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Research article

Pharmaceutical allergy pattern for antibacterial of some specified species of bacterial which diagnosed by vitek-2 technology isolated from the infection of the yolk sac in the meat breeders in Al-Diwaniyah

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Abstract

For the first time in Iraq, the study was used electronic vitek-2 apparatus for detection some bacteria that were isolated from omphalitis cases of broiler chicks of meat breeders in AL-Diwaniyah city. Thirty swaps were taken from thirty of meat broiler chicks infected by yolk sac infection or omphalitis then the samples tested biochemical tests by use electronic vitek-2 apparatus after culture all the isolates on media. E.coli, Staphylococcus aureus, and Enterococcus faceuim were recorded by our study from the samples with percentages (60%), (23.3%) and (16.6%) respectively; then the antibiotics sensitivity test has done for all the isolates where the antibiotics that have more effect were trimethoprim(TR)(5MG), Amikacin(AK)(30mg) and Tetracycline(TE)(30mg) respectively. E.coli was prevalent from other bacteria, the subject was required more studies about all aspects like pathological, molecular, and immunological.

Keywords: Vitek, Yolk sac, Sensitivity test, Broiler chicks

Introduction

Omphalitis or known yolk sac infection or navel ill, sometimes called mushy chick disease considered a major cause of increased first-week chick mortality. The disease is a hatchery-born disease, the chicks infected by this disease and its common disease, causing high economic losses in the brooding period, occur due to penetration the wall of the eggshell by bacteria (1). Omphalitis is a bacterial disease resulting from its failure to close completely following get in bacteria resides in around the environment. The infection happens due to poor hygiene in farms and mismanagement of the hatchery. Yolk sac infection (YSI) is the main cause of chick mortality during the first week of the post-hatching period and cause high economic losses (2-4). Omphalitis is one of the causes of the mortality in hatching period caused by many of bacteria one or mixed infection like *E.coli*, *staphylococcus spp*, *salmonella spp*, *enterococcus spp. Pseudomonas aruegenosa* (5-7). Many from researchers detected *E.coli* as most common in yolk sac disease in chicks in many of countries (8). Omphalitis is infectious disease transmitted between the chicks usually accompanied with cases of unhealed navels (9, 10). Navel inflammation is common causes increase of mortality in chicks in the first week following the hatching, many of bacteria like *Proteus* spp., *Pseudomonas* spp., *Staphylococcus* spp., *Enterobacter* spp, *Klebsiella* spp *Clostridium* spp., *Bacillus cereus*, *Streptococcus* spp and *Enterococcus spp* isolated from omphalitis and isolated, *Staphylococcus aureus* is a microorganism causes omphalitis, infections of yolk sac and liver in the first period of chicks old; *Staphylococcus aureus* seemed weak, huddled together and had watery diarrhea. The umbilicus was open (11) (12).

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This study aimed to determine the main bacterial causes of omphalitis in broiler chicks' .With determination resistance and sensitivity of these bacteria then confirmed the diagnosis by vitek-2.

Materials and Methods

Ethical approval

The Animal Ethical Committee of Veterinary Medicine College, University of Al-Qadisiyah, Iraq, has approved the present study under permission No: 424

Sampling and bacterial culture:

Thirty Broiler chicks (1-5) day old, with symptoms of yolk sac infections were collected from AL-Diwaniyah hatchery and veterinary center. All chicks were sent to the microbiology laboratory, and swabs were taken from unabsorbed yolk sac content was collected under aseptic technique. Each sample was initially inoculated on Nutrient broth and incubated at 37C° for 18-24 hour, then a by loop use broth was streaked on selective media including MacConkey Agar, Eosin- methylene blue agar, Chrome agar and mannitol salt agar. Primary cultures were evaluated by visual examination of the morphology of the bacterial colonies and were subculture again. Appeared of the bacterial colonies, *E.coli*, *Staphylococcus aureus*, Enterococcus *spp*. detection of the isolated colonies was done by vitek-2 by using biochemical tests as described by (13) and (14).

Biochemical Identification and VITEK 2:

Important Biochemical tests were conducted according to (15), and standard biochemical tests for Identification of *E.coli*, *Staphylococcus aureus*, and *Enterococcus faceuim* isolates using biochemical tests (lactose test, mannitol test, motility test, catalase test and H_2S production test) and the results were interpreted according to the manufacturer's instructions as table(1):

Table (1): biochemical results of *E.coli; Staphylococcus aureus, Enterococcus.*

isolates Biochemical tests	*E. coli*	*staphylococcus aureus*	*Enterococcus faceuim*
Lactose	+	+	+
mannitol	+	+	±
Motility	_	_	_
Catalase	+	+	_
H_2S production	–	–	–

