

Studies on Corynebacterium pseudotuberculosis, Staphylococcus aureus and Staphylococcus aureus subsp. anaerobius isolated from sheep skin abscesses in Beni Suef Governorate.

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Clinical examination of 380 rearing sheep revealed that 30 animal were suffering from skin abscesses with an incidence of (7.89%). Bacteriological examination of 30 swabs from affected sheep revealed isolation of 30 isolates of *C. pseudotuberculosis* (48.39%) , 18 isolates of *S. aureus* (29.03%) and 14 isolates of *S. aureus subsp. anaerobius* (22.58).The isolated bacteria were identified morphologically and biochemically.The results of animal pathogenicity test showed that *C. pseudotuberculosis* isolates were 100% pathogenic to guinea pigs and 80% of *S.aureus* isolates were pathogenic to mice, while all isolates of *S. aureus subsp. anaerobius* were pathogenic to mice. The dead animals showed haemorrhage and symptoms of septicaemia, *C. pseudotuberculosis*, *S.aureus* and *S. aureus subsp. anaerobius* were reisolated from the dead animals. Antimicrobial sensitivity of *C. pseudotuberculosis* , *S. aureus* and *S. aureus subsp. anaerobius* isolates to some antimicrobial agents which usually used in farms showed that from 90% to 100% of *C. pseudotuberculosis* isolates were sensitive to erythromycin, amoxicillin, tetracycline ,streptomycin, ampicillin and rifampicin while *S. aureus* isolates (from 55% to 66%) were sensitive to rifampicin,tetracycline ,erythromycin and streptomycin, while *S. aureus subsp. anaerobius* isolates were moderately resistant to all used antimicrobial agents.

Corynebacterium pseudotuberculosis, the causative agent of Caseous lymphadenitis (CLA) which is a contagious bacterial disease that affects sheep and goats ,continues to cause considerable economic losses in ovine and caprine herds worldwide (Dorella *et al.*, 2009).The disease is characterized by abscess formation in the skin, internal and external lymph nodes, and internal organs, when the disease become endemic in a herd or flock it is difficult to eradicate by virtue of its poor response to therapeutics, its ability to persist in the environment, and the limitations in detecting subclinically affected animals. The disease causes significant economic impact on the small ruminant industry through decreased meat yield, damaged wool and leather, decreased reproductive efficiency, and mortality from the internal environment (Paton *et al.*, 1988; Williamson, 2001; Baired and Fontain, 2007; O'Reilly *et al.*, 2008).

The prevalence of CLA increases with age and the incidence increases only in young sheep after shearing. Sheep are sheared under unhygienic conditions, which may be a contributing factor in increasing both the prevalence and the incidence of CLA (Serikawa

et al.,1993; Al-Rawashdeh and Qudah 2000; Paton *et al.*, 2002). Bacteriological studies revealed that *C. pseudotuberculosis* was the pathogen which most frequently isolated, followed by *Staphylococcus aureus* (Tadayon *et al.*, 1980; Unanian *et al.*, 1985; Gezon *et al.*, 1991; Moller, *et al.*, 2000), and *Staphylococcus aureus subsp. anaerobius*, which is the etiological agent of abscess disease, a specific chronic condition of sheep and goats, which is characterized by formation of necrotic lesions that are located typically in superficial lymph nodes (Ben SaÃ *et al.*, 2002; De la Fuente *et al.*, 2010). This work was aimed to, threw light on *C. Pseudotuberculosis*, *S. aureus* and *S. aureus subsp. anaerobius* as a causative agents of skin abscess in sheep in Beni Suef Governorate, studying the pathogenicity of the isolated *C. Pseudotuberculosis*, *S. aureus* and *S. aureus subsp. anaerobius* to laboratory animals and the in vitro sensitivity of the isolated bacteria to some chemotherapeutic agents commonly used in farms.

Materials and methods

Bacteriological examination.

Samples. Out of 380 examined sheep for skin abscesses , 30 bactreological swabs were taken

from sheep showing skin abscesses randomly in different localities in Beni Suef Governorate and collected in sterile containers and transported directly to the laboratory in ice box.

Isolation and identification.

Media. Tryptic soya agar, tryptic soya broth, mannitol salt agar, sheep blood agar, Muller Hinton agar and Muller Hinton broth.

Isolation and identification. All samples were cultured onto different media in duplicate plates and incubated under aerobic and anaerobic conditions (in anaerobic jar containing 5% carbon dioxide) at 37°C for 24 to 48 h, isolated colonies were purified and identified morphologically and biochemically according to (Cruick Shank, *et al.*, 1975; Collee, *et al.*, 1996)

Coagulase test. Human, sheep and rabbit plasma were used for coagulase test of *Staphylococcus spp.* according to (Cruick Shank *et al.*, 1975; Collee *et al.*, 1996)

Animal pathogenicity.

