



SIGNIFICANCE OF STAPHYLOCOCCUS AUREUS IN RABBITS IN ASSIUT GOVERNORATE

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ABSTRACT :

A total of 67 rabbits (18 apparently healthy and 49 freshly dead rabbits were collected from different private rabbitaries at Assiut Governorate. All cases were subjected to post-mortem and bacteriological examinations for possible recovery of *Staphylococcus aureus* microorganisms. Only 15 positive cases of *Staph. aureus* were isolated with an incidence of 22.38% (out of 18 apparently normal rabbit, 2 (11.11%) rabbits were affected with *Staph. aureus* while, from 49 freshly dead rabbits 13 (26.53%) were positive for *Staph. aureus*. Experimental infection in 6-8 week-old rabbits via the subcutaneous (S/C) injection of isolated *Staph. aureus* led to 80% mortality with post-mortem lesions similar to a great extent to those of natural infection. Reisolation of inoculated bacteria was also done. The in vitro sensitivity testing of the isolated *Staph. aureus* against different antimicrobial agents showed that the examined isolates were highly sensitivite to ciprofloxacin, enerofloxacin, amikacin and gentamycin.

INTRODUCTION:

Staphylococcosis is a commonly occurring disease resulting in a fatal generalized septicemia or in a localized suppurative inflammation. *Staph. aureus* is common disease in both domestic and wild rabbits (Flatt, 1974). The disease is usually sporadic and of little economic importance for commercial rabbitries. Most often, suppurative lesions with Staphylococcal infection, often lead to chronic abscessation in affected sites. The acute septicemia from occurs mostly in neonatal kits and can have lesions ranging from few and nonspecific to multifocal and suppurative in various organs, including the lung, kidney, spleen, heart and liver.

In Egypt, the effect of *Staph. aureus* in rabbits was studies by Saad (1970); Abd-EL-Motelib (1982), Ali (1991) and Abd-EL-Motelib and Salem (1991). Abd-EL-Motelib (1982) isolates *Staph. aureus* in addition to *Pasteurella lepisepctica* from dead rabbits in Assiut Governorate, Ali (1991) recovered that 48 isolates (23.1%) were *Staph. aureus* from dead rabbits and (25.1%) from diseased rabbits from respiratory stress. Abd-EL-Motelib and Salem (1991) found that six strains of *B. haemolytica* and coagulase-positive *Staph.aureus* were isolated from infecting lactating does and their suckling rabbits. Micheal and Eckhaus (1997) isolated *Staph. aureus* from an intra uterine site of adult female pregnant rabbits with metritis and fatal death. Shirliff and Mader (1999)

concluded that the Zithromycin and Clarithromycin may be attractive partners for Rifampin for treatment of Osteomyelitis in rabbits. Richard (1997) isolated Staph from all 5-month old New Zealand four rabbit with mild mucosuppurative discharge. Renquist and Soave (2001) found that Staphylococcosis caused by *Staph. aureus* may be manifest as fat pneumonia in rabbits Ajuwape and Aregbesola (2002) isolated *Staph.aureus* from upper respiratory tract of healthy rabbits with an incidence of 100%. Dalia *et al* (2003) recovered that *Staph. aureus* is frequently associated with bacterial conjunctivitis and keratitis in rabbits.

To the best of our knowledge there is no complete data about the problem of rabbit Staphylococcosis in Egypt, inspite of its importance as a common disease in rabbit farms. Therefore the present work was designed to cover the fallowing items:

- Isolation and identification of *Staph. aureus* from apparently health and dead rabbits in Assiut Governorate.
- Experimental infections using the isolated organism in 6-8 weeks old rabbits by subcutaneous route.
- In vitro sensitivity testing of the isolated *Staph.aureus* strains against different antibiotics.

MATERIALS AND METHODS:

Materials:

1-Samples:

A total of 67, rabbits (18,apparent healthy slaughtered and 49, freshly dead rabbits) of various ages, sex and of different breeds, were collected from different private farms at Assiut Governorate, and subjected to post-mortem examination. Tissue samples from liver, heart, lung, kidney, spleen, uterus as well as samples from suppurative lesions were collected from

these cases and subjected to bacteriological examination.

2- Media:

a-Liquid: Sodium chloride broth, Pepton water, nutrient broth, glucose phosphate broth, semi-solid agar and sugars (glucose, lactose, lactose, maltose, sucrose, galactose and xylose)

b-Solid: Barid-parker agar, blood agar, nutrient agar, simmon' s citrate agar, and triple sugar iron agar and urea agar base.

3-Reagents, chemical and stains:

Kovac's, urea, methyl red, oxidase, andrade' s indicator, rabbit sera and Gram' s stain.

4- Experimental animals:

Sixteen, 6-8-week-old balady rabbits obtained from private farms, Assiut were used for pathogenicity tests.

5-Antimicrobial sensitivity discs:

were produced by Oxoid-Laboratories including: Gentamycin (10/ug), Kanamycin (30ug), Steptomycin (10/ug), Neomycin (30/ug), Amoxicillin (25/ug), Ampcillin (10/ug), Erythromycin (15/ug), Ciprofloxacin (5/ug), Penicillin (10/ug), Amikacin (30/ug), Cloxacillin (5/ug), Enrofloxacin (5/ug), Linco-spectin (15/ug), and Tobramycin (5/ug).

