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RESEARCH ARTICLE
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Detection and molecular characterization of Shiga toxin producing *Escherichia coli* (STEC) autoagglutinating adhesion gene (*saa*) from piglets in Mizoram

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Abstract

Aim: The study was carried out to detect and characterize the Shiga toxin producing *Escherichia coli* (STEC) auto agglutinating adhesion gene (*saa*) from Piglets in Mizoram.

Materials and Methods: A total 100 fecal samples from 0–3 month old piglets with (60) or without (40) diarrhoea were collected from different parts of Mizoram and screened for the presence of *E. coli*. All the locus of enterocyte (LEE) negative STEC strains was tested for the presence *saa* gene by polymerase chain reaction (PCR).

Results: In this study, a total of 254 *E. coli* isolated and identified. Altogether, 51 *E. coli* were found to be positive for at least one virulence gene tested, of which 30 and 21 were classified as STEC and EPEC, respectively. A total of 4 *E. coli* isolates were found to be positive for *saa* gene, of which one was from diarrhoeic and three were from healthy piglets.

Conclusions: STEC and EPEC are prevalent in pig population of Mizoram. Presence of saa gene positive E. coli in pigs in this region may pose a threat to public health.

Keywords: locus for enterocyte effacement, STEC autoagglutinating adhesion, Shigatoxigenic *Escherichia coli* , STEC.

Introduction

Shiga-toxin producing *Escherichia coli* (STEC) are serologically diverse, emerging food borne pathogens and leading cause for a spectrum of human illness ranging from haemorrhagic diarrhoea to even fatal consequences such as hemolytic uraemic syndrome (HUS), thrombotic thrombocytopenic purpurea (TTP) and haemorrhagic colitis (HC) [1, 2, 3]. STEC infection is commonly acquired through the consumption of faecal contaminated food or water, through direct or indirect contact with animal carriers or via secondary person to person transmission [4]. Healthy domestic ruminants are recognised as the main natural reservoir of STEC and large game animal maybe healthy carriers of STEC [5, 6]. Fresh meat and ready-to-eat meat products obtained from deer have been implicated in food borne transmission of STEC to humans in United States [7, 8]. In May 2011, strains belonging to the hitherto rarely detected STEC serotype O104:H4 were identified as causative agents of one of the world's largest outbreak of disease with high incidence of HC and HUS in the infected patients [9].

The STEC family is very diverse, and strains belonging to a broad range of O:H serotypes have been associated with human diseases. However, epidemiological evidence indicates that certain STEC subsets (for example, strains belonging to serotype O157:H7) account for a disproportionately large number of serious infections. STEC strains produce one or both of two major types of Shiga toxin, designated Stx_1 and Stx_2, and the production of the latter is associated with an increased risk of developing HUS. In addition, a subset of STEC strains considered to be highly virulent for humans has the capacity to produce attaching and effacing lesions on intestinal mucosa, a property encoded on a pathogenicity island termed the locus for enterocyte effacement (LEE). LEE encodes a type III secretion system and *E. coli* secreted proteins, which deliver effector molecules to the host cell and disrupt the host cytoskeleton. LEE also carries *eae*, which encodes an outer membrane protein (intimin) required for intimate attachment to epithelial cells; *eae* has been used as a convenient diagnostic marker for LEE positive STEC strains. However, the presence of *eae* is not absolutely linked to human virulence, as some sporadic cases of severe STEC disease, including HUS, as well as occasional outbreaks have been caused by LEE-negative strains [10, 11].

Recently, another virulence gene has been reported as STEC autoagglutinating adhesion gene (*saa*), which is carried on the large plasmid of certain LEE negative strains and this genes encode a novel outer membrane protein, which functions as an adhesin [12,13,14]. Till date, only two reports are available on detection of *saa* gene, one from captive Yaks [15, 16] and another from sheep [17].

