DETECTION OF SOME VIRULENCE GENES OF *STAPHYLOCOCCUS AUREUS* ISOLATED FROM RABBITS BY POLYMERASE CHAIN REACTION.

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A B S T R A C T

The present study was performed on a total of 260 rabbits (48 diseased and 212 freshly dead ones) from rabbit farms at Kaliobia Governorate that inspected for *S. aureus*. Samples were taken from these rabbits (liver; heart blood; lung; intestine; kidney and spleen from each one) after clinical and postmortem examinationfor bacteriological examination. The results revealed that 314 out of 1560 samples (20.1%) were positive for *S. aureus* isolation, where 30 isolates (1.9%) from 288 samples of 48 diseased rabbits and 284 isolates (18.2%) from 1272samples of212 freshly dead ones.Moreover, higher rates of isolation of *S. aureus* from; liver (28.0%); heart blood (23.6%) ; lungs (22.0%) ; intestine (16.9%); kidneys (6.7%) and finally spleen (2. 9%) .Ciprofloxacin, Gentamycin, Norfloxacin and Sulpha trimethoprim were the most proper antibiotics with the highest in vitro efficiency against isolated *S. aureus.* PCR results showed that spa and clfA virulence genes were detected in 9 studied strains. While hlyA virulence gene was detected in 4 strains, ica A virulence gene was detected in 5 strains and Enterotoxins Sea, sec were detected in 4 out of 5 studied strains. Moreover, leukocidine (pvl) and enterotoxins (seb, sed, see) virulence genes were not detected in all studied strains. In addition, these results conceded the results obtained by dry spot Staphytect plus card test for spa and clfA.

Keywords: *Staphylococcus Aureus,* RABBITS**,** PCR

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1. INTRODUCTION

Staphylococcosis is one of the most serious problems that affect rabbits causing high economic losses not only due to high mortality in young rabbits but also for the debilitating effect, which predisposes for many other diseases (Corpa et al., 2009). The pathogenesis of Staphylococcosis in rabbit was previously described as the organism (*S. aureus*) may be residue in the nasal sinus or lungs and may be spread by direct contact or by aerosol. Infection of skin wounds is a common route of infection and result in supportive inflammation of the skin; subcutaneous abscesses and podo-dermatitis. Septicemia may also result from skin infection and in cases of acute septicemia; there may be fever, anorexia, depression and death. Septicemia may results in per acute death with only few nonspecific lesions however, if the rabbit survives this phase abscesses may be developed in many internal organs as heart, kidney, lungs, liver, spleen, testes and in joints leading to osteomyelitis(David and Partrick 1994; Vancraeynest et al., 2004). The Staphylococci are gram-positive cocci in the family Micrococcaceae, form grape-like clusters on Gram's stain, non-motile, non-spore forming facultative anaerobes that grow by aerobic respiration or by fermentation. Most species have a relative complex nutritional requirement, however;

in general, they require an organic source of nitrogen, supplied by 5 to 12 essential amino acids, e.g. arginine, valine and B vitamins, including thiamine and nicotinamide (Wilkinson, 1997). Members of this genus are catalase-positive and oxidase-negative, distinguishing them from the genus Streptococci, which are catalase-negative, and have a different cell wall composition to Staphylococci (Wilkinson, 1997). Staphylococci are tolerant to high concentrations of salt (Wilkinson, 1997) and show resistance to heat (Kloos and Lambe 1991). *S.aureus* produces a wide spectrum of virulence factors and many of the diseases caused by this bacterium in livestock, including rabbits, could be attributed to the virulence factors the bacteria produce. These virulence factors include , adhesion factors (collagen - binding protein, fibronectine-binding protein A/B, clumping factors A and intracellular adhesion A); toxins (enterotoxins, toxic shock syndrome toxin-1, Panton-Valentine Leukocidine); haemolysins; coagulase, thus clot blood; protease and protein A (Tenover and Gaynes, 2000; Etz et al., 2002; Vancraeynest et al., 2006; Meulemans et al., 2011; Tirpude and Batra, 2012). Though *S. aureus* contributes significantly to a variety of infections in rabbits, very little information is available on staphylococcal virulence factors in rabbit strains of staphylococci and their epidemiological relationship with Staphylococcosis in rabbits. On the contrary, most of the research and epidemiological surveillance is centered on staphylococcosis in man, cattle and goats. Thus, the present study was planned for bacteriological characterization of rabbit *S. aureus* isolates and detection of some virulence genes of the isolated strains by using Polymerase Chain Reaction.

