MOLECULAR CHARACTERIZATION OF STREPTOCOCCUS AND ENTEROCOCCUS SPECIES ISOLATED FROM BROILER CHICKENS

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ABSTRACT

Streptococcus and Enterococcus infections in chickens may result in significant negative effect on economy. In the current study, the prevalence of Streptococcus and Enterococcus species was planned in different broiler chickens farms in Beni-Suef Governorate. A total of 272 samples were collected from lesions (septicemic organs, enlarged organs, necrotic focci) of the affected organs including heart, lung, liver and kidney of diseased broiler chickens and freshly dead ones. Out of 272 samples a total of 49 isolates were recovered with incidence of 18% including 26 Streptococcus spp. (53.1%) and 21 Enterococcus spp. (42.8%) meanwhile 2 isolates (4.1%) were unidentified. Streptococcus isolates were identified as S. gallinaceous (24.5%), S. dysgalactiae (16.3%) and S. durans (12.2%). Meanwhile all Enterococcus isolates were identified as E. faecalis. The in-vitro antibiotic sensitivity testing showed that all isolates were highly sensitive to amoxicillin (77.6%), sulfamethoxazole-trimethoprim (73.5%) and amoxicillin-clavulanic acid (65.3%). Meanwhile, all isolates were resistant to cephalaxin, cefotaxime sodium, cefotaxime, tetracycline, kanamycin and apramycin while 87.8 and 63.2% of isolates showed resistance against gentamicin and enrofloxacin, respectively. Moreover, multidrug resistant were detected in all isolates. Polymerase chain reaction (PCR) was applied to identify 4 resistance-associated genes including (tetO, aac(6)aph(2”) , blaz and Pbp1A) as well as 6 virulence-associated genes including (cylE, brpA, hyl, cylA, asa1 and gelE). The results indicated that tetO, aac(6)aph(2”), blaz, Pbp1A, cylE, brpA, cylA and asa1 genes were recovered from all the tested isolates (100%). Meanwhile, none of streptococcus isolates had hly gene also, gelE gene not detected in enterococcus isolates.

Keywords: Streptococci, Enterococci, Broiler chickens, Resistance genes, Virulence genes.

INTRODUCTION

The poultry industry is considered one of main sources of animal protein (meat and egg) to man also it is a good source of manure for crops. (Mohammed and Sunday, 2015). Streptococci and Enterococci are intestinal inhabitants of birds and mammals and they may accidentally enter circulation and causing disease in poultry (Smyth and McNamee, 2001). Streptococci are Gram-positive cocci, arranged in short chains catalase-negative organisms. Recently more than 40 species are documented, most of these species are
contributed with causing disease in human and animals (Collins et al., 2001). Enterococci are Gram-positive cocci facultative anaerobes and non sporulated also, they are able to hydrolyze esculin in the presence of bile salts, and are catalase negative (Dubin and Pamer, 2017). Since 2000, several new species have been identified and currently more than 50 species of streptococci and at least 21 species of enterococci are recognized and the most common species isolated from poultry are Streptococcus gallinaceus, Streptococcus zooepidemicus, Enterococcus durans, Enterococcus faecalis and Enterococcus hirae (Smyth and McNamee, 2001).

In chicken husbandry, antimicrobial agents used for treatment and growth promotion in broilers more than layers, so resistant enterococci usually recovered from broilers (Klare et al., 1995; Butaye et al., 1999). Enterococcus isolates from poultry subsequently acquired resistance against macrolides, chloramphenicol, β-lactams, and tetracycline has been described (Maasjost et al., 2015). High resistance to aminoglycosides recorded in Enterococci found related to different genes such as (aac(6′) aph(2″), ant(6)) (Hegstad et al., 2010). Moreover, tetM and tetO were the most common tetracycline resistance genes detected in different Streptococcus species (Oppegaard et al., 2020). Some recent studies established that, the genes encoding certain Enterococcus virulence factors such as asa1, geLE and cytA in addition to different antibiotic resistance genes are associated with causing nosocomial infection (Ngbede et al., 2017). The present study was designed for detection of genotypic characterization of Streptococcus and Enterococcus species isolated from broiler chickens achieved by determination of some virulence and antimicrobial resistance associated genes in the MDR isolates using PCR technique.