To the recent knowledge of the authors, no report

Table-1: Details of the oligonucleotide primers used in the present study-

Primer	Sequence	Amplicon size	Reference
stx1F	5¨ATAAATCGCCATTCGTTGACTAC-3		
stx1R	5¨AGAACGCCCACTGAGATCATC-3	180bp	[20]
stx2F	5¨GGCACTGTCTGAAACTGCTCC-3		
stx2R	5¨TCGCCAGTTATCTGACATTCTG-3	255bp	[20]
eaeAF	5¨GACCCGGCACAAGCATAAGC-3		
eaeAR	5¨CCACCTGCAGCAACAAGAGG-3	384bp	[20]
hlyAF	5¨GCATCATCAAGCGTACGTTCC-3		
hlyAR	5¨AATGAGCCAAGCTGGTTAAGCT-3	534bp	[20]
saaF	5¨CGTGATGAACAGGCTATTGC-3		
saaR	5¨ATGGACATGCCTGTGGCAAC-3	119bp	[19]

has been published on association of *saa* gene in pigs in India. So, the present study was conducted to detect the *saa* gene in *E. coli* isolated from piglets in Mizoram.

Materials and Methods

Ethical approval: The present study was approved by the Institutional Animal Ethics Committee vide Order No. CVSC/CAU/IAEC/11-12/R17.

Sampling and isolation of *E. coli*: In the present study 100 fecal samples originating from 40 healthy and 60 diarrhoeic piglets (0–3 month) were collected from different parts of Mizoram, India. The samples were collected directly from rectum using swabs and processed immediately by inoculating on Mac Conkeys Agar (Hi-Media, Mumbai, India) plates. After 24 hours incubation at 37ºC, five rose pink colonies were randomly picked up and subcultured on eosin methylene blue (EMB) agar (Hi-Media, Mumbai, India) plates to observe the metallic sheen characteristics of *E. coli*. A well separated presumptive *E. coli* single colony was picked up on nutrient agar slants as pure culture and subjected to standard morphological and biochemical testing as described by Ewing [18].

Templates DNA preparation: The *E. coli* isolates confirmed by conventional tests were grown in Luria Bertani broth (Hi-media, Mumbai, India) at 37ºC overnight. One ml of the broth culture was pelleted by centrifugation at 8000 rpm for 10 minutes, washed twice with 500µl of PBS (pH 7.4). The bacterial pellet was finally, re-suspended in 300µl sterile nuclease free water and lysed by boiling for 10 minutes in a water bath followed by immediate chilling for 10 minutes on ice. The lysates were centrifuged again at 6000 rpm for 10 minutes and the supernatant was used as template DNA.

Detection of STEC and Enteropathogenic *Escherichia coli* (EPEC) marker genes and *saa* gene by PCR: A multiplex Polymerase chain reaction (PCR) was carried out using four sets of oligonucleotide primers for stx_1, stx_2, *eaeA* and *hlyA* genes (Table-1) and all the LEE negative STEC strains were further tested for the presence *saa* gene (Table-1). The PCR protocol was followed as per the method described in previous works [19, 20] with slight modification. The multiplex PCR mixture of 25µl contained 1X PCR buffer, 1.5 mM of $MgCl_2$, 40 nM of primer, 200 µM dNTPs, 1.0 U of *Taq* DNA polymerase and 2.0µl of template DNA. The PCR reaction was performed in a thermal cycler (Thermo Electron, Germany) using the following standard cycling procedure: an initial denaturation at 95^0C for 5 min, followed by 30 cycles of denaturation at 94^0C for 45 sec, primer annealing at 65^0C for 45 sec and extension at 72^0C for 42 sec and a final extension at 72^0C for 5 min.

Amplified products were analyzed by agarose gel (2% in 1X TBE) electrophoresis at 5v/cm for 2 h and documented using gel documentation system (Alpha imager, Germany).

Results

Bacterial isolation: A total of 254 *E. coli* strains were isolated from 100 faecal samples collected from piglets, of which 132 isolates were from piglets with diarrhoea (60) and 122 from non-diarrhoeic piglets (40).

PCR for STEC and *EPEC* genes: Of 254 *E. coli* isolates tested for 4 virulence genes (stx_1, stx_2, *eae*A and *hly*A), 51 (20.08%) were carried at least 1 virulence gene, of which 30 (11.81 %) and 21 (8.26 %) were detected as STEC and EPEC, respectively. Of 21 EPEC isolates, 17 (80.95%) isolates carried both *hly*A and *eaeA* genes and 4 (19.05%) carried *eae*A gene only. A total of 2(0.78%), 9(3.54%), 2(0.78%), 7(2.75%), 1(0.39%), 9(3.54%), 4(1.57%), 17(6.69%) *E. coli* isolates were carried stx_1 only, stx_2 only, stx_1 and stx_2, stx_2 and *eae*A, stx_2 and *hly*A, stx_2, *eae*A and *hly*A, stx_2, *eae*A and *hly*A, *eae* and *hly*A, respectively. None of the isolates were carried *hly*A gene only.