The VITEK 2 is electronic laboratory automatic and electric apparatus used for microbial identification have high accuracy and provide the result during relatives sort period and take more than (10) card in same time, used card is electronic and have readable bar code after testing the isolates. A reagent card of VITEK 2 Compact has 64 wells for each card, there are two type of card was used in this study (GP) for gram positive and (GN) for gram negative, in this study thirty (GN) card used for examine and confirm the isolates showed (18) out of (30) *E.coli*, while the rest isolates (GP) used for (7) for *Staphylococcus spp* and (5) for *Enterococcus spp*. The apparatus used the card to the analysis of the data need to 6 hours to finish the results, the card contains a substance for measuring several metabolic activities (biochemical tests) like oxidase, catalase, and fermentation of some sugar etc. Each card contains on micro slides for allows for a known level of oxygen changed. Each card has bar codes that contain information on product type, lot number, expiration date, (16).

Antibacterial susceptibility testing:

Antibiotic susceptibility test was performed by the standard disk diffusion method in Mueller-Hinton agar (Hi-Media) according to (17). Moreover, the results were interpreted in accordance with the criteria of the CLSI. The isolates were screened for resistance to

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the following antimicrobial agents as a table (2). The sensitivity of Antimicrobial was compared by using McFarland solution (50%), also use Mueller-Hinton media (made in the UK) with ten common antibiotics. (18) Isolates of *E.coli*, (7) *Staphylococcus aureus* isolates, and (5) *Enterococcus faceuim* isolates selected for making this test. This test was done depend on Kirby-Bauer method (18). The results were shown as resistant (R), sensitive (S) according to (18).

Table (2): antibiotics disc used and their concentration in study

N	Antibiotic disc	Concentration
1	Amikacin	30mg
2	Ciprofloxacin	5mg
3	Erythromycin	15mg
4	Gentamycin	10mg
5	Chloramphenicol	30mg
6	Sulphafurazole	300mg
7	Tetracycline	30mg
8	Streptomycin	10mg
9	Nitrofurantoin	30mg
10	Trimethoprim	5mg

All antibacterial discs were purchased from Hi- Media Laboratories.

Results

Thirty samples were taken from infected chick meat broiler at (1-5) day old and examine chick and yolk sac infection and take swap and sent to the lab for culture on several media see figure (1) and (2). As a next step, the swap culture on several media like MacConkey agar and chrome agar see figure (3) (4) and (5).

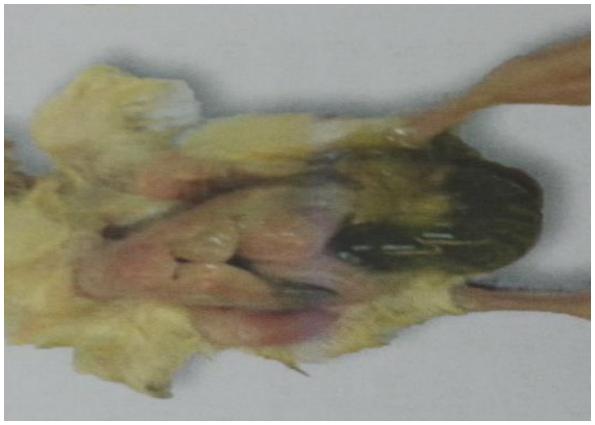

Figure (1): Distended, unabsorbed abnormal color yolk sac of one-day old broiler chick.

Figure (2): Yolk sac of five-day old broiler chick.

Figure (3): Colonial morphology of *Enterococcus spp*. Isolates on chrome agar.

Figure (4): Colonial morphology of *Staphylococcus aureus* isolation on chrome agar.

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Figure (5): Colonial morphology of *E. coli Isolates* on MacConkey agar.

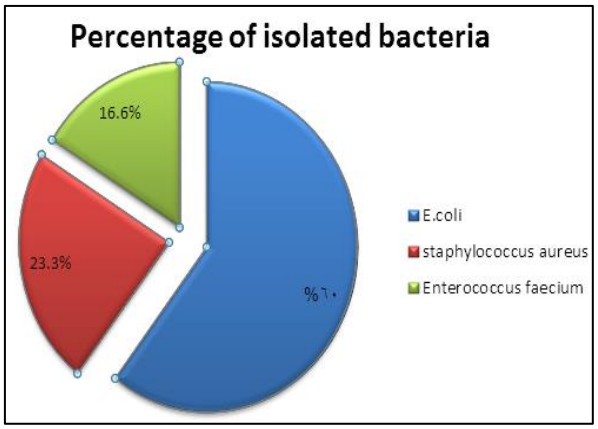

Table (3): species and number of isolates

Species (*spp.*)	n = number of isolates(30)	(%) = percentage
E. coli	18	60 %
Staphylococcus aureus	7	23.3%
Enterococcus faecium	5	16.6 %

Our results found the percentage were (60%),(23.3%) and (16.6%) in *E.coli*, *Staphylococcus aureus* and *Enterococcus faecium* respectively *E.coli* was prevalent as the chart below.

