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MOLECULAR CHARACTERIZATION OF *STREPTOCOCCUS* AND *ENTEROCOCCUS* SPECIES ISOLATED FROM BROILER CHICKENS

AHMED H. ABED [1]; ASMAA S. A. SEDIK [1] AND SALAMA A. S. SHANY [2]

[1] Bacteriology, Mycology and Immunology Department, Faculty of Veterinary Medicine, Beni-Suef University, Beni-Suef 62511, Egypt.

[2] Poultry Diseases Department, Faculty of Veterinary Medicine, Beni-Suef University, Beni-Suef 62511, Egypt.

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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 cephalexin, cefotaxime sodium, cefipime, cefotriaxone, tetracycline, kanamycin and apramycin while 87.8 and 63.2% of isolates showed resistance aganist 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 (*tet*O, *aac(6')aph(2''), bla*Z and *Pbp*1A) as well as 6 virulence-associated genes including (*cyl*E, *brp*A, *hyl*, *cyl*A, *asa*1 and *gel*E). The results indicated that *tet*O, *aac(6')aph(2''), bla*Z, *Pbp*1A, *cyl*E, *brp*A, *cyl*A and *asa*1 genes were recovered from all the tested isolates (100%). Meanwhile, none of streptococcus isolates had *hly* gene also, *gel*E 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

Corresponding author: Asmaa S.A. Sedik
E-mail address: asmaa.saad91@yahoo.com
Present address: Bacteriology, Mycology and Immunology Department, Faculty of Veterinary Medicine, Beni-Suef University, Beni-Suef 62511, Egypt.

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, *tet*M and *tet*O 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 *asa*1, *gel*E and *cyl*A 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 untill December 2019. The pooling samples were collected aseptically from lesions (septicemic organs, enlarged organs, necrotic focci) 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 ngative 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. Antibiograms sensetivity testing

The isolated Enterococci and Streptococci were investigated for their susceptibility aganist 12 different antimicrobial agents of veterinary and human significance. Antimicrobial discs included amoxicillin (10µg), apramycin (15µg), cefotaxime sodium (30µg), ceftriaxone (30µg), cephalexine (30µg), cefepime (30µg), enrofloxacin (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 reults 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 (*tet*O, *aac(6')aph(2'')* and *pbp*1A) and 3 virulence-associated genes (*cyl*E, *hyl* and *brp*A) in 3 streptococci isolates. Moreover, it was applied on 3 enterococcus isolates for detection of 3 resistance genes (*tet*O, *aac(6')aph(2'')* and *blaZ)* and 4 virulence genes (*hyl, cyl*A, *Asa*1 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.

Bacteria	Gene	Primer sequence (5'-3')	Length of amplified product	Reference
Enterococcus spp.	*hyl*	*ACAGAAGAGCTGCAGGAAATG*	276 bp	Vankerckhoven *et al*., 2004
		GACTGACGTCCAAGTTTCCAA		
	*cyl*A	*ACTCGGGGATTGATAGGC*	688 bp	
		GCTGCTAAAGCTGCGCTT		
	*gel*E	*TATGACAATGCTTTTTGGGAT*	213 bp	
		AGATGCACCCGAAATAATATA		
	*asa*1	*GCACGCTATTACGAACTATGA*	375 bp	
		TAAGAAAGAACATCACCACGA		
	*bla*Z	*ACTTCAACACCTGCTGCTTTC*	173 bp	Duran *et al*., 2012
		TGACCACTTTTATCAGCAACC		
Enterococcus and *Streptococcus* spp.	*aac(6')aph(2'')*	*GAAGTACGCAGAAGAGA*	491 bp	
		ACATGGCAAGCTCTAGGA		
	*tet*O	*AACTTAGGCATTCTGGCTCAC*	515 bp	Malhotra-Kumar *et al*. 2005
		TCCCACTGTTCCATATCGTCA		
Streptococcus spp.	*brp*A	*TGA AGC TAA GTT GAA TGC TGC*	534 bp	Alves-Barroco *et al*., 2019
		GAA CCA CCA TCA GAC AAG GT		
	*pbp*1A	*AAACAAGGTCGGACTCAACC*	430 bp	Mosleh *et al*., 2014
		AGGTGCTACAAATTGAGAGG		
	hyl	*CATACC TTAACAAAGATATATAACAA*	950 bp	Krishnaveni *et al*., 2014
		AGATTTTTTAGAGAATGAGAAGTTTTTT		
	*cyl*E	*TGACATTTACAAGTGACGAAG*	248 bp	Bergseng *et al*., 2007
		TTGCCAGGAGGAGAATAGGA		

Table 2: Cycling conditions of the different primers during PCR.

