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Clinical Features of Methicillin-Resistant *Staphylococcus aureus* (MRSA) Infection in Rabbits and its Zoonotic Potentials

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Abstract: Staphylococcosis caused by methicillin-resistant *Staphylococcus aureus* (MRSA) infection in rabbitry is neglected as a cause of rabbit production impairment and zoonoses. This study aimed to monitor a rabbit flock problem associated with MRSA infection by following a rapid and simple sampling and diagnostic scheme. Resistance to antibiotics and zoonotic risk posed to human contact and food contamination were assessed. The identification of *S. aureus* was carried out by traditional bacteriological methods that confirmed with PCR. Results showed that a total of 80 (39.21%) out of 204 animals from apparently healthy rabbit were MRSA positive. The isolation rate was highest from nose/eye (26.47%), followed by skin affections (8.82%) and vaginal/perineum (3.92%) sampling sites. In post-mortem examination, MRSA was positive in 55 (26.96%) out of 204 animals. Isolation rate from lung was (22.05%) that was higher than from uterus (7.81%). Resistance to antibiotics was shown in 59.9% of the isolates. All tested isolates were methicillin and oxacillin resistant strains. Vancomycin and oxytetracycline also were resistant in 91.66% of strains. Ciprofloxacin is considered the drug of choice for treating multidrug resistant MRSA infections. MRSA was isolated from nasal swab of attendant and slaughterhouse workers (42.6%). In conclusion, high mortalities and infertility of rabbits caused by multi-drug resistant strains of MRSA with dissemination to environment and contamination of rabbit meat shed the light on its impacts on rabbit production and public health. Thus, large-scale epidemiological investigations of *MRSA* in rabbitry in Egypt are needed.

Key words: *Staphylococcus aureus*, MRSA, infertility, rabbit, antimicrobial, zoonoses

INTRODUCTION

Staphylococcus aureus (*S. aureus*) is an opportunistic pathogen able to persist and multiply in a variety of environments and to cause a wide spectrum of diseases in humans and animal species (Cucarella *et al*., 2004). In particular, methicillin-resistant *S. aureus* (MRSA) infections are major problem in rabbitries (Mork *et al*., 2005). Rabbit infections with *S. aureus* could be distinguished into low and high-virulent strains, in low virulent strains the disease remains limited to a small number of animals with minor economic significance, while infection caused by high-virulence strains causes an epidemic spread of disease in the rabbitry (Devriese *et al*., 1981; Hermans *et al*., 2000). International dissemination of high virulent *S. aureus* clone in rabbits has been reported (Vancraeynest *et al*., 2006).

Clinical diseases caused by *S. aureus* infections in rabbits are dermal lesions, pododermatitis, abscesses and mastitis; consequently lead to poor production and mortality in young and infertility of the breeders (Corpa *et al*., 2009; Hermans *et al*., 2003). As a result of agalactia in the doe caused by *S. aureus*, suckling rabbits acquire infection during lactation that lead to high mortality rates in offspring. Therefore, staphylococcal mastitis is considered to be one of the main reasons for culling adult does from rabbitries (Rosell and de la Fuente, 2009; Segura *et al*., 2007). Moreover, chronic staphylococcosis infection renders infected rabbitries unremunerative, administration of antibiotics, disinfection of the environment and vaccination are not able to solve the problems (Hermans *et al*., 2003; Lee *et al*., 1996) and the only feasible method is to cope with rabbit staphylococcosis.

From the public health perspective, although *S. aureus* transmission appears to be primarily between animals, indistinguishable isolates have been isolated from those with occupational exposure to animals (Armand-Lefevre *et al*., 2005; Voss *et al*., 2005). It has been reported that human biotypes of *S. aureus* can survive and colonized in rabbitries (Devriese, 1984; Hermans *et al*., 1999). Food chain contamination by *S. aureus* could be occur (Mead and Dodd, 1990). Moreover, there is an

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additional concern over the potential transmission of resistant organisms and resistance genes from livestock to man through consumption and handling of foods derived from animals (Rodriguez-Calleja *et al*., 2006; Tollefson and Karp, 2004).

