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Isolation of *Staphylococcus aureus* from chicken and study sensitivity to antibiotic

Maysoon. S. Abbas

Zoonotic Diseases Unit, College of Veterinary Medicine, University of Baghdad, Iraq

Corresponding author email: maysoon.s.abbas@gmail.com

Abstract---Aim: This study was designed to determine the prevalence of *Staphylococcus aureus* in fresh and frozen chicken and study sensitivity of bacterial isolates to antibiotics. Methods: A total of (100) liver samples were collected from different market in Baghdad city during period from (10/2020-2/ 2021). The samples were subjected for isolation of following standard bacteriological examinations such as cultural and morphological characteristics, biochemical properties, antibiotics sensitivity (Vancomycin 30 mg, Tetracycline 10mg, Penicilin 10U, Gentamicin 10mg, Amoxicillin 25 mg, Ampicillin 25 mg, Trimethoprim/Sulphamethaxazole 25mg, Streptomycin 25 mg). Results: the results showed that 30 / 100 (30%) of *Staphylococcus aureus* isolates, 10/50 (20%) for fresh and 20/50 (40%) for frozen. The growth of colony with different characteristics biochemical test indicated positive results, the results showed that *Staphylococcus aureus* isolates were sensitive (90%) to Gentamicin and Amoxicillin (80%) to Streptomycin and Vancomycin, (70%) to Trimethoprim/Sulphamethaxazole, (10%) to Ampicillin, and Resistant (90%) to Penicilin (80%) to Ampicillin (60%) to Tetracycline (20%) to Trimethoprim/Sulphamethaxazole (10%) Vancomycin, Gentamicin, Streptomycin. Conclusion: this study concluded that there is a need for monitoring of poultry and its products in markets and abattoirs, good cooking to minimize the possibility of human infections, do not use antimicrobial as growth promoters in poultry production to prevent the resistance to antibiotic in human.

Keywords---*Staphylococcus aureus*, chicken, antibiotics, fresh and frozen liver.

Introduction

Staphylococcus are ubiquitous in the environment, including the water, soil and air, and are isolated from various animal species, including poultry. the genus

Staphylococcus currently numbers more than 70 species. ¹ From a clinical perspective, particularly harmful species include coagulase positive staphylococci, such as *S. aureus*, *S. intermedius*, *S. schleiferi* subsp. *coagulans*, *S. pseudointermedius*, *S. lutrae*, *S. delphini* and some strains of the species *S. hyicus* ². The most important of these is *S. aureus*, which apart from organ infections can produce as many as 25 different toxins causing severe food poisoning³. The remaining Staphylococcus species, which are coagulase-negative (CNS), usually induce infections in individuals with reduced immunity. Data indicate that the species most frequently isolated from samples of animal origin are coagulase-positive *S. intermedius*, and coagulase-negative species: *S. sciuri* and *S. hyicus* ³. Infections caused by Staphylococcus spp. are taking on increasing importance in the pathology of poultry as well. The most important associations of CNS with poultry are: *S. lentus*, *S. xylosus*, *S. sciuri*, *S. cohnii*, *S. chromogenes* and *S. gallinarum* ⁴. *S. aureus* "grape-cluster berry", Latin *aureus*, "golden") is a facultative anaerobic, Gram-positive coccid (round) bacterium also known as "golden staph" and "oro staphira". *S. aureus* is as *S. aureus*, *Staph aureus* or *Staph a.* *S. aureus* appears as staphylococci (grape-like clusters) when viewed through a microscope, and has large, round, golden-yellow colonies, often with hemolysis, when grown on blood agar plates. *S. aureus* reproduces asexually by binary fission. Complete separation of the daughter cells is mediated by *S. aureus* autolysin, and in its absence or targeted inhibition, the daughter cells remain attached to one another and appear as clusters. ⁵ *S. aureus* is catalase-positive (meaning it can produce the enzyme catalase). Catalase converts hydrogen peroxide (H₂O₂) to water and oxygen. Catalase-activity tests are sometimes used to distinguish staphylococci from enterococci and streptococci. Previously, *S. aureus* was differentiated from other staphylococci by the coagulase test. However, not all *S. aureus* strains are coagulase-positive and incorrect species identification can impact effective treatment and control measures ⁶

