among the values ($p < 0,05$).
RB– Rappaport-Vassiliadis Broth + Brilhant Green Agar
SB– Selenite-Cystine Broth + Brilhant Green Agar
RM–Rappaport-Vassiliadis Broth + MacConkey Agar
SM– Selenite-Cystine Broth + MacConkey Agar
RS– Rappaport-Vassiliadis Broth + *Salmonella-Shigella* Agar
SS– Selenite-Cystine Broth + *Salmonella-Shigella* Agar

The media SM, followed by the SS, were superior ($p < 0.05$), considering the growth of *Proteus* spp., and were the worst to the other genus except for *Salmonella* spp., as would be expected. The higher number of enterobacteria isolates was obtained in the SM, followed by the RM and the SS media.

DISCUSSION

Contamination of poultry products by enterobacteria is one of the greatest problems regarding world food industry, and animal feces are their main contaminating source (RASMUSSEN et al., 2004). OLIVEIRA et al. (2004) verified the following frequency of enterobacteria in broiler feces: *Proteus* sp., 86.7%, *Arizona* sp., 6.7%, *Enterobacter* sp., 66.7%, *Citrobacter* sp., 6.7%, *Edwardsiella* sp., 3.3%, *E. coli*, 60%, *Klebsiella* sp., 6.7% and *Shigella* sp. 13.3%.

The role of *Proteus* spp in foodborne diseases is a controversy, despite its potential as a pathogen. According to FRANCO; LANDGRAF (1996), *Proteus* spp is related with food deterioration and its presence is not directly related to infections, but as a contaminant

agent. It is involved in the decomposition of carcasses and can be found in feces, sewer water, putrefied meat, suppurating wounds, among others (Bier, 1981). Hobbs, Roberts (1999) reported that this bacteria is related to intoxication of scombroid fishes, making humans susceptible, presenting symptoms as: nausea, vomiting, peri-ophthalmic swelling, cyanotic tongue and gum, itching, headache and respiratory problems.

Proteus spp. and *Escherichia* were the genus with the highest number of isolations in all carcass categories. Yogaratnam, (1995) reported that in 43% of broiler carcasses condemned for disease at processing plants had lesions consistent with colisepticemia. The chickens are susceptible to colonization by *E. coli* O157:H7, an important shiga toxin-producing, enterohemorrhagic pathogen of humans, but low occurrence of natural infection has been found in both chickens and turkeys in different geographic areas (Guo et al., 1998; Heuvelink et al., 1999).

In the present study, the lowest level of contamination was found for *Citrobacter* spp. Iversen; Forsythe (2004) isolated 49 samples from food destined to children. Out of all, 6 were contaminated with the genus *Enterobacter*, three with *Escherichia* spp. and none with *Citrobacter* spp and *Klebsiella* spp. In the same study, one isolate of *Citrobacter* spp, *Serratia* spp and *Klebsiella* spp, three of *Enterobacter* spp and none of *Escherichia* spp were detected in 82 samples of milk prepared for children's consumption.

Species of *Enterobacter* have been associated with rare cases of infantile meningitis, newborn death and bacteremia. This genus has been often isolated from food, such as: meat, cheese, vegetables and condiments (Lai, 2001; Iversen; Forsythe, 2003).

Bacteria of the genus *Shigella* were, in the present study, isolated from four samples. They are responsible for the shigellosis or bacillary dysentery, involving invasion and destruction of the epithelial layer of the mucosa causing intense inflammatory reaction (Campos, 2002).

Shigella infections remain a global public health concern, causing diarrhea in both the developing and developed regions (Guerrant et al., 1990). The bacteria are readily transmitted through the fecal-oral route, with the majority of illnesses arising through the consumption of fecally contaminated food and water. Poor personal hygiene and sanitation are the common sources of such food and water contaminations (Sapsford et al., 2004). Mead et al. (1999) estimated that 20% of the total number of cases of shigellosis involve food as the vehicle of transmission. The food involved was contaminated during handling by infected workers (Bryan, 1978).

Centers For Disease Control And Prevention (1999) estimates that the number of humans cases for *Shigella*

and *Salmonella* is, annually, 89.648 and 1.341.873, with 1.246 and 15.608 hospitalizations and 14 and 553 deaths, respectively. The selective enrichment is an essential step, because inhibits the competitive microbiota, favoring growth of *Salmonella* (Busse, 1995; Rhodes et al., 1985).

Selenite-Cystine broth and MacConkey agar combination showed the best results, due to two factors: the aminoacid cystine establishes oxide-reduction potential that favors the enrichment and recovery of the enterobacteria in scarce number and the MacConkey agar has, in its composition, biliar salts that inhibit the growth of Gram-positive bacteria. According to Michael et al. (2003), the Rappaport-Vassiliadis broth, as a selective enrichment, is superior to Selenite-Cystine in order to obtain *Salmonella* isolation.

Usage of different culture media combinations is better than using a single medium because there are a number of factors that can affect the bacterial isolation, choosing many different culture media is an essential requirement for isolation of any microorganisms (Albuquerque et al., 2000).

It was verified a significant occurrence of enterobacteria, including human pathogens, in broiler carcasses in Ceara State. The fresh carcasses samples were more contaminated than the refrigerated and frozen ones. We also observed that no combination of culture media was able to detect all enterobacteria contamination in broiler carcasses. Thus, it may be necessary the use of several combinations of culture media to determine the real microbiological quality of broiler carcasses.

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