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ANTIBIOGRAM AND MOLECULAR CHARACTERIZATION OF METHICILLIN RESISTANT *STAPHYLOCOCCUS AUREUS* (MRSA) IN PET DOGS

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ABSTRACT

Staphylococcus aureus and Methicillin resistant *Staphylococcus aureus* (MRSA) are widely spread in companion animals. The study was to investigate the Prevalence of MRSA in both nasal and wound swabs from pet dogs by conventional method and molecular characterization for detection of *mecA* gene using specific primers to confirm MRSA. A total of 101 pet dog samples (67nasal swabs and 34 wound swabs) were collected from 101 pet dogs. All samples were cultured for the isolation of *Staphylococcus aureus* using selective media and biochemical tests. Isolates were identified as MRSA after antimicrobial susceptibility testing. PCR for *mecA* gene was applied. Nineteen nasal MRSA isolates were recovered from 67 (28%) nasal swabs and 6 wound isolates from 34 (17.6%). All isolates were resistant to cefoxitin and sensitive to both polymyxin B and vancomycin. All the 25 MRSA isolates were positive to *mecA* gene. The aim of this study is to Investigate Methicillin-resistant *Staphylococcus aureus* (MRSA) through antibiogram using Cefoxitin disc and molecular detection of *mecA* gene in pet dogs. The result of this study indicated the possible role of pet dogs in the epidemiology of MRSA zoonosis in the community to be considered as an emerging veterinary pathogen of pets with a public health burden.

Key words:

Staphylococcus aureus, MRSA, Pet dogs, *mecA,* PCR.

INTRODUCTION

Since 1880, *S. aureus* has been a potential pathogen, when it was isolated by Alexander Ogston from a surgical wound infection. The isolated organism was injected into guinea pigs, mice and it was able to produce abscesses **(Ogston, 1881)**. Methicillin-resistant *Staphylococcus aureus* (MRSA) has emerged, spreaded globally and became a leading cause of bacterial infections (**Lee *et al*., 2018)**. Due to the presence of *mecA* gene, borne on the *Staphylococcal* cassette chromosome *mec* (SCC*mec*) that codes for a 78-kDa penicillin binding protein (PBP2a), with

decreased affinity to methicillin and all beta-lactam antibiotics, the Methicillin resistance occurred **(Chambers,1997)**. From epidemiological point of view, MRSA isolates are classified based on their characteristics into hospital acquired (HA-MRSA), community acquired (CA-MRSA), and livestock associated (LA-MRSA). This is according to the location from which the isolate is obtained. These epidemiological classifications are important for the selection of antimicrobial therapy and play a role in understanding the virulence of the isolate **(David *et al*., 2008).** The highest incidence of MRSA was reported not only in Egypt, but in all Mediterranean region. CA-MRSA is know as a causative agent of wide variety of diseases in human and animals including skin and soft tissue infections, blood stream infection, and necrotizing pneumonia. In Egypt, more complicated conditions caused by CA-MRSA were recorded such as brain abscess and pericarditis **(Abdel-moein *et al*., 2012)**. Human-animal relationships shared a long history from the agricultural sector to modern day by keeping companion animals like pet dogs at home, pet shop owners, veterinarians, and workers, also it helps the police to sniff out illegal substances and explosives, etc. Cases of animal-to-human transmissions of MRSA were reported and MRSA is gradually emerging as an important veterinary pathogen **(Weese *et al*., 2006).**

The aim of this study was the Investigation of Methicillin-resistant *Staphylococcus aureus* (MRSA) through antibiogram using Cefoxitin disc and molecular detection of *mecA* gene in pet dogs to determine the possible role of pet dogs in the epidemiology of MRSA zoonosis in the community.

MATERIAL AND METHODS

2.1. Samples:

A total of 101 samples were obtained from pet dogs of different breeds and ages, they were collected from Egypt (Cairo, Giza) shelters and veterinary clinics during Jan and Nov. 2019. The samples were collected from nose and wound using sterile bacteriological swabs; each one was immersed in 5 ml saline (0.9% Sodium Chloride), labelled with details of the animal and kept cold until transported to Laboratory of Microbiology, Faculty of Veterinary Medicine, Cairo University. The sample collection was done according to **Loffler *et al*. (2005)**.

