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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]