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Identification and antimicrobial resistance of isolated bacteria from trachea of laying hens

Identificación y resistencia antimicrobiana de bacterias de tráquea de gallinas ponedoras

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ABSTRACT

Bacterial respiratory problems cause economic losses due to production decreases and the increasing cost of antibiotic treatment. In poultry production, they are the leading cause of death in laying hens. The presence of pathogens implies a distribution in production units, and their identification by biochemical tests allows the characterization at the species level and its adequate treatment. The present study aimed to identify the bacteria isolated from the trachea of laying hens and determine their antibiotic resistance profile. In an intensive production farm, trachea samples were taken from hens; the bacterial detection was carried out with isolation, colonial and microscopic identification, and biochemical tests; the antimicrobial susceptibility test was also determined. Thirty-two isolates corresponding to five types of bacterial colonies with the morphology of cocci and Gram-positive bacilli (*Staphylococcus aureus, Staphylococcus epidermidis and Corynebacterium spp*) and Gram-negative coccobacilli (*Pasteurella multocida and Gallibacterium anatis*) were obtained. *S. aureus*, *S. epidermidis*, and *Corynebacterium* spp showed resistance to glycopeptides (100 %), in addition to *P. multocida* and *G. anatis,* to ampicillin (beta-lactams) and quinolones (100 %). Isolated bacteria showed antibiotic resistance and multi-resistance, with implications for poultry farming and public health.

Keywords: resistance, susceptibility, antibiotic, bacteria, laying hens.

RESUMEN

En la producción avícola los problemas respiratorios bacterianos son causa de pérdidas económicas por disminución de la producción, aumento del costo por tratamiento con antibióticos y primera causa de muerte en gallinas ponedoras. La presencia de patógenos implica una distribución en las unidades de producción y su identificación por pruebas bioquímicas permite la caracterización a nivel especie y su tratamiento adecuado. El objetivo del presente estudio fue identificar las bacterias aisladas de tráquea de gallinas ponedoras y determinar su perfil de resistencia a antibióticos. En una granja de producción intensiva, se tomaron muestras de tráquea de gallinas; la detección bacteriana se realizó con el aislamiento, identificación colonial, microscópica y pruebas bioquímicas, además se determinó la prueba de susceptibilidad antimicrobiana. Se obtuvieron 32 aislados correspondientes a cinco tipos de colonias bacterianas con morfología de cocos y bacilos Gram positivos (*Staphylococcus aureus*, *Staphylococcus epidermidis* y *Corynebacterium* spp) y cocobacilos Gram negativos (*Pasteurella multocida* y *Gallibacterium anatis*). *S. aureus*, *S. epidermidis* y *Corynebacterium* spp, mostraron 100 % de resistencia a glucopéptidos, además *P. multocida* y *G. anatis*, a ampicilina (betalactámicos) y quinolonas (100 %). Las bacterias aisladas

mostraron resistencia y multirresistencia a antibióticos, con implicaciones para la avicultura y la salud pública.
Palabras clave: resistencia, susceptibilidad, antibiótico, bacterias, gallinas ponedoras.

