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Studies on *Staphylococcus aureus* Infection in rabbit farms

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ABSTRACT

The present study was carried out to spotlight on the presence of Staphylococcus aureus (Staph aureus) among rabbit farm and to characterize the bacterial isolates for the presence of blaZ, coa, mecA, tetK and nuc gene, It also aimed to demonstrate the antimicrobial susceptibility of S. aureus in vitro and detect its pathogenicity in weaned rabbits. A total of 426 samples were collected included 98, 257 and 71 samples from apparently healthy rabbit, diseased rabbits and freshly dead rabbits. Staph aureus was isolated in 20(20.4%) out of 98 apparently healthy rabbits, 78(30.4%) out of 257 diseased rabbit and 22(31%) out of 71 dead rabbits and its presence was confirmed through cultural and biochemical characterization. Multiplex PCR was performed on the isolated strains of S. aureus which showed the presence of blaZ, coa, mecA, tetK and nuc virulent genes. All isolates were positive for the gene coa, mecA, tetK and nuc virulent genes. This results indicates that the coagulase-positive of S. aureus was the most predominant cause of skin disease in rabbits disease and carried the mecA and nuc virulent gene. Antibiotic sensitivity against conventional antimicrobials revealed that Staphylococcus aureus strains were sensitive to streptomycine (70%) and penicillin, gentamycin (63.3%) in addition to doxycycline (60%) and resistance to ciprofloxacin and Amoxicillin (75%). As well, high degree of resistance to Trimethoprim /Sulphathoxazol, Cefotaxime, Cephradin (100%) and Colstine sulphate were detected 72.3%. The pathogenicity test conducted on of 5 week old weaned rabbits showed that S. aureus was highly pathogenic resulting in mortality rates of 65 and 35%, respectively. The rabbits exhibited septicaemia with congestion and peticheal hemorrhage throughout the intestine, sever congestion of the liver, spleen and kidneys, as well as the congestion of the intestine blood vessels and lung. Pneumonia or abscesses were also observed in the lungs or heart. Histopathological changes support-

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ed the so observed lesions. In conclusion, the detection of S. aurues virulent genes with overlapping functions and the reproduction of antibiotic resistance genes in the Egyptian field is very importance additionally, in vivo pathogenicity studies. S. aureus was identified as the most significant pathogen causing death of rabbits in rabbit farms. Therefore, accurate, rapid and molecular diagnostic methods of must be implemented. Finally, it is essential to conduct in vitro sensitivity tests of S. aurues to different antimicrobial agents due to the observed resistance to the commonly used antibiotics for prevention and control S. aureus in rabbits.

INTRODUCTION

Staphylococci are Gram-positive bacteria, non-motile, non-spore forming with diameters of 0.5 – 1.5 µm. They are facultative anaerobes that grow by aerobic respiration or by fermentation. Staphylococci are characterized by individual cocci, which divide in more than one plane to form grape-like clusters **(Wilkinson. 1997)**. there are over 30 different types of staphylococci and eight sub-species in the genus Staphylococcus that can infect humans and animals **(Cucarella et al. 2004),** most infections are caused by Staphylococcus aureus **(Kuroda et al. 2001).**

Staphylococcus aureus is a common disease in both domestic and wild rabbits **(Vancraeynest et al. 2007).** Moreover, staphylococcal infections lead to substantial economic losses in the livestock industry worldwide **(Espinosa et al. 2020 and Mork et al. 2005).**

Staphylococcus aureus is one of the most common causes of infections in people worldwide. It also a versatile opportunistic pathogen affects rabbits of different ages, resulting in various pathologies including dermal lesions, suppurative dermatitis, multisystemic abscessation. pododermatitis, mastitis and pneumonia **(Viana et al. 2007; Vancraeynest et al. 2004),** in Additionally meteritis can lead to poor production, infertility and death **(Shirtliff and Mader. 1999)** .

Rabbits can be infected by two types of S. aureus strains: high- and low-virulence strains, Severe outbreaks of the disease in young rabbits are attributed to specific high virulence (HV) S. aureus strains **(Hermans et al. 2000,** The main manifestations are subcutaneous abscesses, mastitis, pododermatitis and septicaemia. Severe economic losses have been reported in the USA **(Hagen. 1963),** Italy, Belgium and France **(Okerman et al. 1984)** and England **(Holliman and Girvan. 1986).**

Staphylococcus is a dangerous pathogen that causes a localized suppurative inflammation or a fatal generalized septicemia, The virulence of S. aureus as a pathogen stems from the fact that S. aureus strains possess virulence factors, including the production of biofilms, Adherence may be mediated by a variety of adhesions, such as the autolysin/adhesins Atl and Aaa and secreted toxins **(Kreikemeyer et al. 2002; Hagar et al. 2003).**

Staphylococcus aureus is a major pathogen of increasing importance due to the propagation of antibiotic resistance strains such as methicillin resistant Staphylococcus aureus (MRSA) **(Yu et al. 2015 and Nemet et al. 2020).**

Staphylococcus aureus poses a hazard to human health as it causes a number of diseases including abscesses as endocarditis, osteomyelitis, pneumonia, and sepsis, so is a frequent cause of serious and life-threatening infections, They are therefore of great public concern **(Higgins et al. 2006 and Li et al. 2010).**

Therefore, the present study is aims to evaluate the prevalence of Staph aureus strains from a rabbit farm in Damietta governorate, and to detect some virulent genes (blaZ, coa, mecA, tetK and nuc virulent gene), test the susceptibility of the isolated strains to various antimicrobials *in vitro* and examine their pathogenicity in weaned rabbits.

MATERIALS AND METHODS

Samples collection

Samples were taken from 15 rabbit farms in Damietta governorate, Egypt during the period from January to October, 2022. A total of 426 samples were collected including 350 rabbits (98 swabs from apparently healthy rabbit, 257 samples from diseased rabbit and 71 samples from dead rabbits (Table 1). The samples were taken from the skin and infections using sterile cotton swabs. Milk sample were aseptically collected in a sterile tube. lungs or uteruses were taken from Rabbits with genital tract or lung infections were euthanized at laboratories and necropsied under aseptic condi-tions in laboratories. Lung samples were also taken from the dead rabbits with severe respiratory disease. Samples taken at farms were collected in sterile plastic containers, kept in ice box and transported as soon as possible to the laboratory.