2. 2. MATERIAL AND METHODS

2.1. Samples collection

A total of 260 rabbits of different ages and Sexes were examined in different rabbit farms at Kaliobia Governorate for bacteriological examination. Samples were taken from 48diseased rabbits and 212 freshly dead ones (liver; heart blood; lung; intestine; kidney and spleen from each rabbit) after clinical and postmortem examination. Each examined organ was taken alone in sterile plastic bag, kept in icebox and transferred with minimum delay to the laboratory for bacteriological examination.

2.2. 2.2. Bacteriological examination

The surface of organs was seared by hot spatula, and then a sterilized loopfuls were inoculated onto nutrient broth and incubated aerobically at 37ºC for 24 hours. A loopful from incubated nutrient broth was streaked into:7% salted nutrient agar; Baird parker agar; Mannitol salt agar; Milk salted agar and Blood agar.All plates were incubated for 24-48 hours at 37ºC.The developed colonies were picked up and subcultured for purification. The purified colonies were morphologically identified by Gram stain and biochemical tests (Quinn et al., 2002 and Arora, 2003), PCR and enterotoxin examination.

2.3. 2.3. In-Vitro anti-microbial sensitivity test:

The isolated *S. aureus* strains were subjected to the sensitivity test against different antibiotics, using the disc and agar diffusion method (Finegold and Martin, 1982).

2.4. Detection of Virulence genes of isolated S. aureus

2.4.1. Detection of clumping factor,

Protein A and capsular polysaccharide by Dry spot Staphytect plus card (Essers and Radebold, 1980).

2.5. 2. Detection and typing of enterotoxins of S. aureus strains:

By optimum sensitivity plate method (OTSP) recorded by Robbins et al., (1974).

2.4. 3. Virulence genes of S.aureus detection by PCR

PCR was applied by using ten sets of primers for detection of ten virulence genes that may play a role in virulence of ***S. aureus***. These genes were protein (spa) , clumping factor(clfA) , leukocidine (pvl), haemolysin(hlyA),intra –cellular adhesion (ica A) and enterotoxins (sea,seb,sec, sed,see). It was applied on 10 random isolated ***S.aureus*** following QIAamp® DNA Mini Kitinstructions (Catalogue no. M501DP100);Emerald Amp GT PCR mastermix (Takara) with Code No. RR310A and agarose gel electrophoreses (Sambrook et al., 1989).

3. RESULTS

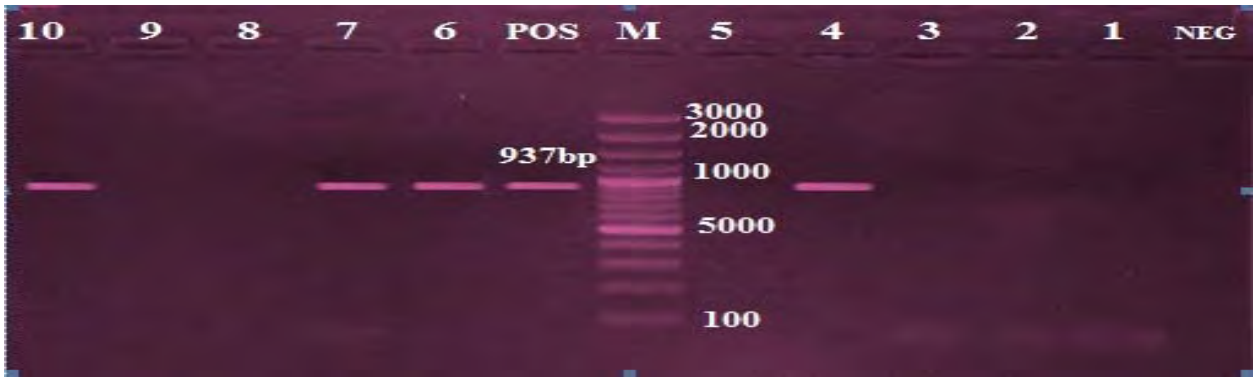
The clinical examination of studied rabbits showed clinical manifestations as anorexia, ruffed fur, depression, disinclination to move, diarrhea, slight respiratory manifestation with coughing, sneezing, catarrhal nasal discharge, podo dermatitis and subcutaneous abscess. Meanwhile, The postmortem lesions of freshen dead and scarified rabbits from which *S. aureus* were isolated are signs of septicemia including congestion with petechial hemorrhages in internal organs as liver, lung, spleen, kidneys, heart and intestine in young rabbits while abscessation in lung, liver, subcutaneous observed in adult rabbits.