MATERIALS AND METHODS

1. Ethical approval
The present study was approved by the Institutional Animal Care and Use committee Beni-Suef University (BSU-IACUC, 021-191), Egypt.

2. Chicken Samples
A total of 272 pooling samples were aseptically collected from 272 diseased broiler chickens aged from 2-5 weeks from different farms in Beni-Suef Governorate during duration from December 2018 until December 2019. The pooling samples were collected aseptically from lesions (septicemic organs, enlarged organs, necrotic foci) in the internal organs; liver, lung, heart, and kidney of diseased slaughtered chickens and freshly dead ones.

3. Bacteriological isolation
Isolation of both Streptococci and Enterococci was done according to Collee et al. (1996) and Quinn et al. (2002).

4. Identification of Streptococci and Enterococci isolates
4.1. Morphological identification
Pure culture from each isolate was identified morphologically according to its staining reaction, shape, size, and arrangement. these colonies that revealed to be Gram positive cocci medium size and non- sporulated were further examiened biochemically.

4.2. Biochemical identification
1. catalase test: used to differentiate between catalase positive and catalase negative cocci. Colonies which revealed to be catalase negative were further examiened.

2. Other non-biochemical tests: were performed on catalase negative colonies including,
- growth on MacConkey agar
- cultivation on bile aesculin agar
- detection of hemolytic activity of isolates using sheep blood agar (7%) this was done according to Collee et al. (1996).

4.3. Biochemical identification of isolates using Vitek2 compact system: (Using ID-GP kits) according to (BioMérieux, 2013)
The Vitek2 compact system using ID-GP (Gram positive cocci) identification kits was
applied on pure cultures for complete identification according to BioMérieux (2013).

5. Anti biograms sensetivity testing
The isolated Enterococci and Streptococci were investigated for their susceptibility against 12 different antimicrobial agents of veterinary and human significance. Antimicrobial discs included amoxicillin (10μg), apramycin (15μg), cefotaxime sodium (30μg), ceftriaxone (30μg), cephalexine (30μg), cepfoxacin (5μg), sulphamethoxazol-trimethoprim (25μg), amoxicillin-clavulanic (30μg), tetracycline (30μg), gentamicin (10μg) and kanamycin (30μg). All antimicrobial discs used in this study were obtained from (Oxoid, Basing Stoke, UK). Antimicrobial susceptibility profiling and results interpretation were performed according to (CLSI, 2019). Resistance to more than three antimicrobials of different classes was recorded as multidrug resistance (MDR) according to Chandran et al. (2008).

6. Polymerase chain reaction (PCR) for Streptococcus and Enterococcus isolates
PCR used for detection 3 resistance-associated genes (tetO, aac(6')aph(2'') and pbp1A) and 3 virulence-associated genes (cyl/E, hyl and brpA) in 3 streptococcus isolates. Moreover, it was applied on 3 enterococcus isolates for detection of 3 resistance genes (tetO, aac(6')aph(2'') and blaZ) and 4 virulence genes (hyl, cyl/E, Asa1 and gel/E). Extraction of Genomic DNA was done by using QIAamp DNA extraction Mini prep Kit. Extracted DNA was stored at -80°C for later using in PCR amplification. Table (1) reveals the used Primers sequences and amplified products for the targeted genes for Streptococcus and Enterococcus isolates. Cycling conditions (temperature & time) of the primers during PCR were displayed in table (2).

### Table 1: Primers of virulence and resistance genes used in PCR.

<table>
<thead>
<tr>
<th>Bacteria</th>
<th>Gene</th>
<th>Primer sequence (5'-3')</th>
<th>Length of amplified product</th>
</tr>
</thead>
<tbody>
<tr>
<td>Enterococcus spp.</td>
<td>hyl</td>
<td>ACAGAAGAGCTGCAGGGAATG GACTGAGCTCCAAGTTCCAA</td>
<td>276 bp</td>
</tr>
<tr>
<td>Enterococcus and Streptococcus spp.</td>
<td>aac(6')aph(2'')</td>
<td>GAATACCGGAGAAGGAAGA ACATGGCAAGCTCTAGGA</td>
<td>491 bp</td>
</tr>
<tr>
<td>Streptococcus spp.</td>
<td>tetO</td>
<td>TGA AGC TAA GTT GAA TGC TGC TGA CCA CCA TCA GAC AAG GT</td>
<td>534 bp</td>
</tr>
<tr>
<td></td>
<td>brpA</td>
<td>AAAACAGTGGCGGACCTCAACC AGGTGCTCAAATTTGAGAGG CATACCCATTGCATAATCAGTC</td>
<td>430 bp</td>
</tr>
<tr>
<td></td>
<td>hyl</td>
<td>TGAATTTTATACAGGAAATGAGAAGTTTTTT</td>
<td>950 bp</td>
</tr>
<tr>
<td></td>
<td>cyl/E</td>
<td>TGACATTACATGGACCGAAAG TCGCCAGGAGGAGAATAGGA</td>
<td>248 bp</td>
</tr>
</tbody>
</table>

