

Methicillin-resistant *Staphylococcus Aureus* (MRSA) isolated from dogs with recurrent pyoderma

Abstract

Methicillin Resistant *Staphylococcus aureus* (MRSA) has been recently growing problem in small animal clinical practice due to increasing resistance to multiple number of antimicrobials. Present study reports about the antimicrobial susceptibility pattern of MRSA isolated from the dogs with recurrent pyoderma. Based on the cultural and biochemical studies 12 isolates were identified as *Staphylococcus aureus*. Methicillin resistant *Staphylococcus aureus* was identified based on the disc diffusion method. Fifty percent of the dogs showed the methicillin resistant *Staphylococcus aureus* isolates which were resistant to almost all the antimicrobials used in the present study. Other fifty percent dogs had methicillin susceptible *Staphylococcus aureus* which were susceptible to amoxicillin with sulbactam, cefpodoxime with clavulanic acid, enrofloxacin, cephalexin, ciprofloxacin, chloramphenicol, amoxicillin and clavulanic acid, cephadroxil and cefpodoxime. Most of the cultures were resistant to tylosin, lincomycin, erythromycin, azithromycin, gentamicin, amikacin. The alarming antimicrobial resistance and methicillin resistant *Staphylococcus aureus* recorded in the present study of dogs with recurrent pyoderma suggest maintenance of personal hygiene by the small animal owners.

Keywords: *S. Aureus*, skin infections, dog, antibiogram, MRSA

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Sudhakara Reddy B,¹ Nalini Kumari K,² Sivajothi S³

¹Teaching Veterinary Clinical Complex, College of Veterinary Science, India

²Department of Veterinary Medicine, College of Veterinary Science, India

³Department of Veterinary Parasitology, College of Veterinary Science, India

Correspondence: S Sivajothi, College of Veterinary Science, Sri Venkateswara Veterinary University, Andhra Pradesh, India, Tel 871 223 4284, Email sivajothi579@gmail.com

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Introduction

Staphylococcus species are the normal skin microflora of different animals and these organisms are responsible for skin infections in the majority of domestic animals.¹ *Staphylococcus aureus* infection causes mild skin infection to life threatening invasive infections and had the ability to rapidly develop resistance to different antibiotics which are in clinical use.² *Staphylococcus* that are resistant to semi-synthetic penicillins such as methicillin and oxacillin are considered to have methicillin resistance. Methicillin resistance is an alarming condition for treatment because it implies resistance not only to beta-lactam antibiotics including cephalosporins, but also to a wide range of antibiotics.³ MRSA isolates, have been generally isolated from human beings, but increasing numbers of reports have documented the occurrence of MRSA in various animal species also.⁴ Previous studies suggested that MRSA can cause infections in dogs and dogs can also act as reservoirs of MRSA.⁵ Recurrent pyoderma is an important clinical skin problem in dogs and frequently occurs as a result of uncorrected underlying cause(s) or use of inappropriate antibiotics or improper duration of antibiotic therapy.⁶ In recent years, due to increase the number of MRSA has become a therapeutic challenge in veterinary dermatology.⁷ Hence, present study was undertaken to ascertain the occurrence of methicillin resistant *Staphylococcus aureus* in dogs with recurrent pyoderma and their susceptibility to various antimicrobials.

Materials and methods

Present study was conducted at College Hospital of College of Veterinary Science, Tirupati from 2009 to 2011. A total of 50 dogs belong to the different breeds with a history of recurrent skin problems were included in the study (Figure 1). Dogs were examined clinically and diagnosis of recurrent pyoderma was confirmed by cytological examination of the smears made from the skin lesions.^{8,9} After

confirmation of recurrent pyoderma, pus samples were collected from the pustules aseptically. Samples were inoculated in nutrient broth, mannitol salt agar for the growth of *Staphylococcus*. *Staphylococcus* isolates were identified by gram's stain, catalase test, coagulase test and mannitol salt agar as per the standard routine procedure.¹⁰ Based on the fermentation of maltose in purple agar, sugar fermentation tests, differentiation of *staphylococcus* was done.¹¹



Figure 1 Dog with recurrent deep pyoderma.