The results of *S.aureus* isolation (Table1) showed that 314 out of 1560 samples (20.1%) were positive for *S.aureus* isolation, where 30 isolates (1.9%) were isolated from 288 samples of 48 diseased rabbits and 284isolates (18.2%) from 1272samples of212 freshly dead ones.

The bacteriological examination of studied organs revealed that, a total of 314 *S. aureus* strains were isolated, 88 from liver samples (28.0%);74 from heart blood samples (23.6%);69 from lung samples(22.0%) ; 53 from intestine samples (16.9%) ;21 from kidney samples (6.7%) and 9 from spleen(2.9%) as shown in Table (2) .

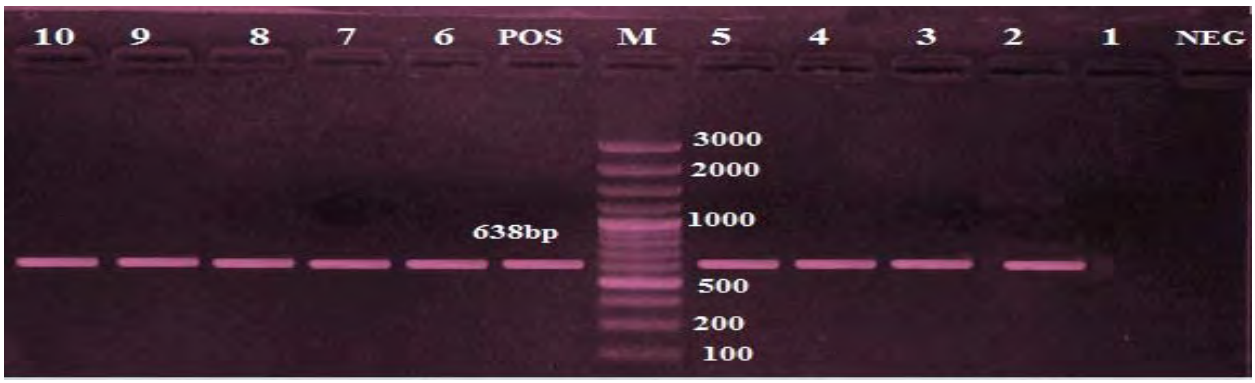
The in- vitro sensitivity tests (Table, 3) showed the isolated *S. aureus* were highly sensitive for Norfloxacin, Gentamycin, Sulpha trimethoprim and Ciprofloxacin but they were resistant to Vancomycin, Ampicillin, Doxycycline and Oxacilline.

The results of virulence genesdetection appeared that 19 out of 20 tested *S. aureus* strains had clumping factor, protein A and capsular polysaccharide by Dry spot Staphytect plus card; absence of enterotoxins inall 20 *S. aureus* strains tested by optimum sensitivity plate method (OTSP) and PCR results (Table,4) recovered that spa and clfA virulence genes were detected in 9 studied strains (90.0%). Meanwhile, hlyA virulence gene was detected in 4 studied strains (40.0%) and Enterotoxins Sea, sec were detected in 4 out of 5 studied strains and ica A was detected in 5(50%) studied strains . Moreover, leukocidine (pvl) and enterotoxins (seb, sed, see) virulence gene were not amplified in all studied strains. The hlyA gene was amplified in 4(40.0%) *S.aureus* strains giving product of 937 bp(photo, 1).The clfA gene was amplified in 9(90.0%) *S.aureus* strains giving product of 638 bp (photo, 2).The pvl gene was not amplified in all *S.aureus* strains and giving no product at 433bp bp(photo, 3). The spaA gene was amplified in 9(90.0%) *S.aureus* strains giving product of 226bp (photo, 4).The ica A gene was amplified in 5(50.0%) *S.aureus* strains giving product of 103 bp(photo, 5).The sea gene was amplified in 4(80.0%) *S.aureus* strains only and giving product at 102 bp(photo ,6).The seb gene was not amplified in all tested *S. aureus* strains and giving no product at 164 bp (photo,6).The sec gene was amplified in 4(80.0%) *S.aureus* strains only and giving product at 451 bp(photo ,6).The sed gene was not amplified in all 5 *S.aureus* strains and giving no product at 278 bp(photo, 7).The see gene was not amplified in all 5 *S.aureus* strains and giving no product at 209 bp (photo, 7).