Methods

This study was conducted in different markets in Baghdad, (100) Samples of liver from chicken (50) Samples from each fresh and frozen liver were collected during the period between (10/2020 to 2/2021). Each liver sample from chicken was submitted to bacterial culture by using standard methods

Culturing of specimen

Inoculation on 5% blood agar Incubation at 37 for 24-48 hours observation of colony characteristics Sub culturing on nutrient agar Incubation at 37 C for 24 hours The growing isolates were identified according to their colonial morphology primary, identification by gram staining, biochemical reaction (Catalase test, DNase test, gelatinase test). Secondary identification by Growth on mannitol salt agar and Drug sensitivity. The colonies that were confirmed by staining reaction, catalase test, were streaked on mannitol salt agar plate and incubated at 37 C and examined after 24-48 h for growth. The presence of growth and change of pH in the media (red to yellow color) regarded as presumptive identification for *S. aureus* ⁷

Antibiotics sensitivity test

The tested for bacterial isolates were: Vancomycin (30mg) ,Tetracycline(10mg), Penicilin (10U) ,Gentamicin (10mg), Amoxicillin (25 mg), Ampicillin (25 mg),Trimethoprim /Sulphamethaxazole(25mg), Streptomycin (25 mg) .Inoculation of distinct colony in to 5ml nutrient broth incubated at 35-37°C for about 2 hours. Then the turbidity is compared with MacFarland standard. a sterile cotton swab on a wooden applicator stick is used to transfer the diluted bacterial suspension to *Muller- Hinton Agar* excess fluid must be squeezed out by rotating the swab against the sides of the tube. The plate is seeded uniformly by rubbing the swab against the entire agar surface in three different planes roughly 60 degrees to each others. Within 15 minutes (time used to dry the inoculums) after the plates are inoculated, antibiotic impregnated discs are applied to the surface of the inoculated plates by hand using a sterile forceps. All discs gently pressed down on to the agar with forceps to ensure complete contact with the agar surface. The disc should no closer than 1.5 cm to the edge of the plate and they should rest 24 mm apart from each other. Incubate the plates inverted aerobically for 24 hours .Zone of inhibition is measured in millimeters using a transparent ruler on the under surface of the Petri dish ⁸

Results

Cultural and microscopic characteristics

S. aureus colonies were yellow in colour fermented mannitol producing an acid which changes the indicator color from red to yellow light to golden yellow colonies surrounded by zone of clear beta hemolysis colonies of *S. aureus* on blood agar .Microscopically coccus or spheroid shape Gram positive purple and found either single cells in pairs or more in clusters

Biochemical tests

Conventional tests of the isolated bacteria gave different results, the isolates gave positive result in (catalase test , dnase test,gelatinase test) The results of this study were showed that the overall identification rate of *S. aureus* from chicken in in Baghdad city were 30/100 (30 %). Fresh reported a higher percentage 10/50 (20%) and 20/50 (40%) isolates Frozen liver sample Table (1) .

Table (1) The percentage of *S. aureus* isolated from different market in Baghdad city

Type of sample	No.of samples	Positive	%
Fresh liver	50	10	20%
Frozen liver	50	20	40%
Total	100	30	30%

In antibiotic sensitivity Some isolates of *S. aureus* are sensitive, other Intermediate, and Resistant to antibiotic Table 2

the results showed that *Staphylococcus aureus* isolates were sensitive (90%) to Gentamicin and Amoxicillin (80%) to Streptomycin and Vancomycin, (70%) to Trimethoprim/Sulphamethaxazole, (10%) to Ampicillin, and Resistant (90%) to Penicillin (80%) to Ampicillin (60%) to Tetracycline (20%) to Trimethoprim/Sulphamethaxazole (10%) Vancomycin, Gentamicin, Streptomycin. Table 2

Table 2: Results of antibiotic sensitivity test

Type of Antibiotic	Number sensitive	Sensitive %	Number Intermediate	Intermediate %	Number Resistant	Resistant %
Vancomycin 30mg	24	80%	3	10%	3	10%
Tetracycline 10mg	-	0%	12	40%	18	60%
Penicilin 10U	-	0%	3	10%	27	90%
Gentamicin 10mg	27	90%	-	0%	3	10%
Amoxicillin 25 mg	27	90%	3	10%	-	0%
Ampicillin 25 mg	3	10%	3	10%	8	80%
Trimethoprim/Sulphamethaxazole 25mg	21	70%	3	10%	6	20%
Streptomycin 25 mg	24	80%	3	10%	3	10%