References:
- Vankerckhoven et al., 2004
- Duran et al., 2012
- Malhotra-Kumar et al., 2005
- Alves-Barroco et al., 2019
- Mosleh et al., 2014
- Krishnaveni et al., 2014
- Bergseng et al., 2007
Table 2: Cycling conditions of the different primers during PCR.

<table>
<thead>
<tr>
<th>Bacteria</th>
<th>Gene</th>
<th>Primary denaturation</th>
<th>Secondary denaturation</th>
<th>Annealing</th>
<th>Extension</th>
<th>No. of cycles</th>
<th>Final extension</th>
</tr>
</thead>
<tbody>
<tr>
<td>Enterococcus</td>
<td>Hyl</td>
<td>94˚C/5 min.</td>
<td>94˚C/30 sec.</td>
<td>55˚C/30 sec</td>
<td>72˚C/30sec.</td>
<td>35</td>
<td>72˚C/7 min.</td>
</tr>
<tr>
<td></td>
<td>cylA</td>
<td>94˚C/5 min.</td>
<td>94˚C/30 sec.</td>
<td>50˚C/40 sec</td>
<td>72˚C/45sec.</td>
<td>35</td>
<td>72˚C/10 min.</td>
</tr>
<tr>
<td></td>
<td>gelE</td>
<td>94˚C/5 min.</td>
<td>94˚C/30 sec.</td>
<td>50˚C/30 sec</td>
<td>72˚C/30sec.</td>
<td>35</td>
<td>72˚C/7 min.</td>
</tr>
<tr>
<td></td>
<td>AsaI</td>
<td>94˚C/5 min.</td>
<td>94˚C/30 sec.</td>
<td>53˚C/40 sec</td>
<td>72˚C/40sec.</td>
<td>35</td>
<td>72˚C/10 min.</td>
</tr>
<tr>
<td></td>
<td>blaZ</td>
<td>94˚C/5 min.</td>
<td>94˚C/30 sec.</td>
<td>54˚C/30 sec</td>
<td>72˚C/30sec.</td>
<td>35</td>
<td>72˚C/7 min.</td>
</tr>
<tr>
<td>Enterococcus and streptococcus</td>
<td>aac(6’)aph(2&quot;)</td>
<td>94˚C/5 min.</td>
<td>94˚C/30 sec.</td>
<td>54˚C/40 sec</td>
<td>72˚C/40sec.</td>
<td>35</td>
<td>72˚C/10 min.</td>
</tr>
<tr>
<td></td>
<td>tetO</td>
<td>94˚C/5 min.</td>
<td>94˚C/30 sec.</td>
<td>56˚C/40 sec</td>
<td>72˚C/45sec.</td>
<td>35</td>
<td>72˚C/10 min.</td>
</tr>
<tr>
<td>Streptococcus</td>
<td>brpA</td>
<td>94˚C/5 min.</td>
<td>94˚C/30 sec.</td>
<td>42˚C/40 sec</td>
<td>72˚C/45sec.</td>
<td>35</td>
<td>72˚C/10 min.</td>
</tr>
<tr>
<td></td>
<td>Pbp1A</td>
<td>94˚C/5 min.</td>
<td>94˚C/30 sec.</td>
<td>57˚C/40 sec</td>
<td>72˚C/45sec.</td>
<td>35</td>
<td>72˚C/10 min.</td>
</tr>
<tr>
<td></td>
<td>Hyl</td>
<td>94˚C/5 min.</td>
<td>94˚C/30 sec.</td>
<td>54˚C/30 sec</td>
<td>72˚C/30sec.</td>
<td>35</td>
<td>72˚C/7 min.</td>
</tr>
<tr>
<td></td>
<td>cylE</td>
<td>94˚C/5 min.</td>
<td>94˚C/30 sec.</td>
<td>55˚C/30 sec</td>
<td>72˚C/30sec.</td>
<td>35</td>
<td>72˚C/7 min.</td>
</tr>
</tbody>
</table>