Bacteria	Gene	Primary denaturation	Secondary denaturation	Annealing	Extension	No. of cycles	Final extension
Enterococcus spp.	*Hyl*	94˚C/ 5 min.	94˚C/ 30 sec.	55˚C/30 sec	72˚C/30sec.	35	72˚C/7 min.
	cylA	94˚C/5 min.	94˚C/30 sec.	50˚C/40 sec	72˚C/45sec.	35	72˚C/10 min.
	gelE	94˚C/5 min.	94˚C/30 sec.	50˚C/30 sec	72˚C/30sec.	35	72˚C/7 min.
	Asa1	94˚C/5 min.	94˚C/30 sec.	53˚C/40 sec	72˚C/40sec.	35	72˚C/10 min.
	blaZ	94˚C/5 min.	94˚C/30 sec.	54˚C/30 sec	72˚C/30sec.	35	72˚C/7 min.
Enterococcus and streptococcus spp.	*aac(6')aph(2'')*	94˚C/5 min.	94˚C/30 sec.	54˚C/40 sec	72˚C/40sec.	35	72˚C/10 min.
	*tet*O	94˚C/5 min.	94˚C/30 sec.	56˚C/40 sec	72˚C/45sec.	35	72˚C/10 min.
Streptococcus spp.	*brpA*	94˚C/5 min.	94˚C/30 sec.	42˚C/40 sec	72˚C/45sec.	35	72˚C/10 min.
	Pbp1A	94˚C/5 min.	94˚C/30 sec.	57˚C/40 sec	72˚C/45sec.	35	72˚C/10 min.
	Hyl	94˚C/5 min.	94˚C/30 sec.	52˚C/40 sec	72˚C/50sec.	35	72˚C/10 min.
	cylE	94˚C/5 min.	94˚C/30 sec.	55˚C/30 sec	72˚C/30sec.	35	72˚C/7 min.

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%.

According to Vitek2 compact system, the bacterial isolates were arranged as 26 *Streptococcus* spp. (53.1%) and 21 *Enterococcus* spp. (42.8%) 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* (12.2%). On the other hand, all Enterococcus isolates (*n=21*) were identified as *E. faecalis* (Table 3).

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

Genus	Species	Isolation	
		No.	%
Streptococcus	*S. gallinaceous*	12	24.5
	S. dysgalactiae	8	16.3
	S. durans	6	12.3
	Total	26	53.1
Enterococcus	*E. faecalis*	21	42.8
Unidentified		2	4.1
Total isolates		49	100

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

2. Antibiogram sensetivity 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 cephalexin, cefotaxime, cefipime, cefotriaxone, 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.

Antimicrobial type	Symbol	Disc content (µg)	Tested isolates (*n= 49*)					
			R		I		S	
			No	%	No	%	No	%
Amoxicillin-clavulanic acid	AMC	30	17	34.7	0	0	32	65.3
Cephalexin	CL	30	49	100	0	0	0	0
Cefotaxime	CTX	30	49	100	0	0	0	0
Cefipime	FEP	30	49	100	0	0	0	0
Cefotraxione	CRO	30	49	100	0	0	0	0
Enrofloxacin	ENR	5	31	63.2	9	18.4	9	18.4
Tetracyclin	TE	30	49	100	0	0	0	0
Gentamicin	CN	10	43	87.8	1	2	5	10.2
Sulfamethoxazole-trimethoprim	SXT	25	11	22.4	2	4.1	36	73.5
Kanamycin	K	30	49	100	0	0	0	0
Apramycin	APR	15	49	100	0	0	0	0
Amoxicillin	AML	10	11	22.4	0	0	38	77.6

% 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 (*tet*O, *aac(6')aph(2'')* and *pbp*1A) were detected in all the tested isolates (*n=3*; 100%). On the other hand, among the tested virulence genes; *cyl*E and *brp*A 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.

Gene / Sample	Resistance genes			Virulence genes		
	***tet*O**	***aac(6')aph (2'')***	***pbp*1A**	***cyl*E**	***hyl***	***brp*A**
1	+	+	+	+	-	+
2	+	+	+	+	-	+
3	+	+	+	+	-	+
Total (%)	**3 (100%)**	**3 (100%)**	**3 (100%)**	**3 (100%)**	**0 (0%)**	**3 (100%)**

%: 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 (*tet*O, *aac(6')aph(2'')* and *blaZ)* and 4 virulence genes (*hyl*, *cyl*A, *Asa*1 and *gel*E).

