

Serotyping of *Escherichia coli* isolated from piglet diarrhea

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Abstract

Aim: The aim was to investigate the different strains of *Escherichia coli* isolated from diarrheic piglets by serological typing.

Materials and Methods: A total of 150 isolates consists of 66 from diarrheic feces and 84 from intestinal contents were subjected to serological typing. The isolates were referred to National *Salmonella* and *E. coli* Center, CRI, Kasauli, Himachal Pradesh, India for serotyping.

Result: Of 150 isolates, 90 isolates were serotyped into 20 different serogroups, 4 isolates were rough and remaining 56 isolates were refractory to serotype. The most frequently encountered serogroups were O76 (25 strains), O60 (18 strains), O120 (11 strains), O87 (6 strains), O107 and O80 (5 strains each), O84 and O64 (3 strains each), and O117 and O158 (2 strains each). The other serogroups identified were O3, O5, O24, O25, O36, O42, O100, O116, O132, and O140 (1 strain each), 3 rough and 56 strains were untypable.

Conclusion: The results in the present study showed that variable strains of *E. coli* are responsible for diarrhea in piglets.

Keywords: diarrhea, *Escherichia coli*, piglet, serotype.

Introduction

Gastrointestinal disorders in pigs are a great economic challenge to intensive pig farming. They cause substantial economic losses due to mortality, stunted growth, and prolonged time for reaching slaughter weight [1]. Pathogenic *Escherichia coli* is a common agent responsible for a variety of intestinal disorders. Porcine neonatal diarrhea and post weaning diarrhea caused by enterotoxigenic *E. coli* (ETEC) result in significant mortality and morbidity, and are economically important diseases of pigs [2].

Secretory diarrhea is associated with ETEC infection and is mediated by any one of the several enterotoxin including heat-labile enterotoxin, heat stable enterotoxin-a, and heat stable enterotoxin-b [3]. The ETEC colonization in the small intestine is mediated by fimbriae that attach to the receptors on the villous enterocytes [4]. Fimbriae including K88 (F4), K99 (F5), 987P (F6), F18, and F41 are commonly found and porcine *E. coli* strains expressing these fimbriae are clinically important for porcine diarrhea [5,6]. Certain serotypes of *E. coli* are far more likely to be associated with some of the virulence factor of diarrheagenic *E. coli* than other serotypes.

The purpose of the present study was to find out the frequency of different serotypes among diarrheal cases in piglets below 2 months of age.

Materials and Methods

Ethical approval

Collection of samples was done as per standard procedure.

Collection of samples

The samples were collected from piglets below 2 months of age with the history of diarrhea viz. yellowish watery feces with the offensive smell, dehydration, and weakness. Rectal swabs were collected aseptically and diluted in phosphate buffered saline and preserved in the refrigerator until used. Then the intestinal contents from dead piglets were also collected aseptically and preserved in the refrigerator until used. Piglets died of diarrhea revealed typical lesions of gastroenteritis viz. stomachs contained undigested material, watery to blood mixed fluid in the intestine and mild catarrhal enteritis to severe hemorrhagic gastroenteritis.

Isolation and identification of *E. coli*

The rectal swabs and intestinal contents were inoculated into MacConkey's lactose agar for primary isolation by streak plate following a standard protocol [7]. Pink-colored colonies representing coliforms were picked and subcultured in eosin methylene blue agar plates for purification of the isolates. Characterization and identification of the organisms were made by a standard protocol described earlier [8] on the basis of the criteria viz. colony morphology, morphological characteristics in Gram's staining, and biochemical characteristics.

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Serotyping of *E. coli*

The purified colonies were subcultured into nutrient agar slants and preserved at 4°C. The duplicate cultures were referred to National *Salmonella* and *Escherichia coli* Research center, Kasauli, Himachal Pradesh for serological typing.

Results

Of 150 isolates consisted of 66 from diarrheic feces and 84 from intestinal contents, were subjected to serological typing. Altogether 90 (60%) isolates could be serotyped into 20 different serogroups, 4 (2.6%) isolates were rough and remaining 56 (37.33%) isolates were refractory to serotype as shown in Table-1. The most frequently encountered serogroups were O76 (25 strains), followed by O60 (18 strains), O120 (11 strains), O87 (6 strains), O107 and O80 (5 strains each), O84 and O64 (3 strains each), and O117 and O158 (2 strains each). The other serogroups identified were O3, O5, O24, O25, O36, O42, O100, O116, O132, and O140 (1 strain each).