RESULTS

1. Prevalence of bacterial isolation from different samples
Out of 272 samples from broiler chickens, a total of 49 bacterial isolates suspected (morphologically and by biochemical tests) to be streptococci or enterococci were recovered; with a total prevalence of 18%.

Table 3: Prevalence of Streptococcus and Enterococcus isolated from the diseased broiler chickens.

<table>
<thead>
<tr>
<th>Genus</th>
<th>Species</th>
<th>Isolation No.</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Streptococcus</td>
<td>S. gallinaceous</td>
<td>12</td>
<td>24.5</td>
</tr>
<tr>
<td></td>
<td>S. dysgalactiae</td>
<td>8</td>
<td>16.3</td>
</tr>
<tr>
<td></td>
<td>S. durans</td>
<td>6</td>
<td>12.3</td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td>26</td>
<td>53.1</td>
</tr>
<tr>
<td>Enterococcus</td>
<td>E. faecalis</td>
<td>21</td>
<td>42.8</td>
</tr>
<tr>
<td></td>
<td>Unidentified</td>
<td>2</td>
<td>4.1</td>
</tr>
<tr>
<td></td>
<td>Total isolates</td>
<td>49</td>
<td>100</td>
</tr>
</tbody>
</table>

%: was calculated according to the corresponding number (No.) of isolates

2. Antibiogram sensitivity testing
The in-vitro antimicrobial susceptibility testing revealed that the tested isolates (n=49) showed high sensitivity to amoxicillin (77.6%), sulfamethoxazole-trimethoprim (73.5%) and amoxicillin-clavulanic acid (65.3%). On the other hand, they were completely resistant to cephalxin, cefotaxime, cefipime, cefotiaxone, tetracycline, kanamycin and apramycin (100% for each) and were highly resistant to gentamicin (87.8%) and enrofloxacin (63.2%) (Table 4). More over, all investigated isolates showed presence of multidrug resistance.
Table 4: Results of in-vitro antimicrobial susceptibility testing of recovered isolates.

<table>
<thead>
<tr>
<th>Antimicrobial type</th>
<th>Symbol</th>
<th>Disc content (µg)</th>
<th>Tested isolates (n= 49)</th>
<th>R</th>
<th>%</th>
<th>I</th>
<th>S</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Amoxicillin-clavulanic acid</td>
<td>AMC</td>
<td>30</td>
<td>17</td>
<td>34.7</td>
<td>0</td>
<td>0</td>
<td>32</td>
<td>65.3</td>
</tr>
<tr>
<td>Cephalexin</td>
<td>CL</td>
<td>30</td>
<td>49</td>
<td>100</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Cefotaxime</td>
<td>CTX</td>
<td>30</td>
<td>49</td>
<td>100</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Cefipime</td>
<td>FEP</td>
<td>30</td>
<td>49</td>
<td>100</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Cefotaxione</td>
<td>CRO</td>
<td>30</td>
<td>49</td>
<td>100</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Enrofloxacin</td>
<td>ENR</td>
<td>5</td>
<td>31</td>
<td>63.2</td>
<td>9</td>
<td>18.4</td>
<td>9</td>
<td>18.4</td>
</tr>
<tr>
<td>Tetracyclin</td>
<td>TE</td>
<td>30</td>
<td>49</td>
<td>100</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Gentamicin</td>
<td>CN</td>
<td>10</td>
<td>43</td>
<td>87.8</td>
<td>1</td>
<td>2</td>
<td>5</td>
<td>10.2</td>
</tr>
<tr>
<td>Sulfamethoxazole-trimethoprim</td>
<td>SXT</td>
<td>25</td>
<td>11</td>
<td>22.4</td>
<td>2</td>
<td>4.1</td>
<td>36</td>
<td>73.5</td>
</tr>
<tr>
<td>Kanamycin</td>
<td>K</td>
<td>30</td>
<td>49</td>
<td>100</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Apramycin</td>
<td>APR</td>
<td>15</td>
<td>49</td>
<td>100</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Amoxicillin</td>
<td>AML</td>
<td>10</td>
<td>11</td>
<td>22.4</td>
<td>0</td>
<td>0</td>
<td>38</td>
<td>77.6</td>
</tr>
</tbody>
</table>

% was calculated according to the number of the tested isolates (n=49).