Regarding Enterococcus isolates (*n=3*), all the tested resistance genes (*tet*O, *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*, *cyl*A and *Asa*1 genes were detected in all the tested isolates (*n=3*; 100%) while *gel*E 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.

Gene / Sample	Resistance genes			Virulence genes			
	***tet*O**	***aac(6')aph(2'')***	***blaZ***	***hyl***	***cyl*A**	***Asa*1**	***gel*E**
1	+	+	+	+	+	+	-
2	+	+	+	+	+	+	-
3	+	+	+	+	+	+	-
Total (%)	3 (100%)	3 (100%)	3 (100%)	3 (100%)	3 (100%)	3 (100%)	0 (0%)

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

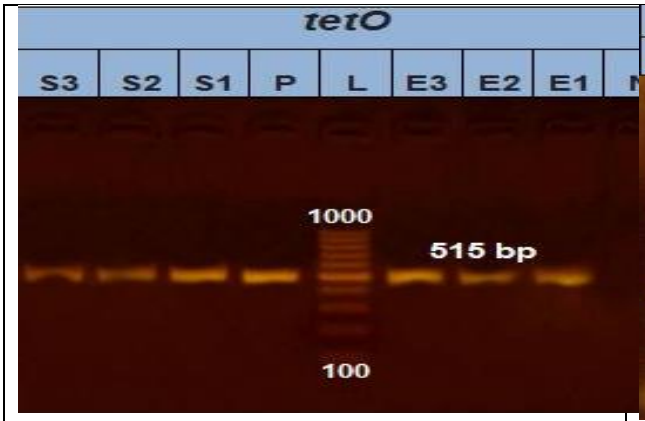

Fig. (1): PCR amplification of the 515 bp fragment of *tet*O resistance gene from 3 Streptococci (S1-S3) and 3 Enterococci (E1-E3) showing positive amplicons migrates with the molecular DNA size ladder (L)., P (control positive), and N (control negative).

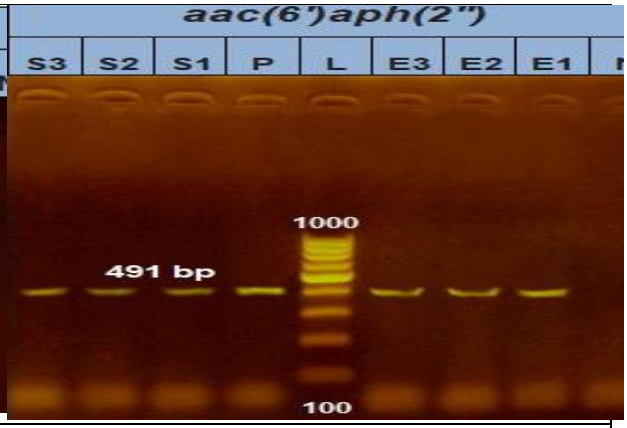

Fig. (2): PCR amplification of the 491 bp fragment of *aac(6') aph(2'')* resistance gene from 3 Streptococci (S1-S3) and 3 Enterococci (E1-E3) showing positive amplicons migrates with the molecular DNA size ladder (L)., P (control positive), and N (control negative).

