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Multidrug resistance and ESBL-producing *Salmonella* spp. isolated from broiler processing plants

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ABSTRACT

The aim of this study was to investigate the occurrence of multidrug-resistant, extended spectrum beta-lactamase (ESBL) producing *Salmonella* spp. isolated from conveyor belts of broiler cutting rooms in Brazilian broiler processing plants. Ninety-eight strains of *Salmonella* spp. were analyzed. Multidrug resistance was determined by the disk diffusion test and the susceptibility of the isolated bacteria was evaluated against 18 antimicrobials from seven different classes. The double disk diffusion test was used to evaluate ESBL production. Of the 98 strains tested, 84 were multidrug resistant. The highest rates of resistance were against nalidixic acid (95%), tetracycline (91%), and the beta-lactams: ampicillin and cefachlor (45%), followed by streptomycin and gentamicin with 19% and 15% of strain resistance, respectively. By contrast, 97% of the strains were sensitive to chloramphenicol. 45% of the strains were positive for the presence of ESBL activity. In this study, high rates of multidrug resistance and ESBL production were observed in *Salmonella* spp.

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Introduction

Salmonella spp. is one of the main agents responsible for several infections, e.g., food borne gastroenteritis. Most of these infections cause self-limiting diarrhea and do not require antimicrobial treatment. However, in certain cases, such as when the bacterium is spread via blood stream leading to complications such as meningitis, antibiotic therapy is necessary. Such complications are most commonly observed in children and elderly and immune compromised patients. Fluoroquinolones and cephalosporins are the drugs of first-choice in such cases.[1]

Food of animal origin, especially poultry derived items, are the main sources of infection by *Salmonella* spp.[2] Given the importance to *Salmonella* spp. as causative agents in food borne diseases, reduction of its contamination on broiler carcasses is of extremely high priority for industry as well as regulatory agencies.[3] The occurrence of *Salmonella* spp. on the broiler carcasses might be a result of contamination either at the farm or cross-contamination within the processing plant.[4] Cross-contamination can be attributed partly to residual bacteria remaining on surfaces and equipment after sanitization.[5]

Studies on *Salmonella* spp. are usually focused on analyzing resistance of the bacterium to antimicrobials such as fluoroquinolones; but, in the last decade or so, bacterial production of large spectrum beta-lactamases has also been evaluated.[6,7] In Enterobacteriaceae, resistance to cephalosporins is generally attributed to the production of large spectrum beta-lactamases such as ESBL (extended spectrum beta-lactamase) and AmpC beta-lactamase.[8] ESBL is the term used for any beta-lactamase that is acquired and not intrinsic to a species. Such lactamases quickly hydrolyze and confer resistance against oxyimino-cephalosporins. Mutant beta-lactamases that have similar activity are also referred to as ESBLs.[9,10]

Extensive studies have been undertaken to analyze ESBL production by *Klebsiella* spp., *Enterobacter* spp., and *Escherichia coli* isolated from human clinical samples. These studies are prompted by the lack of therapeutic success against these microorganisms, which includes, but not restricted to, cephalosporins. Treatment is rendered unsuccessful by the acquisition of resistance genes that reside on mobile genetic elements. For example, plasmids that transmit resistance genes for cephalosporins are frequently found to carry resistance to other antibiotics, such as fluoroquinolones.[11] Some authors have also reported ESBLs in microorganisms isolated from food products.[12–14] According to Blanc et al. (2006), these findings are more recent in the case of *E. coli* and *Salmonella* spp. as compared to *Klebsiella* spp. and *Enterobacter* spp. ESBL production by *Salmonella* spp. isolated from animals has also been reported[15,16]; although, the reports on ESBL from animal origin are less frequent.[17,18]

Due to the public health risk of cross-contamination, it is important to have sufficient information on the occurrence of pathogens, e.g., *Salmonella* spp., in the cutting rooms for broiler processing and slaughtering facilities. It is especially important to have knowledge of the behavior of these strains against antimicrobials. Therefore, the objective of this study was to determine the occurrence of multidrug resistant in ESBL-producing *Salmonella* spp. isolated from conveyor belts in the cutting rooms of broiler processing plants.

