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Incidence of Macrolide-lincosamide-streptogramin-B Resistance Phenotypes of Methicillin Resistance *Staphylococcus aureus* and Methicillin Sensitive *Staphylococcus aureus* Among Animals in Saudi Arabia

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ABSTRACT

Macrolide-lincosamide-streptogramin-B (MLS_B) are important antibiotic family for treatment of staphylococcal infections in both humans and animals. Clindamycin is widely used in veterinary medicine to treat a variety of bacterial infections. Thus, this study was conducted to investigate the incidence of MLS_B resistance phenotypes among the *Staphylococcus aureus* isolated from animals by agar disk diffusion (D-test) method. A total of 158 coagulase positive staphylococci were isolated from different stock animals. The incidence rate of MLS_B resistance phenotype was 57 (36.1%) in Methicillin-resistant and Methicillin-sensitive *Staphylococcus aureus* (MRSA and MSSA). Thirty two (20.2%) isolates showed constitutive resistance ($cMLS_B$ phenotype) and 12 (7.6%) isolates showed inducible clindamycin resistance ($iMLS_B$ phenotype) while the remaining thirteen (8.2%) isolates showed MS phenotype. The rate of constitutive $cMLS_B$ phenotypes resistance (32.2%) and inducible clindamycin $iMLS_B$ (10%) resistance phenotypes was high in MRSA when compare to MSSA isolates. The higher incidence of inducible clindamycin $iMLS_B$ resistance was observed in camel isolates while the lower rate was observed in sheep's isolates. Detection of erythromycin-induced clindamycin resistance in *S. aureus* by routine screening is necessary so that it will help in guiding therapy and therapeutic failures may be avoided.

Key words: Clindamycin, resistant phenotypes, antimicrobial, antibiotics

INTRODUCTION

Methicillin-resistant *Staphylococcus aureus* (MRSA) strains have been associated with infections and colonization in human and many animal species (Lowy, 1998; Reacher *et al*., 2000). Macrolide-lincosamide-streptogramin-B (MLS_B) is important antibiotic for treatment of staphylococcal infections in humans and animals. The development of antimicrobial resistance among *S. aureus* has been associated as important public health concern which causes high morbidity and mortality worldwide. Emergence of methicillin resistance in *S. aureus* has left us with very few therapeutic alternatives available to treat staphylococcal infections. MLS_B family of antibiotics serves as one such alternative. Among this family Clindamycin is a good alternative for the treatment of both methicillin-resistant and -susceptible staphylococcal infections, being the preferred agent due to its excellent pharmacokinetic properties (Frank *et al*., 2002).

However, the widespread use of MLS_B antibiotics has led to an increased number of staphylococcal strains acquiring resistance to MLS_B antibiotics. The most common mechanism for such resistance is target site modification mediated by *erm* genes which can be expressed either

as constitutive (constitutive MLS_B phenotype) or inducible expression (inducible MLS_B phenotype) (Deotale *et al*., 2010). Strains with inducible resistance to clindamycin are difficult to detect in the routine laboratory as they appear erythromycin resistant and clindamycin sensitive *in vitro* when not placed adjacent to each other (Fiebelkorn *et al*., 2003).

There are few epidemiological studies have revealed the occurrence of indistinguishable MRSA clones in animals and in humans exposed to animals, such as veterinarians and farmers (Loeffler *et al*., 2005; Voss *et al*., 2005; Weese *et al*., 2005). Similarly, dogs affected by pyoderma and their owners have been reported to share identical *S. intermedius* strains in the nasal cavity (Guardabassi *et al*., 2004). These findings indicate that staphylococci colonizing the nasal mucosa can be transmitted between animals and humans, including clinically relevant bacteria such as MRSA. Clindamycin is a good alternative for the treatment of both methicillin-resistant and -susceptible staphylococcal infections, Veterinarians should be aware of the potential for clinical failure when clindamycin is used to treat staphylococcal infections due to isolates with *in vitro* inducible clindamycin resistance.

MLSB resistance phenotype frequently is recognized among *S. aureus* and may be constitutive or inducible. Resistance due to MLS_B results from alterations in ribosomal antibiotic binding sites mediated by erythromycin ribosomal methylase (*erm*) genes. *S. aureus* with the inducible MLS_B phenotype are susceptible to clindamycin and resistant to erythromycin on initial *in vitro* testing, but may become resistant to clindamycin following exposure to clindamycin. Inducible Clindamycin Resistance (ICR) has been recognized as a cause of treatment failure (Sibery *et al*., 2003).

Current knowledge on species distribution, diversity and antimicrobial resistance of animal staphylococci is an epidemiological importance. So, the aim of this study was to assess the prevalence and detection of inducible resistance to CL in ER-resistance (ER-R) among animal isolates of *S. aureus* by D-test.

