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Contamination and antimicrobial resistance profile of *Escherichia coli* in chicken carcasses from public markets in Maranhão

Contaminação e perfil de resistência antimicrobiana de Escherichia coli *em carcaças de frango de mercados públicos maranhenses*

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ABSTRACT: Contamination of chicken meat sold in public markets is a public health concern. The objective of this study was to identify contamination and evaluate the antimicrobial resistance profile of *Escherichia coli* in chicken carcasses from public markets in the North Mesoregion of Maranhão. A total of 160 freshly slaughtered chicken carcasses were collected in 16 markets in six municipalities in the microregions of Itapecuru-Mirim and São Luís. The samples were analyzed for the presence of *E. coli* using counting thermotolerant coliforms and classified according to the ANVISA microbiological standard. Of all the samples, 134 (83.75%) were considered unacceptable for consumption, according to Brazilian health legislation. Bacteria were isolated from the positive samples, and 50 isolates were tested for susceptibility to 15 antimicrobial principles using the disc diffusion method. The results confirm the presence of *E. coli*, with counts ranging from 10^1 to 10^8 NMP/g. The isolates showed resistance to neomycin (49/50, 98%), streptomycin (48/50, 96%), sulfonamides (47/50, 94%), nitrofurantoin (45/50, 90%), cefazolin (43/50, 86%), and tetracycline (43/50, 86%). No antibiotic was effective against the isolates, which were resistant to more than 3 antimicrobial classes considered resistant to multiple drugs (MDR). Therefore, chicken meat sold in public markets in Maranhão presents unsatisfactory conditions for consumption and risk of transmission of E. coli with an MDR profile.

KEYWORDS: Contamination; enterobacteria; chicken carcass; antibiotics

RESUMO: A contaminação da carne de frango comercializada em mercados públicos é uma preocupação de saúde pública. Nesse sentido, objetivou-se avaliar a contaminação e perfil de resistência antimicrobianas de *Escherichia coli*das carcaças de frango de mercados públicos da Mesorregião Norte do Maranhão. Foram coletadas 160 amostras de carcaças de frango recém-abatidas e comercializadas em 16 mercados de seis municípios das microrregiões de Itapecuru-mirim e São Luís. As amostras foram analisadas quanto à presença de *E.coli* por meio da contagem de coliformes termotolerantes e classificadas segundo o padrão microbiológico da ANVISA. A bactéria foi isolada de amostras positivas. Testou-se 50 isolados quanto à suscetibilidade a 15 princípios antimicrobianos, seguindo o método de Difusão em Disco.Os resultados confirmam a presença de *E.coli* com contagens de 10^1 a 10^8 NMP/g. De todas as amostras 134 (83,75%) foram consideradas inaceitáveis para consumo, conforme legislação brasileira sanitária. Os isolados mostraram alto índice de resistência atimicrobiana aos princípios neomicina (49/98%), streptomicina (48/96%), sulfanomidas (47/94%), nitrofurantoína (45/90%), cefazolina (43/86%) e tetraciclina (43/86%). Nenhum antibiótico foi eficaz contra os isolados, sendo resistente a mais de 3 classes antimicrobianas considerados resistente a múltipla a drogas (MDR). A carne de frango comercializada nos mercados públicos maranhenses apresenta condições insatisfatórias para consumo e risco de transmissão de *E. coli* com perfil MDR.

PALAVRAS-CHAVE: contaminação; enterobactérias; carcaça de frango; antibióticos.

INTRODUCTION

Chicken meat is recognized for its beneficial effects to human health, due to its high protein content and low cholesterol, calorie, and fat contents (KRALIK et al., 2018). In addition, chicken meat is more competitive due to its lower commercial value as compared to pork, beef, and lamb (KIM et al., 2020).

Chicken meat with satisfactory quality for consumption must be preserved in a good state, should follow the processing procedure in accordance with the specific legislation, and

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have adequate hygienic and sanitary conditions (FARIAS et al., 2021). Since the requirements of the national and international markets in relation to the sanitary safety of products of animal origin are increasing, offering a product with guaranteed quality to the consumer is a great challenge for the contemporary meat production chain (WAHYONO; UTAMI, 2018).