Isolation and identification of Staphylococcus aureus isolates

Each sample was mixed with sterile phosphate buffer saline and homogenized to create 50% suspension. A 100 µl of suspension was inoculated on sheep blood agar plate for cultivation at 37 °C for 24 h. Presumptive *Staph. aureus* isolates were then sub-cultured mannitol salt agar plates. The suspected S. aureus colony was initially identified through colony morphology and gram staining and biochemical testing [catalase, coagulase and sugar fermentation (glucose, sucrose, lactose and mannotol)]. Five colonies typical and atypical were selected from each plate and inoculated into 5ml Brain Heart Infusion broth. The tubes were incubated at 37oC for 24 hours. From which 0.1 ml was transferred to tubes containing 0.3 ml of sterile citrated rabbit plasma. Inoculated tubes were incubated at 37 °C and examined for clot formation after 4 hours **(Tchamba et al. 2019**. Isolation of *Staph. aureus* isolates were conducted according to **Jarraud et al. 2002**.

Molecular detection of Staphylococcus aureus virulent genes

DNA extraction and PCR

DNA extraction from samples was performed using the QIAamp DNA Mini kit (Qiagen, Germany, GmbH) with modifications from the manufacturer's recommendations. Briefly, 200 µl of the sample suspension was incubated with 20 µl of proteinase K and 200 µl of lysis buffer at 56OC for 10 min. After incubation, 200 µl of 100% ethanol was added to the lysate. The sample was then washed and centrifuged following the manufacturer's instructions. Nucleic acid was eluted with 100 µl of elution buffer provided in the kit.

Oligonucleotide Primer. Primers used were supplied from Metabion (Germany) are listed in table (1).

PCR amplification.

Uniplex PCR. Primers were utilized in a 25 - µl reaction containing 12.5 µl of EmeraldAmp Max PCR Master Mix (Takara, Japan), 1 µl of each primer of 20 pmol with concentration, 5.5 µl of water, and 5 µl of DNA template. The reaction was carried in an Applied biosystem 2720 thermal cycler.

Analysis of the PCR Products.

The products of PCR were separated by electrophoresis on 1.5% agarose gel (Applichem, Germany, GmbH) in 1x TBE buffer at room temperature using gradients of 5V/cm. For gel analysis, 20 µl of the uniplex products and 30 µl of the multiplex products were loaded in each gel slot. Gelpilot 100 bp plus Ladder (Qiagen, Germany, GmbH) was used to determine the fragment sizes. The gel was photographed by a gel documentation system (Alpha Innotech, Biometra) and the data was analyzed through computer software. The positive DNA samples for blaZ, coa mecA, tetK and nuc gene were obtained from reference laboratory for veterinary quality control on poultry production. (Animal Health Research Institute of Cairo, Egypt). Distilled water was used as negative control.

In vitro-antibiotic susceptibility test of *Staph. aureus*

The pre-incubated 24 hours cultures of *S. aureus* were diluted in sterile buffer peptone water and matched with the 0.5 MacFarlane turbidity standards to get 1×10^{8} cfu/ml as total count. Culture with 0.5 McFarland density were distributed onto the Müller-Hinton

agar (Merck, Germany). The antibiotic discs were distributed on the agar surfaces, and the zones of inhibition were measured after 24 hr **(CLSI protocol, Cockerill, 2012).**

Pathogenicity test of Staphylococcus aureus in rabbits.

The experiment was done according to the National Regulations on Animal Welfare and Institutional Animal Ethical Committee (IAEC). A total of 65, five rabbits were subjected for bacteriological examination to confirm absence of Staphylococcus aureus. Rabbits were reared in thoroughly cleaned and disinfected houses. Rabbits were kept for a week under observation for adaptation and to ensure the absence of any clinical signs, mortalities. Rabbits were divided into 3 equal groups at 38 days old, each containing 20 rabbits. Rabbits were challenged at 38 day old, rabbits in group (1) were challenged with1 ml subcutaneously of bacterial suspension containing 1×10^8 cfu/ml of viable virulent S. aureus **(Reinoso et al. 2002),** while rabbits of group (2) were challenged in the nose with1 ml of a bacterial suspension containing 1×10^8 cfu/ml of viable virulent S. aureus **(Kildow et al. 2012).** Rabbits in group (3) were kept as blank control negative non challenged group, those in group (3) were inoculated with sterile phosphate buffer saline. All rabbits were kept under observation for 3 weeks to detect clinical signs, mortalities and post-mortem lesions. Samples were collected from dead rabbits for re-isolation of S. aureus. At the end of the study, samples including the liver, lung, spleen and heart were collected from sacrificed rabbits for gross lesions, re-isolation of Staph. aureus.

Histopathological examination:

Tissue specimens from lung, skin, liver and heart were collected and fixed in 10% neutral buffered formalin for 72 hrs. Samples were processed in serial grades of ethanols, cleared in Xylene, then infiltrated and embedded into Paraplast tissue embedding media.5µn thick serial tissue sections were cut by rotatory microtome for demonstration of different samples and mounted on glass slides. Tissue sections were stained with Hematoxylin and Eosin as a standard staining method for blinded light microscopic examination using Full HD microscopic imaging system (Leica Microsystems GmbH, Germany). All standard procedures and protocols for samples fixation and staining according to **Culling, C.F.A. 2013**.

Table 1. Primers sequences, target genes, amplicon sizes and cycling conditions.