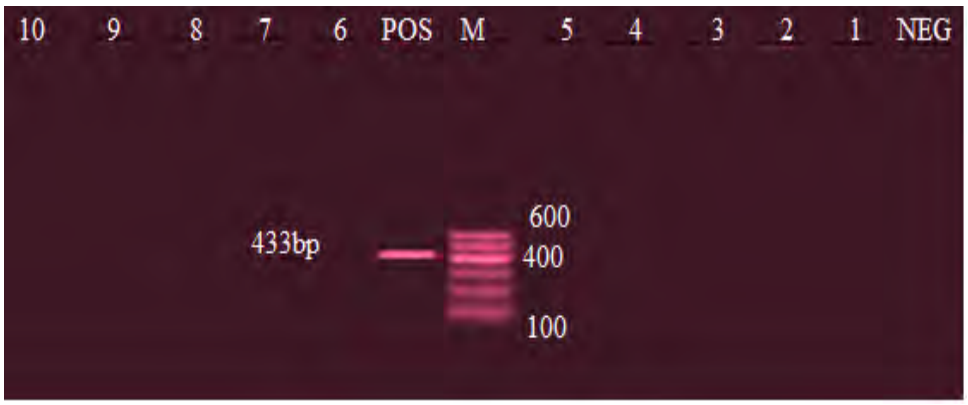
hlyA

Photo (1): Haemolysin (*hlyA*) gene. Lane L: 100-3000bpDNA Ladder. Neg.: Negative control. Pos.: Positive control (at 937 bp).Lane 1; 2; 3; 5; 8&9: *S. aureus* (Negative). Lane4; 6; 7&10: *S. aureus* (Positive).



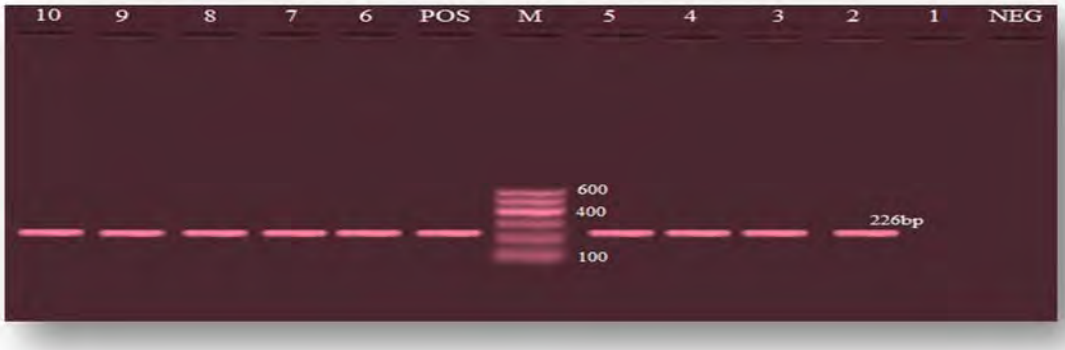
clfA

Photo (2): Clumping factor (*clfA*) gene. Lane L: 100-3000bpDNA Ladder.Neg.: Negative control.Pos.: Positive control (at 638 bp).Lane 1:*S. aureus* (Negative).
Lane2to Lane10: *Staph. aureus* (Positive).