The Brazilian poultry industry is intensely growing, but chicken meat is still sold in open fairs and public markets in Brazilian municipalities, especially in the North and Northeast regions (BRITO et al., 2019). These places have a large flow of consumers and attract attention for selling fresh food and wide variety of products, and having diversity of prices (SANTOS et al., 2019). In the case of poultry, there are numerous slaughterhouses, without a sanitary inspection service, that slaughter animals with precarious physical and hygienic conditions, exposing the meat to contamination during processing (ZAKKI et al., 2017).

Contamination of chicken meat can occur through contact with different surfaces during processing, contaminated water and/or lack of care in poultry slaughter operations (SOARES; SILVA; GÓIS, 2017; MONTEZANI et.al., 2017). Among the bacterial contaminants that can impact the sanitary quality of chicken meat, the pathogenic species belonging to the Enterobacteriaceae family, such as *Salmonella* enterica and *Escherichia coli*, are the most relevant for public health (ROUGER; TRESSE; ZAGOREC, 2017). . E. coli has a natural habitat in the intestinal tract of homeothermic animals, including humans, being a good microbial indicator of contamination of fecal origin, with some strains capable of causing gastrointestinal and extra-intestinal infections (JANG et al., 2017). This bacterium can be found in poultry litter, dust, water, feed and especially in the intestine of healthy birds, and can contaminate chicken carcasses during processing (HUSSAIN et al., 2017).

The emergence of antimicrobial resistance among foodborne pathogens has been widely reported and is attributed to the use of antimicrobials in animal production (CANIÇA et al., 2018). *Escherichia coli* suffers strong selective pressure from the use of these antimicrobials, and this bacterium can act as a reservoir of resistance genes and transfer them horizontally to other bacterial species (HUSSAIN et al., 2017). Thus, multidrug-resistant *E. coli* is a growing public health concern, as it can directly transfer resistance genes to humans through the food chain, making it difficult to treat infectious diseases in humans and animals (NHUNG; CHANSIRIPORNCHAI; CARRIQUE-MAS, 2017).

E. coli is a commensal bacterium that is a member of family Enterobacteriaceae. Investigating the selective pressure exerted by antimicrobials on *E. coli* isolates from carcasses or meat of animals is important in monitoring the occurrence of multidrug resistance (MDR) phenotypes (EFSA, 2013; JIMÉNEZ-BELENGUER et al., 2016). The objective of this study was to investigate contamination and evaluate antimicrobial resistance profile of *E. coli* in chicken carcasses from public markets in the North Mesoregion of Maranhão.

MATERIAL AND METHODS

This study was conducted in artisanal slaughterhouses in public markets in the North Mesoregion of Maranhão, represented by the microregions of the urban agglomeration of São Luís and Itapecuru-Mirim, from 2014 to 2015.

Sixteen public markets were selected from the cities with the highest population: São Luís, São José de Ribamar, Paço do Lumiar, Itapecuru-Mirim, Vargem Grande, and Nina Rodrigues. Then, 160 freshly slaughtered chicken carcass samples were collected, representing each artisanal slaughterhouse located in the selected markets (Table 1).

All samples were collected in the morning, between 0 and 2 h after slaughter of broiler, identified, and transported in cool condition, under refrigeration to the Microbiology Laboratory of the Federal Institute of Maranhão (IFMA), São Luís – Maracanã campus. Samples were immediately analyzed.

In the laboratory, the samples were analyzed using a superficial washing technique (COX et al., 1978). Briefly, samples were weighed and aseptically transferred to a sterile clear bag, and 300 ml of sterile buffered peptone water was added. Then, hand massages were performed on the carcass for 2 min, and the rinsing solution was aseptically transferred to a sterile glass bottle (100 dilution). Serial dilutions until 10^{-5} were performed using 0.1% buffered peptone water.

