Microbiological Contamination and Tetracycline Resistance of Enterococci Isolated from Gastrointestinal Tract and Abdominal Cavity of Chickens

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Abstract

The objective of this study was to determine the microbiological contamination of broiler chicken’s abdomen (n=83) by mesophilic, psychrotrophic bacteria and enterococci. Determined the counts of enterococci in gastrointestinal tract of chickens (crop, ileum and caecum) and found the resistance of enterococci isolated from gastrointestinal tract and abdominal cavity of chickens to tetracycline by disc diffusion method. Species identification of enterococci was determined by commercial biochemical ENCOCCUS test and by species specific PCR method. The average value of mesophilic, psychrotrophic bacteria and enterococci counts in abdominal cavity of chickens were 3.64, 2.08 respectively 2.77 log cfu.cm⁻². E. faecalis was predominant species out of 150 isolates (80%) from chicken’s abdomen, followed by E. faecium (10%) and others sporadically identified isolates (E. gallinarum, E. casseliflavus, E. durans). The average counts of enterococci in crop, ileum and caecum were 4.81, 3.76 respectively 6.81 log cfu. g⁻¹. From the gastrointestinal tract was isolated mainly E. faecalis (n=54). Eighty percent of enterococci isolate from chicken’s abdominal cavity and 87 % from gastrointestinal tract were resistant to tetracycline. Our study suggests that broiler chicken’s abdomen is potential source of enterococci resistant to tetracycline.

Keywords: broiler chicken’s abdomen, enterococci, resistance, tetracycline

1. Introduction

Enterococci are part of the normal microbiota belonging to the gastrointestinal and genitourinary tract of humans and animals [1]. Their ubiquitous nature and resistance to adverse environmental conditions account for their ability to colonise different habitats and underlie their potential to easily spread through the food chain [2]. Poultry enterococci carrying antimicrobial resistance genes may not only transfer these genes to other, possibly pathogenic bacteria in the chicken gut, but upon transfer to zoonotic bacteria they also may pose a human health hazard. Furthermore, these enterococci may be transferred, directly or indirectly, to man, where they might be able to cause disease or further disperse their antimicrobial resistance genes among the gastrointestinal bacterial community [3]. Poultry litter has been found to contain large amounts of antibiotic resistant bacteria and resistance genes associated with the use of antibiotics in poultry production [4]. From studies in which patterns of antibiotic resistance to a broader range of therapeutic agents were determined, it appears that tetracycline resistance is one of the most common phenotypes of acquired antibiotic resistance in food isolates of the genus Enterococcus [5, 6].

The objective of this study was to determine microbiological contamination of chicken’s abdominal cavity after evisceration and rinsing by mesophilic, psychrotrophic bacteria and enterococci. The tetracycline resistance of
enterococci isolated from abdomen of broiler chickens was also determined.

2. Materials and methods

Sample collection

Broilers (n=86) were processed by stunning, bleeding, scalding. Sampling was carried out with sterile cotton swabs after evisceration procedure which including rinsing step with drinking water. The abdomen swabs from chickens were taken from the area of 25 cm². The cotton swabs were vigorously swirled 10 ml of peptone water for 10 minutes. Then submitted to serial 10-fold dilutions in 0.1% (wt/v) peptone water and 1 ml of each dilution was plated to the Petri dishes. Cultivation for mesophile counts enumeration was carried out on Plate count agar (Himedia, India) after 72±2 hours incubation at temperature 30±1°C. Also cultivation of psychrotrophic bacteria was on the Plate count agar during 10 days at temperature 4±1°C. Count of enterococci was determined on selective diagnostic Slanetz–Bartley agar at temperature 37±1°C for 48 resp. 24±2 hours.

The chyme from crop (n=10), ileum (n=10) and caecum (n=10) were aseptically removed and weighed and properly homogenized in ten-fold amount of saline. Serial dilutions for count of enterococci determination were made also in saline and cultured on selective diagnostic Slanetz–Bartley agar and Bile esculin azide agar at the at temperature 37±1°C for 48 resp. 24±2 hours.

Isolation of enterococci genomic DNA

The isolation of genomic enterococci DNA from the overnight cultures at 37°C was prepared according to previous study [7].