MATERIALS AND METHODS

Sampling: In the period between January and April, 2010, nasal swabs were collected from camels, sheep, goats and cattle. All nasal swabs were obtained from healthy animals in different areas of Qassim region, Saudi Arabia. For all animals, cotton swabs were rolled on the nasal mucosae of both nostrils and transported in Stuart medium (Oxoid, UK) prior to laboratory analysis as previously described (Alzohairy, 2011).

Bacterial isolates and identification of *Staphylococcus aureus*: All the samples received in the laboratory were cultured onto various microbiological media. Suspected colonies of *Staphylococcus* isolates were characterized to species level by Gram stain, catalase, slide and tube coagulase tests (Oxoid, UK) and by biochemical tests (API Staph; bioM´erieux, France).

Identification of MRSA: For the identification of the MRSA among the isolates of *S. aureus*, ChromID MRSA (Oxoid, Hants, UK) was used. The MRSA only grew on this Hi Chrome MRSA agar, while the MSSA was inhibited on the same agar plate. All cultures showing bright blue colored growth were taken as MRSA positive strains, while all others are recorded as MSSA strains. As controls, all strains were also inoculated on MH Agar and incubated simultaneously.

Additionally, Methicillin-resistance was confirmed via penicillin binding protein 2a (PBP2a) latex agglutination test (PBP20 Test Kit, Oxoid, Hants, UK).

Antimicrobial susceptibility testing: Antimicrobial susceptibility testing was performed by the disk diffusion method according to Clinical and Laboratory Standards Institute (CLSI, 2007)

guidelines. The following antimicrobial disks (Oxoid, Hants, UK) were used: chloramphenicol (30 mg), ciprofloxacin (5 mg), clindamycin (2 mg), erythromycin (15 mg), fusidic acid (10 mg), kanamycin (30 mg), mupirocin (5 mg), penicillin (10 U), quinupristin/dalfopristin (15 mg), rifampicin (5 mg), tetracycline (30 mg) and trimethoprim/sulfamethoxazole (1.25+23.75 mg). The reference strain *S. aureus* ATCC 25923 was used for quality control.

Detection of MLS_B phenotypes by double-disc diffusion (D-test) test: Double-disc diffusion testing (D-test) was performed for each isolate according to (CLSI 2007) guidelines. A 0.5 McFarl and suspension was prepared in normal saline for each isolate and inoculated on Mueller-Hinton agar plate. Clindamycin (CLI)-2 μg and erythromycin (ER)-15 μg disks were placed 15 mm apart edge to edge manually. Following overnight incubation at 37°C, flattening of zone (D shaped) around clindamycin in the area between the two discs, indicated inducible clindamycin resistance. Three different phenotypes were appreciated after testing and the interpretation was done as follows:

Interpretation of the D-test:

- **Inducible MLS_B phenotype:** Staphylococcal isolates showing resistance to erythromycin (zone size = 13 mm) while being sensitive to clindamycin (zone size = 21 mm) and giving D shaped zone of inhibition around clindamycin with flattening towards erythromycin disc were labelled as having this phenotype
- **Constitutive MLS_B phenotype:** This phenotype was labelled for those Staphylococcal isolates which showed resistance to both erythromycin (zone size = 13 mm) and clindamycin (zone size = 14 mm) with circular shape of zone of inhibition if any around clindamycin
- **MS phenotype:** Staphylococcal isolates exhibiting resistance to erythromycin (zone size = 13 mm) while sensitive to clindamycin (zone size = 21 mm) and giving circular zone of inhibition around clindamycin was labelled as having this phenotype (Fig. 1)

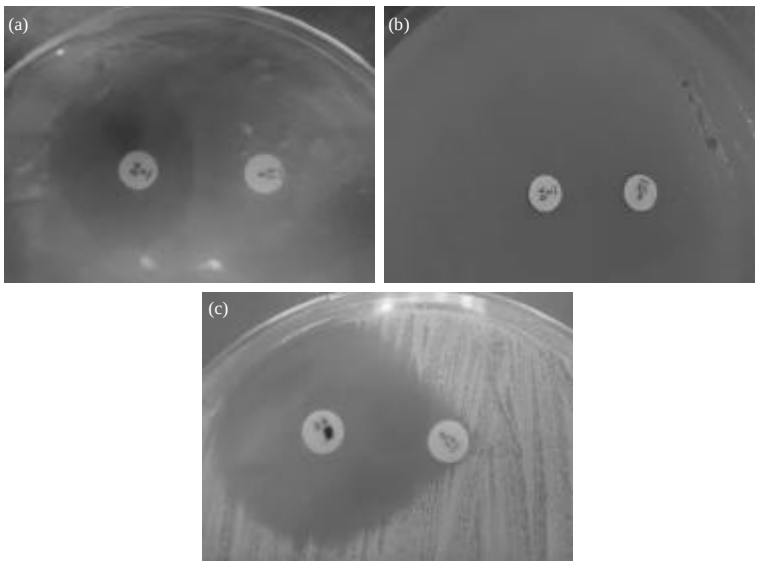

Fig. 1(a-c): Antibiotic susceptibility pattern of isolates, (a) Inducible MLS_B phenotype, (b) Constitutive MLS_B phenotype and (c) MS phenotype