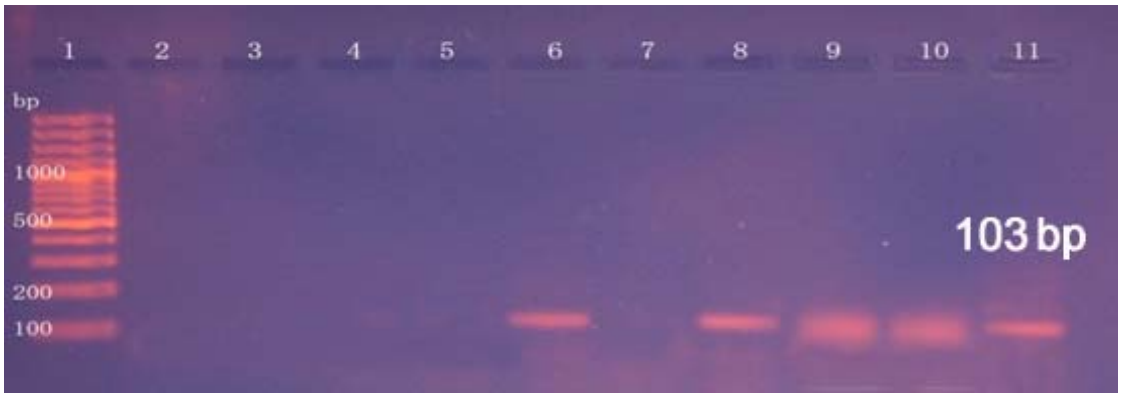
pvl

Photo (3): Leukocidine (pvl) gene. Lane M: 100-600bpDNA Ladder.Neg.: Negative control. Pos.: Positive control (at 433bp). Lane 1to Lane 10: *S. aureus* (Negative).



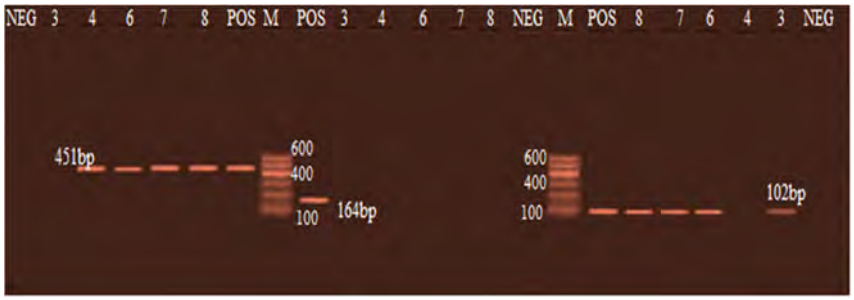
spa

Photo (4): protein A (spa) gene. Lane M: 100-600bpDNA Ladder. Neg.: Negative control. Pos.: Positive control (at 226bp). Lane 1: *S. aureus* (Negative).Lane2to Lane10:S. aureus (Positive).



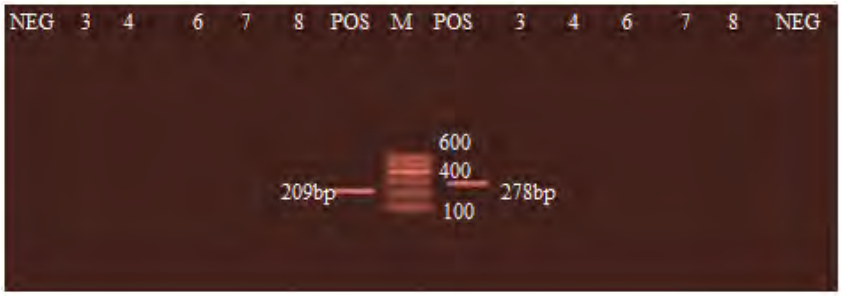
icaA

Photo (5):Intra-cellular adhesion (icaA) geneLane1 M: 100-1000 bp DNA Ladder.Lane2 ,3,4,5, &7: ***S.aureus*** (Negative). Lane 6, 8, 9, 10&11:***S.aureus*** (Positive at 103bp).



sea, seb, sec

Photo (6): Enterotoxins (*sea*, seb, sec) genes. Sea: Lane M: 100-600bpDNA Ladder. Neg.: Negative control. Pos.: Positive control (at102 bp). Lane 3, 6, 7&8: *S. aureus* (Positive).Lane 4:*S.aureus* (Negative). seb:Pos.: Positive control (at 164 bp). Lane 3, 4, 6, 7&8: *S. aureus* (Negative). Sec: Pos.: Positive control (at 451 bp). Lane 4, 6, 7 & 8: *S. aureus* (Positive).Lane 3: *S. aureus* (Negative).



sed, see

Photo (7): Enterotoxins (sed, see) genes. A. Sed: Lane M: 100-600 bp DNA Ladder. Neg.: Negative control. Pos.: Positive control (at 278 bp). Lane 3, 4, 6, 7&8: *S. aureus* (Negative). B. See: Pos.: Positive control (at 209 bp). Lane 3, 4, 6, 7&8: *S. aureus* (Negative).

