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Article

Characterization of diarrheagenic *Escherichia coli* isolated from pig in Dinajpur, Bangladesh

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Abstract: Pig diarrhea is a common problem in pig farms and scattered areas of Bangladesh. This study was subjected to characterize *Escherichia coli* isolated from fecal sample of pig diarrhea and to find out therapeutic possibilities in the treatment of the disease. Isolation and characterization of the microorganisms were confirmed on the basis of their morphology, staining, cultural and biochemical properties. The study was conducted on 125 fecal samples of diarrheic pig. The prevalence of *E. coli* infection was higher in 2 years and above aged pigs (62.50 %). Prevalence of E. coli was higher in diarrheic pigs 61.00 % (100) and non diarrheic 32.00% (25) were also found to be positive for *E. coli.* The results of MR and Indole test of *E. coli* isolates were positive. The antibiogram study revealed that this isolates were highly sensitive to ciprofloxacin, levofloxacin and gentamicin; moderately sensitive to colistin sulphate and azithromycin; less sensitive to amoxicillin, tetracycline, cloxacillin, ceftraexon, neomycin, erythromycin and ampicillin. The findinings of the experiment indicates that the use of ciprofloxacin, levofloxacin and gentamicin may have the preference to be choice in clinical control of *E. coli* causing pig diarrhea.

Keywords: antimicrobial agents; *Escherichia coli*; isolation; characterization; pig diarrhea

1. Introduction

Diarrhea is one of the most likely reasons in young and aged pigs become sick or dies. Pig diarrhea is a complex disease, with many interrelated causes, agent, host and environmental factors collectively explain diarrhea and these factors interact dynamically over the course of time (Smith, 2007). The first week of life is a critical period for the newborn piglets and is generally associated with a mortality rate of 10%. Diarrhea is one of the major causes of mortality in newborn piglets, the incidence of diarrhea in pig under one year ranges between 15 to 20%, the greatest risk occurring during the first two weeks of life (Adesiyun *et al.*, 2001). Young pig diarrhea is a multifactorial disease which despite decades of research on the topi born alive in 2002, and diarrheic remains the most common cause of death of pig. The overall mortality in preweaned piglets was 10.5% and diarrhea accounted for 62.1% of pig losses (Lorenz, 2006).

In Bangladesh, pig diarrhea remains the most often reported clinical problem in pig management and rearing system (Debnath *et al.*, 1990). Prevalence of *Escherichia coli* in fecal samples was higher in yearling pig than in cull pigs (Van Donkersgoed *et al*., 2005). There are not less than forty diseases or manifestations which cause diarrhea in pig. The agents responsible for most pig diarrhea are mainly bacteria, viruses, fungi, protozoa,

helminthes, chemical agents, nutritional factors (deficiency) and also conditions like indigestion and faulty management, hepatic cirrhosis and other toxic factors. A large number of different bacterial species were isolated from diarrheic pigs, mainly enterotoxigenic *Escherichia coli, Streptococcus* spp., *Staphylococcus* spp., *Shigella* spp., *Klebsiella* spp., *Proteus* spp. (Shome *et al*., 1996); *Campylobacter jejuni, Campylobacter coli, Pasteurella multocida* (Ahmed *et al*., 1986); *Yersinia enterocolitica, Clostridium perfringens* and *Bacillius* spp. (Rycke *et al*., 1986). Discontinuation or incomplete course of treatment and continuous indiscriminate uses of antibacterial drugs against diarrhea infection of' man and animal might have influenced to produce a new generation of virulent and resistant type of bacteria (Kaura *et al*., 1988; Marshall *et al*., 1990). Although routine laboratory isolation and drug sensitivity testing are expensive and impractical, the periodical check of the pattern of the drug sensitivity of organisms is more significant (Sondgrass, 1982; Joshi *et al*., 1986). With a great consideration given to the above facts in view, the present study was undertaken to isolate and characterize *E. coli* of associated with pig diarrhea and to study the antibacterial sensitivity of isolated *E. coli* to different antibiotics.

It is, therefore, important that sensitivity of dit-Teren bacteria isolated from diarrheic pigs need to be studied from time to time 11 orders to formulate appropriate therapeutic measures.