INTRODUCTION

In production poultry, respiratory diseases are important because of the economic impact due to the decrease in productive parameters and increased cost for the use of drugs. This depends on the productive species, birds age, cost for treatments, pathogenic microorganism, mortality, biosecurity, stress due to climatic factors or management and immune response (Espinosa *et al*., 2011; Colas *et al*., 2011[a]; Colás *et al*., 2011[b]; Bagust, 2013; Ataei *et al*., 2017). In the commercial cage-based layer system, treatment consists of the use of antimicrobials; however, antibiotic resistance is largely due to their inappropriate use (Vanegas-Múnera & Jiménez-Quinceno, 2020), which represents one of the greatest risks we face as a global community (Davies & Davies, 2010; Nhung *et al*., 2017; ONU, 2019). Braykov *et al*. (2016) evidenced the presence of *E. coli* in water, soil and poultry house samples, as well as in small-scale free-range hens and chickens in northwestern Ecuador, finding antimicrobial resistance in all cases. It is truly alarming in intensive production systems the presence of respiratory problems diagnosed with single or multiple etiology caused by various pathogenic organisms (viruses, bacteria, fungi and immunosuppressive agents) (Glisson, 1998) and emerging pathogens with multi-resistance (Nworie *et al*., 2016). In the case of *S. aureus*, compromises the performance of an organism preventing it from showing its genetic potential in production, this pathological complex is known as *Avian Respiratory Syndrome* or *Avian Respiratory Complex (ARC)* or *Complicated Chronic Respiratory Disease (CRD)* (Colas *et al*., 2010; Ammar *et al*., 2016; De la Cruz, 2016; Brochu *et al*., 2019). The proper management of the flock or the production system acquires a primary role in the occurrence of respiratory processes, as they will always be higher in flocks where there is no proper management (Colás *et al.,* 2011[b]). Bacterial phenotypic identification is based on morphological characteristics, developmental, biochemical and metabolic properties, these serve in the differentiation of bacterial genera, since their realization and cost makes them more affordable, however, sometimes complementary methodologies are required for species identification (Bou *et al*., 2011; El-Adawy *et al*., 2018). Due to the high incidence of carriers of respiratory disease-causing bacteria in laying hen and broiler breeder flocks, the identification of the causative agents is substantial, as this means a widespread distribution of these microorganisms among poultry. It causes a decrease in egg production and represents the first cause of laying hen death (Colás *et al*., 2011[b]; Nworie *et al*., 2016; Elbestawy *et al*., 2018). A failure in ventilation influences the onset of the presence of respiratory tract diseases. In it various bacteria such as *Mycoplasma* spp, *Escherichia coli*, *Pasteurella multocida*, *Ornithobacterium rhinotracheale*, *Bordetella avium*, *Avibacterium paragallinarum*, *Staphylococcus* spp, *Streptococus* spp,

Corynebacterium spp, among others, cause ARC (Espinosa *et al*., 2011; De la Cruz, 2016; Singh *et al*., 2016; Nworie *et al*., 2016). In addition, age is included as a related factor (Glendinning *et al*., 2017). Regarding antibiotic resistance in *P. multocida*, erythromycin, chloramphenicol and clindamycin are found with susceptibility to penicillin derivatives and other beta-lactams (Espinosa *et al.,* 2011; Atere *et al.,* 2016). Methicillin-resistant *Staphylococcus aureus* isolates and reduced susceptibility to vancomycin have been reported (Jorgensen & Ferraro, 2000). Likewise, resistance profiles have been reported where they include up to 12 antibiotics in coagulase-negative Staphylococcus species, among which *S. epidermidis* stands out (Osman *et al*., 2015). Regarding *Corynebacterium* spp, resistance to macrolides, clindamycin, trimethoprim/sulfamethoxazole, quinolones and/or rifampicin was described (Yang *et al*., 2018). On the other hand, resistance to beta-lactams (penicillin and ampicillin), tetracycline, tylosin, tylosin, novobiocin, sulfonamide, lincomycin, enrofloxacin, florfenicol, cefotaxime, clindamycin, sulfathiazole, penicillin, norfloxacin and cephalothin was reported in *Gallibacterium anatis* (formerly *Mannheimia haemolytica*) (Osuna *et al.*, 2017; El-Adawy *et al*., 2018; Elbestawy *et al*., 2018; Nassik *et al*., 2019; Krishnegowda *et al*., 2020). The aim of the present study was to identify bacteria isolated from trachea of laying hens and determine their antibiotic resistance profile.

MATERIAL AND METHODS

The present work was carried out at the Bacteriology and Mycology Laboratory belonging to the Faculty of Veterinary Medicine and Zootechnics of the Autonomous University of Sinaloa (FMVZ-UAS), in Culiacán municipality, Sinaloa. From an intensive production system farm in the north of Sinaloa state (Ahome municipality), tracheal samples were taken from apparently healthy laying hens, houses 1 and 2 of 59 weeks of age and house 3 of 111 weeks of age, all kept in cages from rearing to production, with approximately 20 thousand birds in each. This work was a cross-sectional, observational and descriptive cohort study; the sample size was determined by convenience (5 swabs per house). The hens were treated with enrofloxacin (10 mg/kg body weight or 0.5 mL/L water) between 10 and 32 weeks prior to sampling, due to the presence of rales and drop in production. For sample collection, the technique of manual physical restraint of the bird was used, the swab was introduced and an intra-tracheal drag was performed. Samples were transported in Stuart medium in a container at 4 °C.