3. Polymerase chain reaction (PCR) analyses of streptococcus and enterococcus isolates

Concerning Streptococcus isolates (n=3), all the tested resistance associated genes (tetO, aac(6')aph(2'') and pbp1A) were detected in all the tested isolates (n=3; 100%). Among the tested virulence genes, cyl-E and brpA genes were detected in all the tested isolates (n=3; 100%) while hyl gene was not found in any isolates (Table 5 and Figs. 1, 2 & 3).

Table 5: Distribution and prevalence of resistance and virulence -associated genes in the examined Streptococcus isolates.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Gene</th>
<th>Resistance genes</th>
<th>Virulence genes</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>tetO</td>
<td>aac(6')aph(2'')</td>
</tr>
<tr>
<td>1</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>2</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>3</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Total</td>
<td>3 (100%)</td>
<td>3 (100%)</td>
<td>3 (100%)</td>
</tr>
</tbody>
</table>

% was calculated according to the number (No.) of the tested isolates (n=3).

Moreover, it was applied on 3 enterococcus isolates for detection of 3 resistance genes (tetO, aac(6')aph(2'') and blaZ) and 4 virulence genes (hyl, cylA, Asa1 and gelE).

Regarding Enterococcus isolates (n=3), all the tested resistance genes (tetO, aac(6')aph(2'') and blaZ) were detected in all the tested isolates (n=3; 100%). On the other hand, among the tested virulence genes; hyl, cylA and Asa1 genes were detected in all the tested isolates (n=3; 100%) while gelE gene was not found in any isolates (Tables 6 and Figs. 1, 2, 4, 5 & 6).

Table 6: Distribution and prevalence of resistance and virulence -associated genes in the examined Enterococcus isolates.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Gene</th>
<th>Resistance genes</th>
<th>Virulence genes</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>tetO</td>
<td>aac(6')aph(2'')</td>
</tr>
<tr>
<td>1</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>2</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>3</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Total</td>
<td>3 (100%)</td>
<td>3 (100%)</td>
<td>3 (100%)</td>
</tr>
</tbody>
</table>

% was calculated according to the number (No.) of the tested isolates (n=3).
**Fig. (1):** PCR amplification of the 515 bp fragment of *tetO* resistance gene from 3 Streptococci (S1-S3) and 3 Enterococci (E1-E3) showing positive amplicons migrates with the molecular DNA size ladder (L), P (control positive), and N (control negative).

**Fig. (2):** PCR amplification of the 491 bp fragment of *aac(6')aph(2'″)* resistance gene from 3 Streptococci (S1-S3) and 3 Enterococci (E1-E3) showing positive amplicons migrates with the molecular DNA size ladder (L), P (control positive), and N (control negative).

**Fig. (3):** PCR amplification of the 534 bp fragment of brpA resistance gene and 248, 950 and 430 bp fragments of *cylE*, *hyl* and *pbp1A* virulence genes, respectively, from 3 Streptococci (S1-S3) showing positive amplicons migrates with the molecular DNA size ladder (L), P (control positive), and N (control negative).

**Fig. (4):** PCR amplification of the 173 bp fragment of *blaZ* resistance gene from 3 Enterococci (E1-E3) showing positive amplicons migrates with the molecular DNA size ladder (L), P (control positive), and N (control negative).

**Fig. (5):** PCR amplification of the 213 and 688 bp fragments of *gelE* and *cylA* virulence genes, respectively, from 3 Enterococci (E1-E3) showing positive amplicons migrates with the molecular DNA size ladder (L), P (control positive), and N (control negative).
Fig. (6): PCR amplification of the 276 and 375 bp fragments of *hyl* and *asa1* virulence genes, respectively, from 3 Enterococci (E1-E3) showing positive amplicons migrates with the molecular DNA size ladder (L), P (control positive), and N (control negative).