Pathogenicity test for *C. pseudotuberculosis* isolates. Twenty-two guinea pigs (350-400 g weight) were used for testing the pathogenicity of 10 *C. pseudotuberculosis* isolates, two guinea pigs were used for each isolate and two controls. The animals were inoculated subcutaneously inside the lateral aspect of the hind limb with one dose of 1ml of 1×10^{13} c.f.u / ml according to method described by (Barakat *et al.*, 1973; Basma, *et al.*, 2003), the control G.pigs were injected with sterile saline.

Pathogenicity test for *S. aureus* and *S. aureus* subsp. anaerobius isolates. Sixty-three Swiss albino mice (15 – 20 g) were divided into three groups, group one (30 mice) for testing 10 *S. aureus* isolates, group two (30 mice) for testing 10 *S. aureus* subsp. anaerobius isolates, (three mice for each isolate) and group three (3 mice) were kept as control. Group one and two were injected intraperitoneally with 0.5ml of 5×10^9 C.F.U/ ml of *S. aureus* and *S. aureus* subsp. anaerobius isolates respectively according to (Calvinho and Dodd, 1994; Nevine, 2001), the control mice were injected with sterile saline.

The mice and guinea pig were kept under observation for 2 weeks before they were inoculated and kept under observation for 7 days post-infection.

Antimicrobial sensitivity of *C. pseudotuberculosis*, *S. aureus* and *S. aureus* subsp. anaerobius isolates to some chemotherapeutic agents. The sensitivity of *C. Pseudotuberculosis*, *S. aureus* and *S. aureus* subsp. anaerobius isolates to some antibacterial agents which usually used in farms was examined by disc diffusion method using Muller Hinton agar and Muller Hinton broth media according to (Collee *et al.*, 1996).

Results

Out of 380 examined sheep 30 animals were suffered from skin abscesses with an incidence of (7.89%). Bacteriological examination of 30 swabs collected from affected sheep revealed isolation of 30 isolates of *C. pseudotuberculosis* 18 isolates of *S. aureus* and 14 isolates of *S. aureus* subsp. anaerobius as shown in Table (1) and Table (2).

Regarding to microscopic examination and biochemical reaction *C. pseudotuberculosis* were small pleomorphic Gram-positive rods, aerobic and facultative anaerobic bacteria, colonies were white to cream in colour, regular, haemolytic, catalase and urease positive, fermented glucose and maltose. *S.aureus* and *S. aureus* subsp. anaerobius isolates were Gram-positive cocci oxidase negative coagulase positive, fermented mannitol, DNase and Lecithinase positive.

Animal pathogenicity. All injected guinea pigs dead within few days post injection with *C. Pseudotuberculosis* isolates and the post-mortem examination showed haemorrhage at the site of injection and haemorrhagic necrosis .On the other hand 80% of mice injected with *S. aureus* isolates were dead and showed symptoms of septicaemia, while all mice injected with *S. aureus* subsp. anaerobius were dead.

Table (1): Incidence of some bacterial isolates recovered from skin abscesses in sheep.

Bacterial isolates	No	%
<i>C. pseudotuberculosis</i>	30	48.39
<i>S. aureus</i>	18	29.03
<i>S. aureus</i> subsp. anaerobius	14	22.58
Total	62	100.00

% was calculated according to the total No of isolates

Table (2): Incidence of mixed bacterial infection in swabs from skin abscesses in sheep.

Bacterial isolates	No	%
<i>C. pseudotuberculosis</i> + <i>S. Aureus</i>	16	53.33
<i>C. pseudotuberculosis</i> + <i>S. aureus</i> subsp. <i>Anaerobius</i>	12	40
<i>C.pseudotuberculosis</i> + <i>S. aureus</i> + <i>S.aureus</i> subsp. <i>Anaerobius</i>	2	6.67
Total	30	100

Table (3): Coagulase activities of *S. aureus* and *S. aureus* subsp. *anaerobius* isolated from skin abscesses in sheep.

Species	No	Rabbit plasma		Sheep plasma		Human plasma	
		No	%	No	%	No	%
<i>S. aureus</i>	18	18	100	15	83.33	16	88.89
<i>S. aureus</i> subsp. <i>anaerobius</i>	14	14	100	13	92.86	14	100

Table (4): Pathogenicity test of some isolates of *C. pseudotuberculosis*, *S.aureus* and *S. aureus* subsp. *anaerobius* recovered from skin abscesses in sheep to the susceptible laboratory animals.