Materials and methods

Isolation and identification of Salmonella spp.

Strains of *Salmonella* spp. were obtained from the cutting rooms of four different Brazilian broiler processing and exporting plants having a slaughtering capacity in excess of 160,000 broilers/day. For the isolation of *Salmonella* spp. from the surface of the conveyor belts, the sponges (Nasco Whirl-Pak™), pre-moistened with 10 mL of 0.1% saline peptone water, were utilized on a 400 cm^2 area. *Salmonella* spp. detection was carried out according to the Food and Drug Administration (FDA – USA) methodology as published in the Bacteriological Analytical Manual.[19] Subsequent to these tests, isolates of *Salmonella* spp. were confirmed by genus identification using polymerase chain reaction (PCR) for the *sifB* gene as per the protocol described by Almeida et al.[20]

Antimicrobial susceptibility test

The susceptibility to antimicrobials was determined using the agar diffusion test as per the documents M31-A3[21] and M100-S23[22] of the Clinical and Laboratory Standards Institute. Eighteen antimicrobial agents from seven different classes were tested: (1) Beta-lactams, divided into 3 subclasses: (a) Penicillins: ampicillin (AMP; 10 μg), (b) Cephalosporins: cefachlor (CFC; 30 μg) and ceftiofur (CTF; 30 μg), and (c) Carbapenems: meropenem (MER;10 μg) and imipenem (IPM; 10 μg); (2) Aminoglycosides: streptomycin (EST; 10 μg), tobramycin (TOB; 10 μg), gentamycin (GEN; 10 μg), amikacin (AMI; 30 μg) and neomycin (NEO; 30 μg); (3) Quinolones: enrofloxacin (ENO; 5 μg), nalidixic acid (NAL; 30 μg), and ciprofloxacin (CIP; 5 μg); (4) Sulfamethoxazole and Trimethoprim: sulfamethoxazole/trimethoprim (SUT; 25 μg); (5) Tetracyclines: tetracycline (TET; 30 μg); (6) Phenicols: chloramphenicol (CLO; 30 μg) and florfenicol (FLF; 30 μg) and (7) Polymyxins: polymyxin B (POL, 300 UI). Strains were considered multidrug resistant if they were resistant to at least three classes of antimicrobials (at least one antimicrobial of each class).[23] The quality control test was based on *E. coli* ATCC 25922.

ESBL production

ESBL production was analyzed by the double disk diffusion test.[24] The central disk was having amoxicillin plus clavulanic acid (AMX/AC; 20/10 μg). Four other disks were placed within a 20 mm radius of the first one: ceftazidime (CAZ; 30 μg), ceftriaxone (CRO; 30 μg), cefepime (CPM, 30 μg) and aztreonam (ATM; 30 μg).[25] Samples were considered positive for ESBL when the inhibition zone around any cephalosporin increased toward the central disk with AMX/AC, and when the inhibition zone around at least one of the cephalosporins was smaller than 19 mm.[26]

Results

Out of the 98 strains evaluated (26, 23, 19, and 30 from each of the four cutting rooms, respectively), three were sensitive to all antimicrobials tested, four were resistant to one class, seven were resistant to two classes, and 84 (86%) were considered multidrug resistant.

Fig. 1 shows the resistance profiles of the multidrug resistant strains arranged according to the room of origin, and the corresponding percentage profiles in each room. ESBL activity was detected in 45% of the strains. From the strains positive for ESBL production, the most frequent resistance profile was against beta-lactams, quinolones, and tetracyclines (38/44); the ESBL-negative strains were resistant to aminoglycosides, quinolones, and tetracyclines (36/54). The results for *Salmonella* spp. antimicrobial susceptibility test are presented in Fig. 2.