Target gene	Primers sequences	Amplified segment (bp)	Primary denaturation	Amplification (35 cycles) Secondary denaturation	Anneal-ing	Exten-sion	Final ex-tension	Reference
S. aureus 23S rRNA	ACGGAG-TTACAAAGGAC GAC AGCTCAGCCTTA ACGAGTAC	1250 bp	94˚C 5 min.	94˚C 30 sec.	55˚C 1 min	72˚C 1.2 min.	72˚C 12 min.	Bhati et al., 2016
MecA	TCTGCAGTT-GCTTCACTTCGC GTA GAA ATG ACT GAA CGT CCG ATA A CCA ATT CCA CAT TGT TTC GGT CTA A	310 bp	94˚C 5 min.	94˚C 30 sec.	50˚C 30 sec.	72˚C 30 sec.	72˚C 7 min.	McClure et al., 2006
Coa	ATA GAG ATG CTG GTA CAG G GCT TCC GAT TGT TCG ATG C	Four differ-ent types of bands may be detected 350 bp 430 bp 570 bp 630 bp	94˚C 5 min.	94˚C 30 sec.	55˚C 40 sec.	72˚C 45 sec.	72˚C 10 min.	Iyer and Kumosani, 2011
blaZ	TACAACTGTAA-TATCGGAGGG CATTACAC-TCTTGGCGGTTT C	833 bp	94˚C 5 min.	94˚C 30 sec.	50˚C 40 sec.	72˚C 50 sec.	72˚C 10 min.	Bagcigil et al. 2012
nuc	ATATGTATGG-CAATCGTTTCAA T GTAAATGCAC-TTGCTTCAGGAC	395 bp	94˚C 5 min.	94˚C 30 sec.	55˚C 40 sec.	72˚C 40 sec.	72˚C 10 min.	Gao et al., 2011
tetK	GTAGCGACAA-TAGGTAATAGT GTAGTGACAA-TAAACCTCCTA	360 bp	94˚C 5 min.	94˚C 30 sec.	54˚C 40 sec.	72˚C 40 sec.	72˚C 10 min.	Duran et al., 2012

RESULTS

Based on cultural, morphological and biochemical characteristics of the isolates, a total of 20.4%, 30.4% and 31% Staph. aureus isolates were recovered from 98 apparently healthy rabbit, 257 diseased rabbit samples and 71 dead rabbit, respectively, We recovered isolates from different clinical back-grounds: 37 from respiratory infections, 22 from skin infections, 5 from mastitis and 16 from genital tract infec-tions (Table 2).

Morphologically, Staph. aureus is usually beta hemolytic as it causes lyses of red blood cells on blood agar plates (hemolytic staphylococci), Staphylococci are able to tolerate the high salt concentration found in Mannitol Salt agar and thus grow readily. If mannitol is fermented, the acid produced turns the phenol red pH indicator from red (alkaline) to yellow (acid). Microscopically, the organism is Gram-positive round shape and it is found as either single cells, in pairs, or more frequently, in clusters that resemble a bunch of grapes. Biochemically, it is positive oxidase, catalase, citrate, coagluase, methyl red, voges Proskauer, gelatin hydrolysis, urea hydrolysis and negative for indole, oxidase and protease activity on milk agar medium, lipase production on egg yolk agar medium (HiMedia, Mumbai), It shows sugar fermentation for Fructose, Galactose, Glucose, Lactose, Maltose, Mannitol, Mannose, ribose and sucrose, but not xylose, salicin, raffinose, cellobiose, arabinose.

The bacterial isolates which were found to be Staphylococcus aureus based on specific

phenotypic features were further confirmed through PCR using specific primer pairs of 23S rRNA (Figures 1). These primers amplify 1250 bp region of 23S rRNA gene fragment of Staphylococcus aureus, which is highly conserved at species level. PCR programming was began with an initial denaturation step at 94°C for 5 min followed by 35 cycles of 94°C for 30 sec, 55°C for 1 min, and 72°C for 1.2 min, ending with a final extension step at 72°C for 12 min (Table 1& Figure 1).

The molecular detection of virulent genes of Staphylococcus aureus showed the presence of coa, mecA, tetK and nuc genes in all strains and blaZ in almost strains (Figures 2 and 3).

The 23S rRNA, mecA, blaZ, tetK, and virulence genes encoding thermonuclease (nuc), coa genes were screened by PCR. The primers for 23S rRNA, mecA, coa, blaZ, nuc and tetK were reported previously (Table 1).

In 60 Staphylococcus aureus strains, *in* the *vitro* sensitivity test revealed susceptibility to streptomycine (70%) and penicillin, gentamycin (63.3%) in addition to doxycy-cline (60%). resistance was detected to ciprofloxacin and Amoxicillin (75%), while, high degree of Resistance to Trimethoprim\Sulphathoxazol (100%), Cefotaxime colstine sulphate and Cephradin was 83.3% (Table 5 and Figure 4).

Clinical signs of Staphylococcus aureus challenged rabbit were off food, depression, off food, ruffled fur, disinclination to move, respiratory signs, rhinitis and sinusitis. Nasal discharge and mucopurulent conjunctivitis are sometimes present. Pus from nose and the exudate adheres to the fur around the nares leading to bouts of sneezing. Abscesses can be palpated in subcutaneous tissue and at the site of inoculation. The observation of body weight along the period of the study which showed a significant decrease ($P<0.05$). Post-mortem examination of the dead and surviving rabbits revealed septicemia, peticheal hemorrhage throughout the intestine, sever congestion of the liver, spleen and kidneys, intestine and lung. Pneumonia or abscesses in the lungs or heart. Mortalities began in rabbits of group (2) were challenged in the nose 48hr post challenge (PC) with mortality rate of (13/20, 65%), while rabbits of group (1) that were challenged subcutaneously appeared at 73 hr PC with mortality rate of (7/20, 35%). No clinical signs or mortalities was recorded on phosphate buffer saline inoculated rabbits.

The histopathological lesions of liver of dead and sacrificed rabbit that were challenged at 38 days old are represented in plate (1).The liver of the normal control rabbit demonstrated normal morphological structures of rabbit liver parenchyma with many apparent intact well organized hepatocytes with intact subcellular details (arrow), intact hepatic vasculatures (star) as well as hepatic sinusoids were shown (fig. A). The liver of rabbist that were challenged intranasally showed apparent intact hepatic parenchyma with abundant records of intact well- organized hepatocytes (blue arrow), sporadic focal records of peri portal inflammatory cells infiltrates (red arrow) (fig. B). The liver of rabbits that were challenged subcutaneously showed the same records as intranasal samples (fig. C,D).