Bacterial isolation. The swab was taken from the medium and continuous streaking was performed on plates with blood and chocolate agar medium. Plates were then incubated for 24 h at 37 °C, in aerobiosis and anaerobiosis (Thermo Scientific anaerobic incubator with a 5 % CO_2 atmosphere). Once plates were seeded, the swabs were taken and were placed in tubes with peptone water in order to preserve the samples at 4 °C.

Bacterial identification by phenotypic methods. In the identification of the characteristics of colonies in the culture, the size, shape, color, texture and edges of the colonies were observed, as well as the presence of hemolysis. For microscopic identification, Gram staining was used and shapes and clusters were observed (Kaiser, 2017).

Identification by biochemical tests. The biochemical tests used for bacterial identification were iron and triple sugar agar (TSI), iron and lysine agar (LIA), Simmons citrate agar and indole sulfide motility (SIM), oxidase, catalase, coagulase and mannitol fermentation tests. In TSI, the ability to ferment carbohydrates such as glucose, sucrose and lactose was evaluated. In LIA, the presence of the enzyme decarboxylase (decarboxylation of lysine) was evaluated. In Simmons citrate, the utilization of sodium citrate as the only carbon source was tested. In SIM, motility, hydrogen sulfide (H_2S) and indole production were tested. In the oxidase test, the presence of enzymes of the cytochrome oxidase system was determined; in the catalase test, the presence of this enzyme, which decomposes hydrogen peroxide, was tested. The coagulase test evidenced the presence of this enzyme by coagulating plasma and transforming fibrinogen into fibrin; finally, some microorganisms ferment mannitol (Kaiser, 2017). The biochemical differentiation of each bacterium obtained was performed based on that described by Hoover *et al*., 1983; Funke *et al*., 1997; Weinstein *et al*., 1997; Arce *et al*., 2011; Castillo *et al*., 2014; Sanz-Rodríguez *et al*., 2014 and Zendejas-Manzo *et al.*, 2014.

Antibiotic susceptibility. Based on phenotypic and metabolic characteristics, one isolate from each group of bacteria was randomly selected and *in vitro* inhibition tests were performed in triplicate using the Kirby-Bauer disk diffusion method. The bacteria were grown in trypticasein soy broth, and readings were taken in a Bandwidth UV-5200 spectrophotometer at an optical density of 600 nm to estimate bacterial growth until a 0.5 McFarland standard was established. Subsequently, the bacteria were seeded on Müeller-Hinton agar plates by continuous cross streaking with the aid of a sterile swab until the inoculum was impregnated in the medium. Subsequently, multidiscs for Gram-negative (PT-35Nmultibac I.D.) and Gram-positive bacteria (PT-34Nmultibac I.D.) contained antibiotics such as ampicillin (10 µg), cephalothin (30 µg), cefotaxime (30 µg), ciprofloxacin (5 µg), clindamycin (30 µg), dicloxacillin (1 µg), erythromycin (15 µg), gentamicin (10 µg), and penicillin (10 U). Also, contained trimethoprim-sulfamethoxazole (25 µg), tetracycline (30 µg), vancomycin (30 µg), chloramphenicol (30 µg), carbenicillin (100 µg), netilmicin (30 µg), nitrofurantoin (300 µg), norfloxacin (100 µg) and amikacin (30 µg). The chemotherapeutics clindamycin, dicloxacillin, erythromycin, penicillin, tetracycline and vancomycin were tested only on Gram-positive bacteria; while chloramphenicol, carbenicillin, netilmicin, nitrofurantoin, norfloxacin and amikacin were tested only on Gram-negatives. Finally, the plates were incubated at 37 ± 1 °C for 18-24

h. After the incubation period, the inhibition halo was measured with a ruler (mm) to determine susceptibility or resistance according to the recommendations of CLSI (*Clinical and Laboratory Standards Institute*) and the manufacturer Diagnostic Research Diagnostic Reagents Laboratory (CLSI, 2015; CLSI, 2017; CLSI, 2018; ID, 2020; CLSI, 2021; EUCAST, 2022). Isolates with intermediate susceptibility were considered resistant, as these bacterial populations have subpopulations of resistant bacteria that will transmit this phenotype to susceptible bacteria (Flores-Hernández *et al*., 2020).

