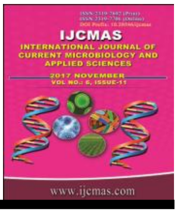
Int.J.Curr.Microbiol.App.Sci (2017) *6*(11): 1983-1987

EXCELLENT PUBLISHERS

International Journal of Current Microbiology and Applied Sciences
ISSN: 2319-7706 **Volume 6 Number 11 (2017) pp. 1983-1987**
Journal homepage: http://www.ijcmas.com



Short Communicaitons

https://doi.org/10.20546/ijcmas.2017.611.235

Virulence gene(s) Gamut of Enteropathogenic *Escherichia coli* (EPEC) and Shiga Toxin-Producing *Escherichia coli* (STEC) in Piglets with or without Diarrhoea in Mizoram (India)

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ABSTRACT

Keywords

Growth, Yield, Quality, TSS: acid ratio.

Article Info

Accepted:
17 September 2017
Available Online:
10 November 2017

Entero pathogenic *Escherichia coli* (EPEC) and Shiga toxin-producing *Escherichia coli* (STEC) are food-borne pathogens of public health concern. EPEC causes infantile watery diarrhoea and is more prevalent in developing countries than in the industrialized country. STEC, conversely are responsible for both outbreaks and sporadic cases of human diseases, ranging from mild diarrhea to hemorrhagic colitis (HC) and life-threatening hemolytic uremic syndrome (HUS) leading to kidney failure and death. Various transmission routes of EPEC and STEC have been identified, such as contaminated food and water, animal contact, person to person contact etc. Animals, such as cattle, wildlife species and swine are recorded as important reservoirs.

Introduction

Entero pathogenic *Escherichia coli* (EPEC) and Shiga toxin-producing *Escherichia coli* (STEC) are food-borne pathogens of public health concern (Ercoli *et al.*, 2015). EPEC causes infantile watery diarrhoea and is more prevalent in developing countries than in the industrialized country (Baliere *et al.*, 2015). STEC, converselyare responsible for both outbreaks and sporadic cases of human diseases, ranging from mild diarrhea to hemorrhagic colitis (HC) and life-threatening hemolytic uremic syndrome (HUS) leading to kidney failure and death (Baranzoni *et al.*, 2016).

Various transmission routes of EPEC and STEC have been identified, such as contaminated food and water (Kaspar *et al.*, 2010), animal contact (Keen *et al.*, 2007), person to person contact (Rowe *et al.*, 1993) etc. Animals, such as cattle, wildlife species (Singh *et al.*, 2015) and swine (Tseng *et al.*, 2014) are recorded as important reservoirs.

Materials and Methods

A total of 100 rectal swabs from piglets (0-3 months) with or without diarrhoea were collected from different places of Mizoram, India. All the swabs were processed for isolation and identification of *E.coli* (Ewing, 1986). All the isolates were subjected to serotyping based upon somatic antigens and were further assessed by polymerase chain reaction (PCR) assay for the presence of putative virulence gene(s)(*eae*A, *hly*A, stx_1 and stx_2) (Paton and Paton, 1998) and STEC autoagglutinating adhesion gene (*saa*) (Paton and Paton, 2001). All the isolates were subjected to antimicrobial sensitivity assay by disc diffusion method using 14 commonly used antimicrobial agents (Bauer *et al.*, 1966).

A total of 254 *E. coli* were isolated, of which 55 (21.65%) isolates were found to be positive for at least one virulence gene (Table 1). As depicted in Table 1, a total of 2 (0.78%), 9 (3.54%) and 4 (1.54%) isolates were positive forstx_1, stx_2 and *eae*A gene, respectively. A total of 40 (15.74%) isolates were carrying multiple genes. Four (1.56%), isolates in combination of stx_1 and stx_2 genes were recorded as positive for *saa* gene. Based upon the virulence genes profile, 34 (12.20%) and 21 (8.27%) isolates were characterized as STEC and EPEC, respectively. Among STEC isolates, stx_2 (54.5%) was predominant over by stx_1 (14%), which was in accordance to the finding of earlier workers (Islam *et al.*, 2016; Bandhopadhyay *et al.*, 2012). Isolation of stx_2 *E. coli* positive from piglets may be a public health concern. *saa* and *eae*A genes appears to be mutually exclusive to each other and our results was also in accordance to earlier workers (Miko *et al.*, 2014; Paton *et al.*, 2001). Similarly, there was no association between *saa* and *hly*A gene, which was also in corroboration to the result of Beraldo *et al.*, (2014). However, the present result is in contrast to earlier workers (Olivera *et al.*, 2007; Wani *et al.*, 2004), who reported the mutually inclusiveness of *saa* and *hly*A gene, which can be explained by the high variability of the large STEC plasmids (Brunder *et al.*, 1997) and it may also be possible that *hly*A gene is not located on the same plasmid with *saa*. To the best of our knowledge, this is the first report on detection of *saa*gene from piglets with or without diarrhoea in India.

