RESEARCH ARTICLE

Virulence Characterization of Faecal *Escherichia coli* Isolates from Wild Animals

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Abstract

The present study was undertaken to characterize virulence factors associated with fecal *Escherichia coli* (*E.coli*) isolates (n=22) from wild animals. Isolates were phenotypically characterized for haemolytic, haemagglutination and gelatinase activity, whereas presence of adhesion genes,*viz., fimH* and *papG1* was detected genotypically by PCR. Further, whole blood survival assay of *E. coli* isolates (n=6) was performed using human and goat blood. Isolates exhibited haemolytic (45.45%) as well as gelatinase activity (27.27%) and led mannose sensitive agglutination of human (45.45%), dog (40.90%) and chicken (27.27%) RBCs. *fimH* gene was detected in 27.27% of the isolates, whereas none of the isolates was found positive for *PapG1* gene. Isolates survived, got adapted and then grew successfully in human as well as goat blood. It can be concluded from the present study that wild animals are potent reservoir of virulent *E. coli* that could adversely affect in-contact human and livestock population.

Key words: Faecal *E.coli*, *fimH,* Gelatinase, Haemagglutination, Wild animals.

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Introduction

The role of the zoo has evolved to prioritize research, education and conservation. Zoos are the important place of research and education in addition to conservation of wild animals. In Chhattisgarh, Kanan Pendari and Nandanvan are popular zoological parks and are home to wide range of animals including fox, Jackal, hyena, tiger and lion. Because of frequent interaction of visitors with wild animal species, zoos become an important component of one health concept. Captive wild animals are reservoirs of many microbial pathogens. Among which, *E. coli* present in the gut microbiomes of these animals has the major inferences on public health (Guenther *et al.*, 2011). *E. coli* can express a broad variety of virulence factors involved in the colonization, adhesion, invasion, and survival of host defenses. The most important of these factors include adhesins or fimbriae and haemagglutination; serum resistance, gelatinase enzyme and toxins such as haemolysin (Shruthi *et al.*, 2012; Mittal *et al.*, 2014). Fimbriae mediate specific binding to host receptors. Type I fimbriae are produced by *E. coli* that enable the transitory colonization of the oropharyngeal tract and allow faecal / oral transmission between hosts. *FimH* gene is responsible for the mannose-specific or receptor-specific adhesin of the type 1 fimbriae (Sauer *et al.*, 2019). Another adhesin, P fimbriae encoded on *PapG* genes are hair-like filaments, which facilitate the binding and colonization of the urinary mucosa by *E. coli* (Flores-Mireles *et al.*, 2015).

Considering the pathogenic importance of faecal *E.coli* of wild animals on human and livestock health, current study was designed to investigate major virulence determinants in *E.coli* isolated from captive wild animals in Chhattisgarh (India).

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Materials and Methods

E.coli Isolates

Twenty two faecal *E.coli* isolates of wild animals available in the laboratory of Department of Veterinary Microbiology of the College of Veterinary Science & AH, Anjora, Durg, Chhattisgarh (India) were used. Isolates were revived in nutrient broth and further subcultured on MacConkey Lactose agar as well as Eosine Methylene Blue agar for the current study.

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Phenotypic Characterization

Haemolytic Activity on 5% Calf Blood Agar: Each of the *E.coli* isolates was inoculated on the blood agar plate by streak plate method and incubated aerobically at 37ºC for 24 h and observed for zone of complete lysis of the erythrocytes around the colony and clearing of the medium (Shruthi *et al.*, 2012).

Haemagglutination Test: Haemagglutinating ability of *E. coli* isolates was assessed using RBC from different species such as human, dog and chicken. The haemagglutination test was performed by direct bacterial haemagglutination test-slide method as per protocol of Mittal *et al.* (2014). An equal volume of 3% (v/v) solution of RBC was added to well of cavity slide with or without 2% D-mannose and gently mixed with the bacterial suspension (10^9CFU/mL). After incubation at room temperature for 10 min, the haemagglutination results were observed. Haemagglutination was designated as mannose resistant haemagglutination (MRHA) when it occurred in the presence of D-mannose and mannose sensitive haemagglutination (MSHA) when it was inhibited by D-mannose.

Gelatinase Activity: The gelatinase activity of *E.coli* was detected on gelatin nutrient agar plate (Shruthi *et al.,* 2012). The plate was inoculated with overnight culture of *E.coli* and incubated at 37ºC for 18-24 h. Thereafter plate was flooded with 1 N solution of HCl. The development of opacity in the medium and a zone of clearing around the colonies were considered positive for gelatinase activity.

Whole Blood Survival Assay: Whole blood survival assay of *E. coli* isolates using human and goat blood was performed as described by Sannat (2021) and bacterial load (CFU/mL of blood) was determined.