With a great consideration given to the above facts in view, the present study was undertaken with the following objectives:

(i) To isolate and identify *Escherichia coli* spp. of associated with pig diarrhea.

(ii) To characterize the isolated *Escherichia coli* spp.

(iii) To study the antibacterial sensitivity of isolated *Escherichia coli* spp. to different antibiotics study of their pathogenicity in the laboratory animal.

2. Materials and methods

2.1. Study area and sample collection

The loose feces samples were collected from the selected pig suffering from diarrhea and enteritis in different areas of Dinajpur, Bangladesh and brought to the laboratory for conducting the experiment. Apparently healthy pig's with non diarrheic samples also collected to conduct laboratory tests. A total 125 samples were collected where 100 were diarrheic samples and 25 were non diarrheic samples.

2.2. Morphological study by Gram's staining

At first, samples were stained by using Gram's stain and observed under light microscope. Large rod shaped, single or paired arranged with pink colored organisms containing samples were suspected Gram negative bacteria and these samples were selected for further isolation and characterization and Gram positive (violate color) organisms containing samples were discarded.

2.3. Isolation of *Escherichia coli* in cultural media

Fecal material was placed into the test tubes containing nutrient broth and incubated at 37^{o}C for 24 hours. If there was found turbidity which indicated presence of bacteria (Carter, 2003). For primary culture on ordinary solid media (Nutrient agar) the selected broth containing samples were directly inoculated into nutrient agar and incubated at 37^{0}C for 24 hours. Smooth, glistening and opalescent colony were found on nutrient agar (Carter, 2003).

Above suspected sample of positive growth smooth, glistening and opalescent colonies were taken and sub-cultured on selective solid media (EMB agar) and they were incubated at 37°C for overnight. Smooth circular colonies with dark centers and metallic sheen in light found in Positive cases (Cowan, 1985). After characteristics colonies were found three representative colonies were chosen and in order to get a pure culture of the organisms, EMB agar plates were employed for subsequent sub culture. Finally, the organisms obtain from pure culture were grown on differential solid media (MacConckey agar) and incubated at 37°C for overnight. The organisms suspected for presence of *Escherichia coli*, those were shown rose pink color colony (lactose fermenter) and in Salmonella-Shigella agar media, the suspected organisms did not produce any growth (Cowan, 1985).

2.4. Characterization of isolated *Escherichia coli* by using biochemical tests

After an aerobic incubation of 24 hours at 37°C the TSI agar tubes were examined for changes in the slant or in the butt or in both places. Butt and slant both were yellow due to acid production and all sugar fermentation. Gas bubbles were found in the butt due to gas production but did not produce hydrogen sulfide (H_2S) because media did not turn into black color (Cowan, 1985). For sugar fermentation tests the tubes containing different

sugar media such as sucrose, maltose, dextrose, lactose, and mannitol were inoculated with a loopful of broth culture of the isolated organisms and incubated at 37°C for 18 hours and produced acid and gas in each sugar (Cheesbrough, 1985).

Furthermore, the organisms were inoculated into MR (Methyl Red)-VP (Voges-Proskauer) medium. It was incubated at 37°C for 48 hours. (i) When 2-3 drops of methyl red solution was added and immediately red color was found in the upper part of test tube indicated *Escherichia coli* positive (Cowan, 1985). (ii) And when 3 ml of α-napthol was added followed by 1 ml of 40% potassium hydroxide, then they were mixed well and wait for 30 minutes. No color change indicated VP test negative test and suspected presence of *Escherichia coli* (Cowan, 1985). For indole test one colony from a pure culture bacterium was inoculated into peptone water and incubated at 37^0C for 24 hours. After incubation 1 ml Kovac's reagent was added then examined. Development of bright pink color in the top layer indicated *Escherichia coli* positive (Cheesbrough. 1985).

2.5. Bacterial motility test

Before performing the test, a pure culture of the organism was allowed to grow in nutrient broth. One drop of cultured broth was placed on the cover slip and was placed invertedly over the concave depression of the hanging drop slide to make hanging drop preparation. Vaseline was used around the concave depression of the hanging drop slide for better attachment or the cover slip to prevent air current and evaporation of the fluid. The hanging drop slide was then examined carefully under 100 power objective of a compound microscope using immersion oil. The motile Escherichia coli organisms were identified by observing their motility, so they were flagellated organism (Cowan, 1985).