The histopathological lesions of the pulmonary tissue in the dead and sacrificed rabbit that challenged at 38 days old are represented in plate (2). Normal control pulmonary tissue samples showed apparent intact histological features of pulmonary parenchyma with almost apparent intact alveolar epithelium with thin inter alveolar septa (arrow) showing minimal inflammatory cells infiltrates and normal vasculatures. Intact bronchiolar structures with few peribronchiolar inflammatory cells infiltrates (red arrow) were also observed (fig. A). pulmonary tissue samples of rabbits that challenged subcutaneously showed marked diffuse hemorrhagic pneumonia (blue star) with significant thickening of interalveolar walls and abundant inflammatory cells infiltrates (blue arrow) as well as marked congested and dilated pulmonary vasculature(fig. B). The pulmonary tissue samples of rabbit that challenged intranasal showed moderate focal records of hemorrhagic pneumonia with intraluminal bloody exudates, with sever congested blood vessels (black star), peribronchiolar inflammatory cells infiltrates (red arrow) and intra bronchiolar desquamated epithelial cells mixed with bloody exudates (blue star) (fig. C,D).

The histopathological lesions of the skin / muscle samples of dead and sacrificed rabbits that were challenged at 38 days old are collected in plate (3). Normal control samples showed apparent intact, normal and organized histological features of skin layers including thin epidermal layer and dermal layers with normal organized collagen fibers, as well as hair follicles with minimal inflammatory cells infiltrates (fig. A, B). samples of rabbits that challenged subcutaneously demonstrated sever mixed inflammatory cells infiltrates in deep dermal and subcutaneous tissues with abundant necrotic tissue depress (star) and a few records of intact subcutaneous and deeper muscular tissue with significant fibroblastic activity. Intact epidermal layers (fig. C,D).

Table 2.The incidence of Staphylococcus aureus from surveyed rabbit's farms in Damietta governorate,Egypt

Type of samples	Apparently healthy rabbit			Diseased rabbit			Dead rabbit		
	Number of samples	Number of positive	% positive	Number of samples	Number of positive	% positive	Number of samples	Number of positive	% positive
Lung	20	0	0	80	25	31.3	40	12	30
Skin swaps	20	5	25	50	14	28	9	3	33,3
Milk	7	1	14.3	13	4	30.8	-	-	-
Uterus	20	5	25	30	8	26.7	10	3	30
Nasal swaps	31	9	29	84	27	32.1	12	4	33.3
Total	98	20	20.4	257	78	30.4	71	22	31

Table 3. Results of antibiogram test against Staph aureus.

Antibiotic Disc (Code)	Disc content/ µg	Antimicrobial efficacy (%) against 60 strain of Staph aureus		
		Susceptible	IntermediateSusceptibility	Resistant
Amoxicillin (AX)	25	0	15 (25%)	45 (75%)
Cefotaxime (CTX)	30	0	0	60 (100%)
Cephradin (CE)	30	0	0	60 (100%)
Ciprofloxacin (CIP)	5	1 (1.7%)	14 (23.3%)	45 (75%)
Gentamycin (CN)	10	38 (63.3%)	2 (3.3%)	20 (33.3%)
Colistinsulphate (CT)	10	0	10 (16.7%)	50 (83.3 %)
Streptomycin (S)	10	42 (70%)	8 (13.3%)	10 (16.7%)
Doxycycline (Do)	30	36 (60%)	3 (5%)	21 (35%)
Trimethoprim /Sulphathoxazole (SXT)		0	7 (21.2%)	60 (100%)
Penicillin G (P)	10	38 (63.3%)	7 (11.7%)	15 (25%)

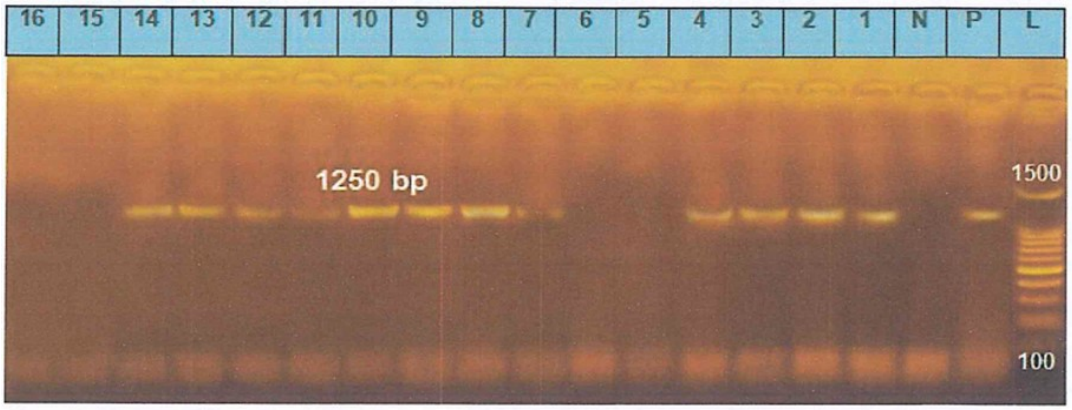

Figure (1): PCR amplification using Staph aureus 23S rRNA), Pos= positive control, L= ladder, lines 1-4, 7-14 = clinical isolates of Staph aureus, lines 5-6, 15-16 = negative isolates of Staph aureus, Neg= negative

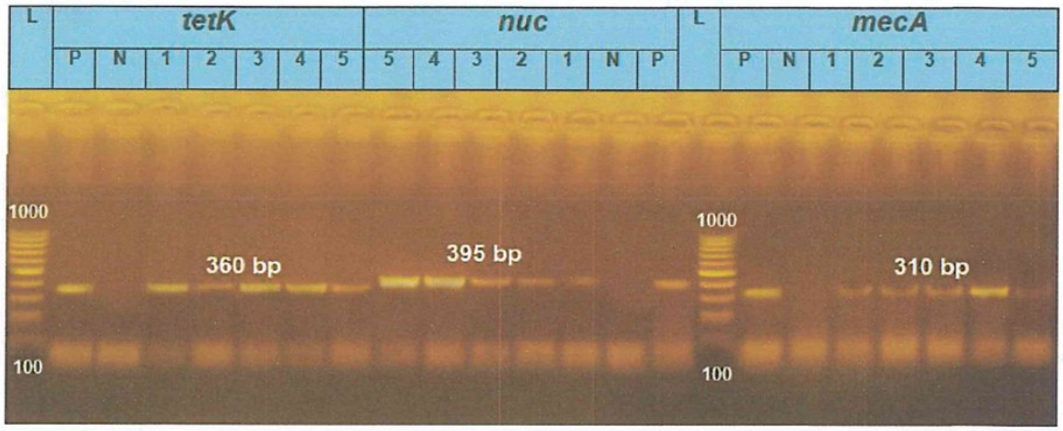

Figure (2): PCR amplification using Staph aureus genus-specific primers (tetK, nuc, mecA genes) Pos= positive control, L= ladder, lines 1-5 = clinical isolates of Staph aureus tetk *genus*, lines 1-5 = clinical isolates of Staph aureus nuc *genus*, lines 1-5 = clinical isolates of Staph aureus mecA *genus*, Neg= negative control