Table (1): Percentage of ***S.aureus*** isolated from studied rabbits

Rabbit case	Number of rabbits	Number of sample	Positive samples numbers	Positive percentage of S.aureus		
				%[1]	%[2]	%[3]
Diseased	48	288	30	10.4	9.6	1.9
Freshly Dead	212	1272	284	22.3	90.4	18.2
TOTAL	260	1560	314	20.1	100.0	20.1

[1]Percentage in relation to total number of samples in each row
[2] Percentage in relation to total number of positive samples (314)
[3] Percentage in relation to total number of collected samples (1560)

Table (2): Total number and percentage of *S. aureus* isolated from different organs of studied rabbits' cases

Rabbit case		Number of rabbits	Positive Samples						Total			
			Liver	Heart Blood	Intestine	Kidney	Spleen	Lung				
			NO.	NO.	NO.	NO.	NO.	NO.	NO. of samples	NO. of Positive samples	Positive percentage %[1]	Positive percentage %[2]
Diseased		48	11	6	4	2	2	5	288	30	10.4	5.9
Freshly Dead		212	77	68	49	19	7	64	1272	284	22.3	55.8
TOTAL	NO.	260	88	74	53	21	9	69	1560	314	20.1	61.7
	%[3]	-	28.0	23.6	16.9	6.7	2.9	22.0	-	100.0	-	-

[1]Percentage in relation to total number of samples in each row
[2] Percentage in relation to total number of positive samples (509)
[3]Percentage in relation to tot al number of positive samples for ***S.aureus*** (314) .

Table (3): In-Vitro anti-microbial Sensitivity test for isolated *S.aureus*

Antibacterial agent	Disc content	*Staph.aureus*
Ampicillin	10 ug	R
Ciprofloxacin	5ug	S
Enrofloxacin	10 ug	S
Erythromycin	15 ug	R
Norfloxacin	5ug	S
Gentamicin	10 ug	S
Doxycycline	30 ug	R
Penicillin G.	10 units	R
Sulpha trimethoprim	25 ug	S
Amoxicillin	30 ug	R
Oxacilline	30ug	R
Vancomycin	30 ug	intermediate

N. B.: The beta lactamase antibiotics don't use in rabbit as it causes toxicity.

Table (4): The results of PCR amplifications of different used genes of *S. aureus*

Serial	Virulence genes									
	*hly*A	*clf*A	*pvl*	*spa*	ica A	Enterotoxins				
						Sea	*Seb*	*Sec*	*Sed*	*See*
1	-	-	-	-	-	Not done	Not done	Not done	Not done	Not done
2	-	+	-	+	-	Not done	Not done	Not done	Not done	Not done
3	-	+	-	+	-	+	-	-	-	-
4	+	+	-	+	-	-	-	+	-	-
5	-	+	-	+	+	Not done	Not done	Not done	Not done	Not done
6	+	+	-	+	-	+	-	+	-	-
7	+	+	-	+	+	+	-	+	-	-
8	-	+	-	+	+	+	-	+	-	-
9	-	+	-	+	+	Not done	Not done	Not done	Not done	Not done
10	+	+	-	+	+	Not done	Not done	Not done	Not done	Not done
Total NO.	4	9	0	9	5	4/5	0/5	4/5	0/5	0/5
%	40.0	90.0	0.0	90.0	50.0	80.0	0.0	80.0	0.0	0.0

- hlyA (haemolysin) clfA (clumping factor)
- pvl (leukocidine) spa (protein A)
- icaA (intera-cellar adhesion)sea, seb, sec, sed, see (enterotoxins)