**DISCUSSION**

A great attention has been paid to poultry-based industries due to its importance as a source of animal protein in Egypt. Poultry are regarded the most appropriate source of animal protein supply of high nutritive value for humans all over the world. This is due to the efficiency cost of production.

Infectious diseases such as (*Streptococci* and *Enterococci*) are important in the broiler industry due to high mortality, retardation of growth, as well as the preventive and therapeutic use of antimicrobials. Moreover, economic losses may result from the loss of uniformity of the flock and condemnations in the slaughterhouse (McKissick, 2006). *Streptococcus* and *Enterococcus* are considered to cause disease in human and animals (Collins et al., 2001). Also, the enterococci are important agents in human nosocomial infections (Cardona et al., 1993). *Streptococcus* and *Enterococcus* have been considered as normally inhabitant, Gram-positive fastidious microorganisms of chickens. Additionally, they may cause disease conditions as endocarditis and urinary tract, intra-abdominal infections in broilers. (Tankson et al., 2001). In the present study, the incidence of *Streptococci* and *Enterococci* were identified in broilers in Beni Suef Governorate. The data illustrated in the table (3) revealed that the total prevalence of *Streptococcus* and *Enterococcus* species in the diseased broiler chickens was 18% where 49 isolates were recovered from 272 diseased broiler chickens. According to Vitek2 compact system, the bacterial isolates were arranged as 26 *Streptococcus* spp. (53.1%) and 21 *Enterococcus* spp. (42.8%) and isolates while there were 2 unidentified isolates (4.1%). *Streptococcus* isolates (*n* = 26) were identified as 12 *S. gallinaceous* (24.5%), 8 *S. dysgalactiae* (16.3%) and 6 *S. durans* (12.3%). On the other hand, all *Enterococcus* isolates (*n* = 21) were identified as *E. faecalis*. These results were higher than those recovered by Cauwerts et al. (2007) who found *E. faecalis* with a prevalence rate of 13.6% in broilers, Diarra et al. (2010); who remarked that prevalence rate of *E. faecalis* was 10.1%. While the lowest result recorded by (Chadfield et al., 2004) who collected 227 samples from broiler chickens and recovered 15 *E. faecalis* isolates (6.6%). Also, Aslantaş (2019) isolated *E. durans* with prevalence of 2.4% and Cauwerts et al. (2007) who recorded *E. durans* with prevalence of 9.5%. Results in present study was noted to be less than those recorded by (Chadfield et al., 2004) who documented *S. gallinaceous* with prevalence of 37.4% and Abd El-Hafeez et al. (2018) who recorded *S. dysgalactiae* with prevalence rate 34.7%. Higher rates of isolation were achieved by Petersen et al. (2008); 77.5%, and 46.5%. Meanwhile, much higher prevalence was recorded by Aslantaş (2019); 87.8%.

In poultry rearing Antimicrobials are used for treatment infectious microbial diseases also they play an important role in growth promotion. Its excessive use in animal production leads to spread of antibiotic resistance (Gosh and LaPara 2007). In-vitro antimicrobial susceptibility testing of different veterinary pathogens helps the veterinarian in the choice of the most suitable drug for treatment (Radwan et al., 2016). In the present study, the isolated *Enterococci* and *Streptococci* were investigated for their susceptibility against 12 different antimicrobial agents of veterinary and human significance.
The in-vitro antibiogram senstivity testing results for both Streptococcus and Enterococcus isolates were showed in table (4). Isolates were highly sensitive to amoxicillin (77.6%) followed by sulfamethoxazole- trimethoprim (73.5%) and amoxicillin- clavulanic acid (65.3 %). Meanwhile, they were completely resistant to cephalaxin, cefotaxime sodium, cepipime, cefotrixone, tetracyclin, kanamycin and apramycin (100%) and were highly resistant to gentamicin (87.8%) and enrofloxacin (63.2%). Also, growing of resistance was observed by the intermediate behavior of the tested isolates against the tested antimicrobial agents. The percentages of the intermediate zones were 2, 4.1 and 18.4 % against gentamycin, trimethoprime- sulfamethoxazole and enrofloxacin, respectively. Additionally, multidrug resistance was detected in all tested isolates. Higher prevalence rates of resistance were reported against tetracycline and kanamycin by Diarra et al. (2010) 91.3, 59.4%; (Tremblay et al., 2011) 95.6, 25.2% and Nowakiewicz et al. (2017) 60.5, 42.1%, respectively. Also, Hershberger et al. (2005) recorded resistance against gentamycin in 32% of isolates. On the other hand, Rehman et al. (2018); Aslantaş (2019); Obeng et al. (2013) and Liu et al. (2013) reported closely matching resistance rates with those detected in present study. B. casuse of misuse antimicrobials which might leads to high resistance rates, it was difficult to found an effective drug against the Streptococci and Enterococci infections. (Sharada et al., 2001). More over, all investigated isolates showed presence of multidrug resistance. Our results were nearly similar to previous reports all over the world. Aslam et al. (2012) founded that multidrug resistance were detected in 91% of isolates. Meanwhile, lower percentages of MDR were recorded by Nowakiewicz et al. (2017); 56.8% and (Ngbede et al., 2017); 53.1%.