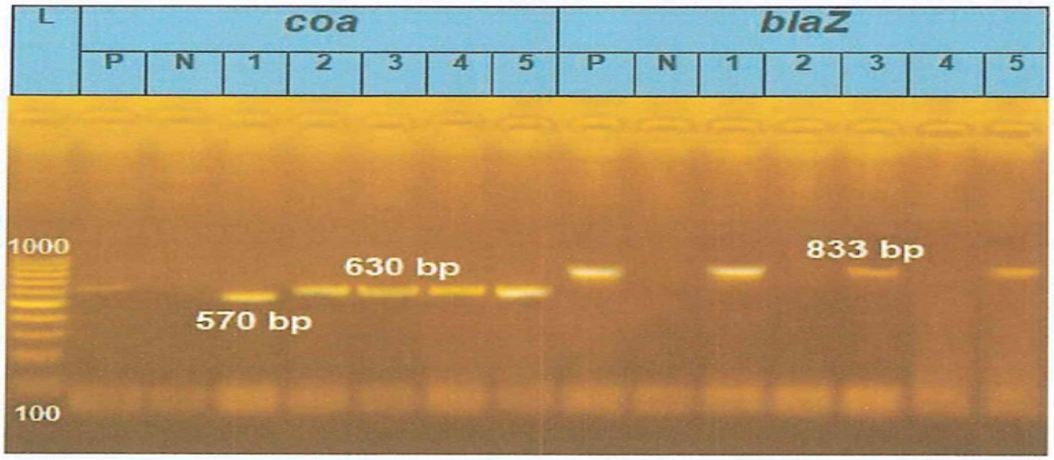

Figure (3): PCR amplification using Staph aureus genus-specific primers (coa and blaZ genes), Pos= positive control, L= ladder, lines 1-5 = clinical isolates of Staph aureus coa *genus*, 1,3,5= clinical isolates of Staph aureus blaZ *genus*, lines 2,4 (negative blaZ), Neg= negative control

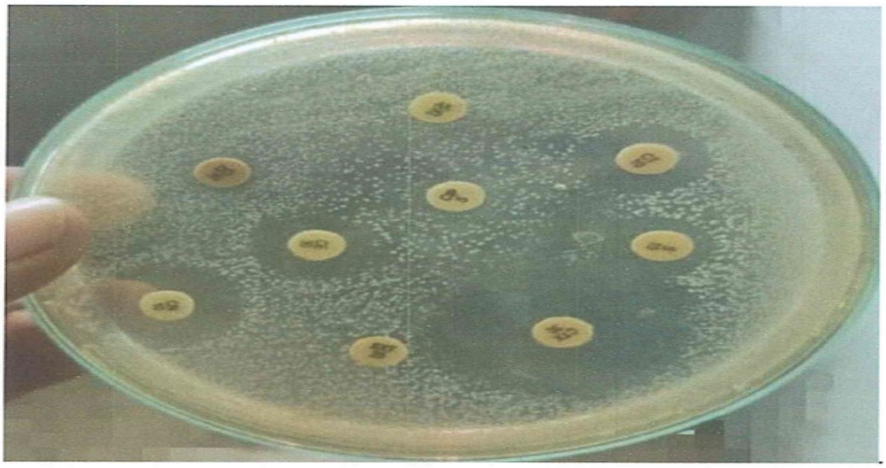

Figure (4): Results of antibiogram test against Staphylococcus aureus

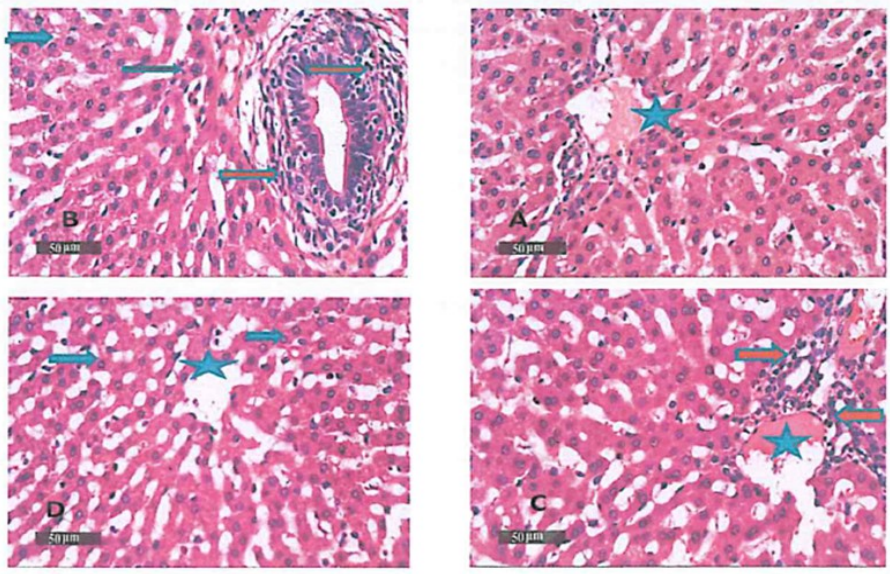

Plate (1): Showing the histopathological lesions in liver organs of 38 day old rabbit.

