

Microbial profiling of enteric pathogens among post weaned piglets and analysis of antibiotic sensitivity patterns

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Abstract

The enteric colibacillosis is a major reason for death of post weaned piglets worldwide. Porcine diarrhoea is commonly caused by different types of *E. coli* particularly colonization of the small intestine by enterotoxigenic strains of *Escherichia coli*. The Shiga toxin producing *E. coli*, neurotoxicogenic *E. coli*, enterohaemorrhagic *E. coli*, entero aggregative *E. coli* and entero invasive *E. coli* are the major group of *E. coli* affecting young piglets. A total of 52 faecal swab and 35 post mortem samples were collected from 3 piggery farms in Tamil Nadu having history of post weaning diarrhoea. The post mortem lesions show severe dehydration and distention of the small intestine with yellowish, watery mucoid fluid and the colon dilated with mucoid fluid. The stomach is dilated with partially digested food. The samples were subjected in to culture isolation and identification with Mac Conkey, XLD, EMP,MSA and BHI agar. Among the 87 samples 48 samples shows *E. coli* and 2 samples shows Salmonella isolates by colony morphology and biochemical test. The intestinal content and rectal swab were subjected into parasitic examination which shows negative for Ascariasis and other parasitic eggs. The samples were subjected into DNA extraction and PCR with specific primers. The clinical examination and the post mortem were performed with suspected piglets and the clinical examination of suspected piglet shows severe emaciation and swelling of head The antibiotic sensitivity pattern shows sensitivity towards chloramphenicol and levofloxacin and moxifloxacin resistance to most of the antibiotics.

Key words: post weaned piglet; enteric colibacillosis; *E. coli*; shiga toxin; PCR-ABST

Introduction

Escherichia coli is one among the Enterobacteriaceae group of organism and it's a rod-shaped, gram-negative bacteria which exhibits pathogenicity and it is a commensal and a common inhabitant in the gut and intestines of humans as well as animals. Some Pathogenic strains of *E. coli* are known to cause intestinal infections such as diarrhea and edema disease syndrome in pigs. Recently the increased rate of mortality in pigs due to severe diarrhea and intestinal complications by *E. coli* infection thereby affecting global swine production. The disease-causing strains are categorized into various types namely enterotoxigenic *E. coli* (ETEC) and Verotoxigenic *E. coli* (VTEC) or Shiga Toxin producing *E. coli* (STEC). The Enterotoxigenic *E. coli* induces diarrhea by colonizing intestinal mucosa using surface proteins called fimbriae and producing enterotoxins which are heat-stable (Sta, Stb, EAST1) or heat-labile (LT) or both forms. The shiga toxin producing *E. coli* of two groups Shiga toxin 1 (stx₁) and Shiga toxin 2 (stx₂) are responsible for causing death in piglets by edema of intestinal endothelium, subcutaneous and brain tissues (Vukhac et al., 2006, Begum et al., 2014 and Selva et al., 2014).

E. coli Post weaning diarrhea (PWD) also termed as enteric colibacillosis is a major reason for death of weaned piglets worldwide. Porcine diarrhea is commonly caused by different types of *E. coli* strains ETEC, Vero or Shiga toxin producing *E. coli*, necrotoxicogenic *E. coli*, enterohaemorrhagic *E. coli*, enteroaggregative *E. coli* and enteroinvasive *E. coli*. Apart from the effects of the *E. coli* toxins, sometimes physiological characteristics of small intestine and the influence on immune system of weaned pigs also causing severe infection in young piglets (Kaetz et al., 2002). The 3-4 weeks old young piglets are highly susceptible to PWD due to antibody deprivation from sow milk, stress after weaning and dietary changes resulting in dehydration, weight loss causing death leading to major economic losses to swine industries (Fair Brother et al., 2005). Typically, the primary adhesive virulent factors of ETEC linked with PWD are F4 and F18 fimbriae and the pre weaning diarrhea is associated with F4 ETEC infection. (Sun et al., 2017). Enteropathogenic *E. coli* (EPEC) in humans is also associated to cause diarrhea in pigs. EPEC possesses virulent gene which encodes adhering protein called intimin for the attachment of bacteria to the intestinal tract to cause enteric diseases (Begum et al., 2014, Kim et al., 2019). Some of the countries the prophylactic and metaphylactic use of the antibiotics is practiced whereas probiotics are used to increase the immune status after weaning (Ovcharova et al., 2022).