In Egypt, *S. aureus* infections of rabbits are neglected as cause of production impairment and zoonoses and there is a lack of reports concerning these issues. Therefore, this study aimed to investigate a rabbit flock for staphylococcosis by simple sampling for diagnostic scheme and estimating zoonotic risk posed to human contact and food poisoning risk as well as antibiotic resistance of the isolates.

MATERIALS AND METHODS

Study population: The rabbits included in this study were 204 of New Zealand white rabbits (72 dams, 8 bucks and 124 young rabbits rabbit), the average ages of the adults was from 22 to 28 month and off spring aged from one to three months, the flock belongs to the farm of faculty of Veterinary Medicine, Suez Canal university, Ismailia, Egypt. Flock was regularly vaccinated with recommended doses for viral hemorrhagic disease and formalin-inactivated *Pasteurella multocida* vaccines (Veterinary vaccine and Anitsera Institute, Abbasia, Cairo, Egypt). In addition anthelmentics (Ivermictine) 1% was administered regularly.

Flock showed high mortality rate reached 30% in newly born (from birth to one month) and 10.4% in broiler rabbit, low body gain in growing young rabbit were noticed. In dams, delayed in conception rate and infertility (prevalence of complete infertile female reached 82.4%) were recorded. In addition, intermittent conjunctivitis, nasal discharge and skin abscesses, pododermatitis and mastitis with teat inflammations were observed as a general problem in the flock. The postmortem lesions recorded during slaughtering rabbits. Generally carcasses showed congestion of visceral organs in most cases, engorgement of heart and intestine blood vessels. Pneumonia was predominant, while catarrhal enteritis and subcutaneous abscess were occasionally observed.

The flock was medically followed for six months with trial for treatment with recommended doses of antibiotics including oxytetracycline, enrofloxacin, erythromycin without progress or curing the clinical cases.

Specimen collection

Swabs from live and slaughtered rabbits: Swab samples were collected using sterile cotton swabs from two body sites from all rabbits (N = 204), eye and nose as one sample, vagina in female or perineum in male and from skin lesions found in 18 rabbits. In addition, lung of all rabbits (N = 204) and uterus of 128 females were collected from slaughtered rabbit carcasses and transported in sterile containers in ice to the laboratory with minimal delay and swabs were collected from uterine contents and deep bronchi of lung under complete aseptic conditions.

Swabs from rabbit attendants and cages and slaughterhouse places: Nasal and hands swabs (swabs from both hands were mixed in one sample) from persons contacting the rabbits; including two attendants, two veterinarians and three slaughter house workers were collected. A total of ten swabs, including four from cages, four floors and walls of slaughterhouse places and two from knife blades after slaughtering were also collected.

Bacteriological examination: Swabs were inoculated into 2 mL of enrichment tryptone Soya broth containing 7% (w/v) sodium chloride, Following 24 h incubation, at 35°C, a loopful of vortexed broth was inoculated onto selective Mannitol salt agar and cultured at 37°C for 24-48hr.

Colonies were identified according to morphology; yellow-colored colonies as a result of mannitol fermentation and subsequent drop in the medium's pH were collected. Doubtful colonies were re-incubated in Columbia Agar Base medium added with sheep red blood cells (5% v/v). The obtained colonies were picked up and stored in semisolid agar for further identification according to their characteristics according to Kloos and Bannerman (1999). Any rabbit with at least one positive sampling was considered a “true positive”. Control positive Strain *Staphylococcus aureus*, Designation; NCTC7447/ATCC ®6538P.

Polymerase chain reaction amplification: To confirm microbiological and biochemical techniques, the positive isolates were further tested by PCR. Briefly, DNA was extracted by taking one colony of fresh bacterial growth from a blood or Manitol Salt agar plates, washed 3 times then suspended in 800 µl of sterile distilled water and boiled for 10 min and centrifuged at 7, 500 g for 5 min and the supernatant was used as DNA template. The PCR was performed in a 25 µl volume with 5 µl of 5X Master Mix (Jena Bioscience, GMBH, Germany) and 50 pmol concentrations of each of the primers, distilled water was added to bring the final volume to 25ul with a primer set that anneals to the *S. aureus* 16S rRNA gene according to Monday and Bohatch (1999) that generates a 228-bp amplicon during the amplification process using primers forward primers 5-GTAGGTGGCAAGCGTTATCC-3 and reverse primers 5-CGC ACATCAGCGTCAG-3. PCR conditions were started by initial denaturation step of 2 min at 95°C, followed by 30 cycles of 95°C for 30 min, 60°C for 30 s and 72°C for 30 seconds. The reaction was terminated

with a 10-min incubation at 72°C. PCR products were resolved by electrophoresis in 1.5% agarose Gel and visualized on a transilluminator. Product sizes were determined by using the 100 pb DNA molecular weight ladder.