2.6. Antibacterial sensitivity discs

To determine the drug sensitivity and resistance patterns of *Escherichia coli*, different types of antimicrobial discs were used. By measuring the diameter of the zone of inhibition resulted from different diffusion of the agent into the medium surrounding the disc. In this study various antibiotics that were tested against selected organisms with their disc concentration (Table 1).

3. Results and Discussion

This study was carried out to isolate and characterize, determine cultural and biochemical characteristics, multiple drug resistance (antibiotics sensitivity) of enterovirulent *Escherichia coli* from diarrheic pig fecal samples.

During present study total 125 fecal samples were collected from different areas of Dinajpur district in Bangladesh. 100 samples were collected from Diarrheic pig in which 61 *E. coli* positive samples (Percentage of *E. coli* positive samples were 61.00%) and 25 samples were collected from non diarrheic pig in which 08 *E. coli* positive samples (Percentage of *E. coli* positive samples were 32.00%). Total number of *E. coli* positive samples were 69 among 125 samples (Table 1). From the analysis of the results of positive samples, both diarrheic and non diarrheic pigs were 55.20% (Table 1). It was demonstrated that the reservoir infection due to *E. coli* was almost identical (±55%). Ateba *et al.* (2008) indicates that the overall prevalence of *E. coli* O157 in pig feces (44-50%) is higher than that in cattle (5.4-20%) and human stool samples (7.5%). In an earlier study on several enteropathogens, (Adesiyun and Kaminjolo, 1994) reported that only *E. coli* was detected at a statistically significantly higher frequency in diarrhoeic compared with non-diarrhoeic cattle, pigs and sheep in Trinidad. The present longitudinal study shows that there were no significant differences between the prevalence of *E. coli* in diarrhoeic and non-diarrhoeic animals. The prevalence of *E. coli* in pig with diarrhea and non-diarrheal faeces may be attributable in the hygiene management practices of the respective animals and it indicates the risk to diseased animals pose to human health.

Regardless of animal species and types of enteropathogens assayed for, age appeared to be a significant factor in the occurrence of diarrhea. It was found that the prevalence of *E. coli* infection was higher in 2 years and above aged pigs 62.50% compared to one to two years aged pigs 58% and day old to one year's aged pigs 42.86%. The result shows of greater risk of infection in two years or above aged pigs in Dinajpur, Bangladesh (Table 2). Our present result differs with Adesiyun *et al.*, 2001, who revealed that the prevalence of infections by enteropathogens was higher among the young than in older animals. It is well established that morbidity and mortality due to enteropathogens are most prevalent in this age group.

The inhibition of the growth was demonstrated by a clear zone of growth around the antimicrobial discs due to the result of two processes viz. (a) diffusion of the antibiotics and (b) growth of the bacteria. Sensitivity was expressed as '+++', '++', '+', and '-' chronologically expressing 'high', 'moderate', 'less sensitive' and 'resistant' levels of susceptibility (Table 3).

In the present study we found that the isolates of pig *E. coli* was highly sensitive to Ciprofloxacin, Levofloxacin and Gentamicin while moderately sensitive to Colistin and Azithromycin and less sensitive to Ceftraexon and Tetracycline. The isolates of pig *E. coli* were resistant to Amoxycillin, Ampicillin, Erythromycin, Neomycin and Cloxacillin (Table 3). Our results of antibiotic sensitive to *E. coli* are almost similar to other findings (Bunner *et al*., 2007).

The cultural characterization of all positive pig *E. coli* revealed greenish black colony with metallic sheen in Eosine methylene blue agar, rose pink color smooth transparent colony in MacConkey agar, slight pinkish smooth inhibited growth colony in *E.coli*-Shigella agar and smooth, glistening and opalescent colony in nutrient agar (Table 4) which were nearly similar to the findings of other authors (Carter, 1979). In biochemical examination all the isolates fermented different sugars with the production of acid and gas after incubation. The isolates also revealed positive reaction in MR test, negative reaction in VP test and differential results in Indole test (Table 5). Most of the clinical signs and bacterial isolates that are recorded in the present study are correlated with the study in pig by the authors like (Blanco *et al.*, 1983).