Table.1 Virulence gene(s) profile and somatic type of *E. coli* isolated from piglets of Mizoram, India

Sl.No	Virulence gene(s)	Serotype and number of strains of the isolates (Figures in parenthesis indicating the number)	Number of isolates		Total N (%)
			With diarrhoea N (%)	Without diarrhoea N (%)	
1.	stx_1	O24 (1), O174 (1)	1 (0.81%)	1 (0.75%)	2 (0.78%)
2.	stx_2	O24 (2), O75 (2), O119 (3), O120 (1), UT (1)	6 (4.9%)	3 (2.2%)	9 (3.54%)
3.	stx_1 + *saa*	O24 (2)	-	2 (1.5%)	2 (0.78%)
4.	stx_1+ stx_2+*saa*	O24 (2)	1 (0.81%)	1 (0.75%)	2 (0.78%)
5.	stx_1 + stx_2	O119 (1), O161 (1)	-	2 (1.5%)	2 (0.78%)
6.	stx_2+*eae*A	O2 (2), O24 (1), O51 (1), O97 (1), O132 (1), O142 (1)	3 (2.4%)	4 (3.0%)	7 (2.75%)
7.	stx_2+*hly*A	O24 (1)	_	1 (0.75%)	1 (0.39%)
8.	stx_2+*eae*A +*hly*A	O2 (1), O69 (1), O75 (1), O119 (3),O159 (1), O165 (1), UT (1)	5 (4.0%)	4 (3.0%)	9 (3.54%)
9.	*eae*A	O60 (1), O75 (1), O141 (1), UT (1)	2 (1.6%)	2 (1.5%)	4 (1.57%)
10.	*eae*A + *hly*A	O56 (1), O60(1), O103 (1), O119 (10), O132 (1), O162 (1), O163 (1), O168 (1)	9 (7.30%)	8 (6.0%)	17 (6.69%)
TOTAL			27 (22.13%)	28 (21.21%)	55 (21.65%)

All the 55 EPEC/ STEC isolates from piglets carrying virulence gene(s) were belonged to 23 different 'O' serogroups (Table 1).

O119 was the predominant serotype which belonged to one of the 12 'O' serogroups of EPEC recognised by World Health Organisation (WHO, 1987). The other serotypes identified in this study were not reported earlier workers in India, nonetheless, as several new serotypes of *E. coli* such as O178 (Mika *et al.*, 2014) and O104 (Frank *et al.*, 2011) are being established as potential pathogens for human and animals.

All the 55 EPEC / STEC isolates showed 100% drug resistance to bacitracin, methicillin and novobiocin. Cefazolin and ofloxacin for EPEC and cefixime, gatifloxacin and cefazolin for STEC were the better antimicrobial agents in terms of sensitivity. Multiple drug resistance in EPEC and STEC strains may result from spread of genetic elements, such as plasmids, prophages or transposons, allowing horizontal transfer within and between bacterial species, predominantly in environments such as the gut micro-biome (Amaechi *et al.*, 2015). In Mizoram, pig rearing is the most common practice and pork is the major source of protein. Emergence of multi drug resistant EPEC and STEC isolates in high proportion indicates that they may act as an important reservoir posing a possible threat to public health and may complicate future therapeutic options.

Acknowledgement

The author are thankful to Dean, College of Veterinary Science and Animal Husbandry, Central Agricultural University, Selesih, Aizawl, Mizoram, India for providing necessary funds and facilities to carry out the investigation.

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How to cite this article:

Kataria, J.L., T.K. Datta and Roychoudhury, P. 2017. Virulence Gene(S) Gamut of Entero Pathogenic *Escherichia coli* (EPEC) and Shiga Toxin-Producing *Escherichia coli* (STEC) in Piglets with or without Diarrhoea in Mizoram (India). *Int.J.Curr.Microbiol.App.Sci.* 6(11): 1983-1987. doi: https://doi.org/10.20546/ijcmas.2017.611.235