Discussion

Staphylococcus aureus has the ability to induce severe diseases of animals and humans, that are associated with numerous virulence factors produced by these bacteria, such as toxins, cell adhesins and secreted exoproteins⁹. The liver is had a high percentage of unsaturated fatty acids, proteins, vitamins and minerals this organ is eaten partially or wholly cooked of food-borne disease. Because it is highly spoilage dietary material contains many non-pathogenic or pathogenic pollution if it stored in bad circumstance There is combination between the incidence of food-borne outbreaks and consumption of the poultry meat. Liver could be contaminated during the slaughter of animals with many microorganisms. High significant morbidity and mortality in the poultry industry were responsible from pathogenic. The wide occurring outbreaks of Staphylococcal food poisoning may be due to inclusive handled and insalubrious cooked meat products¹⁰. The cooking may decrease or avert the earnest risk, from another hand microorganism origin may enter inside liver during slaughter, dressing and cutting of carcass from intestinal tract, knives, cloths, air, workers, carts, boxes and equipment in general, and huge variety of organisms types are supplement and it could be created the most kinds of subsidence ones. This contamination increased inside cutting meat, and the bacterial count redoubled more than six times after hand dealing and reach eight times when it present in markets¹¹. Contamination during processing during storage may later change

foods microflora quantitatively and qualitatively, Also the gut is the most important source of *Staphylococcus* bacteria ¹²

This study were showed that rate of isolation of *S. aureus* from livers of chicken in Baghdad city were 10 / 30 (33.33%). Several studies were done to investigate the microbes' contamination in the chicken product in Iraq. Due to the presence of many types of frozen chicken livers from different sources without health control, this study was designed to modernize our knowledge about microbial contaminants in chicken liver inside markets.

throughout in study of ¹³ reported a high prevalence of *S. aureus* in chicken livers (63.8%) and chicken gizzards(58.1%) after enrichment , also other study reported out of 100 staphylococcal isolates from chicken, *S. aureus* (15/100), Also the result of mean numbercount was below the Iraqi standard specification for frozen poultry meat from Central Organization for Standardization *Staphylococcus aureus*1×10⁴. Those outbreaks were listed with variety of foods including poultry, egg, beef,fish, chicken, dairy products and chocolate ¹⁴.

¹⁵who recorded 56.7% isolation rate of *S.aureus* from apparently healthy and diseased layer chicken, while nearly similar results were recorded by ¹⁶who isolated *S.aureus* in incidence of 51.2% and ¹⁷ isolated *S.aureus* 51% from layer chickens in Nigeria. ¹⁸ reported that 55% of laying hen farms isolates confirmed as *S.aureus*. Partially agreed with ¹⁹ who recorded that 42% of isolates from laying hens in Algeria to be *S.aureus*, while the prevalence was 57%,also ²⁰ recorded 40% isolation rate of *S.aureus* from backyard eggs . On the other hand, many authors isolated *S.aureus* in lower rates as ²¹ who isolated 35% *S.aureus* from layer chickens and ²² who detected 26.7 % of *S. aureus* from layer farms, but disagree with ²³who isolated *S.aureus* in high percentage (68%) from egg layer chickens. the occurrence of *S. aureus* was only in 32 (23%) samples using a conventional culture method *S. aureus* ²⁴ *S. aureus* isolated from many food products, including meat products reported in many studies, to be as a potential risk for public health²⁵ It was also detected in retail chicken meat in Japan ¹³. Only few of studies included data from poultry such as ground turkey²⁶Studies investigating the prevalence of *S. aureus* and MRSA in chicken livers and gizzards are very limited ¹³ and to our knowledge none is available to date from the US. Chicken livers in particular are usually undercooked to preserve taste and texture which can be risky if contaminated with enterotoxin producing strains of *S. aureus* specially that most of these enterotoxins are heat stable.. ²⁷reported the frequency of isolation of *Staphylococci* was 23 (39.7 %), ²⁸the prevalence of *S. aureus* in meat was 24.6% from samples of domestic and imported meat from local retail markets. On the other hand, many authors isolated *S.aureus* in lower rates ²⁹.who detected 26.7 % of *S. aureus* from layer farms, but disagree with ³⁰ who isolated *S.aureus* in (20) isolates of *Staphylococcus aureus* were examined, chickens. ¹³ reported a higher prevalence of *S. aureus* in chicken livers (63.8%) and chickengizzards (58.1%) . ³¹ reported *S. aureus* isolates (48.3%) from 572 samples collected from various species of poultry. ³² showed a relatively high rate of *Staphylococcus* infection in the poultry (10.8%). ³³ was reported a higher prevalence of *S. aureus* (67.6%) in retail chicken livers and gizzards. isolates ,were highly resistant to ampicillin, tetracycline, doxycycline, penicillin, and erythromycinabout . ³⁴. Reported the prevalence of *S. aureus*, in poultry and