Appearance of yellow colour of the media within 24 hours of incubation indicated quick fermentation of maltose which was a characteristic of *Staphylococcus aureus*.¹² Coagulase positive *Staphylococcus aureus* isolates were screened for methicillin resistance using Oxacillin disc diffusion test recommended by Jorgensen & Konia et al.^{13,14} Phenotypic methicillin resistance was

determined by a disk diffusion method on Mueller-Hinton agar. Suspected sample colonies from blood agar base containing 5% sheep blood incubated at 37°C for 24h were suspended in sterile saline to a density approximately equal to McFarland Opacity Standard No. 0.5. The bacterial suspension was covered with swab over the Mueller-Hinton agar containing 2% NaCl. The oxacillin (1µg, Oxd) and cefoxitin discs (30µg, cx) disks were dispensed on the surface of the media and were incubated aerobically at 35°C for 24h. The results were recorded as susceptible (≥ 13 mm), intermediate susceptible (11-12mm) or resistant (≤ 10 mm) by measurement of the inhibition zone diameter according to the interpretive standards of NCCLS & Collee et al.^{14,15}

The antibiotic sensitivity of the individual isolates was carried out *in-vitro* by disc diffusion method on Muller Hinton Agar plates with different antibiotic discs i.e., Cephalexin (30mcg), Cephadroxil (30mcg), Cefpodoxime (10mcg), Cefpodoxime and Clavulanic acid (10/5mcg), Enrofloxacin (10mcg), Ciprofloxacin (10mcg), Amoxicillin and Clavulanic acid (10mcg), Amoxicillin and sulbactam (30/15mcg), Lincomycin (15mcg), Co-Trimoxazole (25mcg), Amikacin (10mcg), Gentamicin (30mcg), Erythromycin (10mcg), Azithromycin (30 mcg), Chloramphenicol (10mcg) and Tylosine (15mcg). The sensitivity patterns of isolates to different antibiotic discs were read by measuring the diameter of zone of inhibition in millimeter as per the chart provided by manufacturer and classified as Sensitive, Intermediate and Resistant based on CLSI guidelines.¹⁶

Results and discussion

Clinical samples collected from the dogs with recurrent pyoderma, showed growth on mannitol salt agar and fermented mannitol in mannitol salt agar were preliminarily taken as *Staphylococcus aureus* (Figure 2). However, further confirmation was done based on isolates which fermented one per cent maltose in purple base agar within 24hours, tested positive for acetoin production by Barritt method, yielded positive sugar fermentation tests (sucrose, maltose, mannitol, trehalose and lactose) and were resistant to Polymyxin B. Sterile swabs collected from the 50 dogs affected with recurrent pyoderma cases all the dogs had *Staphylococcus*. Out of 50, 12 showed *Staphylococcus aureus* (24%).



Figure 2 Growth of *Staphylococcus* on mannitol salt agar.

Out of twelve *Staphylococcus aureus* isolates six were MRSA and remaining were Methicillin susceptible *Staphylococcus aureus* (MSSA). Differentiation was done based on the results of oxacillin

and cefoxitin disc diffusion tests. For MRSA there is growth of the isolates were noticed surround the oxacillin discs. In case of MSSA, there is no growth of the organisms were noticed surrounded by the oxacillin disc (Figure 3).

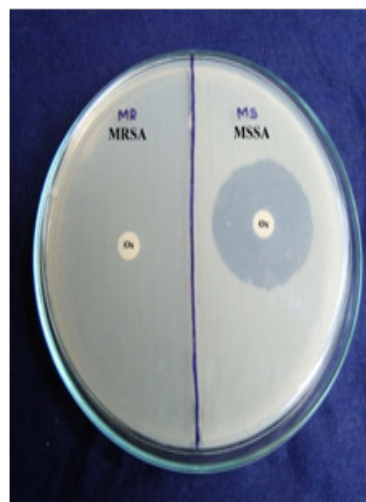


Figure 3 Oxacillin disc diffusion test

Antibiotic sensitivity pattern of isolates of *Staphylococcus aureus* were mentioned in the Table 1. Most of the MSSA isolates in the study were susceptible to amoxicillin with sulbactam, cefpodoxime with clavulanic acid, enrofloxacin, cephalexin, ciprofloxacin, chloramphenicol, amoxicillin and clavulanic acid, cephadroxil and cefpodoxime. Most of the cultures were resistant to tylosin, lincomycin, erythromycin, azithromycin, gentamicin, amikacin. Antibiotic sensitivity pattern of the MRSA isolates showed multidrug resistant and were resistant to the most of the anti microbial in the present study (Figure 4). Almost 66.7 to 100% isolates were resistant to the sixteen anti microbial used in the present work. Resistance to anti microbial like tylosine, chloramphenicol, erythromycin and gentamicin was not effective against recurrent pyoderma. Other antibiotics like cephalexin, cephadroxil, lincomycin, co-trimoxazole, enrofloxacin, ciprofloxacin are regular drugs in dermatology. Due to improper dosage and duration leads to development of the resistant noticed to these drugs.