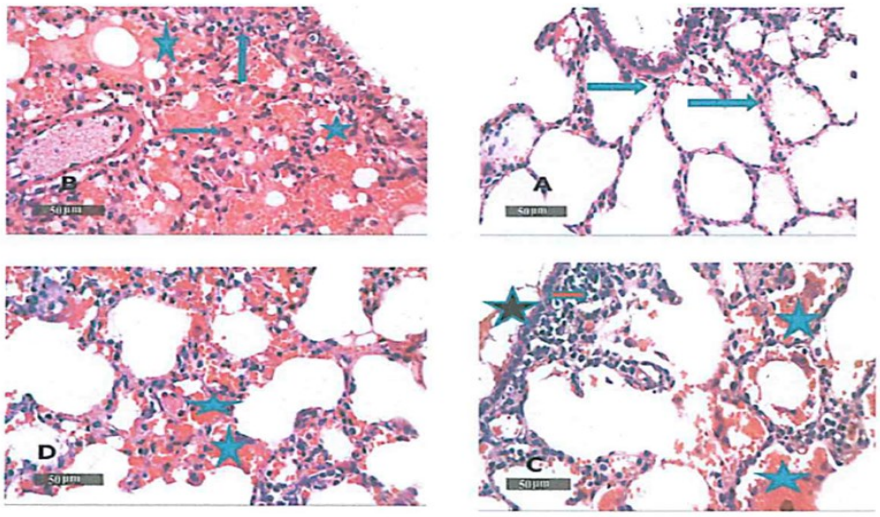

Plate (2): Showing the histopathological lesions in pulmonary tissue samples of 38 day old rabbit

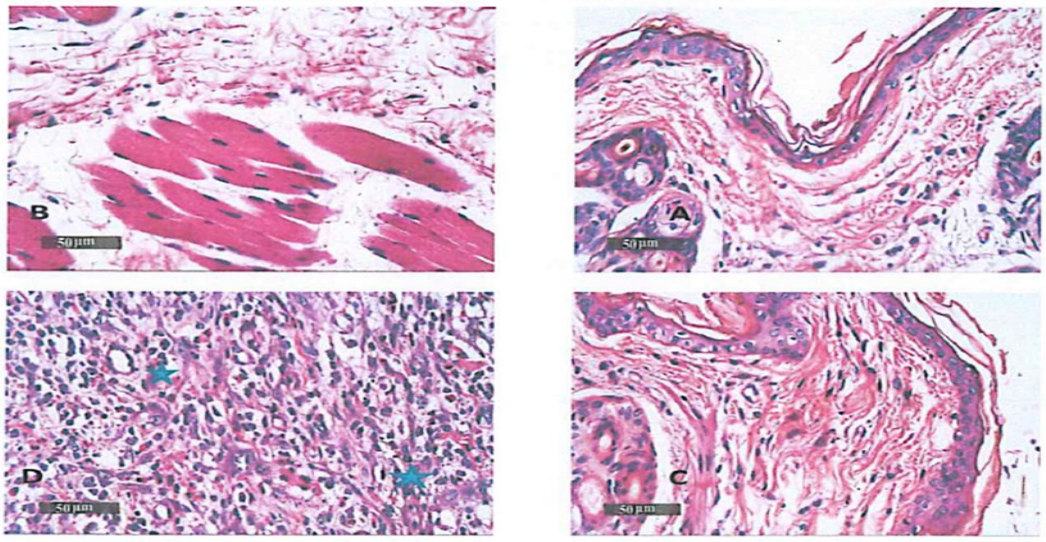

Plate (3): Showing the histopathological lesions in skin /muscle samples of 38 day old rabbit.

DISCUSSION

Staphylococcus aureus remains one of the most prominent human bacterial pathogens worldwide **(Davis et al. 2014 and Sato et al. 2017),** In addition, S.aureus is able to cause a variety of infections in numerous other host species (**Schaumburg et al. 2013and Brady et al. 2018).** Staphylococcus aureus is the cause of serious infections in rabbits, including a highly fatal disease of neonatal rabbits, pododermatitis, subcutaneous abscesses in rabbits of all ages, infertility and mastitis in lactating does, causing significant economic losses **(Hermans et al. 2003 and Vancraeynest et al. 2006**). The severe respiratory disease in rabbits is often caused by Bordetella bronchiseptica, Pasteurella multocida, Staphylococcus species, Pseudomonas species, and other bacteria as well as rabbit haemorrhagic disease virus **(Rougier et al. 2006 and Fisher and Graham. 2023).**

Preliminary identification tests including; cultural, microscopic and biochemical identifications of Staphylococcus aureus, showed that the organism was recovered from 20 (20.4%) out of 98 apparently healthy rabbit, 78 (30.4%) out of 257 diseased rabbit and from 22 (31%) out of 71 dead rabbits. The incidence of Staphylococcus aureus from surveyed rabbit's farms in Damietta governorate, Egypt was detected as follow 5 (25%) out of 20 skin swaps from from apparently healthy rabbits, 1(14.3%) out of 7 milk samples from apparently healthy rabbit, 5 (25%) out of 20 uterus sample from apparently healthy rabbits, 9 (29%) out of 31 nasal swabs from apparently healthy rabbits, 25(31.3%) out of 80 lung samples from diseased rabbits, 14 (28%) out of 50 skin swaps from diseased rabbit, 4 (30.8%) out of 13 milk samples from diseased rabbits, 8 (26.7%) out of 30 uterus sample from diseased rabbits, 27 (32.1%) out of 84 nasal swaps from diseased rabbits and 12 (30%) out of 40 lung samples from dead rabbits, 3 (33.3%) out of 9 skin swabs from dead rabbit, 3 (30%) out of 10 uterus samplse from dead rabbits,4 (33.3%) out of 12 nasal swaps from dead rabbits. Similar findings were observed by **Hermans et al. 1999** who reported that healthy rabbits may be carriers of pathogenic strains, spreading through direct contact or aerosol, and that it is important in maintaining infection within the herd with no previous history of the disease. This is in agreement with **Ali. 1991** who found staphylococcus aurues in percentages of 23.1% in Egypt. Lower isolation percentages were recorded by **Abd-EL-Motelib and Salem. 1991** who recovered six strains of coagulase positive B. hemolytic s. aurues from infecting lactating does and their suckling rabbits. However, higher rates of s. aurues recovery were detected by **Ajuwape and Aregbesola .2002)** who found s. aurues in percentages of100% from the upper respiratory tract of