PCR for *saa* gene: The PCR assay yielded amplified products of 119bp, specific for *saa* genes (Figure-1). In this study, a total of four (1.5%) *E. coli* strains were found to be positive for *saa* gene of which three were from healthy piglets and one was from diarrhoeic piglet. Out of four *saa* gene positive strains, stx_1 gene was detected in two isolates, whereas, other two isolates were positive for both stx_1 and stx_2 genes.

Discussion

This study, reports for the first time the detection and characterization of *saa* genes among piglets with or without diarrhoea in Mizoram. Till now, in India there are only two reports on detection of *saa* gene, one from captive Yaks [15, 16] and another from sheep [17]. They have reported the presence of *saa* gene in 20.63% fecal samples from captive Yaks and 44%

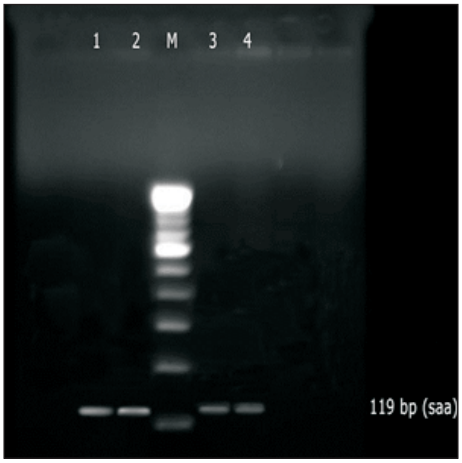

Figure-1: *saa* gene specific PCR analysis of STEC isolates. Lane 1: *saa* positive, Lane 2: *saa* positive, Lane M: Marker (100bp), Lane 3*: saa* positive, Lane 4: *saa* positive

samples from sheep. In every case, it was associated with stx_1, stx_2 and *ehx* genes.

Liu *et al.* [21] could not detect any *saa* gene from 206 isolates obtained from suckling pigs with diarrhea in China. Prevalence of *saa* gene in buffalo was 8% [22] and 83% [23].

The *saa* gene is present exclusively in LEE-negative STEC strains as reported in Yaks [15], sheeps [17], buffaloes [22, 23] and humans [19]. They have also indicated a strong association of *saa* and *ehx* genes in *eae*A negative strains of different serotypes.

Our result indicates the non-association of *eae*A and *saa* genes, which was in accordance to result with the above workers and at the same time it was showing the difference in association of *saa* and *ehx* genes, which can be explained by the high variability of the large STEC plasmids [24] and this result was in accordance to the result of Beraldo *et al.* [25], who found that there was no correlation between *saa* and *ehx* genes. It is possible that *ehx* gene is not located on the same plasmid as *saa*.

The *saa* genes encode a novel outer membrane protein, which appears to function as an auto-agglutinating adhesion and the introduction of cloned *saa* confers a semi localized adherence phenotype on *E. coli* K12 strains [26]. *saa* gene has been recorded from a variety of pathogenic LEE negative STEC strains from HUS cases from different parts of the world [19]. It is, therefore possible that *saa* is a marker for the hitherto ill-defined subset of LEE negative STEC strains capable of causing life threatening disease in humans [20]. Further studies to be carried out to examine the role of *saa* gene associated with diarrhoea in piglets.

Conclusion

STEC and EPEC are prevalent in healthy and diarrhoeic pig populations of Mizoram. Presence of saa gene in EPEC isolates increases the probability of piglet diarrhoea as it helps in AE lesions. It is also a public health concern in the region.

Authors' contributions

JLK and TKD planned and designed the study, JLK, JGT and PR performed the research experiment. JLK and TKD drafted and revised the manuscript. All authors read and approved the final manuscript.

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Competing interests

The authors declare that they have no competing interests.

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