RESULTS AND DISCUSSION

From 15 samples obtained from hen trachea, belonging to three laying houses (PH1-3), 32 isolates were obtained. From these were grouped into five types of bacterial colonies. They were identified by phenotypic and biochemical methods as described in Table 1 as *Pasteurella multocida*, *Corynebacterium* spp, *Staphylococcus aureus*, *Gallibacterium* anatis and *Staphylococcus epidermidis* (Hoover *et al*. 1983; Funke *et al*., 1997; Weinstein *et al*., 1997; Arce *et al*., 2011; Castillo *et al*., 2014; Sanz-Rodríguez *et al*., 2014; Zendejas-Manzo *et al*., 2014). In PH1, all five-colony types were detected, where 14 isolates were obtained. In contrast, in PH2, 3/5 types of colonies were found, with eight isolates. Finally, in PH3, 4/5 were obtained, with 10 isolates (Table 1). *S. aureus*, *P. multocida*, *G. antis*, *S. epidermidis* and *Corynebacterium* spp were observed in 60, 53, 47, 40 and 13 % of the hens, respectively.

Findings of this study coincide with that reported by Osuna *et al.* (2017) where they isolated *G. anatis* and *P. multocida* from 600 laying hens from different farms in Sonora. However, the samples were obtained from diverse tissues such as turbinates, trachea, lungs, cleft palate, liver, spleen, kidneys, follicles and peritoneum of layers selected for the presence of respiratory clinical signs. In addition, they also reported the presence of *E. coli*, *Streptococcus* sp, *Pseudomonas* sp and *Salmonella* sp, while in this study these bacteria were not isolated. Likewise, it differs in microorganisms found and the selection of samples from the work carried out by Mendoza *et al.* (2014), since 96 isolates of *G. anatis* were identified in commercial poultry (38 broilers, 37 layers, 19 breeders and 2 fighting cocks) with respiratory signs and Gram staining showed the morphology of Gram-negative cocobacilli. In the present study, both Gram-positive and Gram-negative bacteria were observed and the samples were taken from birds without clinical signs, apparently healthy with previous treatment for health problems.

Table 1. Frequency of bacteria isolated per laying house based on phenotypic and biochemical tests

Bacteria	PH 1	PH 2	PH 3	Total isolates	Phenotypical tests	Biochemical tests
Pasteurella multocida	2	4	2	8	Gram-negative coccobacillus, non-hemolytic	Indole, oxidase, catalase, glucose, lactose and sucrose fermentation positive. Motility, gas, H_2S, lysine decarboxylation, gelatinase, citrate permease negative.
Corynebacterium spp	2	-	-	2	Gram-positive bacillus, non-hemolytic	Oxidase, catalase, glucose, lactose and sucrose fermentation positive. Indole, motility, gas, H_2S, lysine decarboxylation, gelatinase, citrate permease negative.
Staphylococcus aureus	5	1	3	9	Gram-positive, non-hemolytic cocci	Catalase, coagulase, mannitol fermentation, glucose, lactose and sucrose fermentation positive. Oxidase, indole, motility, gas, H_2S, lysine decarboxylation, gelatinase, citrate permease negative.
Gallibacterium anatis	4	-	3	7	Gram-negative coccobacillus, hemolytic	Catalase, glucose, lactose and sucrose fermentation positive. Oxidase, indole, motility, gas, H_2S, lysine decarboxylation, gelatinase, citrate permease negative.
Staphylococcus epidermidis	1	3	2	6	Gram-positive, non-hemolytic cocci	Catalase, glucose, lactose and sucrose fermentation positive. Oxidase, indole, coagulase, mannitol fermentation, motility, gas, H2S, lysine decarboxylation, gelatinase, citrate permease negative.
Total	14	8	10	32		

