Extended Spectrum Beta-Lactamase Production and Biofilm Formation in Salmonella Serovars Resistant to Antimicrobial Agents

ABSTRACT

Antimicrobial resistance is a serious public health problem and *Salmonella* spp. is highly resistant to antimicrobial agents. Biofilms are important in the food industry due to their formation on products, utensils, and surfaces and the difficulty in their removal. The objective of this study was to assess extended spectrum beta-lactamase (ESBL) production, antimicrobial resistance, and biofilm production of *Salmonella* isolated from poultry slaughterhouses. Antimicrobial susceptibility was assessed by the disk diffusion assay and ESBL by double diffusion disk assay using the beta-lactamase inhibitor (amoxicillin+clavulanate). The antimicrobials tested were: ampicillin, amoxicillin+clavulanate, aztreonam, ceftazidime, cefotaxime, chloramphenicol, gentamicin, enrofloxacin, sulfonamide, and tetracycline. Serovars Infantis, Panamá, and Tennessee were found to produce ESBL. All serovars were sensitive to tetracycline, and *S*. Brandenburg was sensitive to all drugs tested. Serovars Panamá, Anatum, Infantis, and Schwarzengrund were moderate biofilm producers at 3 ºC and 9 ºC±1 ºC, respectively, showing possible adaptation of these serovars to these temperatures. Antimicrobials should be used with caution because of the levels of resistance observed and because of ESBL production, and hygiene and sanitary measures should be enhanced to minimize the adhesion of biofilm-forming *Salmonella* serovars at refrigeration temperatures.

INTRODUCTION

*Salmonella* strains adapt to adverse environmental conditions and can survive in the environment for long periods, colonizing domestic animals and humans. The most common sources of infection among humans are products of poultry origin, dairy products, and surfaces in contact with contaminated products reused without proper disinfection, which could stimulate biofilm formation (Murray *et al*., 2015). Biofilm promotes bacteria survival in stressful environments, like slaughterhouses and food-processing plants (Steenackers *et al*., 2012). Biofilms favor greater resistance to antimicrobials and disinfectants (Steenackers *et al*., 2012). Antimicrobial resistance is one of the major public health problems worldwide and microorganisms isolated from foods, especially *Salmonella* spp., show high resistance to these agents (Markle *et al*., 2015). A global increase in extended spectrum beta-lactamase (ESBL) producing is likely to be occurring (Ziech, 2015; ECDC, 2016).

Therefore, the present study assessed antimicrobial resistance, ESBL production, and biofilm formation by *Salmonella* serovars isolated from poultry slaughterhouses.
MATERIALS AND METHODS

Salmonella strains

The tested Salmonella serovars were previously isolated (Santos et al., 2015; Mion et al., 2016) from ten poultry slaughterhouses under federal inspection system between 2012 and 2014 from the northern region of the state of Rio Grande do Sul (Table 1). The samples were stored in brain heart infusion broth (Oxoid®, United Kingdom) and frozen at -18°C.

Table 1 – Salmonella serovars and source of poultry slaughterhouse isolates.

<table>
<thead>
<tr>
<th>Salmonella serovars</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td>S. Agona</td>
<td>Transport cages after cleaning</td>
</tr>
<tr>
<td>S. Anatum</td>
<td>Cloacal swabs</td>
</tr>
<tr>
<td>S. Brandenburg</td>
<td>Chilled broiler carcasses</td>
</tr>
<tr>
<td>S. Bredeney</td>
<td>Cloacal swabs</td>
</tr>
<tr>
<td>S. Infantis</td>
<td>Transport cages after cleaning</td>
</tr>
<tr>
<td>S. Lexington</td>
<td>Transport cages after cleaning</td>
</tr>
<tr>
<td>S. Panamá</td>
<td>Cloacal swabs</td>
</tr>
<tr>
<td>S. Rissen</td>
<td>Cloacal swabs</td>
</tr>
<tr>
<td>S. Schwarzengrund</td>
<td>Cloacal swabs</td>
</tr>
<tr>
<td>S. Tennessee</td>
<td>Chilled broiler carcasses</td>
</tr>
</tbody>
</table>

Antimicrobial susceptibility and ESBL production tests

Antimicrobial susceptibility was assessed by the disk diffusion assay following the Clinical and Laboratory Standards Institute (CLSI, 2015) and the following antibiotics: ampicillin (10μg), amoxicillin+clavulanate (20/10μg), aztreonam (30μg), ceftazidime (30μg), cefotaxime (30 μg), chloramphenicol (30μg), gentamicin (10μg), enrofloxacin (5μg), sulfonamide (300 μg), and tetracycline (30 μg). Interpretation was performed using the criteria described in the approved standards VET01-S2 (CLSI, 2014) and M100-S25 (CLSI, 2015). All strains classified as being moderately susceptible were considered non-susceptible.

The disk diffusion assay was used for testing ESBL production (CLSI, 2015), using amoxicillin+clavulanate placed at the center of the Mueller Hinton and three other disks were placed within a 20 mm radius of the first one: aztreonam, cefotaxime and ceftazidime. ESBL production was considered positive when the formation of “inhibition zones” or an increase in inhibition halo size was observed. The quality control of antimicrobials was made with E. coli ATCC 25922 and the positive control for ESBL was a K. pneumoniae ATCC 700603 strain.

RESULTS AND DISCUSSION

Table 2 displays the susceptibility profiles of Salmonella serovars, ESBL production, and their capacity to adhere to polystyrene at different incubation temperatures.

