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RESISTOTYPING OF CAMEL SKIN WOUNDS ASSOCIATED *Staphylococcus aureus* ON THE BASIS OF MULTIDRUG RESISTANCE PATTERN

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ABSTRACT

The present investigation was attempted to type *Staphylococcus aureus* associated with camel skin wounds on the basis of multidrug resistance pattern against 35 antibiotics of different generations. Beta-lactamase activity was also determined for the isolates. Twenty six *S. aureus* isolates were obtained from camel skin wounds and confirmed by 23S rRNA gene ribotyping. We recorded susceptibility of 100% isolates to azithromycin, netillin, polymixin-B and rifampicin followed by susceptibility of 96.15% isolates to chloramphenicol and gentamicin, 92.30% to bacitracin, novobiocin and cloxacillin, 88.46% to clindamycin, 84.61% to tobramycin, 80.77% to erythromycin, 69.23% isolates were sensitive to ceftriaxone, methicillin, doxycycline hydrochloride, cefaclor, ciprofloxacin, norfloxacin, ofloxacin, amoxicillin, amoxyclav, sparfloxacin and trimethoprim. Nineteen different resistotypes were identified with 0.9508 discriminatory index. This higher number of resistotypes and more discriminatory index may suggest higher diversity and resistance in the isolates. The continuous local surveillance and genotypic explorations should be performed on regular basis in order to have adequate information for antibiotic resistance patterns of *S. aureus* infections.

Key words: Camel, multidrug resistance, resistotypes, skin, *Staphylococcus aureus*, wounds

The skin infections including contagious skin necrosis, dermatitis, wounds, abscesses or similar lesions is a great problem in camel. Most of the skin infections have been found to be caused by staphylococci. The disease is not fatal but due to reduced working efficiency it causes great economic losses. The skin infections are difficult to be treated medically depending on among other factors, the pathogenic quantities of the staphylococcal strain present (Wernery, 2000). The literature regarding microbiology of the skin wounds in camel is very less (Qureshi *et al,* 2002) but *Staphylococcus aureus* has been found to be most common pathogen associated with skin wounds.

Over the last few decades, there was a sudden increase in the use of antibiotics in veterinary as well as human health care not only to control disease but also as prophylactic measure for bacterial infections secondary to viral infections (Lindeman *et al,* 2013). The use of antibiotics in a frequent manner leads to development of resistance in different disease causing bacterial species. So it is very important to know about the resistance or susceptibility of the bacteria prior to administration of the treatment (Wang *et al,* 2008).

The prescription of new antibiotics to manage *S. aureus* has frequently been followed by the uprising of resistant strains (Schito, 2006). Most significantly, *S. aureus* isolates resistant to beta-lactams have become common. The ability of *S. aureus* to survive in the presence of β-lactam antibiotics remains the main problem in the therapy (Pinho, 2008). Due to various mechanisms of acquired β-lactam resistance, several resistance phenotypes have been described so far in *S. aureus* (Chambers, 1997). These include β-lactamase acquisition, modification of penicillin-binding proteins, or acquisition of low-drug-affinity penicillin-binding proteins. Beta lactams such as penicillin are the most widely used antibiotics and beta-lactamases are the greatest source of resistance to penicillins. An understanding of beta-lactamase detection is therefore valuable (Kilic and Cirak, 2006).

Presently there is growing concern among scientists in regards to increasing resistance in pathogens. The concerns are multifaceted *viz.* inaccurate diagnosis, defective dosage, indiscriminate use, development of new drugs etc. Thus the aim of this study was to assess diversification among *S. aureus* in regards to resistance patterns and to

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determine the level of drug resistance to various classes of antibiotics. This study is of significance in improving baseline data on antibiotic resistance shown by *S. aureus* isolated from camel skin wounds for the prudent use of antibiotics and to promulgate antibiotic policies in disease control programs.

Materials and Methods

Bacterial isolates

A total of 41 swabs from skin wound in camels in and around Bikaner were collected and processed for isolation and identification of *S. aureus* (Quinn *et al,* 1994). All phenotypically identified isolates were further confirmed by ribotyping based on 23S rRNA gene (Straub *et al,* 1999).

Beta-lactamase activity (Acidimetric method)

The method described by Livermore and Brown (2001) was used to demonstrate Beta-lactamase activity.

Antibiotic sensitivity test

The antibiogram of isolates against different antibiotics were determined using method of Bauer *et al* (1966). The interpretation for resistant, sensitive and intermediates was drawn as breakpoints defined by Clinical and Laboratory Standards Institute (CLSI).

Discriminatory index

The discriminatory ability of the different typing system i.e. their ability to distinguish between unrelated strains was determined by the number of types defined by the test method and the relative frequency of their types. The numerical index of discrimination was calculated using the formula given by Hunter and Gaston (1988).

$$D = 1 - \frac{1}{N(N-1)} \sum_{j=1}^{S} nj(nj-1)$$

Where,

D = Discriminatory index, S = Total number of type used, nj = Number of strains belonging to j^{th} type, N = Total number of strains.