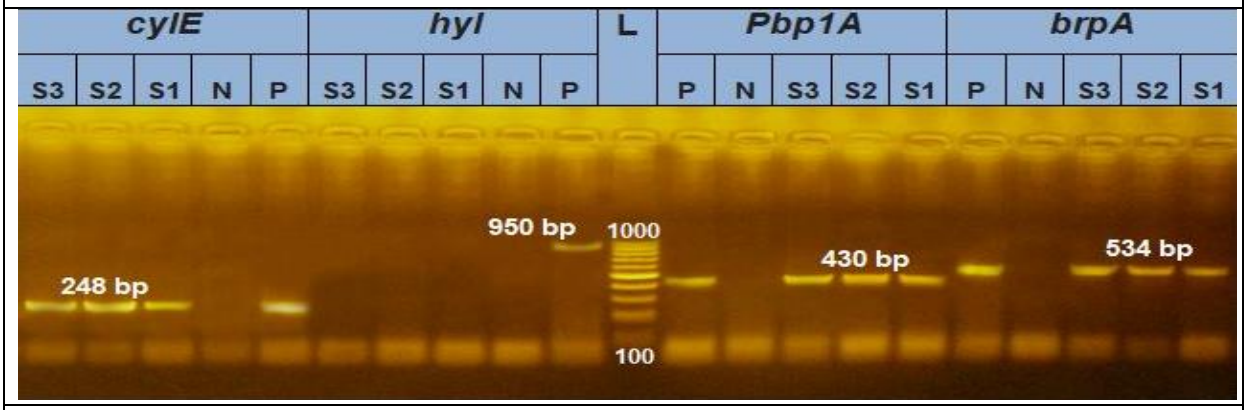

Fig. (3): PCR amplification of the 534 bp fragment of brpA resistance gene and 248, 950 and 430 bp fragments of *cyl*E, *hyl* and *pbp*1A virulence genes, respectively, from 3 Streptococci (S1-S3) showing positive amplicons migrates with the molecular DNA size ladder (L)., P (control positive), and N (control negative).

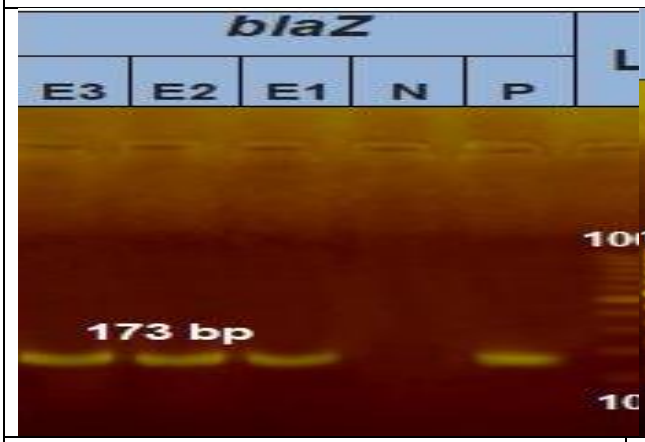

Fig. (4): PCR amplification of the 173 bp fragment of *bla*Z resistance gene from 3 Enterococci (E1-E3) showing positive amplicons migrates with the molecular DNA size ladder (L)., P (control positive), and N (control negative).

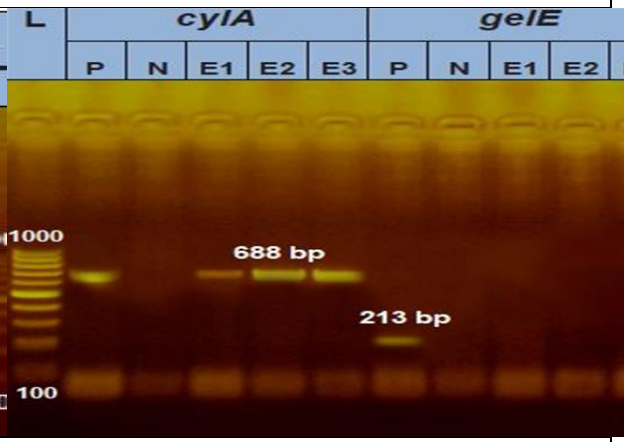

Fig. (5): PCR amplification of the 213 and 688 bp fragments of *gel*E and *cyl*A virulence genes, respectively, from 3 Enterococci (E1-E3) showing positive amplicons migrates with the molecular DNA size ladder (L)., P (control positive), and N (control negative).

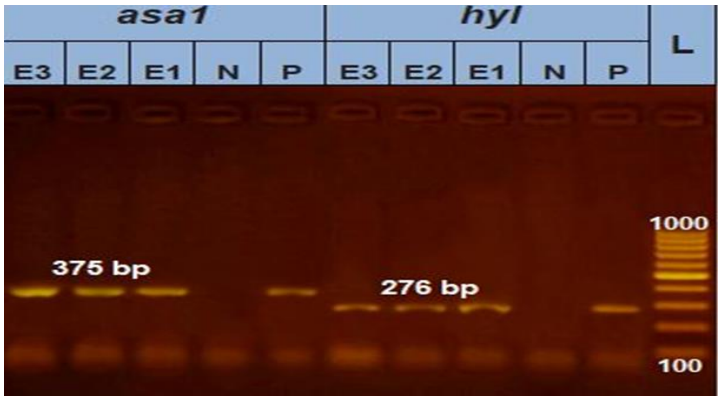

Fig. (6): PCR amplification of the 276 and 375 bp fragments of *hyl* and *asa*1 virulence genes, respectively, from 3 Enterococci (E1-E3) showing positive amplicons migrates with the molecular DNA size ladder (L)., P (control positive), and N (control negative).