E. coli was detected through the quantification of thermotolerant coliforms using the multiple tube method and the results were calculated in most probable number (NMP/g). Briefly, 1 mL of each serial dilution was inoculated in a series of three tubes containing lauryl sulfate tryptose (LST) broth with inverted Durham tubes and incubated in a bacteriological incubator at 37°C for 48 h. Durham tubes that showed gas formation were suggestive of *E. coli*. Then, a loopful of the LST broth suggestive of *E. coli* was inoculated to *Escherichia coli* (EC) broth and incubated in a water bath at 44.5°C for 48 h. The presence of *E. coli* was confirmed by the formation of gas in a Durham tube (KONARCKI et al., 2013).

Table 1. The number of samples collected by public market located in the North Mesoregion of Maranhão, 2015

County	N° of market	N° of samples	N° of slaughterhouse
São Luís	9	90	90
São José de Ribamar	3	20	20
Paço do Lumiar	1	10	10
Itapecuru-mirim	1	20	20
Vargem Grande	1	10	10
Nina Rodrigues	1	10	10
Total	16	160	160

Bacterial quantification was performed, and the samples were classified as acceptable, intermediate, and unacceptable quality, according to the microbiological standard of the National Health Surveillance Agency (ANVISA) subject in Normative Instruction (IN) No. 60 of 2019 (BRASIL, 2019).

E. coli positive tubes were seeded on eosin methylene blue agar (EMB) plates and incubated at 37°C for 24 h. Morphological characteristics of the colonies were then observed. Typical metallic-green colonies were subjected to Gram staining, and short gram-negative bacilli was observed using light microscopy. Five typical colonies from each sample were selected, seeded in tubes containing nutrient agar, incubated at 37°C for 24 h, and used for biochemical tests.

Biochemical tests on glucose fermentation, urease production, motility, H_2S production, and tryptophan degradation were performed to confirm the identity of the isolates. The isolates were identified as *E. coli* species if they grow in triple sugar iron agar (TSI) with yellow base tubes and bevel, and are positive for urease, methyl red, indole, and motility tests and negative for Voges-Proskauer (VM-VP) and H_2S production tests.

Furthermore, two to four *E. coli* isolates were randomly selected from the public markets, having a total of 50 isolates. Disk diffusion method was performed using the protocol recommended by the Manual Clinical and Laboratory Standards Institute (CLSI, 2019). Antimicrobial discs (Laborclin®) representing the classes of penicillin (amoxicillin, 10µg; ampicillin, 10µg), cephalosporins (cefazolin, 30µg), carbapenems (imipenem, 10µg), quinolones (nalidixic acid, 30µg; norfloxacin, 10µg), phenicol (chloramphenicol, 30µg; fluorphenicol, 30µg), aminoglycosides (streptomycin, 300µg; gentamicin, 10µg), folate inhibitors (sulfonamide, 300µg; trimethoprim, 5µg), tetracyclines (tetracycline, 30µg), and nitrofurans (nitrofurantoin, 300µg) were used. The isolates were inoculated in BHI broth and incubated at 37°C for 24 h or until they showed turbidity similar with the 0.5 °MacFarland scale. Seeding was performed on Müller-Hinton (MH) agar plates, and after five minutes, the antibiotic discs were added using flaming forceps. The plates were incubated in a bacteriological incubator at 37°C for 24 h. The zones of inhibition around the antibiotic discs were measured using a millimeter ruler. The results were compared to the laboratory measurement table of the antimicrobial disc manufacturer. Isolates were classified as sensitive, sensitive with increased exposure (intermediate), or resistant. MDR phenotype was considered if the isolate has simultaneous resistance to two or more classes of antimicrobials (RAHMAN et al., 2020). For validation, the reference strain *E. coli* ATCC 25922 was used.

RESULTS AND DISCUSSION

Contamination by *E. coli* was predominant in the 160 chicken carcass samples. Only seven (4.3%) samples from the city of São Luís were not contaminated. *E. coli* counts ranged from 10^1 to 10^8 NMP/g in chicken carcasses, with 8.1% acceptable for consumption, 8.1% with an intermediate standard, and 83.8% considered unacceptable for consumption, according to Brazilian legislation (Table 2).

IN No. 60 of December 23, 2019 of ANVISA established the microbiological quality standard of food. To be considered acceptable for consumption, "in natura" chicken meat, raw giblets, seasoned or not, refrigerated or frozen, must have *E. coli* counts up to 102 NMP/g (BRASIL, 2019b). Using this parameter, it was observed that 83.8% (134/160) of the samples were highly contaminated with *E. coli*, with counts above the permitted level (Table 1).