Methods:

1-Isolation and Identification of Staph. strains:

Samples from individual rabbits including liver, heart, lungs, kidney, spleen, uterus and pus were collected a septicly. Lopopfull from these samples were inoculated into Sodium chloride broth and incubated at 37⁰C for 18-24 hour, followed by sub-culturing on solid media as: Baried-parker agar media at 37⁰C for 48 h as a selective medium for Staphylococci (Baird-

parker, 1962), Blood agar and nutrient agar media at 37°C for 24 h. The obtained colonies were picked up and stored in semisolid agar for further identification, morphologically, microscopically and biochemically according to Cruickshank *et al.* (1975) and Baird-parker (1979).

2- Pathogenicity test:

Sixteen, 6-8 week-old balady rabbits were used in this study. The animals were kept in cages and observed for a period of a week. A random sample of 3 rabbits was slaughtered and exposed to post-mortem, parasitological examination for coccidia and other parasites and bacteriological examinations for *Staph. aureus* and other pathogenic bacteria, which proved their healthy status and free from diseases and the other thirteen, were classified into 2 groups: Group 1 (10) rabbits were inoculated subcutaneously with 0.5 ml of 24h. broth culture (30×10^{10} viable organism/ rabbit) while Group 2 (3) rabbits were kept without inoculation as non-infected control. All animals were kept for 30 days (period of observation) with daily examination for clinical signs, mortality rate and gross P.M. lesions in dead and scarified animals till the end of the observation period as well as trials of reisolation were conducted.

3- Reisolation of infecting organism:

4- Antimicrobiol sensitivity test:

The paper disc technique was carried out after Finegold and Baron (1986) using the identified *Staph. aureus* isolates and 15 chemotherapeutic discs produced by Oxoid. Basingstake, Hampshier, England in order to determine their antibiogram.

RESULTS:

1-Isolation and Identification :

According to morphological and biochemical characters, 15 isolates (22.38%) were identified to be *Staph. aureus*. These isolates were Gram positive cocci usually arranged in clusters and characterized by its typical growth of coagulase positive, black colonies surrounded by faint yellow zone on Baird-Parker agar media and its haemolytic activity on blood agar media. Frequency and percentage of isolates are summarized in Table (1).

2-Experimental infection in rabbits:

The clinical signs noticed were: loss of appetite, ruffled fur, depression, disinclination to move, inclination to separate in the corner of the cage followed by mucopurulent conjunctivitis, sever coughing with pus from nose, diarrhoea. In some cases abscesses may be palpated in site of inoculation and in subcutaneous tissue. In the last stages sick animal showed emaciation followed by death.

The P.M. lesions of dead and survived rabbits include: congestion of internal organs (liver, lung, spleen, and kidney, engorgement of heart and intestine blood vessels. Pneumonia could be observed in most of the examined cases, trachea filled with mucopurulant exudates and in few cases abscesses may be observed in lung, liver. Catarrhal enteritis may be observed in some cases. No symptoms were observed in control group. The results of pathogenicity tract are given in Table (2).

3-Reisolation trials were positive from internal organs and suppurative tissues of dead and scarified rabbits.

4-Antimicrobial sensitivity test: The effect of different antibiotics on isolated *Staph.aureus* isolates is illustrated in Table (3).

Table (1): The frequency percentage of Staphylococcus aureus isolates

General status of examined rabbit	No. of examined	Staph. aureus isolates
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	rabbits	No.	%
Apparently healthy slaughtered	18	2	11.11
Dead rabbits	49	13	26.53
Total	67	15	22.38

Table (2): Showing results of pathogenicity of Staph. aureus in 6-8 week-old rabbits

Group1	No. of inoculated rabbits.	Route of infection	Dose of inoculums	Daily deaths post infection											No. of death	No of survivors	Mortality
				1	2	3	4	5-6	7	8-17	18	19	20-30				
1	10	S/c	30x10 ¹⁰	0	1	0	1	0-0	2	0	3	1	0-0	8	2	80%	
2	3	Cotrol	0	0	0	0	0	0-0	0	0	0	0	0-0	0	3	0.00%	

Table.(3): Showing results of sensitivity test of Staph. aureus isolates against different antimicrobials

Antimicrobial agents	Sensitivity of Staph. aureus isolates
Ciprofloxacin	+++
Enerofloxacin	+++
Amikacin	+++
Gentamycin	+++
Ampcillin	++
Steptomycin	++
Tobramycin	++
Rifampein	++
Linco-spectan	+
Amoxacillin	+
Cloxacillin	-
Neomycin	-
Erythromycin	-
Penicillin	-
Kanamycin	-

+++ = Highly sensitive, ++ = Moderately sensitive,
 + = Weakly sensitive, - = Resistant

DISCUSSION:

The pathogenesis of Staphylococcosis in rabbit was previously described by Richard (1997) as the organism (*Staph.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 suppurative inflammation of the skin and subcutaneous. 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 peracute death with only few non specific 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.