DISCUSSION

Agreat 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 fastidus 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 prevelace 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 prevelace of 37.4% and Abd El-Hafeez *et al.* (2018) who recorded *S. dysgalactae* with prevalace rate 34.7%. Higher rates of isolation were achived 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 infecous microbial diseases also they play an important role in growth promotion. Its exessive use in animal production leads to spread of antibiotic resistance (Gosh and LaPara 2007). In-vitro antimicrobial suscebtibility 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 aganist 12 different antimicrobial agents of veterinary and human significance.

The *in-vitro* antibiogram sensetivity 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 cephalexin, cefotaxime sodium, cefipime, cefotriaxone, 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 gentamicin, trimethoprim- sulfamethoxazole and enrofloxacin, respectively. Additionally, multidrug resistance was detected in all tested isolates. Higher prevelance 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 gentamicin 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 eacuse of misuse antmicrobials which might leads to high resistance rates, it was difficult to found an effective drug aganist 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 (*tet*O), resistance to aminoglycosides (*aac(6')aph(2'')* and resistance to β-lactams (*pbp*1A) in 3 Streptococcus isolates. Moreover, it was applied on 3 Enterococcus isolates for detection of 3 resistance genes including (*tet*O, *aac(6')aph(2'')* and *blaZ)*. The data illsturated in (tables 5-6 and Figs. (1:4) revealed that 100% of the tested isolates harbored *tet*o, (*aac(6')aph(2'')* genes on the other hand 100% of streptococci isolates harbored (*Pbp1A*) gene also, (*bla*Z) gene were detected in all investigated *Enterococci* isolates.

Many genes were detected for tetracycline resistance including *tet*K, *tet*L, *tet*M and *tet*O 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 *tet*A, *tet*B, *tet*C, *tet*D, *tet*E, *tet*G, *tet*H, *tet*K, *tet*L and *tet*A(P) genes which have been identified. All of the following Tet determinants (*tet*A, *tet*B, *tet*C, *tet*D, *tet*E) protected the bacterial ribosomes because they ecoded 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 *tet*O which was detected in all tested isolates (100%), they were higher than those recorded by Cauwerts *et al.* (2007) who found *tet*O and *tet*M 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 *tet*O also, *tet*L and *tet*M 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 *Enterococccci* isolates to detect the following virulence associated genes including β-haemolysin cytolysin gene (*cyl*E), hyalurinidase encoded by (*hly)*and biofilm production (*brp*A) for *Streptococci* isolates. Also, the following genes *asa*1 (aggregation substance), which associated with adherence and conjugation; *cyl*A encodes

(cytolysin-haemolysin) which lyses red blood cells, *hly* (hyalurindase) while gelatinase, encoded by (*gel*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 *cyl*E and *brp*A genes meanwhile no isolates (0%) harbored *hly* gene in case of *Streptococci* isolates. On the other hand, all *Enterococci* tested isolates (100%) harbored *asa*1, *cyl*A and *hly* genes meanwhile no isolates (0%) harbored *gel*E gene. Regarding the obtained results of *cyl*A and *cyl*E which were detected in all tested isolates (100%), this result was higher than those recorded by Diarra *et al.* (2010) who found *cyl*A and *cyl*B genes in 28.5 % of tested isolates. Also, Ngbede *et al.* (2017) recorded *cyl*A gene in 28.3% of tested isolates. Meanwhile, Song *et al.* (2019) found *cyl*A in 16% isolates. The lower prevelances were recorded by (Champagne *et al.*, 2011) who detected *cyl*A and *cyl*B in 6% of isolates and Aslantaş (2019) who found *cyl*A in 0.7 %. On the contrary, Nowakiewicz *et al.* (2017) reported that none of tested isolates (0%) exhibeted genes responsible for haemolysin – cytolysin production. Regarding the obtained results of *gel*E which were not detected in any tested isolate,this result is lower than those detected by Ngbede *et al.* (2017) who found *gel*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 *gel*E in 100% of investegated isolates. Regarding the obtained results of *asa*1 gene which was found in all investigated isolates (100%). This result is higher than those recorded by Aslantaş (2019) who found *asa*1 gene in 6.1% of isolates. While Song *et al.* (2019) found *asa*1 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 exessive 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 cephalexin, cefotaxime, cefipime, cefotriaxone, tetracycline, kanamycin and apramycin. All the tested isolates were MDR. PCR results revealed that *tet*O, *aac(6')aph(2'')*, *bla*Z, *pbp*1A, *cyl*E, *brp*A, *cyl*A *asa*1 genes were detected in all the investigated isolates meanwhile, *hyl* gene was not detected in any Streptococcus isolates and *gel*E gene was not detected in Enterococcus isolates.