The presence of *E. coli* in chicken carcasses may be related to unsafe in slaughtering and handling operations, indicating a low hygienic-sanitary quality of samples offered in public markets in Maranhão. Hygienic deficiencies in artisanal slaughterhouses and exposure of meat to inadequate temperatures for marketing can favor the increase in bacterial counts in the food, making it unacceptable for consumption and causing a risk to consumer health, as highlighted by Vásquez-Ampuero and Tasayco-Alcántara (2020).

The high occurrence of *E. coli* contamination in chicken carcasses from market slaughterhouses observed in this study

Table 2. Contamination by *Escherichia coli* and safety of the 160 chicken carcasses sold in public markets in the Northern Mesoregion of the State of Maranhão, 2015

County	n°*	Microbiological Standard*		
		Acceptable	Intermediate	Unacceptable
São Luís	90	13 (14,4%)	12 (13,1%)	65 (72,2%)
São José de Ribamar	20	0 (0%)	1 (5,0%)	19 (95,0%)
Paço do Lumiar	10	0 (0%)	0 (0%)	10 (100%)
Itapecuru-Mirim	20	0 (0%)	0 (0%)	20 (100%)
Vargem Grande	10	0 (0%)	0 (0%)	10 (100%)
Nina Rodrigues	10	0 (0%)	0 (0%)	10 (100%)
Total	160	13 (8,1%)	13 (8,1%)	134 (83,8%)

*n = number of samples *Microbiological Stander= 10^1, 10^2, 10^3 NMP/g is acceptable, intermediary e and unacceptable quality, respectively for raw chicken or offal, seasoned or not, chilled or frozen, IN°60/2019 ANVISA, Brazil.

is similar to the study by Yulistiani et al. (2019), who found a contamination level of 77.5% in chicken meat from traditional markets. Lower results were found in other surveys evaluating samples from inspected industrial slaughterhouses (FREITAS et al., 2019; MENEZES et al., 2018). The absence of operational standards of hygiene and slaughter techniques, as well as the inappropriate physical conditions of public market slaughterhouses, are factors that increase the risk of cross-contamination of chicken meat by *E. coli* of intestinal origin (YULISTIANI et al., 2019).

In addition, the susceptibility profile of *E. coli* against antimicrobials showed a high frequency of isolates with an antimicrobial resistance phenotype (Figure 1). More than 85% of the isolates were resistant to neomycin (49/50), streptomycin (48/50), sulfonamides (47/50), nitrofurantoin (45/50), cefazolin (43/50), tetracycline (43 /50), ampicillin (42/50), gentamicin (42/50), amoxicillin (39/50), trimethoprim (31/50), imipenem (29/50), and nalidixic acid (28/50).

A high frequency of *E. coli* isolates with resistance to aminoglycoside, sulfonamide, tetracycline, and penicillin classes was observed, possibly related to its permitted use for therapy in broiler chickens in high-production countries such as Brazil (BRASIL, 2009). According to the study by Roth et al. (2019), the average resistance rates of *E. coli* to these classes of antibiotics are above 40% in countries with high poultry meat production. Therefore, the presence of *E. coli* resistance phenotypes in the chicken meat from the studied region suggests possible dissemination of antimicrobial resistance among beef animals and animal products available to consumers, contributing to the global public health problem.

The long- or short-term use of antimicrobials is known to cause drug resistance in bacteria (ELUMBA; ALLERA; TAGANAS, 2018). The administration of antimicrobials in poultry feeds or in treat diseases exerts selective pressure for commensal bacteria such as *E. coli* (IBRAHIM et al., 2019; RONQUILLO; HERNADEZ, 2017).

To contain antimicrobial resistance, several active principles have been banned for use in poultry feed in recent decades; however, resistance in the bacterial population to certain antibiotics can last for years (DAVIS et al., 2018). This assertion is represented by the high frequency of *E. coli* isolates resistant to tetracyclines (86%) and nitrofurantoin (90%) in this study. Tetracyclines and nitrofurantoin were banned as growth promoters two decades ago (PRAXEDES et al., 2013).