Eight out of the 10 serovars were resistant to sulfonamides. High resistance to these drugs is probably related to their extensive use, leading to an increase in the selective pressure on resistant strains and in the dissemination of resistance genes (Machinski Junior et al., 2005; WHO, 2011; ECDC, 2016). Also, 40% of serovars were resistant to enrofloxacin, which belongs to the class of fluoroquinolones and is exclusively used in veterinary medicine; however, despite its restricted use, it has been shown that enrofloxacin-resistant bacteria can develop resistance to ciprofloxacin. Fluoroquinolones and quinolones play a crucial role in human clinical practice, as they are used in the treatment of severe infections and are the main treatment option against salmonellosis (Souza et al., 2010; WHO, 2011).

Resistance to chloramphenicol was found to be 20%, even though it cannot be used in animal production in Brazil since 2003 (Brasil, 2003). As this antimicrobial was used for many years in veterinary medicine, resistance to it might not have been reversed yet, or else, florphenicol, which is exclusively used by veterinarians, could be favoring the transmission of resistance genes that are common to these two antimicrobials (Nógrády et al., 2012; Mattiello et al., 2015).
Salmonella serovars | Antimicrobial resistance | ESBL production | Adhesion to polystyrene
--- | --- | --- | ---
S. Agona | ENO, SUT | - | Non-adherent Non-adherent Non-adherent Weak Weak
S. Anatum | SUT | - | Weak Moderate Weak Weak Moderate
S. Brandenburg | - | - | Weak Weak Moderate Weak Weak
S. Bredene | CLO, SUT | - | Weak Weak Moderate Weak Weak
S. Infantis | ENO, GEN, SUT | Yes | Weak Moderate Moderate Weak Weak
S. Lexington | SUT | - | Non-adherent Non-adherent Non-adherent Weak Weak
S. Panamà | AMP, ENO, SUT | Yes | Moderate Weak Moderate Weak Non-adherent
S. Rissen | SUT | - | Non-adherent Non-adherent Non-adherent Weak Non-adherent Weak
S. Schwarzengrund | CLO, SUT | - | Weak Moderate Weak Weak
S. Tennessee | ENO, GEN | Yes | Weak Weak Moderate Weak Weak

Legend: AMP = Ampicillin 10 μg, CLO = Chloramphenicol 30 μg, GEN = Gentamicin 10 μg, ENO = Enrofloxacin 5 μg, SUT = Sulfonamides 300 μg, TET = Tetracycline 30 μg. ESBL = Ampicillin-Clavulanate 20/10 μg, Aztreonam 30 μg, Cefazidime 30 μg, Cefotaxime 30 μg.

All tested serovars were sensitive to tetracycline. Serovar Brandenburg was sensitive to all tested drugs. Serovars Anatum, Lexington, and Panamà; Agona and Tennessee; Schwarzengrund; and Infantis were considered moderately resistant to sulfonamides, enrofloxacin, chloramphenicol, and gentamicin, respectively. These are alarming findings as the assessed antimicrobials are widely used in veterinary and human medicine and also because the use of drugs with moderate resistance could result in the selection of a resistant bacterial population (Machinski Junior et al., 2005).

The number of Salmonella serotypes showing ESBL production in the current study was lower than in previous studies (Gelinski et al., 2014). Only serovars Infantis, Panamà, and Tennessee phenotypically demonstrated ESBL production, accounting for 30% of the assessed serovars. The prevalence of ESBL production strains varies worldwide. However, the incidence has increased greatly in recent years in several countries. In the study undertaken by Ziech (2015) in Brazilian samples, 45% (44/98) of Salmonella isolates collected from birds were ESBL producers. ESBL production has emerged in serovar Infantis in Italy and Switzerland (Franco et al., 2015; Hindermann et al., 2017). Serovars Panama and Tennessee have also been reported as ESBL producers (Weill et al., 2004; Gelinski et al., 2014). ESBL-producing strains have also been described in serovars Heidelberg, Senftenberg, Newport, Enteritidis, Weltevreden, Indiana, Typhimurium, Litchfield, Oranienburg and Typhi (Jure et al., 2010; González-López et al., 2014; Bae et al., 2015; Djieffal et al., 2017).

Salmonella serovars can adhere to polystyrene, leading to biofilm formation. It is of public health concern, since strains which remain in slaughter plants and in food products could produce biofilms and favor dissemination through breakage of these structures and consequent release of pathogenic microorganisms.

Note that serovars Panamà; Anatum, Infantis and Schwarzengrund were moderate biofilm producers at 3°C and 9°C, respectively, demonstrating a possible adaptation of these serovars to these temperatures. These findings are in line with those of Rodrigues et al. (2017), who observed biofilm formation at these temperatures, with strongly adherent S. Enteritidis strains. These findings are relevant because these serovars adhered moderately to polystyrene at unfavorable temperatures for the multiplication of Salmonella spp., which is recommended as a conventional method for food preservation under refrigeration (Gast, 2008).

The results obtained in the present study allow inferring that even if the recommended temperatures are complied with, there could be multiplication of Salmonella spp. and biofilm formation in slaughterhouses and consequent contamination of the final products, which may lead to foodborne diseases. In addition, serovars Infantis, Panamà, and Tennessee were found to produce ESBL. This is a public health problem, which highlights the need of caution in the use of antimicrobials.

REFERENCES


Ziech RE. Caracterização de Salmonella sp. isolada de indústrias de aves baseada na formação de biofilmes, tolerância a sanitizantes e resistência a antimicrobianos [dissertation]. Curitiba (PR): Universidade Federal do Paraná; 2015.