health rabbits. In addation to **Hagen. 1963** who reported serious economic losses in the USA, in England (**Holliman and Girvan. 1986**) and in Italy, France and Belgium (**Okerman et al. 1984**). The infection of high-virulence strains results in a high mortality rate in young rabbits and an epidemic spread among a rabbit flock, causing significant economic losses (**Hermans et al. 2003**). S. aurues affect rabbits of different ages causing different pathologies including mastitis, suppurative dermatitis and abscessation (**Vancraeynest et al. 2004; Vancraeynest et al. 2006a and Segura et al. 2007),** a severe respiratory disease (**Vancraeynest et al. 2006 and Denayer et al. 2017**). the infection of S. aureus results in a broad variety of diseases including bacteraemia, sepsis, mastitis and fibrinous pneumonia or abscesses in the lungs or heart have been documented in scientific literature**(Hermans et al. 2003; Schmidt et al. 2017 and Hecker et al. 2018)**.

In our search of scientific literature, we discovered that Staphylococcus aureus was confirmed through PCR analysis using 32S rRNA primers. S. aureus Strains were found to have 5 or 6 23S rRNA operons (**Wada et al. 1993, Pillai et al. 2002 and Katsunori et al. 2023**).

Brakstad et al. 1992 who found that identifying the use of the nuc gene for identification of Staphylococcus aureus strains. An extracellular thermostable nuclease (TNase) which is a protein with a molecular mass of 17,000 Da (**Tucker et al. 1978**). TNase cuts both DNA and RNA and is produced by Staphylococcus aureus strains (**Thiele. 1990**). PCR amplification of the nuc gene is used in many laboratories for the identification of S. aureus isolates (**Wilson et al. 1991 and Murray et al. 2003**).Nucleases are known to potentially decrease the antibacterial activity of neutrophil extracellular traps (NETs), which consist of DNA released from lysed neutrophils (**Brinkmann et al. 2012**). The presence of nuc and mecA genes in Staphylococcus aureus isolates from rabbits with dermatological pathologies indicates potential zoonotic behavior (**Silva et al. 2020**). The nuc gene is considered a virulence factor contributing significantly to the pathogenicity of virulent S. aureus and it must be considered in preventive strategies to prevent the transmission of antimicrobial resistance-gene to the human population (**Sahebnasagh et al. 2014**). There is increasing evidence of S. aureus resistance to methicillin including [methicillin-resistant S. aureus (MRSA)] and other antimicrobials of betalactamase group, this resistance is related to the mecA (methicillin resistance)gene (**Baba 2002, Boucher and Corey 2008**). **Hartman et al. 1984 and Ubukata et al. 1989** who reported that the mecA encodes the protein PBP2A (penicillin-binding protein 2A), a transpeptidase that assists in the formation the bacterial cell wall. This complicated the treatment of insidious infections such as those occurring in rabbits with severe abscesses **(Harcourt-Brown 2001**) and severe respiratory disease in rabbits (**Wang J et al. 2019**). Necrotizing pneumonia is frequently associated with community-acquired methicillin-resistant S. aureus (CA -MRSA) (**Harrison 2014 and Sicot et al. 2013**), hospital-acquired(HA) infections (**Rajaduraipandiet al. 2006**) or or other health care settings (**Tyagi et al. 2008 and Sato et al. 2017**). Pathogenic staphylococci are commonly identified by their ability to produce coagulase ,which is a virulence factor of *S. aureus* (**Kloos and Bannerman,1994**), coagulase converts soluble fibrinogen in plasma to insoluble fibrin (**Thomer et al. 2013**), which subsequently coats the bacterial cells and helps resist phagocytic engulfment, In addition to, Fibrin deposition is a critical process to abscess formation (**Cheng et al. 2010 and Kobayashi et al. 2015**). The majority of isolates of Staphylococcus aureus have the blaZ gene, which encoded penicillin resistance in (**Abdellatif et al. 2018**). The gene blaZ, along with its transcription repressor gene blaI, and a signal transducer-sensor protein, encoded by blaR1 are clustered together to control the expression of blaZ gene (**Firth and Skurray. 2000**). Phylogenetic analysis reveals for blaZ three evolutionary lines originating from plasmids, one from chromosome, and one intermediate group (**Schwendener et al. 2019**). **Turner et al. 2019** reported the acquisition of the β-lactamase encoding gene blaZ as amechanism of resistance. High prevalences of penicillin-resistance have been reported for exam-

ple in Korea 78.8% (**Nam et al. 2011**), in Estonia 61.4% (**Hendriksen et al. 2008**) and England 46% (**Kalmus et al. 2011**). Low prevalence of penicillin-resistant S. aureus have been reported in Sweden 7.1% and Norway 11.4% (**Hendriksen et al. 2008 and Persson et al. 2011).** TET resistance genes (tetL, tetK, tetM, tetO) are four major genes associated with tetracycline resistance among Gram positive bacteria (**Lina et al. 1999**).]. The tetK gene, which encodes a tetracycline efflux pump from Staphylococcus aureus (**Truong-Bolduc et al. 2022**), was found to be present in most of the TET-resistant isolates a long with the tetM, gene indicating their responsiblity for resistance to TET genes (**Lim et al. 2012, Emaneini et al. 2013 and Xuehan et al. 2019**). The presence of these genes in rabbit isolates explains the presence of penicillin resistance. Antimicrobial resistance was common, isolates possessed a number of virulence factors consistent with the ability to cause severe abscesses and an understanding of disease pathogenesis in rabbits. Therefore, the indiscriminate use of antimicrobial has generated a selective pressure for the appearance of resistant strains, the efficiency of the treatment is compromising (**Hou et al. 2023**).