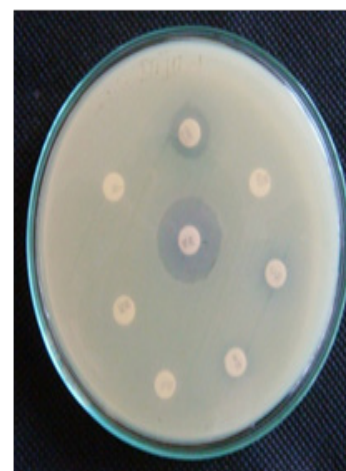


Figure 4 Multiple drug resistant *Staphylococcus aureus* organism while ABST.

Table 1 Antibiotic sensitivity pattern of isolates of *Staphylococcus aureus* in dogs with recurrent pyoderma

S. No	Name of chemotherapeutic agent	Methicillin susceptible <i>Staphylococcus aureus</i> (N:6)				Methicillin resistant <i>Staphylococcus aureus</i> (N:6)			
		Sensitive bacteria		Resistant bacteria		Sensitive bacteria		Resistant bacteria	
		No	Percentage	No	Percentage	No	Percentage	No	Percentage
1	Cephalexin	5	83.3	1	16.7	0	0	6	100
2	Cephadroxil	4	66.7	2	33.3	0	0	6	100
3	Cefpodoxime	4	66.7	2	33.3	1	16.7	5	83.3
4	Cefpodoxime and Clavulanic acid	6	100	0	0	1	16.7	5	83.3
5	Enrofloxacin	5	83.3	1	16.7	2	33.3	4	66.7
6	Ciprofloxacin	4	66.7	2	33.3	2	33.3	4	66.7
7	Amoxicillin and Clavulanic acid	4	66.7	2	33.3	1	16.7	5	83.3
8	Amoxicillin and sulbactam	6	100	0	0	1	16.7	5	83.3
9	Lincomycin	0	0	6	100	0	0	6	100
10	Co-Trimoxazole	3	50	3	50	0	0	6	100
11	Amikacin	2	33.3	4	66.7	1	16.7	5	83.3
12	Gentamicin	2	33.3	4	66.7	0	0	6	100
13	Erythromycin	0	0	6	100	0	0	6	100
14	Azithromycin	1	16.7	5	83.3	1	16.7	5	83.3
15	Chloramphenicol	4	66.7	2	33.3	0	0	6	100
16	Tylosine	0	0	6	100	0	0	6	100

Development of Methicillin resistance in *Staphylococcus aureus* is due to the acquisition of *mecA* gene which encodes new protein (PBP2a) in the bacterial cell wall. The protein (PBP2a) has a very low affinity for β -lactams antibiotics and causes resistance to Methicillin and the other beta-lactams.¹⁷ The Methicillin Resistant *Staphylococcus aureus* (MRSA) recovered from dogs is similar to those affecting humans and dogs have been indicated as potential reservoirs of MRSA.¹⁸ Inter-transmission of MRSA between animals and humans has been reported.¹⁹ The antimicrobial resistance was significantly higher in methicillin resistant *Staphylococcus aureus* than methicillin sensitive *Staphylococcus aureus*. *Staphylococcus* species may develop resistance during prolonged therapy with quinolones.

In the present study, MRSA was identified based on the disc diffusion tests. Due to lack of facilities to conduct other tests confirmation was done by disc diffusion tests but, reliable procedure for detecting MRSA is PCR amplification of the *mecA* gene.^{20,21} MRSA is an important cause of human nosocomial and community-acquired infections in worldwide. Dogs are living in close contact with humans and failure to notice and treat the dogs with MRSA can cause recurrent MRSA infection in humans. So, Pets should be treated systemically to avoid development of MRSA. Due to indiscriminate use of medication rendered the routinely used antibiotics completely ineffective in *Staphylococcus aureus* induced pyoderma.

Conclusion

Recurrent pyoderma is a serious problem when there is presence of multidrug resistant *Staphylococcus aureus* isolates. Present study suggests bacterial cultures with species identification and antimicrobial susceptibility tests are essential for selection of anti microbial agents recurrent pyoderma cases at individual geographical regions.

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Conflict of interest

Author declares that there is no conflict of interest.

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