2.2. Isolation and Identification of *Staphylococcus aureus*:

Mannitol salt agar media (Oxoid Ltd, Hampshire, UK) was prepared in petri dishes, then the swabs were streaked on to the plates and incubated at 37°C for 24-48h. The mannitol fermenting golden yellow round colonies were selected to prepare bacterial films and the Gram's stain was applied to find the Gram-positive cocci arranged in irregular grape like clusters. The colonies were tested for catalase enzyme and the positive isolates were subcultured on 5% sheep blood agar to confirm Beta hemolysis (β-hemolysis). A tube and slide coagulase tests were performed on pure hemolytic colonies using lyophilized rabbit plasma (BD, MD, USA). Isolation and identification of *S. aureus* were performed according to **Forbes *et al*., (2007**).

2.3. The antibiotic disk diffusion test:

Positive *Staphylococcus aureus* isolates were tested for antibiotic resistance through the disc diffusion method using Mueller Hinton agar following the instructions of Clinical and Laboratory Standards Institute (CLSI). During this study 10 antibiotic discs were used namely, (Levofloxacin,Doxycycline,Azithromycin, Gentamicin, Oxacillin, Clindamycin, Trimethoprim, Cefoxitin, Vancomycin and Polymyxin B). The results of antibiotic susceptibility testing were interpreted according to the guidelines of CLSI **(CLSI, 2016).**

2.4. Molecular detection of *mecA* gene:

The phenotypically confirmed MRSA isolates were subjected to molecular confirmation by targeting *mecA* gene. DNA were extracted using the WizPrepTM gDNA Mini extraction kit. The extracted DNA was processed for amplification using *mecA* forward P1: 5-TGGCATTCGTGTCACAATCG-3andreverseprimersP2:5- CT GGA ACTT GTTG AG CA GAG-3'with an expected product size of 310bp **(Jonas *et al.*, 2002).**

A 20 µl volume of reaction mixture contained of water 3 µl, forward primer 2 µl, reverse primer 2 µl, DNA 3 µl and master mix 10 µl. Initial denaturation at 95 °C for 5 minutes was followed by 35 cycles of denaturation (95 °C for 30 sec), annealing (58 °C for 30 sec), extension (72 °C for 30 sec), and final extension at 72 °C for 10 min. The amplicons were analyzed on 2% agarose gel and visualized with UV light transilluminator.

RESULTS AND DISCUSSION

Out of 101 pet dogs 67nasal swabs and 34wound swabs were collected, the isolates were identified according to conventional biochemical tests. The positive *S. aureus* isolates fermented mannitol on Mannitol salt agar, the results of identification with catalase, coagulase and hemolysis tests obtained colonies with the properties of producing catalase and coagulase enzymes, producing acetylmethyl-carbinol and forming b-hemolysis on blood agar. Nowadays in Egypt, the relationship between human and pet animals has a dramatic increase, so that household pets are treated as family members. This enables more contact between human and pets, which may increase the chance for bacterial transmission, including MRSA **(Guardabassi *et al.,* 2004)**. Previously, different studies recorded the occurrence of MRSA in pet dogs in Egypt. The present study confirms those finding. In the current investigation, the recovery rate of MRSA from nasal isolates was 28% (19/67), which is higher than records of nasal isolates by **Loffler *et al*., (2010)** (7.8%) and **Yunita *et al*., (2020)** (6.7%), the results of this study concerning nasal isolates in Egypt is completely agree with those of **Decline *et al.,* (2020)** (28%) in Indonesia. This ensures that the incidence of MRSA has been increasing in the veterinary field. On the other hand, the wound isolates in the current study recorded 17.6% (6/34) against (37.5%) by **Yadav *et al*., (2016).** However, there is a limited data about MRSA from canine wound as compared to nasal carriage**.**

Table (1): *Staphylococcus aureus* and Methicillin Resistant *Staphylococcus aureus* (MRSA) Recovery rate from nasal and wound swabs of pet dogs.

Samples	No. of Samples	*S. aureus* Recovery	MRSA Recovery
Nasal swabs	**67**	**47 (70%)**	**19 (28%)**
Wound swabs	**34**	**17 (50%)**	**6 (17.6 %)**
Total	**101**	**64 (63.2%)**	**25 (24.8%)**

The susceptibility Pattern of *S. aureus* was investigated by the antibiotic sensitivity test using the disk diffusion method. *S. aureus* isolates were confirmed by cefoxitin disc (100%). Resistant for other antibiotics, the nasal and wound isolates showed the following resistant profile respectively: doxycycline (63%) and (66.67%), azithromycin (58%) and (16.67%), clindamycin (50%) and (50%), oxacillin (42%) and (33.3%), trimethoprim (31.6%) and (0%),

gentamycin (21%) and (16.67), levofloxacin (5.3%) and(16.67%) while both vancomycin and polymyxin B (0%).