As evident from our results the bacteriological examination of the specimens revealed that *Staph. aureus* was recovered from 22.38% of the examined rabbits. A nearly similar finding was mentioned by Ali (1991) 23.1% in Egypt. A lower percentage was reported by Abd-EL-Motelib and Salem (1991) who isolated six strains of *B. hemolytic* coagulate positive *Staph. aureus* from infecting lactating does and their suckling rabbits. A much higher percentage was described by Ajuwape and Aregbesola (2002) who isolated *Staph. aureus* from upper respiratory tract of health rabbits with an incidence of 100%.

The P.M. lesions of freshing dead rabbits from which *Staph. aureus* was isolated are signs of septicemia including congestion with pectichial haemorrhages in internal organs as liver, lung, spleen, kidneys, heart and intestine in young rabbits while abscessation in lung, liver, subcutaneous observed in adult rabbits. In few cases endometritis with mucopurulent inflammation of uterus in female pregnant does. Exactly more or less the same observation was recorded by Hagen (1958), Ostler (1961), Saad (1970), Ali (1991), Michael and Eckhaus (1997), Richard (1997) and Dalia *et al* (2003).

Pathogenicity test of *Staph. aureus* conducted on 6-8 week old healthy rabbits by S/C route proved the pathogenic natures of the tested isolate with 80% mortality. The rapid death which occurs in the first week of experimentally infected animals suggesting the highly toxigenic nature of the organism and this similar that reported by Adlam *et al* (1976) while the suppurative lesions occurs in rabbits died in last day and in survivors indicate the chronic form of the organism and this near to some extent to result reported by Michael and Eckhaus (1997). In the present study our results of experimental infection was lower to the results were reported by Abd-EL-Motelib and Salem (1991) who

found that *Staph. aureus* caused 100% mortality in experimentally infected rabbits with the same dose and route of inoculation.

Reisolation of the organism from dead and scarified slaughtered animals were conducted and this proved that the inoculated isolates were responsible for pathogenic affect mentioned before.

In vitro sensitivity testing of the isolated *Staph. aureus* strains to 15 antimicrobial agents revealed that the isolates examined were highly sensitivity to Ciprofloxacin, Enerofloxacin, Amikacin and Gentamycin and moderately sensitivity to Rifampein, Ampcillin, Tobramycin, Streptomycin while Amoxacillin, Linco-spectan are weakly sensitive but Kanamycin, penicillin, Neomycin, Cloxacillin, Erythromycin had no effect at all. Our results agree to some extent with those reported by Carucappa *et al* (1991), Abd-EL-Motelib and El-Zanaty (1993) and Abdel-Hafeez (2002) but to less extent with those reported by Nabila (1982), Ashgan (1988), Abd-EL-Motelib and Salem (1991). Further more, our results completely disagree with those reported by Nader *et al* (1986) who mentioned that no drug was effective against all *Staph. aureus* strains.

Finally it may be concluded from the present investigation that, it is very important to improve hygiene and nutrition as well as early recognition of this sever form of Staphylococcosis by practitioners are of great value for avoidance further spreading of rabbit pathogenic. *Staph. aureus* strains and minimize losses from this diseases in rabbits, Also applied filed treatment with antibiotics in affected rabbits for three successive days gave a satisfactory results.

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دلالات وجود الميكروب المكور العنقودي الذهبي فى الأرناب بمحافظة أسيوط عبد التواب محمد عبد الجواد، عبد الرحمن عبد المجيد عبد الرحمن ، محمد مصطفى على

لدراسة مدى تعرض مزارع الأرناب الخاصة بمحافظة أسيوط للإصابة بالميكروب المكور العنقودى الذهبى . أجريت الفحوص البكتريولوجية لعدد ٦٧ أرنب منها ١٨ أرنب سليم ظاهريا و ٤٩ أرنب حديثة النفوق يشتهبه فى إصابتها بالميكروب المكور العنقودى الذهبى . أوضحت الفحوص البكتريولوجية عزل ١٥ حالة إيجابية لميكروب العنقودى الذهبى بنسبة ٢٢.٣٨%، وكانت نسبة العزل فى الأرناب السليمة ظاهريا ١١.١١% بينما كانت هذه النسبة مرتفعه فى الحالات النافقة ٢٦.٥٣% . وبأجراء العدوى الصناعية بالميكروب المعزول فى الأرناب عند عمر ٦-٨ أسابيع بواسطة الحقن تحت الجلد أتضح شدة ضرورتها للأرناب حيث وصلت نسبة النفوق إلى ٨٠% وقد كانت الآفات التشريحية تشبه إلى حد كبير تلك التي سجلت فى العدوى الطبيعية . هذا وقد تم عزل الميكروب مرة أخرى من الأرناب النافقة . وبإجراء اختبار الحساسية لهذا الميكروب باستخدام أنواع مختلفة من المضادات الميكروبية وجد أنها جميعا عالية الحساسية لكل من السيبروفلوكساسين والأنروفلوكساسين و الأميكاسين والجنتاميسين .