RESULTS

This study was carried out to determine the incidence of inducible clindamycin resistance staphylococci among animals in Qassim region, Saudi Arabia. A total of 158 *S. aureus* isolates obtained from consecutive nasal swabs were included, consisting of 90 MRSA and 68 were MSSA (Table 1) . The total rate of MLS_B resistance phenotype 57 (36.1%) was observed in both MRSA and MSSA isolates. Among this phenotype 32 (20.2%) had the constitutive resistance phenotype, 12 (7.6%) showed the inducible resistance phenotype and 13 (8.2%) was the MS phenotype (Table 2).

Of the 158 animal isolates of staphylococci studied, 12 (7.6%) showed inducible clindamycin resistance and belonged to the $iMLS_B$ phenotypes, Among the $iMLS_B$ phenotypes, 9 isolates were MRSA (10%) and 3 were MSSA (4.4%) isolates. Meanwhile, 32 (20.2%) isolates showed constitutive clindamycin resistance and belongs to the constitutive $cMLS_B$ phenotype, among them, 29 (32.2%) isolates were MRSA and 3 (4.4%) were MSSA isolates. While remaining 13 (8.2%) showed MS phenotype. Among the MS phenotypes, 9 (10%) isolates were MRSA and 4 (5.8%) were MSSA. As shown in (Table 2).

Among MRSA isolates, the constitutive resistance $cMLS_B$ phenotype predominated over the inducible resistance phenotype and susceptible phenotype (32.2, 10 and 10%, respectively). However, the MS phenotype predominated over the inducible resistance $iMLS_B$ phenotype and constitutive resistance $cMLS_B$ phenotype (5.8, 4.4 and 4.4%, respectively) in MSSA isolates. The Erythromycin-Sensitive and Clindamycin-Sensitive phenotypes was predominated in MSSA isolates (85.29%) when compare to MRSA (47.7%) isolates (Table 2).

The overall resistance pattern for all three phenotypes in isolates according to host animal was as follow; Camel isolates: Total MLS_B phenotypes was (53.7%) among these (31.48%) were constitutive resistance $cMLS_B$ phenotype, (9.25%) were inducible resistance $iMLS_B$ phenotype and (12.96%) were MS phenotype. Sheep isolates: Total MLS_B phenotypes (34.21%) that includes

Table 1: Frequency of MRSA and MSSA isolated from animals

	MRSA		MSSA	
Specimens	No.	%	No.	%
Camel	32	35.5	22	32.3
Sheep	26	28.9	12	17.6
Cattle	14	15.5	22	32.3
Goat	18	20	12	17.6
Total	90	100	68	100

MRSA: Methicillin-resistant *S. aureus*, MSSA: Methicillin-susceptible *S. aureus*

Table 2: Susceptibility to clindamycin and erythromycin among MRSA and MSSA isolates

	Bacterial phenotypes									
	Total MLSB		cMLSB ER: R, CL: R		iMLSB ER: R, CL: S		MS phenotype ER: R, CL: S		ER: S, CL: S	
Bacterial isolates	No.	%	No.	%	No.	(%)	No.	%	No.	(%)
MRSA (n = 90)	47	52.2	29	32.2	9	10	9	10	43	47.70
MSSA (n = 68)	10	14.7	3	4.4	3	4.4	4	5.8	58	85.29
Total *S. aureus* (n = 158)	57	36.0	32	20.2	12	7.6	13	8.2	101	63.90

MRSA: Methicillin-resistant *S. aureus*, MSSA: Methicillin-susceptible *S. aureus*, ER: Erythromycin, CL: Clindamycin, R: Resistant, S: Susceptible

Table 3: Prevalence of clindamycin and erythromycin susceptible phenotypes among staphylococci isolated from studied animals