PH= Posture Hut. H_2S= Hydrogen sulfide

Regarding the frequency of bacterial isolates (32), *S. aureus* was the most isolated bacterial agent (28 %), followed by *P. multocida*, *G. anatis* and *S. epidermidis*, with 25, 22 and 19 % of isolates, respectively, while *Corynebacterium* spp was the least frequent (6 %). These results differ from those reported by Espinosa *et al.* (2011), where the most isolated microorganism was *P. multocida* (20 %). However, the frequency of this bacterium is similar in terms of percentage of isolation in a total of 80 samples of nasal and tracheal exudates from laying hens. In that study were also isolated *E. coli* (18 %) and *O. rhinotracheale* (5 %), which were not isolated in this study. Atere *et al*. (2016), in a study conducted in chickens from 23 farms isolated *P. multocida* with a frequency of 12 % (12/97), which differs with this study with a frequency of 53 % (8/15). In addition, of the 12 isolates three were detected in liver. Likewise, the results of the present study differ from those presented by Vargas *et al*. (2010), where they showed *S. aureus* as the fifth most isolated bacterium from 45 wild birds. However, Castillo *et al*. (2014) identified *P. multocida* and *G. anatis* as microorganisms present in the ARC, which agrees with the results obtained. Nassik *et al*. (2019) determined that *G. anatis* is involved in decreased egg production with a frequency of 46 % in samples of ovary, trachea and cloaca from 52

hens, which agrees with these results (47 %); however, the isolates correspond only to trachea. The study by Nworie *et al*. (2016) presents discrepancies in the isolates and the type of samples where they report a frequency of 14 % for *S. aureus* in samples of nares and cloaca. In the present study a frequency of 60 % for this bacterium was found in tracheal exudates, in addition, another species of staphylococcus (*S*. *epidermidis*) was found in 40 % of the birds. Recently, Benrabia *et al*. (2020) found a prevalence of 48.8% for *S. aureus* in laying hens from 840 farms in Algeria, which is similar to these results; in addition, they report that 34 % of these isolates corresponded to methicillin-resistant *S. aureus* (MRSA), which represents a considerable risk for public health.

In two of the Gram-positive isolates obtained (*S. aureus* and *S. epidermidis*), resistance to beta-lactams (dicloxacillin, *Staphylococcus* spp. R:≤12 mm, S:≥13 mm), glycopeptides (vancomycin, *Staphylococcus* spp. R:≤16 mm; S: ≥ 17 mm), macrolides (erythromycin, *Staphylococcus aureus* R: ≤ 21 mm; S: ≥ 22 mm; *S. epidermidis* R: ≤ 20 mm; S: ≥ 21 mm) and tetracyclines (tetracycline, *Staphylococcus aureus* R: ≤ 23 mm; S: ≥ 24 mm; *Staphylococcus* spp. R: ≤ 18 mm, S: ≥ 19 mm), based on the criteria established by CLSI, 2017; ID, 2020; CLSI, 2021 and EUCAST, 2022 (Table 2). Vargas *et al.* (2010), in their study detected resistance of *S. aureus* to macrolides, which coincides with in this study in *S. aureus* and *S. epidermidis*, who showed resistance to erythromycin; however, they differ in terms of resistance to vancomycin and in the type of samples, cloacae and glottis from 30 birds and nasal and rectal swabs from 29 mammals. Likewise, in the study conducted by Nworie *et al*. (2016) in nasal and cloacal samples from poultry they found resistance of *S. aureus* to erythromycin, gentamicin, tetracycline and trimethoprim/sulfamethoxazole, which coincides with the findings obtained. Similarly, Benrabia *et al*. (2020) agree in reporting resistance of *S. aureus* to tetracycline and erythromycin; however, most of the isolates of this bacterium, in nasal swab samples from breeding hens, layers, broilers and turkeys, were resistant to ciprofloxacin and sensitive to vancomycin and gentamicin, which contrasts with the present results. Similarly, Osman *et al*. (2015) agree on the resistance to trimethoprim/sulfamethoxazole but differ in resistance to penicillin and clindamycin in staphylococcal isolates where *S. hyicus*, *S. lugdunensis*, *S. aureus* and *S. epidermidis* stand out. This may be due to the origin of their samples (chicken and beef sold in supermarkets in Cairo), thus highlighting the importance of the origin of the infection for humans. In addition, in *Corynebacterium* spp. resistance was found to the aminoglycoside groups (gentamicin, R: ≤ 14 mm, S: ≥ 15 mm), beta-lactams (ampicillin, R: ≤ 21 mm; S: ≥ 29 mm; cephalothin, R: ≤ 17 mm, S: ≥ 18 mm; cefotaxime, R: ≤ 22 mm, S: ≥ 23 mm), glycopeptides (vancomycin, R: ≤ 16 mm; S: ≥ 17 mm), quinolones (ciprofloxacin, R: ≤ 20 mm, S: ≥ 21 mm) and sulfonamides (trimethoprim/sulfamethoxazole, R: ≤ 15 mm, S: ≥ 16 mm), based on the criteria established by ID, 2020 and EUCAST, 2022 (Table 2).

