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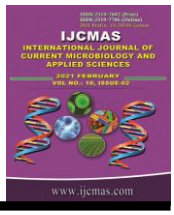

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Molecular Detection of *Staphylococcal* enterotoxin C (SEC) and *Staphylococcal* enterotoxin D (SED) from the Cattle Nares in Aizawl, Mizoram (India)

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A B S T R A C T

Staphylococcus aureus is a gram-positive bacteria commonly found on the skin or mucous membranes of both humans and animals. The bacterium is an opportunistic pathogen that can lead to many human and animal diseases that are self-limiting and even life-threatening. Symptoms such as rapid onset of nausea, vomiting, stomach cramps and diarrhoea are part of staphylococcal food poisoning. The bacteria can be killed when heated at a regular cooking temperature, but the toxins remain active. In foodstuffs, staphylococcal enterotoxins are extremely heat tolerant and are known to be more heat resistant than in a laboratory culture medium. Staphylococcal food toxicity is due to the absorption in the food of staphylococcal enterotoxins.

Introduction

Staphylococcus aureus is a commensal and significant opportunistic pathogen that causes a wide range of diseases in humans and animals, with a high effect on public health and the livestock sector (Rahimi *et al.,* 2015). *S. aureus* develops a large variety of staphylococcal enterotoxins with demonstrated emetic activities (SEs, SEA to SEE, SEG to SEI, SER to SET) as well as staphylococcal-like (SEL) proteins not emetic in the primate model (SElL and SElQ) (Argudin *et al.,* 2010). *Staphylococcal* enterotoxins (SE) can cause toxin-mediated disease, and those that function as superantigens are implicated in the pathogenesis of allergic diseases (Varshney *et al.,* 2009).

An estimated 0.1 µg of SEs can cause staphylococcal food poisoning in humans (le Loir *et al.,* 2003). *S. aureus* has been isolated from the nasal cavities of livestock animals

and hence may be one of the important sources of contamination which may lead to staphylococcal food poisoning (Mourabit *et al.,* 2020).

Materials and Methods

A total of 160 nasal swab samples of cattle were collected from different places of Aizawl, Mizoram, India.

All the swabs were processed for isolation and identification of *S. aureus* (Ewing, 1986). All the isolates were assessed by polymerase chain reaction (PCR) assay for the presence species specific gene (*nuc gene*) (Brakstad *et al.,* 1992).

Those isolates positive for *nuc* gene were further assessed by PCR assay for the presence of classical SE gene(s)(*sea, seb, sec, sed*) (Cremonesi *et al.,* 2005; Johnson *et al.,* 1991) All the isolates were subjected to antimicrobial sensitivity assay by disc diffusion method using 13 commonly used antimicrobial agents (Bauer *et al.,* 1966).

Results and Discussion

A total of 13 out of 160 (8.12%) samples from were found to be positive *nuc* gene which was used for PCR for identification of *S. aureus* (Reddy *et al.,* 2015). All the 13 *nuc* gene positive isolates were screened for the presence of virulence genes (*sec* and *sed).* A total of 1 isolate (6.66%) for *sec* and 2 isolates (13.36%) for *sed* were found to be positive respectively.

From the nasal swabs of goat, 6 (18.75%) isolates were positive for *sec* gene (Zhou *et al.,* 2017). In the food surveillance of South west China, 145 (57.77%) and 43 (17.28%) isolates were positive for *sed* and *sec* genes (Liao *et al.,* 2018). SEC are located in chromosome (Klotz *et al.,* 2003) and commonly isolated from animals (Pinchuk *et al.,* 2010). There are three subtypes of the *sec* gene (*sec1, sec2,* and *sec3*) which are categorised into antigenic properties or diversity in enterotoxin C sequencing (Mousa *et al.,* 2017) and this may be the reason for less detection of *sec* gene in our study.

Table.1 Antibiogram of *sec* and *sed* isolates detected from cattle nares in Aizawl, Mizoram

Sl. no	Antimicrobial agents	*sec* (n=1)		*sed* (n=2)	
		Resistance (%)	Sensitive (%)	Resistance (%)	Sensitive (%)
1	Oxacillin (OC)	100%	-	100%	-
2	Ampicillin (AMP)	100%	-	100%	-
3	Cefoxitin (CX)	100%	-	100%	-
4	Chloramphenicol (C)	100%	-	100%	
5	Gentamicin (GEN)	-	100%	-	100%
6	Ciprofloxacin (CIP)	-	100%	-	100%
7	Tetracycline (TE)	100%	-	100%	-
8	Erythromycin (E)	100%	-	100%	-
9	Ceftriazone (CTX)	-	100%	-	100%
10	Amikacin (AK)	-	100%	-	100%
11	Kanamycin (K)	-	100%	-	100%
12	Amoxyclav (AMC)	-	100%	-	100%
13	Azithromycin (AZM)	100%	-	100%	-

Staphylococcal enterotoxins D on the other hand are carried by plasmid (Klotz *et al.*, 2003) and they are frequently detected in *S. aureus* strains associated with intoxications (Shito *et al.*, 2015). It is the second most enterotoxins associated with staphylococcal food poisoning (SFD) after SEA which was in accordance with other workers (Fisher *et al.*, 2018; Argudin *et al.*, 2010). To the best of our knowledge this may be the first report on detection of *sec* and *sed* genes from cattle nares in India. All the *sec* and *sed* genes isolates showed (Table 1) 100% resistance to ampicillin, tetracycline, erythromycin and azithromycin whereas gentamicin, ciprofloxacin, kanamycin and amoxyclav showed 100% sensitivity. Multiple drug resistance in the isolates may be due to acquired antibiotic resistance such as resistance by mutation, acquisition of resistance genes or it may be due to intrinsic factors like outer membrane permeability, efflux systems etc (Guo *et al.*, 2020). In Mizoram, cattles are regarded as the main source of milk, milk products and meat products and the appearance of *S. aureus* isolates with multi-drug resistance may suggests that they can serve as a significant reservoir that presents a potential public health concern and can complicate possible therapeutic options.

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