	Bacterial phenotypes									
	Total MLSB		cMLSB, ER: R, CL: R		iMLSB, ER: R, CL: S		MS phenotype: ER: R, CL: S		ER: S, CL: S	
Animals	No.	%	No.	%	No.	%	No.	%	No.	%
Camel (n = 54)	29	53.70	17	31.48	5	9.25	7	12.96	25	46.29
Sheep (n = 38)	13	34.21	8	21.05	2	5.26	3	7.89	25	65.78
Cattle (n = 36)	10	27.77	5	13.88	3	8.33	2	5.55	26	72.22
Goat (n = 30)	5	16.66	2	6.66	2	6.66	1	3.33	25	83.33
Total (n = 158)	57	36.00	32	20.25	12	7.60	13	8.20	101	63.92

MRSA: Methicillin-resistant *S. aureus*, MSSA: Methicillin-susceptible *S. aureus*, ER: Erythromycin, CL: Clindamycin, R: Resistant, S: Susceptible

(21.05%) constitutive resistance $cMLS_B$ phenotype, (5.26%) were inducible resistance $iMLS_B$ phenotype and (7.89%) were MS phenotype. Cattle isolates: the total MLS_B phenotypes was (27.77%), among them, (13.88%) showed constitutive resistance $cMLS_B$ phenotype, (8.33%) were inducible resistance $iMLS_B$ phenotype and (5.55%) were MS phenotype. Goat isolates: the total MLS_B phenotypes was (16.66%) including (6.66%) constitutive resistance $cMLS_B$ phenotype, (6.66%) inducible resistance $iMLS_B$ phenotypes and (3.33%) as MS phenotype. Hence, the highest rate of inducible clindamycin resistance $iMLS_B$ phenotype prevalence was observed in camel isolates (9.2%) while the lowest inducible clindamycin resistance $iMLS_B$ phenotype was observed in sheep isolates (5.3%) (Table 3).

DISCUSSION

The development of resistance in *Staphylococcus* species to macrolide, lincosamide and streptogramin B has limited the use of these antibiotics (Lewis *et al*., 2005). Macrolide resistance may be due to enzymes encoded by a variety of *erm* genes-MLS_B phenotype and may be constitutive ($cMLS_B$ phenotype) or inducible ($iMLS_B$ phenotype). Another mechanism is active efflux pump encoded by the *mrs* A gene (MS phenotype) (Daurel *et al*., 2008). Clindamycin is used widely in veterinary medicine to treat a variety of bacterial infections including skin, wound and bone infections, pneumonia, oral cavity infections and infections due to anaerobic bacteria. Clindamycin is a good alternative for the treatment of both methicillin-resistant and -susceptible staphylococcal infections, but therapeutic failures caused by inducible $iMLS_B$ phenotype resistance are being reported more commonly (Sibery *et al*., 2003).

In this study, MRSA isolates had higher constitutive resistance (32.2%) compared to inducible resistance (10%) while in MSSA isolates, constitutive resistance (4.4%) was similar to that of inducible resistance rate (4.4%). These findings go in agreement with several other studies reported before. Azap *et al*. (2005) found that constitutive phenotype is predominant over inducible phenotype in MRSA isolates and MS phenotypes are only found in MSSA (Kader *et al*., 2005) also reported (53%) $cMLS_B$ and 43% $iMLS_B$ (i.e. higher constitutive resistance) among MRSA . Similarly, Gadepalli *et al*. (2006) showed it to be 30% in MRSA and 10% in MSSA.

On the contrary, several other authors have also reported different results such as Schreckenberger *et al*. (2004) and Levin *et al*. (2005) showed higher percentage of inducible resistance in MSSA as compared to MRSA,(7-12% in MRSA and 19-20% in MSSA; 12.5% MRSA and 68% MSSA), respectively. However, taking in account other reports (Rich *et al*., 2005;

Martinez-Aguilar *et al.* (2003) this study reveals that the rate of inducible resistance varies which highlight the variations and importance of inducible clindamycin resistance in different geographical setting.

There have been a number of reports on the clinical failure due to antibiotics resistant. Rao (2000) and Dinkovic *et al.* (2001) reported clinical failures after using clindamycin against S. aureus strain which had been demonstrated to express inducible clindamycin resistance phenotype. Additionally, Sibery *et al.* (2003). also reports another case of clinical failure in which clindamycin resistance developed whilst on therapy for an original clindamycin-susceptible isolate. Therefore veterinarians should be aware of the potential for clinical failure when clindamycin is used to treat staphylococcal infections if the isolates show inducible clindamycin resistance *in vitro* by D-test.

CONCLUSION

This study reported the incidence of inducible clindamycin resistance in *S. aureus* isolated from animals in Qassim region of Saudi Arabia. However this study conclude that the routine screening test for detection of erythromycin-induced clindamycin resistance in *S. aureus* performing by D-test is necessary, so that it will help in guiding therapy and therapeutic failures may be avoided.

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