meat samples, 79 distinct *S. aureus* isolates were characterized and their antibiotic sensitivity pattern was screened against several common antibiotics.³⁵ reported that *S. aureus* organism was widely distributed in human related to poultry which isolated strains from hand swabs in broiler farms with 14.8% (4/27), layer farms with 43.8% (8/23), 25% (2/8) in our study results of antibiotic sensitivity test the results showed that *Staphylococcus aureus* isolates were sensitive (90%) to Gentamicin and Amoxicillin (80%) to Streptomycin and Vancomycin, (70%) to Trimethoprim/Sulphamethaxazole, (10%) to Ampicillin and Intermediate in Sensitivity to (40%) to Tetracycline and (10%) for Vancomycin, Penicillin, Amoxicillin Ampicillin Trimethoprim/Sulphamethaxazole, Streptomycin while Resistant (90%) to Penicillin (80%) to Ampicillin (60%) to Tetracycline (20%) to Trimethoprim/Sulphamethaxazole (10%) Vancomycin, Gentamicin, Streptomycin. The development of resistance has been associated with the extensive therapeutic use of antimicrobial and routine use of it or with their administration as growth promoters in poultry.³⁶ *S. aureus* isolated from chicken meat samples is excessively reported to resist various antibiotics such as penicillin, methicillin oxacillin, chloramphenicol and erythromycin which poses a significant threat to the consumers' health.³⁷ Other study isolated staphylococcal from 21 liver of chicken were examined for antibiotic sensitivity, 11 were found to be methicillin resistant. amoxicillin, cefoxitin, and methicillin in 100% resistance rate, tetracycline 85% and streptomycin 76.2%. While the highest sensitivity rates were detected to vancomycin, tylosin, gentamycin, ciprofloxacin and, colistin as 100%, 100%, 90.5%, 85% and 80% respectively.³⁸ Likewise, Also³⁹ recorded high resistance to methicillin, amoxicillin and cefotaxim, meanwhile high sensitivity to gentamycin and ciprofloxacin. In another study conducted by¹⁵, the highest sensitivity was to vancomycin, amoxicillin+ clavulanic acid by percentages of 84.5%, 83.8%, respectively. other found high resistance against tetracycline (76.8%), ciprofloxacin (60.4%), oxacillin (36.6%), and cotrimoxazole (26.6%) while the isolates showed significant susceptibility to cefoxitin (97.7%), and gentamicin (96.9%)⁴⁰. Meanwhile⁴¹ and⁴² mentioned that all *S. aureus* were sensitive to vancomycin, likewise⁴³ is the antibiotic resistance of *Staphylococcus* strains isolated from poultry. the phenomenon of acquired resistance occurs in all microorganisms that are pathogenic for humans and animals, as well as among saprophytic bacteria⁴⁴. The has been found in poultry and transmission identified in a human who was working with in live and slaughtered chickens were shown to be 100% resistance towards tetracycline, Penicillin, and erythromycin and were additionally resistant to other antibiotics including Vancomycin (46.2%)^{45,46}

Conclusion

This study concluded that there is a need for monitoring of poultry and its products in markets and abattoirs, good cooking to minimize the possibility of human infections, do not use antimicrobial as growth promoters in poultry production to prevent the resistance to antibiotic in human

Author Contribution

The author had the major contribution in practical part implantation, paper writing, editing and reviewing

Declaration of competing interest

the author declare no conflicts of interest.

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