In Brief, the isolates were recorded MRSA positive after resistance to Cefoxitin. On the other hand, all isolates were sensitive to both vancomycin and polymyxin B. Most isolates were resistant to doxycycline, azithromycin and clindamycin, while the other majorities were sensitive to Levofloxacin, Oxacillin and trimethoprim.

Table (2): Antibiogram of methicillin resistant *Staphylococcus aureus* (MRSA) for nasal and wound isolates of pet dogs.

Antimicrobial agent	Nasal Swabs (19)		Wound swabs (6)	
	Sensitive	Resistant	Sensitive	Resistant
Levofloxacin	18 (94.7%)	1 (5.3%)	5 (83.3%)	1 (16.67%)
Doxycycline	7 (36.8%)	12 (63%)	2 (33.3%)	4 (66.67%)
Azithromycin	8 (42%)	11(58%)	5 (83.3%)	1 (16.67%)
Gentamaycin	14 (73.7%)	4 (21%)	5 (83.3%)	1 (16.67%)
Oxacillin	11(58%)	8 (42%)	4 (66.67)	2 (33.3%)
Clindamycin	9 (50%)	9 (50%)	3 (50%)	3 (50%)
Trimethoprim	13(68%)	6 (31.6%)	6 (100%)	0 (0%)
Cefoxitin	0 (0%)	19 (100%)	0 (0%)	6 (100%)
Vancomycin	19 (100%)	0 (0%)	6 (100%)	0 (0%)
Polymyxin B	19 (100%)	0 (0%)	6 (100%)	0 (0%)

Molecular Detection of *mecA* gene in MRSA isolates:

MRSA was confirmed molecularly by the presence of *mecA* gene. All MRSA isolates were positive to *mecA* Fig. (1). The higher the prevalence of *mecA* (MRSA), the higher pattern of risk factors at animal-human-environment interface along with increased variants of antimicrobial resistance **(Shoaib *et al*., 2020).**

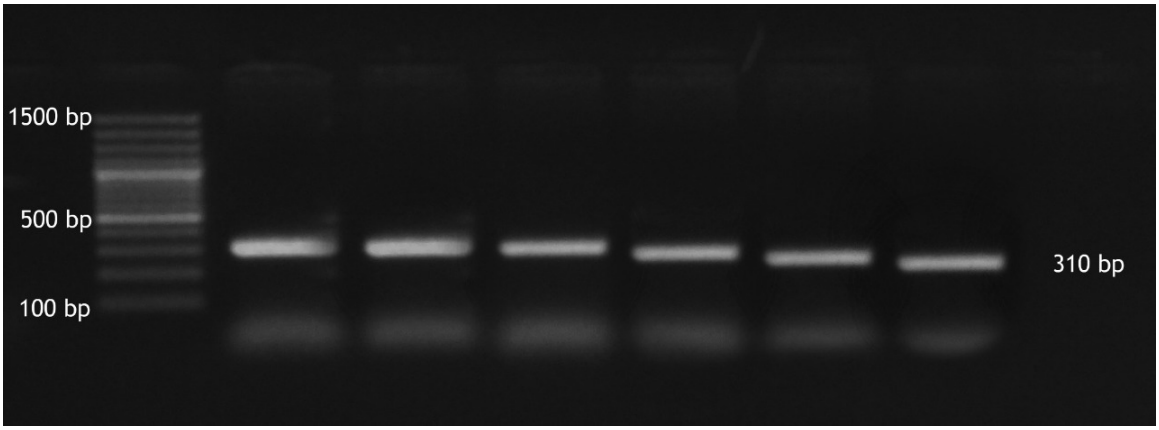

Fig. (1): PCR results of amplified 310bp DNA fragment of MRSA *mecA* gene against a known 100bp.
Lane1: 100pb DNA size ladder, Lane 2: positive control DNA of *mecA* MRSA strain.
Lanes 3-1: DNA of 5 MRSA isolates recverd in the study
Lane : negative control master mix without DNA.

CONCLUSION

The incidence of MRSA in pet animals increased during the last period. Following theantibiogram, the first drugs of choice for MRSA were Polymyxin B and Vancomycin.

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