4. DISCUSSION

The infection of rabbits with *S.aureus* is one of the most serious problems that affect rabbits causing high economic losses. Very little information is available on staphylococcal virulence factors in rabbit strains of staphylococci and their epidemiological relationship with Staphylococcosis in rabbits. Therefore, this study was planned for bacteriological characterization of rabbit *S.aureus* isolates and detection of some virulence genes in isolated strains. The results of clinical and postmortem examinations of studied rabbits were similar to that reported by (Ali, 1991;Hermans et al., 2003; Abd El-Gwad et al., 2004; Vancraeynest et al., 2004; Corpa et al., 2009; Tirpude and Batra2012).The results of *S. aureus* isolation, (Table, 1) revealed that a total of 314 strains (20.1%) were isolated, 30(1.9%) from48 diseased rabbits and 284(18.2%) from 212 freshly dead ones. These results came in accordance with that obtained by(Ali, 1991 ;Abd El-Gwad et al.,2004; Devriese et al.,2004; El-Genaidyet.al. 2006; Rougier et al.,2006; El-Sayed and Moustafa, 2007; Kohler et al .,2008 ; Hassan et al .,2009; Corpa et al.,2009) .Meanwhile, some reported higher incidence of *S. aureus* isolation (Nadung and Buoro, 1994; El-Sayed and Abd El-Latife, 2006; Segura et al.,2007) . Moreover, higher rates of isolation of *S.aureus* from; liver (28.0%) ; heart blood (23.6%) ; lungs (22.0%) ; intestine (16.9%); kidneys (6.7%) and finally spleen (2. 9%) as shown in Table (2)..Nearly similar results were recorded by (Devries et al.,1996;Hermans et al.,2003; Abd El-Gwad et al.,2004 ;Vancraeynest et al.,2006 ; Segura et al.,2007; Tirpude and Batra ,2012).The results of antibiotic sensitivity tests (Table, 3) revealed that, Ciprofloxacin, Gentamycin, Norfloxacin and Sulpha trimethoprim and were the most proper antibiotics with the highest in vitro efficiency against isolated *S.aureus* but they were resistant to Vancomycin, Ampicillin, Doxycycline and Oxacilline. These results go in parallel with those obtained by (Carucappa et al., 1991; Abd El-Gwad et al., 2004; Devriese et al., 2004; Cui et al., 2006; Kowalski et al., 2012). Our results disagreed with that recorded by (Nadung and Buoro, 1994) who reported that Ampicillin, Erythromycin and Chloramphenicol were the sensitive antibiotics.

PCR results (Table, 4) showed that, protease protein A (spa) and adhesion clumping (clfA) virulence genes were detected in (90.0%); intra-cellular adhesion (ica A) virulence gene was detected in (50.0%) and gamma haemolysin (hlyA) toxin virulence gene was detected in (40.0%) of *S.aureus*studied strains. While Enterotoxins sea, sec were detected in 4 out of 5 studied strains. Moreover, Leukocidine (pvl) and enterotoxins (seb, sed, see) toxin virulence genes were not detected in all studied *S.aureu* sstrains. Regarding to the occurrence of haemolysin (hlyA) gene in *S.aureus* isolates. Our result revealed that it was amplified in 4(40.0%) *S.aureus* strains giving product of 937 bp(photo, 1). These results came in accordance with those recorded by (Prêvost, 1995; Feng et al., 2012; Tirpude and Batra, 2012;Viana et al., 2012).The results of PCR for amplification of clumping factor A(clfA)gene in *S.aureus*isolates (photo, 2) showed that, the clfA gene was amplified in 9(90.0%) strains giving product of 638 bp. Similar findings were recorded by (Vancraeynest et al., 2004; Tirpude and Batra ,2012; Viana et al.,2012). Also, these results conceded the results obtained by dry spot Staphytect plus card test.The results of PCR for amplification of Panton-Valentine Leukocidine (pvl) gene of *S.aureus* (photo, 3) revealed that, the pvl gene was not amplified in all *S.aureus* strains and giving no product at 433bp bp . These results were agreed with those obtained by(Tavakol et al.,2012 ; Loncarie and Kunzet, 2013).On the contrary, these results disagreed with the findings of (Prêvost, 1995 ;Parklet etal.,2008 ;Liut et al.,2010 ; Ritz and Curtis,2012) who detect pvl gene in

S.aureus strains. The results of PCR for amplification of spa gene in *S.aureus* isolates (photo, 4) showed that, the spa gene was amplified in 9 (90.0%) *S.aureus* strains giving product of 226bp .Similar findings were recorded by (Parkletetal., 2008; Soong et al., 2011; Tavakol et al., 2012; Tirpude and Batra, 2012; Loncarie and Künzet,2013). In addition, these results conceded the results obtained by dry spot Staphytect plus card test. The results of PCR for amplification of ica A gene in *S.aureus* isolates (photo, 5) showed that, the ica A gene was amplified in 5(50.0%) *S.aureus* strains giving product of 103bp .Similar findings were recorded by (Parklet et al., 2008; Viana et al., 2012).Regarding to the occurrence of Enterotoxins (sea,seb,sec,sed,see) genes of 5 *S.aureus* isolates .Our result revealed that, the sea and sec genes were amplified in 4(80.0%) *S.aureus* strains only, giving products at 102 bp& 451 bp. Meanwhile the seb ,sed and see genes were not amplified in all isolates and giving no product at 164 bp, 278 bp and 209 bp respectively as shown in photo (6&7).Nearly similar results obtained by(Kohler et al.,2008; Argudin et al.,2010;Tirpude and Batra,2012 ; Viana et al.,2012; Mattis et al.,2013).