In vitro sensitivity test conducted on 60 Staphylococcus aureus strains, the revealed Susceptibility was detected to streptomycine (70%) and penicillin, gentamycin (63.3%) and doxycycline (60%). resistance was observed against ciprofloxacin and Amoxicillin (75%). As well as Trimethoprim / Sulphathoxazol, Cefotaxime, Cephradin (100%) and Colstine sulphate was 83.3%.These results a long with similar findings reported by **María et al. 2020** who noted a high percentage of resistance Staphylococcus species isolates to Amoxicillin/Clavulanate, Trimethoprim Sulfamethoxazole, Ciprofloxacin, Enrofloxacin and Vancomycin. Furthermore, **Jamali et al. 2015** reported that S. aureus resistance to gentamicin, chloramphenicol, and tetracycline, but a low incidence of resistance to erythromycin, kankanamycin, streptomycin, penicillin G, and oxacillin. Staphy aureus strains were determined to be resistance to methicillin. Studies conducted by (**Rubinstein et al. 2008, Baba et al. 2002, Davis et al. 2014, Haenni et al. 2017 and Moreno et al. 2018**). Additionally, other studies reported resistance of this genus to cephalexin (**Papichet al. 2018**), quinolones (**Kang et al. 2014**), Cloxacillin (**Abdel-Hafeez. 2002**) and lincosamides (**Larsen et al. 2015**). contradictory to the finding reported by **Wang et al. 2019** the isolate of staph aurues was resistant to ampicillin and streptomycin and susceptible to ciprofloxacin, ofloxacin, florfenicol, levofloxacin doxycycline, enrofloxacin and neomycin.

The observation of body weight during the study period showed a significant decrease ($P<0.05$) which was due to systemic reaction against the bacterial infection and its toxins affecting interal organs. This was confirmed by positive isolation of bacteria and the effect of bacterial toxin on the digestive system impacting the general health condition and absorption of food from intestine (**Emsile and Nade.1983**). Meanwhile, the body temperature showed significant increase if compared with control group which may be attributed to the Neutrophiles ability to produce endogenous pyrogenes that is responsible for increasing the body temperature or due to the effect of bacteria(causing lesions) on hypothalamus that part of the brain responsible for regulating body temperature resulting in an increase in body temperature (**Kobayashi et al. 2015**). The clinical sings that experimental animals show (chock & cough , difficulty in breathing) are considered as normal reaction to the irritant injected material .however, the death of animals occur due to the lethal toxin effect of Staphylococcus aureus which kilsl with in 2-24 h **(Merchant and Parker.1967).**On the other hand, some of Staphylococcus aureus challenged rabbits showed off food, depression, ruffled fur, disinclination to move, respiratory signs, rhinitis and sinusitis are the most common forms, Nasal discharge and mucopurulent conjunctivitis sometimes occur. Then, pus from nose, the exudate adheres to the fur around the nares and bouts of sneezing may occur. Abscesses are able to be palpated in subcutaneous tissue and in site of inoculation. The post-mortem examination of the dead and survived rabbits revealed septicemia, peticheal hemorrhage throughout the intestine, sever congestion of the liver, spleen

and kidneys, intestine blood vessels and lung. pneumonia or abscesses may be present in the lungs or heart. However, congestion of some organs was due to the harmful effect of bacterial toxins on endothelial cells of blood vessels which induce disturbance in the permeability of these blood vessels**(Landrum et al. 2012 and Edelweijin et al. 1976)**. The typical inflammatory response to Staphylococcus aureus infection was characterized by accumulation of inflammatory cells at different part of the organ including (neutrophils, macrophage and lymphocytes) which lead to abscess formation or suppurative inflammation at different organs such as trachea, lung, liver and kidney **(Adlam et al. 1976 and Lowy. 1998)** with degenerative changes**(Bekeredjian-Ding. 2017)**.skin lesions developed due to the effect of staphylococcus aureus on T-regulatory cell which lose their effect in preventing exacerbation of inflammatory response **(Bekeredjian-Ding. 2017 and LaborelPréneron. 2015)**. Staphylococcus aureus bacteria were isolated throughout the entire period of experiment from internal organs and blood due to the distribution of bacteria via the blood stream (bacteremia) **(Emsile and Nade.1983 and DeMaria and Frank.1978)**.

Mortalities began in rabbits of group (2) which were challenged in the nose 48hr post challenge (PC) with mortality rate of (13/20, 65%), While rabbits of group (1) which were challenged subcutaneously appeared 73 hr PC with mortality rate of (7/20, 35%). No clinical signs or mortalities were recorded in phosphate buffer saline inoculated rabbits. Serious economic losses have however been reported in the USA (**Hagen. 1963**), England (**Holliman and Girvan. 1986**), Italy, France and Belgium (**Okerman et al. 1984**) and in the livestock industry worldwide (**Mork et al. 2005**). The pathogenicity test of 5 week old weaned rabbits showed that Staph aureus was highly pathogenic, inducing mortality rates of 65 and 35%, respectively. Generally, the pathogenic capacity of Staphy aureus is clearly dependent on a combination of virulence factors (**Edwards et al. 2010**). In rabbits, isolated Staph aureus strains carried a panel of virulence genes (**Bae et al. 2009 and Viana et al. 2015**). The infection of S. aureus results in a broad variety of diseases including skin and soft tissue infection, which can be complicated and life-threatening (**Robinson et al. 2004, Segura et al. 2007** and **Bae et al. 2009**). Furthermore, bacteraemia, mastitis and pneumonia (**Hermans et al. 2003; Sicot et al. 2013, Schmidt et al. 2017 and Hecker et al. 2018**).

In the last of study, we found that injectable antimicrobials were often used for staphylococcus control, in addition to the hygiene practices such as, the duo system, moving to clean disinfected barns for parturition, culling diseased does and avoiding fostere kits. A single batch is better, or the duo system, which involve moving females near parity to a clean and disinfected room, therefore, the number of batches per maternity barn also influences the occurrence of disease. Biosecurity practices, husbandry, environment and changes in hosts are highlighted to provide health and welfare benefits for breeding rabbits.

CONCLUSION

Accurate, rapid and molecular methods of diagnosis must be applied. There are resistance to the common antibiotics used for prevention and control Staph aureus in rabbits, so it is necessary to carry out in vitro sensitivity test of rabbits Staph. aureus to different antimicrobial agents. The detection of virulent genes of the isolated Staph. aureus strains with overlapping functions and reproduction of antibiotic resistance gene in the Egyptian field are very important, as well as in vivo pathogenicity studies.

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