Antimicrobial susceptibility testing: Susceptibilities to antibiotics commonly administered to rabbits were determined by modified Kirby-Bauer disk diffusion methods in Mueller-Hinton agar according to the Clinical Laboratory Standards Institute, formerly NCCLS guidelines. Strains were screened for resistance to different antibiotics using antibiotic discs. Antibiotic discs used and its concentrations were shown in Table 2. Results were recorded according to the zone size and interpreted in accordance with the criteria of the Clinical and Laboratory Standards Institute (2005).

RESULTS

Bacteriological and molecular identification of the isolates: Results revealed that all isolates were identified to Staphylococcus species (catalase test positive) and to *S. aureus* indicated by positivity of coagulase test, DNAse (zone of clearance on nutrient agar), lipase (a yellow color and rancid odor smell) and phosphatase (a pink color) tests. All the isolates were confirmed to *S. aureus* by PCR amplification of 228-bp amplicon of *S. aureus* 16S rRNA gene.

Total prevalence of MRSA in ante-mortem and post-mortem samples: As tabulated in Table 1 and 2, a total of 80 out of 204 swabs (39.21%) from rabbits were MRSA positive. Regarding the sampling from different anatomical body sites, the isolation rate was higher from nose/eye (26.47%), followed by skin affections (8.82%) and vaginal/perineum (3.92%) sampling sites. Bucks (62.5%) were higher in isolation rate than dams (51.38%) and male broilers were higher than females by percentages of (32.35%) and (28.57%) respectively. In postmortem examination, MRSA was positive in 55 out of 204 organs of slaughtered animals (26.96%). Isolation rate from lung was (22.05%) that was higher as compared to uterus 10/128 (7.8%).

Antimicrobial susceptibility patterns: As shown in Table 3, the *in vitro* antibiotic sensitivity test of 12 isolates from swabbing and organ isolation to 16 antibiotic discs revealed that resistance to antibiotics was shown in 59.9% of the isolates, while moderate and high sensitivity was 30.2 and 9.9% respectively. All tested isolates were methicillin and cloxacillin resistant strains. Vancomycin and oxytetracycline also were resistant in 91.66% of strains, followed by sulbactin-ampicillin, erythromycin, trimethoprim and sulphamethoxazole, gentamicin, clindamycin and ampicillin showed a marked resistant to the *S. auraus* isolates by percentage 66.66% or more. Marked sensitivity was shown to colistin, amoxicillin-claveulinic, Penicillin, cefotaxime and ciprofloxacin. No resistance was shown to ciprofloxacin which considered the drug of choice.

Swabs from human contacts, cages and slaughterhouse places: MRSA was isolated from nasal swab of one attendant and two from three slaughterhouse workers (3/7) 42.6%. All swabs collected from cages and slaughterhouse places and butchers knifes blade after slaughtering were identified to MRSA indicating spreading of infections to environment and contamination of meat.

DISCUSSION

Rabbits are an important source of meat in Egypt. It is estimated that 88-90% of rabbit population in Egypt is in

Table 1: Incidence MRSA isolates from different body sites of carrier rabbit

	Mothers/males		Broilers		Total
Sampling site	Dams n =72	Buck n = 8	Female n = 56	Male n = 68	n = 204
Nose/Eye	21 (29.16%)	5 (62.5%)	12 (21.42%)	16 (23.52%)	54 (26.47%)
Vaginal/perineum	8 (11.11%)	0 (0)	0 (0)	0 (0)	8 (3.92%)
Skin affection	8 (11.11%)	0 (0)	4 (7.14%)	6 (8.82%)	18 (8.82%)
Total	37 (51.38%)	5 (62.5%)	16 (28.57%)	22 (32.35%)	80 (39.21%)