As a final step was done sensitivity test for all the isolates and for all the types , the study found trimethoprim (TR) (5MG) have more effect on all E. *coli* isolates as table (3), While Amikacin (AK) (30mg) was more effectiveness on *Staphylococcus aureus* see table (4) and finally Tetracycline (TE) (30mg) was more effective on *Enterococcus faecium* isolate see table (5).

Table (4): Antibacterial susceptibility pattern of *E. coli* strains isolated from chicks.

***E. coli* (n= 18) isolates**						
No		**Resistant N (%)**			**Susceptible N (%)**	
1	Amikacin(AK)(30mg)	5	(27.7%)		13	(72.2%)
2	Ciprofloxacin (CIP)(5mg)	10	(55.5%)		8	(44.4%)
3	Erythromycin (E)(15mg)	5	(27.7%)		13	(72.2%)
4	Gentamycin (GEN) (10mg)	10	(55.5%)		8	(44.4%)
5	chloramphenicol (C) (30 mg)	3	(16.6%)		15	(83.3%)
6	Sulphafurazole (300mg) (SF)	13	(72.2%)		5	(27.7%)
7	Tetracycline (TE)(30mg)	15	(72.2%)		3	(16.6%)
8	Streptomycin (S) (10mg)	9	(50%)		9	(50%)
9	Nitrofurantoin (NIT) (30mg)	4	(22.2%)		14	(77.7%)
10	Trimethoprim (TR) (5MG)	0	(0%)		18	(100%)

n = number, (%) = percentage.

Table (5): Antibacterial susceptibility pattern of *staphylococcus aureus* strains isolated from chicks.

***staphylococcus aureus*(n=7) isolates**						
No		**Resistant n (%)**			**Susceptible n (%)**	
1	Amikacin (AK)(30mg)	0	(0)		7	(100%)
2	Ciprofloxacin (CIP)(5mg)	4	(57.1%)		3	(42.8%)
3	Erythromycin (E)(15mg)	2	(28.5%)		5	(71.4%)
4	Gentamycin (GEN) (10mg)	4	(57.1%)		3	(42.8%)
5	chloramphenicol (C) (30 mg)	6	(85.7%)		1	(14.2%)
6	Sulphafurazole (300mg) (SF)	2	(28.5%)		5	(71.4%)
7	Tetracycline (TE)(30mg)	3	(42.8%)		4	(57.1%)
8	Streptomycin (S) (10mg)	3	(42.8%)		4	(57.1%)
9	Nitrofurantoin (NIT) (30mg)	1	(14.2%)		6	(85.7%)
10	Trimethoprim (TR) (5MG)	2	(28.5%)		5	(71.4%)

n = number, (%) = percentage.

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Table (6): Antibacterial susceptibility pattern of *Enterococcus faecium* strains isolated from chicks.

Enterococcus faecium (n= 5) isolates						
No		Resistant n (%)			Susceptible n (%)	
1	Amikacin (AK)(30mg)	1	(20%)		4	(80%)
2	Ciprofloxacin (CIP)(5mg)	3	(60%)		2	(40%)
3	Erythromycin (E)(15mg)	5	(100%)		0	(0%)
4	Gentamycin (GEN) (10mg)	1	(20%)		4	(80%)
5	chloramphenicol (C) (30 mg)	4	(80%)		1	(20%)
6	Sulphafurazole (300mg) (SF)	3	(60%)		2	(40%)
7	Tetracycline (TE)(30mg)	0	(0%)		5	(100%)
8	Streptomycin (S) (10mg)	2	(40%)		3	(60%)
9	Nitrofurantoin (NIT) (30mg)	2	(40%)		3	(60%)
10	Trimethoprim (TR) (5MG)	2	(40%)		3	(60%)

n = number, (%) = percentage.