In the present study, PCR was used for detection of 3 resistance-associated genes including resistance to tetracycline (tetO), resistance to aminoglycosides (aac(6’)aph(2’)) and resistance to β-lactams (php1A) in 3 Streptococcus isolates. Moreover, it was applied on 3 Enterococcus isolates for detection of 3 resistance genes including (tetO, aac(6’)aph(2’)) and (blaZ). The data illsrutated in (tables 5-6 and Figs. (1:4) revealed that 100% of the tested isolates harbored tetO, (aac(6’)aph(2’)) genes on the other hand 100% of streptococci isolates harbored (Php1A) gene also, (blaZ) gene were detected in all investigated Enterococci isolates.

Many genes were detected for tetracycline resistance including tetK, tetL, tetM and tetO genes Ngbede et al. (2017). Tet(O) gene which resposible for tetracycline resistance was detected in enterococci isolated from broilers by Aarestrup et al. (2000) and, when studing tetracycline resistance determinants in raw food, Wilcks et al. (2005) founded that this gene only occur in enterococci isolated from poultry meat. Also, this gene has been described in human E. faecalis, but is rare Aarestrup et al. (2000). The efflux proteins have been the best studied of the Tet determinants including tetA, tetB, tetC, tetD, tetE, tetG, tetH, tetK, tetL and tetA(P) genes which have been identified. All of the following Tet determinants (tetA, tetB, tetC, tetD, tetE) protected the bacterial ribosomes because they encoded for energy-dependent membrane associated proteins which release tetracycline out of the cell reducing the intercellular tetracycline concentration (El-Seedy et al. 2019).

Regarding the obtained results of tetO which was detected in all tested isolates (100%), they were higher than those recorded by Cauwerts et al. (2007) who found tetO and tetM in 30% of tested isolates. Meanwhile, much lower prevalence was recorded by Diarra et al. (2010) who founded that 7.2% of tested isolates harbored tetO also, tetL and tetM were detected in 57.15% of isolates. Moreover, (Tremblay et al., 2011; Ngbede et al., 2017; Nowakiewicz et al., 2017) detected this gene in Enterococci isolates from broilers. On the other hand, the obtained results of aminoglycosides resistance encoding gene (aac(6’)aph(2’)) which is detected in all tested isolate (100%), were higher than those obtained by Diarra et al. (2010) who found (aac(6’)aph(2’)) gene in 30.4% of tested isolates. Also, Rehman et al. (2018) recorded (aac(6’)aph(2’)) gene in 8.3% of Enterococci isolates from broilers.