The strains, sensitive to all antimicrobials (3/98), originated from Room 3 and represented 16% (3/19) of the total number of strains isolated from this room. The same percentage of resistance was observed for only one or two antimicrobial classes and 52% (10/19) strains were multidrug resistant. However, in Rooms 1, 2, and 4, 100% (26/26), 87% (20/23), and 93% (28/30), respectively, of the *Salmonella* spp. strains were multidrug resistant. The highest numbers of ESBL-positive strain were isolated from Room 4, corresponding to 54% (24/44) of the positive strains.

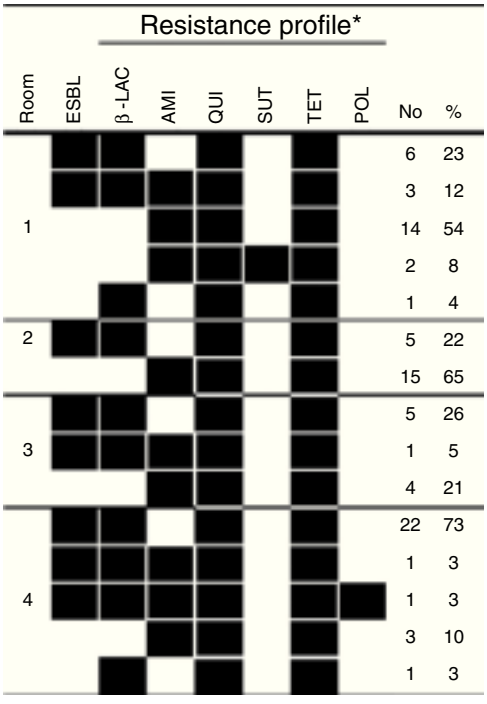

Fig. 1 – Resistance profile of multidrug resistant *Salmonella* spp. strains as per ESBL analysis and cutting room of origin. * B-LAC, B-lactams; AMI, aminoglycosides; QUI, quinolones; SUT, sulfamethoxazole/trimethoprim; TET, tetracyclines; POL, polymixin.

Discussion

Recently, incidence of antimicrobial resistance in *Salmonella* spp. isolated from foods of animal origin, especially poultry products, has increased.[27–29] In this study, all strains were sensitive to ciprofloxacin and 95% of them were resistant to nalidixic acid (Fig. 2). Hamidiam et al.[30] suggested that the low in vitro resistance to ciprofloxacin may be due to a mutation in the *gyrA* gene and that in vitro resistance to nalidixic acid may be used to detect the actual level of resistance to ciprofloxacin. The CLSI[22] recommends that resistance to this class of antimicrobials should be considered as collective, that is, resistance to one drug implies that the microorganism is resistant to the whole class. This information is of great importance because fluoroquinolones are considered as the drug of first choice for the treatment of infections caused by *Salmonella* spp. in human.[31]

High levels of resistance were also observed against tetracycline (Fig. 2). Worldwide incidence of resistance in *Salmonella* spp. isolated from the poultry production chain is variable with percentages ranging from 96.6% to 21.8%.[32–34] In Brazil, Oliveira *et al.*[35] reported low resistance indexes in *Salmonella* Enteritidis isolated from humans, poultry carcasses, poultry-related samples, and food items involved in food borne disease outbreaks in the southern region of the country.

Additionally, 97% of the strains tested were sensitive to chloramphenicol (Fig. 2), which is one of the first drugs used in veterinary medicine and which has been banned from animal production in Brazil since 1998.[36] The use of tetracycline as a growth promoter in poultry production is also prohibited in Brazil.[37]