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التوصيف الجزيئي للمكورات السبحية والمعوية المعزولة من دجاج التسمين

أحمد حسين عابد ، أسماء سعد عبد الصادق صديق ، سلامه أبوحمرة سيد شانى

E-mail: asmaa.saad91@yahoo.com Assiut University web-site: www.aun.edu.eg

قد تؤدي عدوى المكورات السبحيه والمكورات المعوية في الدجاج إلى خسائر اقتصادية كبيرة. في الدراسة الحالية تمت دراسة انتشار المكورات السبحية والمكورات المعوية في مزارع دجاج التسمين المختلفة بمحافظة بني سويف. تم جمع ٢٧٢ عينة من الأعضاء الداخلية المصابة متضمنة القلب والرئة والكبد والكلى لدجاج التسمين المذبوحة والميتة حديثاً. تم الاهتمام بعزل الاعضاء التى تحمل الآفات التشريحية الاتية: البؤر النخرية في الكبد وتضخم الكلى والتهاب الكلية والأعضاء الداخلية التى تعانى من تسمم الدم. من خلال الفحص البكتريولوجي للعينات تم عزل ٤٩ عزلة إيجابية باجمالى معدل انتشار ١٨٪ . تم التعرف على العزلات باستخدام الاختبارات البيوكيميائية (catalase test) ووايضا تم زرع العزلات على أوساط مختلفة (MacConkey agar , bile aesculin agar, sheep blood agar) للتعرف على خصائصها . كما أنه تم تصنيف العزلات باستخدام Vitek2 compact system الى٢٦ Streptococcus spp. (53.1)٪ و ٢١ Enterococcus spp. (42.8)٪ بينما ٢ عزلة (٤,١٪) لم يتم التعرف عليها. تم التعرف على عزلات المكورات السبحية على أنها سلالة *S. gallinaceous* (٢٤,٥٪) ، 16.3)٪*S.dysgalactiae* و *S. durans* (12.2). من ناحية أخرى ، تم تحديد جميع عزلات Enterococcus على أنها *E. faecalis*. أظهر اختبار الحساسية للمضادات الحيوية في المختبر أن جميع العزلات كانت شديدة الحساسية للأموكسيسيلين (٧٧,٦٪) ، سلفاميثوكسازول-تريميثوبريم (٧٣,٥٪) وأموكسيسيلين-كلافولانيك أسيد (٦٥,٣٪). في الوقت نفسه كانت جميع العزلات مقاومة لمضادات سيفاليكسين ، سيفوتاكسيم صوديوم ، سيفيبيم ، سيفوترياكسون ، تتراسيكلين ، كاناميسين وأبراميسين بينما كانت ٨٧,٨ و ٦٣,٢٪ من العزلات مقاومة للجنتاميسين والإنروفلوكساسين على التوالي. علاوة على ذلك ، لوحظ وجود مقاومة متعددة للأدوية في ١٠٠٪ من العزلات. تم استخدام تفاعل البلمرة المتسلسل للكشف عن ٤ جينات مرتبطة بالمقاومة بما في ذلك (*tet*O، *aac (6 ') aph (2' ')* ،*bla*Z و *Pbp*1A) بالإضافة إلى ٦ جينات مرتبطة بالضراوة بما في ذلك (*Cyl*E، *brp*A، *hyl* ، *cyl*E ، *asa*1 و*gel*E). أظهرت النتائج أن جميع العزلات المختبرة (١٠٠٪) تحتوي على جينات *tet*O و *aac (6 aph (2'')* و *bla*Z و *Pbp*1A و *cyl*E و *brp*A و *cyl*A و *asa*1. في الوقت نفسه ، لم يتم الكشف عن جين *hyl* في أيا من عزلات المكورات السبحيه كما لم يتم الكشف عن جين *gel*E في عزلات المكورات المعوية.