Table 2: Incidence MRSA isolates among organs from slaughtered animals

	Mothers/males		Broilers		Total
Organs sampled	Dams n =72	Buck n = 8	Female n = 56	Male n = 68	n = 204
Lung	19 (26.38%)	1 (12.5%)	11 (19.64%)	14 (20.58%)	45 (22.05%)
Uterus	10 (13.88%)	-	(0)	-	10 (7.8%)*
Total	29 (40.27%)	1 (12.5%)	11 (19.64%)	14 (20.58%)	55 (26.96%)

*Incidence positive isolates was calculated to total females no.128

Table 3: Comparative efficacy of antibiotic sensitivity of 16 antimicrobials against 12 MRSA isolates from rabbits

		Degree of sensitivity		
Antibiotic disc	Disk potency	Resistant	Moderate sensitive	Sensitive
Oxytetracycline (T),	30 mcg	11 (91.67%)	1 (8.33%)	-
Colistin (CT)	10 mcg	4 (33.33%)	5 (41.67%)	3 (25%)
Erythromycin (E)	15 mcg	9 (75%)	2 (16.67%)	1 (8.33%)
Gentamicin (CN)	10 mcg	8 (66.67%)	4 (33.33%)	-
Amoxicillin, Claveulinic (AMC)	20+10 mcg	3 (25%)	7 (58.33%)	2 (16.67%)
Trimethoprim				
Sulphamethoxazole (SXT)	1.25mcg 23.75g	9 (75%)	3 (25.0%)	-
Methicillin (ME)	5 mcg	12 (100%)	-	-
Penicillin (P)	10u	4 (33.33%)	5 (41.67%)	3 (25.0%)
Vancomycin (VA)	30 mcg	11 (91.67%)	-	1 (8.33%)
Ciprofloxacin (CIP)	5 mcg	-	10 (83.33%)	2 (16.66%)
Sulbactin-Ampicillin (SAM)	10+10 mcg,	10 (83.33%)	2 (16.66%)	-
Cloxacillin (CX)	1mcg	12 (100%)	-	-
Cefotaxime (CTX)	30 mcg	1 (8.33%)	8 (66.67%)	3 (25.0%)
Clindamycin (DA)	2 mcg	8 (66.67%)	3 (25.0%)	1 (8.33%)
Ampicillin (AM)	10 mcg	8 (66.67%)	4 (33.33%)	-
Enrofloxacin	5 mcg	5 (41.66%)	4 (33.33%)	3 (25.0%)
Total (No = 192)		115 (59.9%)	58 (30.20%)	19 (9.9%)

the hands of smallholders while the rest belongs to the commercial sector which are differing in hygienic measures, However, it is reported that disseminating staphylococcosis can be found in hygienic as well as in less hygienic rabbitries (Hermans *et al*., 1999).

The prevalence of MRSA infections among the studied flock reached 39.21% of healthy carrier. In a previous study by Herman *et al*. (1999), the mean percentages of *S. aureus* infected animals in flocks that suffered from chronic problems of staphylococcosis and clinically healthy flocks were 90 and 43.3%, respectively. The high percentage of positive rabbits can be explained by the fact that *S. aureus* is one of the most common pathogens found in breeders which immediately after birth at lactation can contaminate their newborn through the close physical contact act as direct transmission does and suckling young, between litter mates and between stable mates (Devriese *et al*., 1981).

In this study, results showed 10.4% mortality rate of rabbits aged 1-3 month and mortality of newly born reached 30% that indicate the high virulence of the isolated strains. These results were in agreement with (Holliman and Girvan, 1986) who reported that due to staphylococcosis in does, the loss of complete litters after death of their mother is very common. In addition (Okerman *et al*., 1984) reported that an outbreak of cutaneous staphylococcosis caused high mortality among newborn and very young rabbits; however, the death rate during the fattening period is not usually higher than normal. The high mortality rate was attributed to a high incidence of mastitis among does and a high colonization capacity of the isolates that considered an important virulence determinant in rabbit Staphylococcosis (Hermans *et al*., 2000). No mortality was detected in our breeding does in the current study; this result disagreed with Holliman and Girvan (1986) who detected 35% mortality in does.