Finally, from results of the present work we could conclude that, higher percentage of *S.aureus* infection was detected in rabbits. Ciprofloxacin, Gentamycin, Norfloxacin and Sulpha trimethoprim were the most proper antibiotics with the highest in vitro efficiency against isolated *S. aureus* could be used for treatment in cases of their infections. Also, PCR could indicate that spa and clfA virulence genes were detected in 9 *S. aureus* studied strains. While ica A was detected in 5strains;hlyA virulence gene was detected in 4 strains and Enterotoxins sea, sec were detected in 4 out of 5 studied strains. Meanwhile, leukocidine (pvl) and enterotoxins (seb, sed, see) virulence gene were not detected in all studied *S. aureus* strains. To the best of our knowledge, it may be the first record of studying the virulence genes of *S. aureus* strains isolated from rabbits in Egypt.

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الكشف عن بعض جينات الضراوة في الميكروب العنقودى الذهبى الممرض للارانب بواسطة تفاعل البلمرة المتسلسل

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1. قسم البكتريا والفطريات والمناعة – كلية الطب البيطري – جامعة بنها. [2]معهد بحوث صحة الحيوان – فرع بنها

الملخص العربي

عدوى الميكروب العنقودى الذهبي من أهم العوامل التي تؤثر فى تربية الأرانب والتي تسبب خسائر اقتصادية كبيرة. وبالرغم من ذلك فأنه نادرا ما تقوم الدراسات على العوامل الممرضة في هذا الميكروب. وعلى ذلك فإن هذه الدراسة تلقى الضوء على هذه ا لميكروبات المعزولة من الأرانب و دراسة زراعتها على الأوساط الملائمة و كذلك الخصائص المورفلوجية و البيوكيميائية و عمل اختبارات الحساسية مع تحديد أهم الجينات الأكثر ضراوة بين العترات المعزولة و عمل تتابع نيوكليتيدى لبعض عناصر الضراوة. وقد أجريت هذه الدراسة على 260 أرنب (48 مريضة و 212 نافقة حديثا) وقد جمعت العينات من فئات الأرانب المختلفة من حيث العمر والجنس من مزارع مختلفة بمحافظة القليوبية وأخذت العينات من الكبد والرئة ودم القلب والأمعاء والكلى والطحال من كل حالة بعد إجراء الفحص الإكلينيكي والصفة التشريحية. وقد أظهرت نتائج العزل لميكروبات العنقودى الذهبى تواجد314 معزولة من أجمالي 1560 عينة بنسبة 20.1% حيث كانت 30 عترة بنسبة 1.9% تم عزلها من الارانب المريضة بينما تم عزل 284 عترة بنسبة 18.2% من الارانب النافقة حديثا و سجلت اعلى معدلات للعزل من الاعضاء المختلفة كالاتى من الكبد بنسبة 28.0 % يليها دم القلب بنسبة23.6 %و الرئة بنسبة 22.0 %و الامعاء بنسبة 16.9% و الكلى بنسبة 6.7% و أخيرا الطحال بنسبة 2.9%.أظهرت نتائج اختبارات الحساسية لعترات الميكروب العنقودى الذهبى المعزولة انها شديدة الحساسية لكل من السبرو فلوكساسين و الجنتاميسين و النورفلوكساسين والسالفاتراي ميثوبريم ومقاومة للفانكومايسين و الامبيسلين و الدوكسى مايسين و الاوكساسيلين . و لقد أوضحت نتائج اختبار تفاعل البلمرة المتسلسل لجينات الضراوة (spa, clf A ,icaA, hlyA, pvl, sea ,seb, sec, sed & see) للميكروب العنقودي الذهبي أن clf, spa ثبت تواجدهم بنسبة 90.0% و ica بنسبة 50.0% و hly بنسبة 40.0% و sec, sea في 4 من 5 عترات. بينما لم يثبت تواجد كلا من pvl, seb, sed&see فى جميع العترات.