Bacterial species	No. of tested isolates	No. of infected animals	No. of dead animal per day							Total dead animal	
			1 st	2 nd	3 rd	4 th	5 th	6 th	7 th	No.	%
<i>C. Pseudotuberculosis</i> (guinea pig)	10	20	-	12	5	1	2	0	0	20	100
<i>S. aureus</i> (mice)	10	30	-	5	8	8	3	0	0	24	80
<i>S.aureus</i> -subsp. <i>anaerobius</i>	10	30	-	8	10	12	0	0	0	30	100

Table (5): Antimicrobial sensitivity of *C. pseudotuberculosis*, *S.aureus* *S. aureus* subsp. *anaerobius* isolated from skin abscesses in sheep to different chemotherapeutic agents.

Chemotherapeutic agents	<i>C. Pseudotuberculosis</i> isolates (30)			<i>S. aureus</i> isolates (18)			<i>S. aureus</i> subsp. <i>anaerobius</i> (14)		
	R	S		R	S		R	S	
		No	%		No	%		No	%
Erythromycin (15 µg)	0	30	100	8	10	55.56	6	8	57.14
Amoxicillin (30 µg)	2	28	93.33	10	8	44.44	8	6	42.86
Ampicillin (30 µg)	2	28	93.33	10	8	44.44	9	5	35.71
Streptomycin (30 µg)	3	27	90	8	10	55.56	7	7	50
Penicillin (10 IU)	20	10	33.33	18	0	0	14	0	0
Rifampicin (30 µg)	3	27	90	9	9	50	5	9	64.29
Tetracycline (30 µg)	2	28	93.33	6	12	66.67	5	9	64.29

S = Sensitive

R = Resistant

Discussion

Caseous lymphadenitis (CLA) of sheep, caused by *C. pseudotuberculosis*, has been a significant disease in the majority of sheep-rearing regions for over a century. Because of the chronic and often sub-clinical nature of the infection, it has proved difficult to control and prevalence in many parts of the world, which in turn leads to significant economic losses for farmers (Baired and Fontaine, 2007). Caseous lymphadenitis causes an annual loss of about \$17 million in wool production to the Australian wool industry (Paton *et al.*, 1994). *S. aureus* and *S. aureus* subsp. *anaerobius* were the microorganisms isolated from the affected animals beside *C. pseudotuberculosis*, (Tadayon, *et al.*, 1980; Ben Sañ *et al.*, 2002; De

la Fuente *et al.*, 2010). In this work out of 380 examined sheep 30 animals were suffering from skin abscesses with an incidence of (7.89%). Paton *et al.*, (2003) stated that, the average prevalence of CLA in adult sheep flocks was 26% and varied from 20% in Western Australia to 29% in New South Wales, Ellis *et al.*, (1990) stated that the overall prevalence of *C. pseudotuberculosis*-positive CL lesions in 104 sheep was 31.7% and Amany and Halla, (2008) recorded high percentage from slaughtered animals, the difference in results may be attributed to limitations in detecting subclinically affected animals and the methods used for examination of living animals. Bacteriological examination of 30 swabs from affected sheep revealed isolation of 30 isolates of *C.*

pseudotuberculosis (48.39%) and 18(29,03%) isolates of *S. aureus* as shown in table (1) and (2), Skalka *et al.*, (1998) and Komala *et al.*, (2008) isolated *C. pseudotuberculosis* from affected sheep at different rates and Amany and Halla (2008) isolated *C. pseudotuberculosis* and *S. aureus* from lymph nodes of slaughtered goat at percentage of 9.78 and 16.30 % respectively, the variation in rate of isolation was due to difference in localities and history of the disease. *S. aureus subsp. anaerobius* was isolated from 14 swabs (22.58%) Alhendi *et al.*, (1993) recorded an outbreak of abscess disease in goats in Saudi Arabia caused by *S. aureus subsp. anaerobius* infection, and BenSaA *et al.*, (2002) cleared that *S. aureus subsp. anaerobius* was particularly isolated from abscesses in sheep aged between 3 months and 2 years old. The results of microscopic examination and biochemical reaction of *C. Pseudotuberculosis*, *S. aureus* and *S. aureus subsp. anaerobius* agreed with the finding mentioned by Kathleen *et al.*, (2000), Nevine (2001), and Rosario *et al.*, (2000) respectively.