To avoid false-negative results, proper sampling body sites for detection “high virulence” as well as for “low virulence’’ *S. aureus* strains is very critical for definitive detection of infection, when bacteriological sampling is used as a preventive diagnostic test rabbits may be discretely to highly positive for *S. aureus* carrier at one to nine of body sites (Hermans *et al*., 1999). In this study the prevalence of HV-MRSA was 26.47 and 3.92% from nose/eye and vaginal/perineum consequently. Sampling from nose and vaginal/perineum were demonstrated and selected as they showed more intense colonization as mentioned by (Hermans *et al*., 1999). Experimentally, these sites were highly colonized by virulent strains of staphylococcus after three days of infection (Hermans *et al*., 2000).

In this study, abscesses were detected in 8.8% of broiler rabbits. In rabbits, problems of staphylococcosis arise when *S. aureus* bacteria infect small dermal lesions and invade subcutaneous tissue (Okerman *et al*., 1984) and dermatitis considered the main prominent signs in *S. aureus* infection in rabbits (Segura *et al*., 2007). In this study, 5/72 (6.9%) dams showed mastitis in one or more teats. In a previous study, internal organ abscesses were sporadically observed with predominance in lungs, liver and uterus (Bogaert *et al*., 2003; Holliman and Girvan, 1986; Segura *et al*., 2007). However, in the present work some cases showed inflammatory symptoms of uterus but without any purulent exudation.

In this study, the percentage of antimicrobial-resistant strains was high (59%), with resistance to oxytetracycline and Vancomycin (91.67%) being the most frequently observed. These results agree with previous

reports (Goni *et al*., 2004; Vancraeynest *et al*., 2004). These high drug resistance levels by the rabbit isolates could be explained by the intensive use and misuse of antimicrobial drugs in human and veterinary medicine leads to the selection of resistant organisms (Smith and Coast, 2002; Cizman, 2003; Levy and Marshall, 2004). There is an additional concern over the potential transmission of resistant organisms and resistance genes from livestock to man through consumption and handling of foods derived from animals (Tollefson and Karp, 2004). Antibiotic resistance could be a factor of failure of curing the herd by different antibiotic administered; Another factor could be pathogenic ability of *S. aureus* strains and the endemic pattern of the disease. For all these reasons, Hermans *et al*. (2003) concluded that the only solution for farmers facing problems with high virulence *S. aureus* strains is to slaughter the entire flock.

Transmission of high- and low-virulence *S. aureus* strains from man to rabbit or between rabbits may be direct or indirect, through cages, hairs or food (Matthes, 1995; Rossi *et al*., 1995). Farmers could also be a source of contamination of both animal and human strains himself through his frequent handling of the rabbits that were in accordance with our rate of isolation from human contact which reached 42.6%. Pooled sample from cages and slaughter house were indicative as indirect source reached 60% in this study. Contamination of meat by pathogens is considered an important issue in terms of food safety. Since food animals arriving at abattoirs are frequently carriers of *S. aureus*, the organism is commonly found on carcasses and cuts and staphylococcal contamination may or may not result from lesions (Mead and Dodd, 1990; Khalafalla, 1993). The ubiquity of the virulent phenotype, as well as the high incidence of resistance to antibiotics with application in human medicine, is a matter of concern in public and animal health (Rodriguez-Calleja *et al*., 2006; Smyth *et al*., 2009).

Eradication programs of HV-SA based on "test and cull" to protect public health and the progressive extension of the concept of food quality and increasing the sanitary management of rabbit breeding and optimizing the use of antimicrobial agents. In addition, limited introduction of new HV-SA carrier animals and limited contact between rabbitries may decrease the risk of infection (Devriese *et al*., 1996).

Conclusions: Clinical diseases, particularly, high mortalities and infertility of rabbits caused by multi-drug resistant strains of MRSA with dissemination to environment and contamination of rabbit meat shed the light on its impacts on rabbit production and public health in Egypt. Thus, large-scale epidemiological investigations of HV-SA in rabbitry in Egypt are needed.

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