Table 1. *Escherichia coli* (*E. coli*) isolates from diarrheic and non diarrheic pig.

Samples from condition of pig	No. of sample examined	No. of *E. coli* positive samples	Percentage *E. coli* positive samples
Diarrheic	100	61	61.00 %
Non diarrheic	25	08	32.00 %
Total	125	69	55.20 %

Table 2. Isolation of *Escherichia coli* in the different ages of pig.

Total no. of sample examine	Age wise distribution of pigs					
	Day old to 01 year		After 01-02 years		After 02 years to above	
	No. of Pig	No. & % positive for *E. coli*	No. of Pig	No. & % positive for *E. coli*	No. of Pig	No. & % positive for *E. coli*
125	35	15 (42.86%)	50	29 (58.00%)	40	25 (62.50%)

Table 3. Antibiotics that were tested against isolated *Escherichia coli.*

Sl. No.	Name of the antibiotics	Disc concentration (µg/disc)	Effect of Antibiotics on *E. coli*
01	Tetracycline	30	+
02	Amoxycillin	25	-
03	Ampicillin	10	-
04	Ciprofloxacin	05	+++
05	Gentamicin	30	+++
06	Ceftraexon	30	+
07	Cloxacillin	05	-
08	Colistin	25	++
09	Levofloxacin	05	+++
10	Azithromycin	15	++
11	Erythromycin	15	-
12	Neomycin	30	-

Legends: Sl = Serial; No = Number; µg = Microgram; + = Sensitive

Table 4. Results of cultural characteristics of *Escherichia coli* in different ages pig.

Source of Samples	Cultural characteristics				
	Nutrient Broth	**Nutrient agar**	**EMB Agar**	**MC Agar**	**SS Agar**
Day old to 01 year	Turbidity of the media	Smooth, glistening and opalescent colony	Greenish black colony with metallic sheen	Rose pink color colony	Inhabited growth of pinkish colony
After 01-02 years	Turbidity of the media	Smooth, glistening and opalescent colony	Greenish black colony with metallic sheen	Rose pink color colony	Inhabited growth of pinkish colony
After 02 years to above	Turbidity of the media	Smooth, glistening and opalescent colony	Greenish black colony with metallic sheen	Rose pink color colony	Inhabited growth of pinkish colony

Legends: EMB= Eosine Methylene Blue; MC = MacConckey; SS = Salmonella-Shigella.

Table 5. Results of biochemical characteristics of *Escherichia coli* in pig.

Name of the test		Biochemical Characteristics	Remark
TSI	Butt	Yellow color	Glucose, Maltose, Lactose all are fermented
	Slant	Yellow color	
	Gas bubble throughout the media		Present
	Black color (H_2S production)		Absent
MR-VP	MR	Red color	Positive
	VP	No color change	Negative
MIU	M	Turbid whole medium	Motile
	I	Pink color neck of the medium	Positive
	U	No color change whole medium	Negative

Legends: TSI = Triple sugar iron; MR = Methyl red; VP = Voges proskauer; MIU = Motility, Indole, Ureas.

4. Conclusions

Pigs are potential and economic animal of Bangladesh. A large number of pig populations are decreasing due to diarrhea caused by *E. coli in* every year. Pigs are often victims of diarrheal disease. The syndromes are variedly characterized. This study was undertaken to isolation, characterization and antibiotic sensitivity of *E. coli*. Therefore, the results of this study will help to develop an effective treatment method of pig diarrhea against this microorganism. Although *E. coli*, a normal flora of the gastrointestinal tracts of animals and humans, was detected with a high frequency in both diarrhoeic and non-diarrhoeic livestock, its true pathogenic significance cannot be ascertained from the present results. This would require the determination of the serotypes, virulence and pathogenicity of the isolates.

Conflict of interest

None to declare.