Results and Discussion

The antibiogram developed for 26 *S. aureus* revealed that the most effective antibiotics were azithromycin, netillin, polymixin-B and refampicin against which all the isolates were sensitive followed by chloramphenicol and gentamicin against which 96.15% of the isolates were sensitive, 92.30% isolates were sensitive to bacitracin, novobiocin and cloxacillin, 88.46% to clindamycin, 84.61% to tobramycin, 80.77% to erythromycin, 69.23% isolates were sensitive to ceftrioxane, methicillin, doxycycline hydrochloride, cefaclor, ciprofloxacin, norfloxacin, ofloxacin, amoxicillin, amoxiclav, sparfloxacin and trimethoprim. The other antibiotics were less effective. Nalidixic acid was found to be the most ineffective antibiotic. Interestingly 100% resistance was not recorded for any of the studied 35 antibiotics in the present study (Table 1). In this investigation, acidimetric method was used for detection of beta-lactamase activity and found that only 8 (30.76%) isolates from camel skin wounds were beta-lactamase producer.

In the resistotyping, multidrug resistance was detected among all isolates except 5 (C9, C10, C15, C22 and C23) which were resistant to two antibiotics (cefalexin and nalidixic acid). Nineteen different resistotypes were detected (Table 2) with 0.9508 discriminatory index and resistance pattern against maximum 24 and minimum 2 antibiotics. The more number of resistotypes and higher value of discriminatory index indicate capabilities of resistotyping method as powerful tool to discriminate isolates. Hunter and Gaston (1988) calculated discriminatory index on the basis of total number of unrelated strains and total number of observed patterns to assess discriminatory power of typing method. It was recommended that the method with more than 0.70 discriminatory index would be considered as good discriminatory method and higher diversity among studied isolates.

The results in the present study were almost in accordance with the observations of Rathore and Kataria (2012) for azithromycin, gentamicin, norfloxacin and nalidixic acid and those of Qureshi and Kataria (2004) for gentamicin, chloramphenicol and cloxacillin who also studied *S. aureus* isolates from camel skin wounds and abscesses from the same study area. Yadav *et al* (2015) also reported similar results as in the present study for netillin, rifampicin, gentamicin, azithromycin and bacitracin from the same study area. In the present study the susceptibility of *S. aureus* to gentamicin is almost similar to that recorded by Ebrahimi and Akhavan Taheri (2009) who found 100% of the isolates susceptible to gentamicin. The continuous observations of susceptibility towards gentamicin in all the previous studies in this area suggest that this antibiotic is not being used in most of the treatment regimens in this area.

Table 1. Antibiogram for *S. aureus* isolates associated with camel skin wounds.

S. No.	Antibiotic disc	Percent (%)		
		Sensitive	Intermediate	Resistant
1	Azithromycin (AZM)	100	–	–
2	Netillin (NET)	100	–	–
3	Polymixin-B (PB)	100	–	–
4	Rifampicin (RIF)	100	–	–
5	Chloramphenicol (C)	96.15	3.85	–
6	Gentamicin (HLG)	96.15	–	3.85
7	Bacitracin (B)	92.30	7.69	–
8	Novobiocin (NV)	92.30	3.85	3.85
9	Cloxacillin (COX)	92.30	–	7.69
10	Clindamycin (CD)	88.46	11.53	–
11	Tobramycin (TOB)	84.61	7.69	7.69
12	Erythromycin (E)	80.77	19.23	–
13	Levofloxacin (LE)	69.23	26.92	3.85
14	Ceftriaxone (CTR)	69.23	23.07	7.69
15	Methicillin (MET)	69.23	19.23	11.53
16	Doxycycline hydrochloride (DO)	69.23	19.23	11.53
17	Cefaclor (CF)	69.23	7.69	23.07
18	Ciprofloxacin (CIP)	69.23	3.85	26.92
19	Norfloxacin (NX)	69.23	3.85	26.92
20	Ofloxacin (OF)	69.23	3.85	26.92
21	Amoxicillin (AMX)	69.23	–	30.76
22	Amoxiclav (AMC)	69.23	–	30.76
23	Sparfloxacin (SPX)	69.23	–	30.76
24	Trimethoprim (TR)	69.23	–	30.76
25	Cotrimoxazole (COT)	65.38	3.85	30.76
26	Moxifloxacin (MO)	65.38	3.85	30.76
27	Ampicillin (AMP)	65.38	–	34.61
28	Azlocillin (AZ)	65.38	–	34.61
29	Neomycin (N)	53.85	15.38	30.76
30	Oxytetracycline (O)	53.85	–	46.15
31	Cefotaxime (CTX)	26.92	26.92	46.15
32	Cefixime (CFM)	19.23	38.46	42.30
33	Cephalexin (CN)	11.53	–	88.46
34	Vancomycin (VA)	3.85	53.85	42.30
35	Nalidixic acid (NA)	–	3.85	96.15

In the present study, susceptibility of isolates towards nalidixic acid and vancomycin was very less but Qureshi and Kataria (2004) reported higher susceptibility towards vancomycin. The lower susceptibility of isolates in the present study towards cefixime in the present study is similar to those reported by Upadhyay and Kataria (2009), Rathore and Kataria (2012) and Yadav *et al* (2015). This antibiotic though not being used in the animals but higher resistance of isolates shows that it might have been transferred from human subjects to animals.