In the present study, PCR was applied on 3 MDR Streptococci and 4 Enterococci isolates to detect the following virulence associated genes including β-haemolysin cytolysin gene (cly/E), hyalurindase encoded by (hly)and biofilm production (brpA) for Streptococci isolates. Also, the following genes asa1 (aggregation substance), which associated with adherence and conjugation; cyaA encodes
(cytolysin-haemolysin) which lyse red blood cells, hly (hyaluronidase) while gelatinase, encoded by (ge/E) which can hydrolyze gelatin, were investigated in Enterococci isolates using PCR. The results illustrated in tables (5-6) and Figs. (3, 5& 6) revealed that all the tested isolates (100%) harbored both cyIA and brpA genes meanwhile no isolates (0%) harbored hly gene in case of Streptococcus isolates. On the other hand, all Enterococci tested isolates (100%) harbored asa1, cyIA and hly genes meanwhile no isolates (0%) harbored ge/E gene. Regarding the obtained results of cyIA and cy/E which were detected in all tested isolates (100%), this result was higher than those recorded by Diarra et al. (2010) who found cyIA and cyIB genes in 28.5 % of tested isolates. Also, Ngbede et al. (2017) recorded cyIA gene in 28.3% of tested isolates. Meanwhile, Song et al. (2019) found cyIA in 16% isolates. The lower prevalences were recorded by (Champagne et al., 2011) who detected cyIA and cyIB in 6% of isolates and Aslantaş (2019) who found cyIA in 0.7 %.

On the contrary, Nowakiewicz et al. (2017) reported that none of tested isolates (0%) exhibited genes responsible for haemolysis – cytolysin production. Regarding the obtained results of ge/E which were not detected in any tested isolate, this result is lower than those detected by Ngbede et al. (2017) who found ge/E gene in 11.3 % and Aslantaş (2019) recorded this gene in 40.3% of tested isolates. While (Diarra et al., 2010; Champagne et al. 2011; Nowakiewicz et al., 2017) recorded ge/E in 100% of investigated isolates. Regarding the obtained results of asa1 gene which was found in all investigated isolates (100%). This result is higher than those recorded by Aslantas (2019) who found asa1 gene in 6.1% of isolates. While Song et al. (2019) found asa1 gene in 44% of tested isolates.

CONCLUSION

Streptococcus and Enterococcus spp. are important infectious agents which can cause disease in broilers, and affect on morbidity and mortality rates. The excessive use of antibiotics resulting in multidrug resistance pathogens and this is considered a great problem. The in-vitro antimicrobial sensetivity testing revealed that all tested isolates were highly sensitive to amoxicillin, sulfamethoxazole- trimethoprim and amoxicillin- clavulanic acid meanwhile they were completely resistant to cefalexin, cefotaxime, cefipime, cefotrixone, tetracycline, kanamycin and apramycin. All the tested isolates were MDR. PCR results revealed that tetO, aac(6’)-aph(2’), blaZ, php1A, cyIE, brpA, cyIA asa1 genes were detected in all the investigated isolates meanwhile, hyl gene was not detected in any Streptococcus isolates and ge/E gene was not detected in Enterococcus isolates.

REFERENCES


targeting the sip gene for detection of group B Streptococcus colonization in pregnant women at delivery. *Journal of medical microbiology*, 56(2), 223-228.

BioMérieux Vitek, Inc. 04/ (2013): Vitek2-technology Product Information Manual. Pdf version located with the QC SOPs, a hard copy is available in the laboratory. 4.


توصيف الجزئي للمكورات السبحية والمعوية المعزولة من دجاج التسمين

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قد تؤدي عديد المكورات السبحية والمكورات المعوية في الدجاج إلى خسائر إقتصادية كبيرة. في الدراسة الحالية تم دراسة انتشار المكورات السبحية والمكورات المعوية في مزارع دجاج التسمين المختلفة. تم جمع عينات من الأعضاء الداخلية المصابة مثل عينات الدم من خلال الفحص البكتريولوجي للعينات. تم استخدام تفاعل البلمرة المتسلسل للكشف عن العزلات. تم استخدام Vitek2 compact system لتصنيف العزلات باستخدام اختبار البكتريولوجي وال-users. تم تحديد جينات مرتبطة بالمقاومة بما في ذلك، 

\( \text{aac (6')} \), \( \text{aph (2')} \), \( \text{brpA} \), \( \text{CyII} \), \( \text{E} \), \( \text{hyl} \), \( \text{tetO} \) و\( \text{aac (6)} \). 

في الوقت نفسه، لم يتم الكشف عن جينات سلبية في العزلات المعزولة من الدجاج. 

العنوان: التوصيف الجزئي للمكورات السبحية والمعوية المعزولة من دجاج التسمين

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العنوان الإنجليزي: Determinants among European Enterococcus faecium from cecal contents in broiler chicken and turkey flocks slaughtered in Canada and plasmid colocalization of tetO and ermB genes. Journal of food protection, 74(10), 1639-1648.