Pathogenic strains of *E. coli* can cause diseases such as colibacillosis in poultry farming, and quinolones, amoxicillin, and sulfamethoxazole are used for antibiotic therapy (CARDOSO et al., 2014). In the present study, the lowest resistance indices of *E. coli* isolates were found in norfloxacin and sulfamethoxazole (Figure 1), indicating that there is less selective pressure due to the use of these antimicrobials in the region under study. In contrast, 78% (38/50) of *E. coli* isolates were resistant to penicillin and amoxicillin, which is widely used in the treatment of respiratory diseases in birds (CARDOSO et al., 2014). Jiménez-Belenguer et al. (2016) found that the presence of *E. coli* populations with this phenotype in slaughter-age birds may be a result of the use of amoxicillin in antibiotic therapy, as well as the vertical transmission of *E. coli* to broiler chicks. According to the authors, treatment with amoxicillin increased the resistance of*E. coli* to other antibiotics, particularly those from tetracycline and aminoglycoside classes.

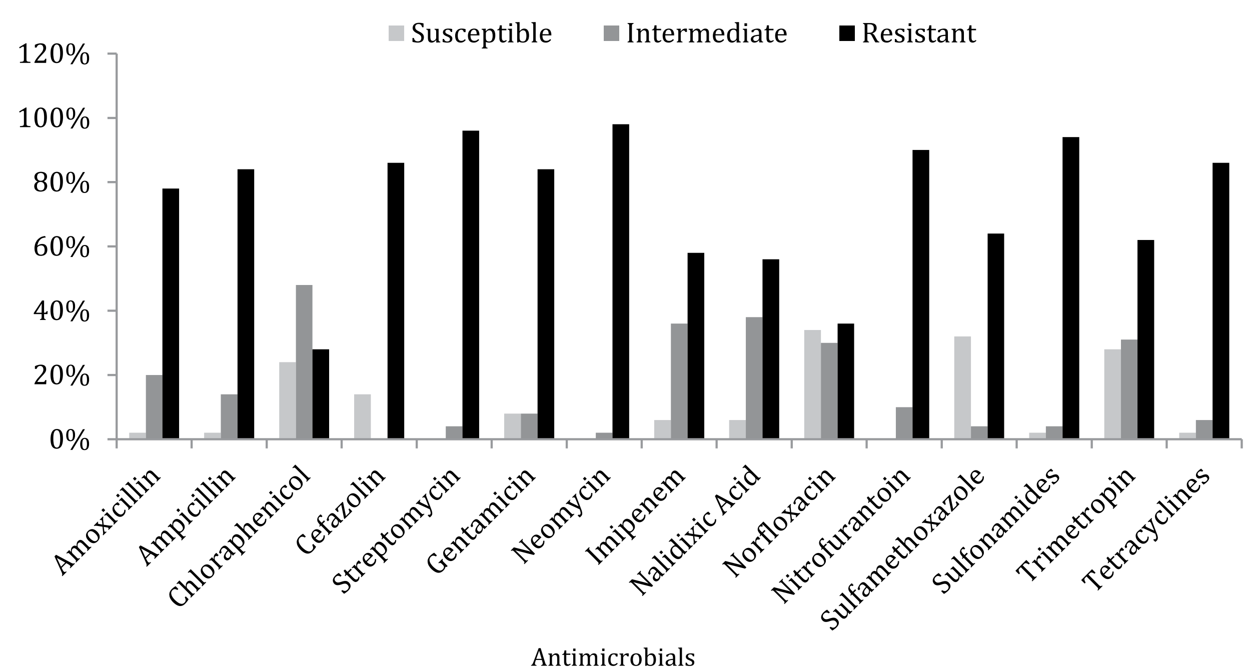

Figure 1. Antimicrobial resistance profile of 50 *Escherichia coli* isolates from chicken carcasses sold in public markets in the Northern Mesoregion of the State of Maranhão, 2015

Most of the isolates were resistant (28/50) and have intermediate sensitivity (19/50) to quinolone nalidixic acid. These results are expected, as they are widely used to control the presence of bacterial agents in animal production, especially *E. coli* in bird infections (XU et al., 2020; KIM; COVINGTON; PAMER, 2017).