Sanjiv and Kataria (2006) and Upadhyay and Kataria (2009) used some similar antibiotics as in this study against *S. aureus* isolates of milk origin from cattle and goats obtained from the same area and reported higher number of isolates susceptible to cloxacillin, gentamicin, bacitracin, chloramphenicol, novobiocin as recorded in the present study. In present investigation, resistance towards methicillin was recorded in 11.53% whereas, El-Jakee *et al* (2010) recorded higher resistance (60%) by *S. aureus* isolates.

Our results are in conformity to earlier observation from same study area made by Yadav *et al* (2015) who reported 34.37% *S. aureus* isolates to be positive for beta-lactamase activity in a lot of 32 isolates obtained from cattle and buffalo mastitic milk. In a study conducted by Oberhofer and Towle (1982), 83.33% of 60 penicillin resistant and intermediate *S. aureus* isolates showed as beta-lactamase producers by acidimetric method. Still a higher percentage of beta-lactamase producing isolates were reported by Kilic and Cirak (2006) who reported as high as 84.3% to 85.5% beta-lactamase producers by using acidimetric method.

The increasing incidence of obtaining antimicrobial resistant pathogens has severe implications for the future treatments and prevention of infectious diseases in both animals and humans (White and McDermott, 2001).

The indiscriminate usage of antibiotics in domestic animals leads to treatment failure, escalated treatment costs and development of resistance to antimicrobials. Such resistance resulted in infections that are more difficult to cure. The efficacy of conventional antibiotic treatments against pathogens such as *S. aureus* is low (Wilson *et al,* 2003). Penicillin and closely related antibiotics of the β-lactam family are the best weapons against staphylococci. However, the massive usage of these antibiotics has led to a dramatic increase in pathogens that can produce an enzyme called β-lactamase that inactivates β-lactam antibiotics, thereby resulting in microbial resistance (Aarestrup and Jensen, 1998). Therefore, there is an urgent need to find new antimicrobials to treat bacterial pathogens and for maintaining optimum health state.

Table 2. Resistotypes of *S. aureus* isolates associated with camel skin wounds.

S. No.	Isolate ID	Isolate No.	Resistance pattern (Resistotype)	No. of antibiotics
1.	C17	1	AMX, AMC, AMP, AZ, CF, CN, CFM, CTX, CTR, CIP, COX, COT, HLG, MET, MO, NA, N, NX, OF, O, SPX, TOB, TR, VA	24
2.	C6	1	AMX, AMC, AMP, AZ, CF, CN, CFM, CTX, CIP, COT, DO, LE, MO, NA, N, NX, OF, O, SPX, TR, VA	21
3.	C20	1	AMX, AMC, AMP, AZ, CF, CN, CFM, CTX, COX, COT, MET, MO, NA, N, OF, O, SPX, TOB, TR, VA	20
4.	C21	1	AMX, AMC, AMP, AZ, CF, CN, CFM, CTX, CTR, CIP, COT, MET, MO, NA, N, NX, OF, O, SPX, TR	20
5.	C5	1	AMX, AMC, AMP, AZ, CF, CN, CFM, CTX, CIP, COT, DO, MO, NA, N, NX, OF, O, SPX, TR	19
6.	C2	1	AMX, AMC, AMP, AZ, CF, CN, CFM, CTX, CIP, COT, MO, NA, N, NX, OF, O, SPX, TR	18
7.	C3	1	AMX, AMC, AMP, AZ, CN, CTX, CIP, COT, DO, MO, NA, N, NX, OF, O, SPX, TR	17
8.	C18	1	AMX, AMC, AMP, AZ, CN, CFM, CTX, CIP, COT, MO, NA, N, NX, O, SPX, TR, VA	17
9.	C1	1	CN, CFM, CTX, NA, VA	5
10.	C7	1	CN, CTX, NA, O	4
11.	C8	1	CN, NA, NV, O	4
12.	C12	1	CN, CFM, NA, O	4
13.	C14	1	CN, CFM, CTX, NA	4
14.	C16	1	CN, CTX, O, VA	4
15.	C25	1	AZ, CN, NA, VA	4
16.	C4	1	CN, CFM, NA	3
17.	C11	1	AMP, CN, NA	3
18.	C13, C19, C24 & C26	4	CN, NA, VA	3
19.	C9, C10, C15, C22 & C23	5	CN, NA	2

The overall analysis of results of previous studies on *S. aureus* isolates from different sources revealed that the susceptibility of the organisms against the antibiotics has greatly reduced, the reason for which appears to be obvious. In this area, the awareness of farmers towards animal care has increased tremendously and they seek veterinary help promptly as and when it is required. The availability of vide variety of antibiotic regime promotes the multidrug resistance and diversification of wide resistance thus the more resistance patterns may exist among *S. aureus* isolates. It requires continuous surveillance of antibiotic susceptibility pattern of isolates. The study may further extend for genotypic characterisation of *S. aureus* isolates to explore various genetic traits involve in resistance mechanisms of organism.

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