However, of 66 isolates from diarrheic feces, 44 (66.66%) isolates could be serotyped into 16 different serogroups, 3 isolates were rough and 19 isolates were refractory to serotype. The serogroups identified were O76 (12 strains), O60 (8 strains), O120 (4 strains), O64 (3 strains), O117, O158, O84 and O80 (2 strains each), O140, O5, O3, O24, O87, O100, O25 O36, and O116 (1 strain each). Of 84 isolates from intestinal contents, 46 (54.76%) isolates could be serotyped into 9 different serogroups, one rough and 37 isolates were refractory to serotype. The serogroups identified were

Table-1: Different serogroups of *E. coli* isolated from diarrheic piglets.

Serogroup	Strain of <i>E. coli</i> from diarrheic feces	Strain of <i>E. coli</i> from intestinal contents	Total number of strains
O3	1	–	1
O5	1	–	1
O24	1	–	1
O25	1	–	1
O36	1	–	1
O42	–	1	1
O60	8	10	18
O64	3	–	3
O76	12	13	25
O80	2	3	5
O84	2	1	3
O87	1	5	6
O100	1	–	1
O107	–	5	5
O116	1	–	1
O117	2	–	2
O120	4	7	11
O132	–	1	1
O140	1	–	1
O158	2	–	2
Rough	3	1	4
Untypable	19	37	56
Total	66	84	150

E. coli = *Escherichia coli*

O76 (13 strains), O60 (10 strains), O120 (7 strains), O87 and O107 (5 strains each), O80 (3 strains), O42, O84, and O132 (1 strain each). Another 3 strains were identified to be rough and 56 strains were refractory to serotyping.

Discussion

Diarrhea is the most common cause of neonatal mortality in pigs in India, particularly in the North Eastern states. In the present study, 90 isolates were serotyped into 20 different serogroups. The most frequently encountered serogroups were O76 (25 strains), O60 (18 strains), O120 (11 strains), O87 (6 strains), O107 and O80 (5 strains each), O84 and O64 (3 strains each), and O117 and O158 (2 strains each), O3, O5, O24, O25, O36, O42, O100, O116, O132, and O140 (1 strain each). Although a variety of serogroups has been associated with diarrhea, a limited number of serogroups has been reported in enteric infections of piglets [9].

The common serogroups of *E. coli* observed in the present study and recorded earlier by different workers from different parts of the world were O5, O24, O25, O76, O120, O80, O84, O100, O116 [10-12]. The other serogroups O60, O100, O116, O117, and O120 were reported from diarrheic piglets in Korea [13]. Serogroup O80 has been reported from 23.56 % of *E. coli* strains isolated from diarrheic piglets in Western China [14]. No reports have been found regarding prevalence of serotype viz. O3, O36, O42, O64, O84, O87, O132, O140, and O158. Thus, further characterization of their virulence factors is the most important. Moreover, there are certain serotypes which were refractory to serotyping, and they are also important for detection of their virulence factors.

The frequencies of serogroups could vary from area to area and over time to time [15,16]. Moreover, the virulence genes in *E. coli* vary with the geographical area [17].

Conclusion

The results indicate that the regional difference or other selective advantages may result in *E. coli* strains with certain O- serogroups adapting to survival in the swine intestine and their environment. Hence, variable serogroups of *E. coli* are associated with diarrhea in piglets causing mortality in young pigs causing heavy economic losses to pig industry. The results provide preliminary information about the various serotypes of *E. coli* responsible for diarrhea in piglets in around organized farms of Guwahati, Assam and constitute an important database for the implementation of prevention, diagnosis, and treatment measures.

Authors' Contributions

MR collected samples for the present study. Rest of the authors equally contributed in the preparation of

the manuscript. All the authors read and approved for the final manuscript.

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Competing Interests

The authors declare that they have no competing interests.

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