All isolates were considered to have MDR phenotypes, as they showed resistance to more than three classes of antimicrobials simultaneously (Figure 2). Most isolates were found to be resistant to six classes (15/50) and seven classes (12/50) of antimicrobials. Five isolates with MDR to nine classes of antimicrobials were observed (Figure 2). The antimicrobial classes that showed the highest levels of resistance were cephalosporins, aminoglycosides, nitrofurans, folate inhibitors, penicillins, and tetracyclines.

The presence of multidrug-resistant *E. coli*chicken carcasses were recorded in other Brazilian regions (NEPOMUCENO et al., 2016; CARDOSO et al., 2015) and in other countries (ADORJÁN et al., 2020; AMIR et al., 2017; RAHMAN et al., 2020), supporting the need for surveillance of this bacterial species as a reservoir of antimicrobial resistance. The study data are similar to those of Vitas et al. (2018), who found MDR phenotypes in *E. coli* isolated from chicken meat samples positive for extended spectrum, a major mechanism of antimicrobial resistance in gram-negative bacteria.

The indiscriminate use of antimicrobials is the main cause of selection for resistant microorganisms in a bacterial population or induction of resistance in certain bacterial species (KHAN et al., 2018). The resistance of *E. coli* to an antimicrobial is due to the acquisition of resistance genes via horizontal transfer mechanisms and mutations followed by selection (CHE et al., 2021). Although *E. coli* is a commensal bacterium, birds and their products can act as reservoirs of four diarrheagenic pathotypes of *E. coli*: enterohemorrhagic, enterotoxigenic, enteropathogenic, and enteroaggregative, that cause intestinal infections in humans, representing a risk of zoonoses (ADORJÁN et al., 2020). Additionally, the high frequency of isolates with MDR profiles found in this study represents the risk of spreading antimicrobial resistance to humans through the consumption of contaminated food.

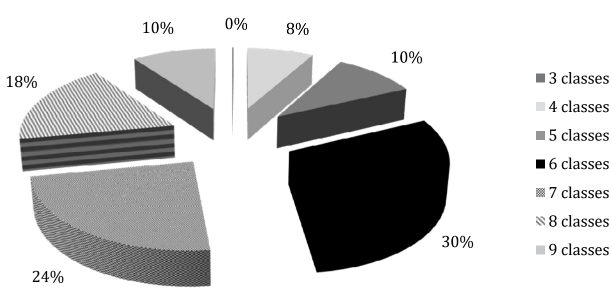

Figure 2. Graph of the percentage of resistance to antimicrobial classes of 50 *Escherichia coli* isolates from chicken carcasses sold in public markets in the Northern Mesoregion of Maranhão, 2015.

A previous study has shown that *E. coli* with an MDR profile is a potential reservoir of resistance genes, being able to transfer them via plasmids to other pathogenic bacterial genera (ROZWANDOWICZ et al., 2018). This observation has major clinical implications, as it limits the therapeutic possibilities for different causative agents of human infections (ADORJÁN et al., 2020).

Contamination of food with multidrug-resistant bacteria may be due to different factors present in the production chain, making it difficult to determine the origin of antimicrobial resistance (ONICIUC et al., 2018). However, similarities have been found between the antimicrobial resistance indices found in chicken meat and live poultry (KUNADU, OTWEY; MOSI, 2020). Several sources of contamination in industrial broiler farming, such as poultry litter and insects, can harbor *E. coli* with MDR phenotypes (GAZAL et al., 2021). Thus, birds can be the initial source of contamination in slaughterhouses, especially from their feathers, skin, and intestine, spreading bacteria with MDR profiles to the meat contact surfaces (AMIR et al., 2017).

CONCLUSION

The "in natura" chicken carcasses sold in public markets in the Northern Mesoregion of Maranhão were highly contaminated with multidrug-resistant *E. coli*, suggesting an unsatisfactory hygienic sanitary conditions and risks to public health. Hygiene and processing practices in artisanal slaughterhouses in public markets should be improved as a preventive measure to reduce the dissemination of MDR enterobacteria to consumers.

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