Detection of Genes Encoding Virulence Factors in *E. coli* Isolates

All the isolates of *E.coli* were subjected to genotypic detection of adhesion genes, *viz., fimH* and *papG1* by PCR using primers procured from Eurofins Genomic Pvt. Ltd., Bangalore (Table 1). DNA was extracted using heat lysis method (boiling and snap chilling method) as per the protocol of Sannat *et al.* (2022). PCR reactions were performed with a total volume of 20 µL containing 10 µLPCR master mix (Ready mix, Sigma), 1.25µL of 10 pmol gene-specific forward and reverse primer, 1.5 µL of template DNA and 6 µL nuclease free water. PCR amplification was performed in gradient thermocycler with 30 cycles of initial denaturation at 94°C for 4 min, final denaturation at 94°C for 4 min, annealing at 60°C for 30 sec (*fimH* gene) and at 64°C for 30 sec (*PapG1* gene); extension at 72°C for 40 sec; and final extension at 72°C for 7 min. Amplified products were visualized and digitized using a Gel Documentation System.

Results and Discussion

Haemolysin Production

Ten (45.45%) out of 22 *E. coli* isolates were haemolytic on calf blood agar, whereas remaining isolates were non-haemolytic (Table 2). In accordance with present observation Mittal *et al.* (2014) and Tabasi *et al.* (2015) reported similar pattern of haemolysis by fecal as well as uropathogenic *E. coli* isolates. Haemolytic activity of *E. coli* is due to α-haemolysin which is a potent cytolysin. It is a significant virulence factor for ascending urinary tract infections and lead to increased proinflammatory cytokines, a substantial fall in circulating thrombocytes, extensive haematuria, and intravascular haemolysis (Johnsen *et al.*, 2019).

Haemagglutination Pattern

45.55% and 40.90% of the *E. coli* isolates were haemagglutination-positive using human and dog erythrocytes, respectively, whereas 27.27% isolates agglutinated chicken erythrocytes (Table 2). Each of the haemagglutinating isolates showed mannose sensitive haemagglutination (MSHA) by using RBCs from all the three species (human, dog and chicken). None of the isolates exhibited mannose resistant haemagglutination. MSHA is mediated by Type 1 fimbriae (Desai *et al.*, 2013). Existence of MSHA in fecal *E. coli* isolates supports the finding of Shruthi *et al.* (2012), who reported higher MSHA in fecal strains than in the urinary isolates. Higher rate of haemagglutination using human RBCs in present study supports the findings of earlier reports (Jadhav *et al.*, 2011; Shruti *et al.*, 2012) and thus human erythrocyte is considered the most commonly used system for studying haemagglutination pattern of *E. coli*. Absence of MRHA in present study might be correlated with absence of *papG* gene in current *E. coli* isolates as P expression represses type 1 fimbrial expression (Lane *et al.*, 2007). Type 1 fimbriae play an important role in pathogenesis of urinary tract infection by inter-bacterial binding and biofilm formation in the center parts of the lumen (Melican *et al.*, 2011).

Table 1: Details of primers used for detection of adhesion genes in *E. coli*

Adhesion Genes	Primer sequence (5'-3')	Amplicon size (bp)	Reference
fimH (Forward)	TGCAGAACGGATAAGCCGTGG	508 bp	Luna-Pineda *et al.* (2018)
fimH (Reverse)	GCAGTCACCTGCCCTCCGGTA		
PapG1 (Forward)	CAACCTGCTCTCAATCTTTACTG	692 bp	
PapG1 (Reverse)	CATGGCTGGTTGTTCCTAAACAT		

Table 2: Virulence factors in *E. coli* isolates

E.coli isolate	Wild animal source	Haemolytic activity on 5% calf blood agar	Haemagglutination			Gelatinase activity on gelatin agar	Adhesion genes	
			Human RBC	Dog RBC	Chicken RBC		*fimH*	*papG1*
1	Fox	Non-Hemolytic	+ (MSHA)	+ (MSHA)	+ (MSHA)	-	+	-
2	Fox	Non-Hemolytic	-	-	-	-	-	-
3	Jackal	Hemolytic	-	-	-	-	-	-
4	Jackal	Non-Hemolytic	-	-	-	-	-	-
5	Jackal	Hemolytic	-	-	-	+	-	-
6	Jackal	Hemolytic	+ (MSHA)	+ (MSHA)	-	+	-	-
7	Jackal	Hemolytic	+ (MSHA)	+ (MSHA)	-	-	+	-
8	Jackal	Non-Hemolytic	+ (MSHA)	+ (MSHA)	+ (MSHA)	-	-	-
9	Jackal	Hemolytic	-	-	-	-	+	-
10	Hyena	Hemolytic	-	-	-	+	-	-
11	Hyena	Hemolytic	+ (MSHA)	-	-	-	-	-
12	Hyena	Non-Hemolytic	+ (MSHA)	+ (MSHA)	+ (MSHA)	-	+	-
13	Hyena	Non-Hemolytic	-	-	-	+	+	-
14	Tiger	Non-Hemolytic	+ (MSHA)	+ (MSHA)	-	-	-	-
15	Tiger	Non-Hemolytic	+ (MSHA)	-	+ (MSHA)	-	-	-
16	Tiger	Non-Hemolytic	-	+ (MSHA)	-	-	-	-
17	Tiger	Hemolytic	-	-	-	-	-	-
18	Tiger	Hemolytic	-	-	-	+	+	-
19	Tiger	Non-Hemolytic	+ (MSHA)	+ (MSHA)	+ (MSHA)	-	-	-
20	Tiger	Hemolytic	-	-	-	-	-	-
21	Lion	Non-Hemolytic	-	-	-	-	-	-
22	Lion	Non-Hemolytic	+ (MSHA)	+ (MSHA)	+ (MSHA)	+	-	-