Species identification of enterococci

Based on positive growth (esculin hydrolysis) negative production of catalase and positive production of pyrrolidonyl arylamidase were isolates identified to genus Enterococcus and further identified at species level by commercial ENCOCCUS test (Czech Republic) and species specific PCR method using specific primers:

For E. faecium (215 bp) - F:5’GAAAAACATAAGAAGAATT AT3’. R:5’ TGCTTTTTGAAAT TCTTCTTTA 3’ and E. faecalis (941 bp) F:5’ATCAAGTACAGTT AGTCTTTATTAG3’. R:5’ACGATTCAAAGCT AACTGAATCA GT3’. One microliter of DNA was added to a mixture containing 2.5 µl of 10 x PCR buffer (Fermentas), 0.5 µl of each 10 mM deoxynucleoside triphosphate (Fermentas), 2.0 µl 25 mM MgCl₂ (Fermantas), 0.25 µl 5 U of DreamTaq polymerase (Fermentas) and 0.5 µl of each 10 pmol primers (IDT, USA). Samples were incubated for 3 min at 95°C to denature the target DNA and were maintained 30 cycles of 95°C for 30 s, 54°C for 40 s and 72°C for 60 s. The samples were then incubated at 72°C for 10 min for a final extension and were maintained at 4°C until they were tested. Gels were stained with GelRed (Biotium, USA) and visualized in UV light. Isolates producing an amplicon band of the appropriate size by agarose gel (1.5%) electrophoresis were considered positive for species identification. The reference strain E. faecalis (CCM 4224) was used.

Antimicrobial susceptibility tests

Inoculum was prepared by suspending of growth colonies from Plate count agar and the suspension was adjusted to equal a 0.5 McFarland standard according to the recommendations of National Committee for Clinical Laboratory Standards (CLSI) [8]. Resistance to tetracycline with concentration 30 µg/disk was tested using the CLSI requirements.

3. Results and discussion

The average value of mesophillic, psychrotrophic bacteria and enterococci counts in abdomen cavity of broiler chickens were 3.64, 2.08 respectively 2.77 log cfu.cm⁻².

With the current poultry slaughtering lines, fecal contamination during scalding, defeathering and evisceration seems unavoidable. Entrapment of bacteria in the skin crevices and feather follicles after defeathering makes the removal of bacteria very difficult in later processing stages [9]. Therefore, even if low levels of enterococci were
obtained in fresh products, it cannot be used as a criterion of good shelf life quality [10]. However enterococci occurred in chicken abdomen cavity can be used as indicators of fecal contamination. According to Wilson and McAfee, (2002) [11] and Busani et al. (2004) [12] enterococci not only contaminate raw meats but are also associated with processed and heat-treated food materials (for example mechanically separated chicken). 

E. faecalis was predominant species out of 150 isolates (80%) from chicken’s abdomen, followed by E. faecium (10%) and others sporadically identified isolates (E. gallinarum, E. casseliflavus, E. durans) (Figure 1). In accordance with our results also Franz et al., (2003) [13] determined E. faecalis as a predominant species of enterococci isolated from chicken’s abdomen. Devriese et al. (1991) [14] found in the samples of chicken’s gastrointestinal tract mainly species of enterococci E. faecalis, E. faecium, E. durans and E. hirae.

The average counts of enterococci in crop, ileum and caecum were 4.81, 3.76 respectively 6.81 log cfu.g⁻¹. From the gastrointestinal tract was isolated mainly E. faecalis (n=54).

Eighty percent of enterococci isolates from chicken’s abdominal cavity and 87% of enterococci from GIT were resistant to tetracycline. Only one strain of E. faecium and twenty nine strains of E. faecalis isolates from chicken’s abdominal cavity were susceptible to tetracycline. All sporadically identified enterococci strains from abdominal cavity were resistant to tetracycline. Out of 12 isolates of enterococci from gastrointestinal tract were resistant to tetracycline 4 from crop, 3 from ileum and 3 from caecum. In work of Brtková et al. (2011) [15] was resistance to tetracycline by disc diffusion method from poultry and environmental samples mainly determined in E. faecalis and E. casseliflavus. In study of Yurdakul et al. (2013) [16] were determined 100% of Enterococcus spp. strains resistant to tetracycline. In another study, Ruzauskas et al. (2010) [17] evaluated the antimicrobial susceptibility of enterococci spread in poultry products. They reported that the most frequent resistance was demonstrated to tetracycline (84.5%). The high percentage of resistant enterococci isolates to tetracycline is probably due to frequently used of tetracycline by farmers for treatment in poultry, being relatively cheap and effective against a wide variety of microorganisms [18].

4. Conclusions

It can be concluded that enterococci was found in the chicken’s abdominal cavity, which is probably due to evisceration procedure. Presence of tetracycline resistant enterococci in the gastrointestinal tract supports a presumption of chicken’s abdomen cavity contamination by these bacteria and during the slaughter process in slaughterhouses and in the production of mechanically separated chicken supports a presumption of meat contamination.

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References

broilers carrying the erm(B) gene, Avian Pathology, 2007, 36, 395-399.