All strains were sensitive to the carbapenems tested; this is an important finding because carbapenems are the drugs of first choice in the treatment of ESBL-producing microorganisms.[38] ESBL production was detected in 45% (44/98) of the strains. These ESBL-positive strains were also multidrug resistant. Clemente et al.[17] analyzed 1120 isolates of *Salmonella* spp. from food items of animal origin and found only five ESBL-producing strains. However, they used a methodology that was different from the present one. Nogueira-Miranda et al.[10] compared six methodologies for the detection of ESBL in *Enterobacter* spp. and concluded that the double disk diffusion test showed sensitivity of 89.2% and specificity of 100% for this species. Due to the absence of a standard methodology approved by any of the international committees for the evaluation of *Salmonella* spp., ESBL production may result in underestimation of the occurrence of this phenotype. It is also difficult to compare different studies. The phenotypic confirmatory tests are highly sensitive and specific as compared to the genotypic confirmatory tests.[39] This study was aimed to screen ESBL producing organisms. Further research is required for the genotypic characterization of ESBL-producing *Salmonella* spp. found in broiler processing plants.

In conclusion, the present study demonstrated multidrug resistance in 86% and ESBL activity in 45% of the *Salmonella* spp. isolated from the studied broiler processing plants. These results should be taken as a precautionary warning for the

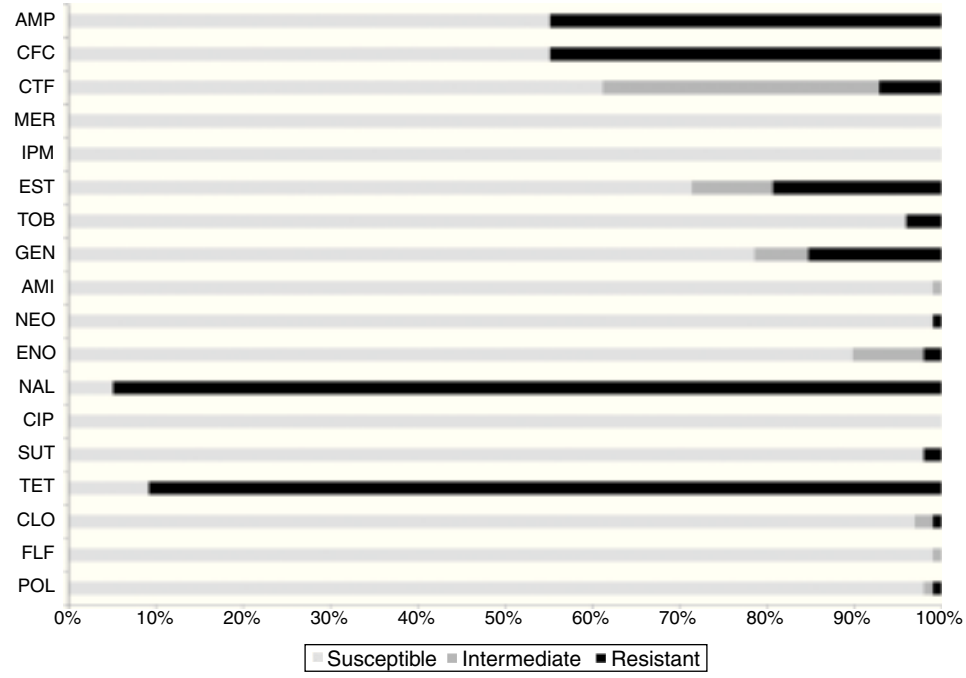

Fig. 2 – Percentages recorded for the antimicrobial susceptibility test of *Salmonella* spp. strains isolated from cutting rooms of broiler processing plants. AMP, ampicillin; CFC, cephachlor; CTF, ceftiofur; MER, meropenem; IPM, imipenem; EST, streptomycin; TOB, tobramycin; GEN, gentamycin; AMI, amikacin; NEO, neomycin; ENO, enrofloxacin; NAL, nalidixic acid; CIP, ciprofloxacin; SUT, sulfamethoxazole/trimethoprim; TET, tetracycline; CLO, chloramphenicol; FLF, florfenicol; POL, polymyxin B.

spread of multidrug resistant *Salmonella* spp. in broiler production industry.

Conflicts of interest

The authors declare no conflicts of interest.

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