MSHA: Mannose sensitive haemagglutination

Gelatinase Activity

A total of six (27.27%) out of 22 *E. coli* isolates produced gelatinase on gelatin agar (Table 2). Present findings corroborated with the observations of Shruthi *et al.* (2012) and Shah *et al.* (2019), whereas Chaturvedi *et al.* (2016) reported 100% deficiency of gelatinase in *E. coli* isolates. Gelatinase is an extracellular metalloendopeptidase capable of hydrolyzing bioactive peptides such as gelatin, pheromone, collagen, casein, fibrinogen, haemoglobin and other bioactive peptides associated with inflammation. Therefore, it plays a very significant role in bacterial pathogenesis by causing direct or indirect damage to host tissue thus facilitating microbial invasion and survival in the host (Shah *et al.*, 2019).

Whole Blood Survival Assay

After an initial drop in bacterial load in blood at 3 h, progressive increase was recorded up to 24 h of incubation at 37°C. Similar growth kinetics of bacterial isolates was reported in human as well as goat blood. Present study reported bacterial resistance to killing by serum that could be attributed to capsular polysaccharide, O polysaccharide and surface proteins (Sande *et al.*, 2019). Growth kinetics of current *E. coli* isolates in human and goat blood corresponds with the observation of Baby *et al.* (2014).

PCR Amplification of Adhesion Genes

Six (27.27%) out of 22 *E. coli* isolates amplified desired amplicons (508 bp) of *fimH* gene (Fig.1), whereas none of the isolates was detected positive for *papG1* gene (Table 2). Absence of *papG1* (P fimbriae) gene in *E. coli* isolates confirms the findings of Luna-Pineda *et al.* (2018), whereas Momtaz *et al.* (2013) found *papG1* in *E. coli* isolates from UTI patients. Kudinha and Kong (2022) reported that the *papG* allele gene is usually observed in isolates from clinical cases as opposed to the lower or nil proportion in fecal isolates. *fimH* gene, encoding the type 1 fimbriae, is the most common adhesions of the uropathogenic isolates and facilitates the colonization of urinary tract (Nam *et al.*, 2013). Al-Yassari *et al.* (2020) also reported similar rate of prevalence of *fimH* genes, whereas

higher prevalence of *fimH* genes in *E. coli* was observed by Nam *et al*. (2013).

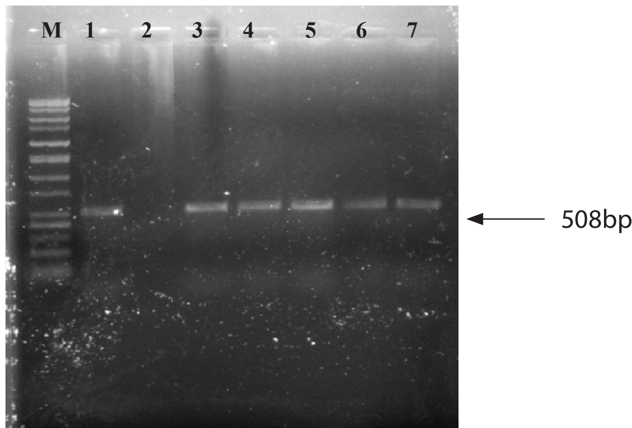

Fig. 1: PCR amplification of *fimH* gene of *E. coli*
Lane M: 100 bp DNA Marker; Lane 1 and 3-7: *E.coli* isolates positive for *fimH* gene, Lane 2: *E.coli* isolates negative for fimH gene

Conclusion

Results of virulence factors of present study indicated that fecal *E. coli* isolates of wild animal origin are potential pathogens. These organisms may enter in human environment and possible complications in human patients are sometimes life threatening. More virulent *E. coli* isolates are untreatable with antibiotics and hygienic practices. Therefore, it has to be dealt immediately by evolving strategy that would allow field veterinarians to treat more successfully and prevent virulent *E. coli* infections.

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ANNOUNCEMENT

X Annual Convention and National Symposium of SVSBT-2023

Extension of Date of Abstract Submission

This is to inform that on request from many participants, the last date of submission of Abstract through **e-mail svsbt2023@gmail.com is extended till 23rd September, 2023 for presentation in the** ***X Annual Convention*** of the Society for Veterinary Science & Biotechnology ***(SVSBT)*** and ***National Symposium on "Recent Biotechnological Advances in Health and Management of Livestock, Poultry and Companion Animals"*** to be Hosted by College of Veterinary Science & Animal Husbandry (NDVSU, Jabalpur), **Mhow, Indore, M.P. during 5th to 7th October, 2023**. The other details floated in Brochure cum Invitation remain unchanged. The abstracts received **after 23rd September, 2023 will not be entertained**.

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