Regarding to virulence factor (coagulase activities) table (3) of *S. aureus* and *S. aureus subsp. anaerobius*, *S. aureus subsp. anaerobius* isolates, showing relatively higher coagulase activities on the all used types of plasma than *S. aureus* isolates, this achieved the results explained by (Ben SaA *et al.*, 2002, and De la Fuente *et al.*, 2010). Table (4) cleared that the tested isolates of *C. Pseudotuberculosis* were 100% pathogenic to guinea pigs the results agreed with Collee *et al.*, (1996) and resemble the results recorded by Basma *et al.*, (2003), post-mortem examination showed haemorrhage at the site of injection and haemorrhagic necrosis may be due to phospholipase D exotoxins produced by the injected *C. Pseudotuberculosis* isolates which explained by Zaki (2004). In case of *S. aureus* isolates 80% were able to killed mice in the first few days, the dead animals showed symptoms of septicaemia similar results were recorded by Nevine (2001) while *S. aureus subsp. anaerobius* isolates were 100% pathogenic to infected mice, this results agreed with Rosario *et al.*, (2000). Table (5) clearing the antimicrobial sensitivity of *C. pseudotuberculosis*, *S. aureus* and *S. aureus subsp. anaerobius* isolated from skin abscess in sheep to different chemotherapeutic agents, *C. pseudotuberculosis* isolates were sensitive to erythromycin, amoxicillin, tetracycline, streptomycin, ampicillin and rifampicin,

Kathleen *et al.*, (2000) recorded similar results on the other hand most of *S. aureus* and *S. aureus subsp. anaerobius* isolates were resistant to all antimicrobial agents used variable rate of resistance were recorded by Amany and Halla, (2008) and Kumar (2008). Variation in susceptibility of the isolates is return to the genetic contents of the isolates and the uses of the different chemotherapeutic agents by farmers. In conclusion the examination of 380 rearing sheep revealed that 30 animal were suffering from skin abscess with an incidence of (7.89%). Bacteriological examination of 30 swabs from affected sheep revealed isolation of 30 isolates of *C. pseudotuberculosis* (48.39%), 18 isolates of *S. aureus* (29.03%) and 14 isolates of *S. aureus subsp. anaerobius* (22.58). *C. Pseudotuberculosis* isolates were highly pathogenic to laboratory animals and sensitive to chemotherapeutic agents used while *S. aureus* and *S. aureus subsp. anaerobius* isolates were pathogenic to laboratory animals and moderately resistant to chemotherapeutic agents used.

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دراسات عن ميكروب السل الكاذب وميكروب المكور العقنقودي الذهبى والمكروب المكور العقنقودي الذهبى اللاهوانى

المعزولة من الأغنام المصابة بخرايج الجلد فى محافظة بنى سويف

فى دراسة عشوائية لبعض الميكروبات المسببة لخرايج الجلد فى الأغنام وجد أن فحص ٣٨٠ حيوان أسفر عن وجود ٣٠ (٧.٨٩٪) حالة مصابة بخرايج جلدية وعند أخذ مسحات بكتيريولوجية من تلك الخرايج وفحصها بالمعمل تم عزل ميكروبات السل الكاذب من جميع العينات (٤٨.٣٩٪) كما تم عزل ميكروب المكور العقنقودي الذهبى من ١٨ عينة (٢٩.٠٣٪) وميكروب المكور العقنقودي الذهبى اللاهوانى من ١٤ عينة (٢٢.٥٨٪) وتم دراسة الصفات الشكلية والبيوكيميائية للميكروبات المعزولة وعند حقن مجموعة من خنازير غنيا بميكروبات السل الكاذب ومجموعة من فئران التجارب بمعزولات الميكروب العقنقودي الذهبى والمكروب اللاهوانى ظهر على هذه الحيوانات أعراض مرضية ونفقت جميع خنازير غنيا المحقونة و ٨٠٪ من فئران التجارب المحقونة بميكروبات المكور العقنقودي الذهبى وجميع الفئران المحقونة بميكروب المكور العقنقودي الذهبى اللاهوانى وأعيد عزل الميكروبات مرة أخرى من هذه الحيوانات وتم إجراء اختبار حساسية الميكروبات المعزولة لبعض المعالجات الدوائية الشائعة الأستعمال فى المزارع ووجد أن ميكروبات السل الكاذب أظهرت حساسية عالية لكل من عقار الريفامبيسين الأريثروميسين والأموكزيسلين والأمبيسلين والتتراسيكلين والستربتوميسين فى حين أن الكثير من ميكروبات المكور العقنقودي الذهبى كانت مقاومة لبعض العقاقير المستعملة إلا أن من ٥٥ إلى ٦٠٪ كان حساس لكل من عقار الريفامبيسين والتتراسيكلين والأريثروميسين والستربتوميسين فى حين أظهر ميكروب المكور العقنقودي الذهبى اللاهوانى درجة مقاومة متفاوتة للعقاقير المستخدمة.