References

Adesiyun A, JS Kaminjolo, M Ngeleka, A Mutani, G Borde , W Harewood and W Harper, 2001. A longitudinal study on enteropathogenic infections of livestock in Trinidad. Rev. Soc. Bras. Med. Trop., 34:29-35.

Adesiyun AA and JS Kaminjolo, 1994. Prevalence and epidemiology of selected enteric infections of livestock in Trinidad. Prev. Vet. Med., 19: 151 - 165.

Ahmad R, M Amin and SE Kazmi, 1986. Studied on the bacterial causes of pig mortality. Pakistan Vet. J., 6: 116-118.

Ateba CN, M Mbewe and CC Bezuidenhout, 2008. Prevalence of *Escherichia coli* O157 strains in cattle, pigs and humans in North West province, South Africa. S. Afr. J. Sci., 104:7-8.

Blanco J, I Bernardez, BR Varela and EA Gonzalez, 1983. Enterotoxigenic and enteropathogenic *Escherichia coli* in Galicia (North-West Spain). Med. Microbiol. Immunol., 172:165-169.

Bunner CA, B Norby, PC Bartlett, RJ Erskine, FP Downes and JB Kaneene, 2007. Prevalence and pattern of antimicrobial susceptibility in *Escherichia coli* isolated from pigs reared under antimicrobial-free and conventional production methods. J. Am. Vet. Med. Assoc., 231:275-83.

Carter GR, and DJ Wise, 2003. Essential of Veterinary Bacteriology and Mycology. 6th edition. Wiley-Blackwell.

Cowan ST, 1985. Cowan and Steel's manual for identification of medical bacteria. 2nd edn. Cambridge University Press, Cambridge, London, pp. 138-139.

Debnath NC, BK Sil, SA Selim, MAM Prodhan and MR Howlader, 1990. A retrospective study of pig mortality and morbidity in small holder traditional farming in Bangladesh. Prev. Vet. Med., 9: 1-7.

Donkersgoed VJ, T Graham and V Gannon, 2005. The prevalence of verotoxins, *Escherichia coli* O157:H7, and E.coli in the feces and rumen of pig at processing. Can. Vet. J., 40: 332-338.

Cheesbrough M, 1985. Culturing of anaerobes in medical laboratory manual for tropical countries. Butricrwortli Co. Kent., 2: 50-56.

Joshi BP, JZ Pociecha and YA Yousif, 1986. Drug sensitivity pattern of organisms isolated from pig colibacillosis in Mosul (Iraq). Indian Vet. J., 63: 783-784.

Kaura YK, DN Bhargava, AK Pruth and S Prasad, 1988. Pathology and isolation of multiple antibiotic resistant strains of *E. coli* from an outbreak of colibacillosis in boar and sow. Indian J. of Poult. Scie., 23: 9-13.

Lorenz I, 2006. Diarrhea of the young pig; an update. World Buiatrics congress XXIV, Nice, France.

Marshall B, D Petrowski and SB Levy, 1990. Inter and intra species spread of *E. coli* in a farm environment in the absence of antibiotic usage. Proceedings the National Academy of Sciences of the United states of America., 87: 6609-6613.

Rycke JRP, N Melik, P Raimbault, J De-Rycke and P Le-Roux, 1981. Frequency of enteropathogenic *Escherichia coli* K99+ heat-stable and rotavirus in diarrhea in newborn pig. A field study in Sarthe, France. Ann. Rech. Vet., 12: 403-411.

Shome R and BR Shome, 1996. Mannose sensitive and mannose resistant haemagglutination of *E. coli* isolated from pig diarrhea cases in Andamans. Indian Vet. J., 73: 998-1000.

Smith DR, 2007. Basic principles used in the "Sand hills calving system" & how they apply to other production environments. Proceedings, the range of beef pig symposium XX. Fort collins, colorado.

Snodgrass DR, D Sherwood, HG Terzolo and BA Synge, 1982. A field survey of the aetiology of neonatal pig diarrhea. Proceedings. X11th World Congress on Diseases of Pig, the Netherlands.,1: 380-384.

Tripathi RD and JL Soni, 1982. Antibiotic sentivity test with *E. coli* isolates from cases of neonatal pig diarrhea (NPD). Indian Vet. J., 59:413-416.