Table 2. Susceptibility to antibiotics in Gram-positive bacteria

Antibiotic	*S. aureus*	*S. epidermidis*	*Corynebacterium* spp	Resistence (%)	Sensitivity (%)	Cited Literature
Aminoglycosides						
Gentamicin	R	S	R	66.66	33.33	CLSI, 2017; CLSI, 2021; EUCAST, 2022
Beta-lactams						
Ampicillin	S	S	R	33.33	66.66	CLSI, 2017; ID, 2020
Cephalothin	R	S	R	66.66	33.33	CLSI, 2017; ID, 2020
Cefotaxime	S	S	R	33.33	66.66	ID, 2020
Dicloxacillin	R	R	S	66.66	33.33	ID, 2020
Penicillin	S	S	S	0	100	CLSI, 2017; CLSI, 2021; EUCAST, 2022
Glycopeptides						
Vancomycin	R	R	R	100	0	CLSI, 2017; EUCAST, 2022
Lincosamides						
Clindamycin	S	R	S	33.33	66.66	CLSI, 2017; CLSI, 2021; EUCAST, 2022
Macrolides						
Erythromycin	R	R	S	66.66	33.33	CLSI, 2017; CLSI, 2021; EUCAST, 2022
Quinolones						
Ciprofloxacin	S	S	R	33.33	66.66	CLSI, 2021; EUCAST, 2022
Sulfonamides						
Trimethoprim/ Sulfamethoxazole	R	S	R	66.66	33.33	CLSI, 2017; CLSI, 2021; EUCAST, 2022
Tetracyclines						
Tetracycline	R	R	S	66.66	33.33	CLSI, 2017; CLSI, 2021; EUCAST, 2022

R= resistant and S= sensitive. ID= Diagnostic Investigation. EUCAST= European Committee on *Antimicrobial Susceptibility Testing*. CLSI= *Clinical and Laboratory Standards Institute*

In this study, based on the criteria established by ID, 2020 and EUCAST, 2022, isolates obtained from *P. multocida* showed resistance to aminoglycosides (amikacin, R: ≤ 16 mm, S: ≥ 17 mm; netilmicin, R: ≤ 14 mm, S: ≥ 15 mm), beta-lactams (ampicillin, R: ≤ 16 mm; S: ≥ 17 mm; Carbenicillin, R: ≤ 22 mm, S: ≥ 23 mm), chloramphenicols (chloramphenicol, R: ≤ 17 mm, S: ≥ 18 mm) and quinolones (ciprofloxacin, R: ≤ 26 mm; S: ≥ 27 mm; norfloxacin R: ≤ 16 mm, S: ≥ 17 mm) (Table 3). It differs from that reported by Zahoor & Siddique (2006) in poultry liver samples from several poultry farms. Isolates of *P. multocida* showed sensitivity to chloramphenicol and ciprofloxacin and resistance to trimethoprim/sulfadiazine.