Discussion

Yolk sac infection is great matter challenge poultry manufactured in the entire world, its occurrence causing a great economic loss in the first week of hatching, many of studied try to resolve this problem by using a different way of control and treatment (19) and (9). Our results show great similarity with (20) and (21) where they record distended abdominal cavity (ascites) with highly inflamed subcutaneous tissue, engorged blood vessels, and vent region is occluded with the spoiled material. Contents of yolk sac were changed from viscid to watery and yellow-green color to yellow-brown due to the multiplication of bacteria; also same with gross lesions observed in chicks died of yolk sac infection included unabsorbed and retained yolk sac edematous yolk, which was, also reported (22-25). Our result record *E.coli* was 60 % that isolated from infected yolk sac in the broiler of meat chicks that has close results with (26) in Egypt where her record percentage of *E.coli* was (57.5%), and was (55%) by (33).While (27) record rate lower than our results he confirms the percentage of *E.coli* was 10.3% in Egypt, Furthermore (28) record lower that our results in Ethiopia where percentage of *Escherichia coli* was (51.2%). Also, there are several studies found an occurrence of *E.coli* by rate more than our results like (29) his record (70%) have been isolated from yolk sac infection of chicks at 5 days old. Our result record *staphylococcus spp* was (23.3%) that isolated from yolk sac in the broiler of meat chicks (30) (31). (28) Have the same score with our rate where the percentage of *Staphylococcus aureus* (23.5%) in Ethiopia, but (20) found the percentage of *Staphylococcus spp*. is (6.3%) were considered lower than results, also (32) recorded percentage of *Staphylococcus aureus, E.coli, Enterobacter faecium* (20%), (19%), (4%) respectively and all that disagreement with our results. (33) Found *Staphylococcus aureus* was 28.5% little higher than our scan. Our result record Enterococcus *faecium* was (16.6%) that isolated from yolk sac in broiler of meat chicks, (1) found Enterobacter *faecium* was (0.4%) that represent less than our percentage. The different between the studies in percentage and the epidemiology causing by several factors, its depend on The majority of disinfectants used in the hatchery like ammonium compounds, phenolic compounds, sanitation in the hatchery, a system of monitoring of microbial levels is important to decrease the occurrence (34). Also, the presence of bacteria in the hatchery depending on deficiencies in hygiene conditions which can result in an increase in first-week chick mortality and growth rate was depressed. hygienic levels very important to decrease the bacteria number and cause an increase in contamination rates and the presence of a large number of the pathogenic bacteria including, *E. coli,*

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Staphylococcus aureus, Pseudomonas aeruginosa and if we don't use antiseptic. These factors work together with all the conditions (temperature, moisture, and organic matters) to allowing the development of several types of bacteria that can effect on performances of the chicks (35). Our study make sensitivity test for all the isolates was taken from omphalitis cases, *E.coli*, *Staphylococcus aureus*, and *Enterococcus faceuim* were found sensitive to antibiotics trimethoprim(TR)(5MG), Amikacin (AK) (30mg) and Tetracycline (TE) (30mg) respectively. And resistance to Tetracycline (TE)(30mg) in (72.2%); chloramphenicol (C)(30 mg) in (85.7%) and Erythromycin (E)(15mg) in (100%) respectively. (28) Make susceptibility to 8 antimicrobial agents to bacterial isolates were showed higher susceptibility to Chloramphenicol, Streptomycin, and Gentamycin, which is recommended for the treatment of yolk sac infection (YSI). *E. coli*, *Staphylococci* submitted to sensitivity test to a group of antibiotics. *E. coli* Isolates were affected by chloramphenicol and resistant to amoxicillin, nalidixic acid, ampicillin, gentamicin, ciprofloxacin, tetracycline, sulphamethoxazole, and erythromycin. *Staphylococci* were sensitive to ampicillin, amoxicillin, ciprofloxacin, chloramphenicol, sulphamethoxazole, gentamicin, erythromycin, and kanamycin and resistant to nalidixic acid and tetracycline (36). *Staphylococcus aureus* resistance to Amoxicillin and Susceptibility to Ciprofloxacin and Gentamicin while *E.coli* resistance to amoxicillin and sensitive to ciprofloxacin and gentamicin (1).Strains examined were largely resistant to the antimicrobials tested with the exception of amikacin and sulfisoxazole. Showed resistance pattern to six antimicrobial agents: nalidixic acid, tetracycline, ampicillin, streptomycin, ciprofloxacin, and trimethoprim-sulfamethoxazole (37). *E.coli* sensitive to gentamycin and chloramphenicol while *staphylococcus aureus* also for gentamycin and chloramphenicol in Ethiopia (28). Antibacterial susceptibility testing showed that *E.coli* isolates were sensitive to Ciprofloxacin (5µg)**,** Gentamicin (10µg), Doxycycline (30µg) and Colistin (10µg), this may be useful in the treatment of Yolk sac infection (26). *E.coli* isolates have resistance to eight of antimicrobial: gentamicin, amoxicillin, erythromycin ampicillin, sulphamethoxazole, nalidixic acid, tetracycline, ciprofloxacin, and sensitive to chloramphenicol, *Staphylococcus aureus* isolates were resistant to tetracycline and nalidixic acid while it was sensitive for Ciprofloxacin (38). There are several studies were showed results of sensitivity test for some similar, but some other different depend on nature the isolates; genetic determinations, acquired resistance gene and finally depend on the type of used antibiotics and its dose (19). It could be concluded that the main bacteria causing omphalitis in chicks according to our study include *E.coli, Staphylococcus aureus*, and *Enterobacter faceuim*.

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