Furthermore, these results differ in terms of resistance reported by Atere *et al*. (2016) in chicken tracheal and liver swabs, where they found resistance to ciprofloxacin, ampicillin, nitrofurantoin and gentamicin in *P. multocida*, while in the present study resistance to ciprofloxacin and ampicillin was observed, in contrast gentamicin and nitrofurantoin had good *in vitro* effectiveness.

In this study, *G. anatis* showed resistance to beta-lactams (ampicillin, FR: ≤ 26 mm; S: ≥ 27 mm), quinolones (ciprofloxacin, R: ≤ 20 mm, S: ≥ 21 mm; norfloxacin, R: ≤ 16 mm, S: ≥ 17 mm) and sulfonamides (trimethoprim/sulfamethoxazole, R: ≤ 23 mm; S: ≥ 24 mm) based on the criteria established by CLSI, 2015 and ID, 2020 (Table 3). It coincides with the resistance reported in different types of samples (turbinates, trachea, lungs, cleft palate, liver, spleen, kidneys, follicles, peritoneum, ovary, proventriculus, larynx, heart, air sac, brain, eye and oviduct) from laying hens, broilers and breeders. In addition, it showed sensitivity to the phenicol and gentamicin family (Osuna *et al.,* 2017; El-Adawy *et al*., 2018; Elbestawy *et al*., 2018; Nassik *et al*., 2019).

Likewise, Mendoza *et al.* (2014) reported resistance of *G. anatis* to ciprofloxacin in bacteria isolated from clinical samples of broilers, laying hens, breeders and fighting cocks, which is in agreement with the results obtained.

CONCLUSION

The pathogenic bacteria isolated and biochemically identified from the trachea of laying hens in northern Sinaloa are represented by 4 bacterial genera, where the Gram-positive bacteria were *Staphylococcus aureus*, *Staphylococcus epidermidis* and *Corynebacterium* spp, and the Gram-negative bacteria were *Pasteurella multocida* and *Gallibacterium* anatis. The Gram-positive microbiota showed high antimicrobial resistance, in the *Staphylococcus* genus, mainly to beta-lactams, glycopeptides, macrolides and tetracyclines and the *Corynebacterium* genus to aminoglycosides, beta-lactams, glycopeptides, quinolones and sulfonamides, likewise, this group of Gram-positive microbiota converged in 100 % for glycopeptides; likewise the Gram-negative *P. multocida* and *G. anatis* showed 100 % resistance to ampicillin (beta-lactams) and to the quinolone group. The presence of these pathogenic bacteria in different poultry houses in intensive production systems may be due to exposure and dispersion, implying in the resistance and multi-resistance found in this type of diseases in poultry production and sanitary production, for which it is necessary to explore alternatives to the use of antibiotics.

Table 3. Antibiotic susceptibility in Gram-negative cocobacilli

Antibiotic	*P. multocida*	*G. anatis*	Resistence (%)	Sensitivity (%)	Literature Cited
Aminoglycosides					
Amikacin	R	S	50	50	ID, 2020
Gentamicin	S	S	0	100	ID, 2020
Netilmicin	R	S	50	50	ID, 2020
Beta-lactams					
Ampicillin	R	R	100	0	CLSI, 2015; EUCAST, 2022
Carbenicillin	R	S	50	50	ID, 2020
Cephalothin	S	S	0	100	ID, 2020
Cefotaxime	S	S	0	100	ID, 2020; EUCAST, 2022
Chloramphenicols					
Cloramfenicol	R	S	50	50	ID, 2020
Nitrofurans					
Nitrofurantoin	S	S	0	100	ID, 2020
Quinolonas					
Ciprofloxacin	R	R	100	0	ID, 2020; EUCAST, 2022
Norfloxacin	R	R	100	0	ID, 2020
Sulfonamides					
Trimethoprim Sulfametoxazol	S	R	50	50	CLSI, 2015; ID, 2020; EUCAST, 2022

R= resistant and S= sensitive. ID= Diagnostic Investigation. EUCAST= *European Committee on Antimicrobial Susceptibility Testing*. CLSI= *Clinical and Laboratory Standards Institute*

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Errata Erratum

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