Pathogenic *E. coli* is controlled in humans and animals by the intervention of antibiotics and antimicrobials. The most common antibiotic used for enterobacterial infections is penicillin and streptomycin in combination or a combination of trimethoprim and sulphonamides (14). However, when subjected to wide range of antibiotics these enteric microorganisms develop antibiotic resistance which causes a threat to animal health affecting livestock farming and production. Hence the study of antibiotic resistance of bacteria following antibiotic treatment is important. The antibiotic multi resistance of *E. coli* strains infecting pigs mostly depends on the age of pigs and level of antibiotic exposure (Burow et al., 2019, Mazurek et al., 2018). In this present study include the isolation of enteric pathogens from weaned piglet and analysis of antibiotic sensitivity patterns for effective treatment

Materials and methods

Experimental study area

This study was performed at Central University Laboratory (CUL), Tamil Nadu Veterinary and Animal Sciences University (TANUVAS), Tamil Nadu for a period of 2023-2025. The samples will be collected from ILFC, MMC, TANUVAS, DLF, Pudukottai and Private piggery farm, Namakkal district of Tamil Nadu

Collection of samples

For the purpose of this study a total of 52 faecal swab from live piglets and 35 post mortem intestinal samples were collected from piglets having history of post weaning diarrhoea. Fecal swabs of 52 large white Yorkshire, Duroc, and Non descriptive diarrheic piglets were collected under aseptic conditions in sterile tubes, labelled and transported in icepacks to the laboratory for identification and characterization of specific pathogens causing diarrhea.

Culturing and Isolation of Bacteria

The primary inoculation for the growth of all kinds of bacteria were performed in brain heart Infusion (BHI) Agar, MacConkey agar, XLD agar and EMP agar. The collected swabs having fecal matter on the sterile cotton buds were gently spread over the BHI Agar and incubated at 37°C overnight to obtain the primary culture. After primary cultivation of the microbe, smears were prepared from each plate and the smears were fixed. The fixed smears were then stained by Gram's staining procedure and observed under oil immersion microscope. Upon visualization of gram-negative rods in smears, subculturing was done and the colonies obtained in the primary culture plates were streaked into MacConkey Agar for selective growth of *E. coli*. Out of the 87 samples collected a total number of 48 *E. coli* isolates were recovered and characterized. The isolates obtained after subculturing were used for further analytical study. In this study the isolation and propagation of microbe by culture methods were performed with EMP, MacConkey agar, BHI, XLD, Hichrome and SDA agar.

The isolates are then morphologically confirmed by Gram staining and the pathogenic microbe is further confirmed by biochemical tests. The molecular aspects of biochemically positive *E. coli* are confirmed by Direct PCR method using specific primers. Among many available detection methods, the molecular diagnostics of *E. coli* by PCR is an advanced method for its good sensitivity and specificity. PCR is useful for the detection of target DNA and provides reliable and rapid results for the clinical diagnosis of various pathogenic species of *E. coli* affecting pigs.

Gram's staining for Morphological Analysis

Gram's staining is one of the reliable initial bacterial identification techniques. It is used to assess culture purity and perform taxonomic studies of organisms (Mayes et al., 2009). A single colony is taken using sterilized bacteriological loop from a single colony and smeared on a glass slide which is followed by gentle heat fixation. The fixed smear was stained using Gram's crystal violet and washed off with running tap water after two minutes. Then Gram's Iodine which acts as a mordant is applied for one minute and again washed with running tap water. To remove the stain Gram's decolorizer is applied for 30 seconds and washed with running tap water. Final step is counter staining with safranin for two minutes and washing with running tap water. The slide is then air dried and then magnified under oil immersion light microscope with high power objective lens (100X) for understanding the colony morphology- color, shape and size of the colony.

Biochemical tests

The biochemical tests were performed based on the method described by Franco-Duarte et al., (2019). The biochemical tests namely Indole, Methyl Red, Voges-Proskauer, Citrate (IMViC) helps in differentiation of enterobacterial species typically *E. coli* and *Klebsiella* group from the isolates. The following Biochemical tests are performed for confirmation of bacteria based on the enzymes produced by the bacteria (fig. 17)

Urease Test

The test was performed using Urea Agar Base for the detection of urease production by the isolates. Suspend 2.58 grams of Urea Agar Base in 100ml distilled water. Heat to boiling temperature until completely dissolved. The media were sterilized by autoclaving at 121°C for 15 minutes. After cooling add 50ml of 40% urea solution was added aseptically and mixed well. Pour the media into sterile test tubes and allow to solidify in slanting position. A single colony was inoculated into the media by streaking and incubated at 37°C for 24 hours. Color change is noted for positive and negative test.

Triple Sugar Iron (TSI) Test

The 6.45 grams of Triple Sugar Iron agar were suspended in 100ml distilled water and boil until completely dissolved and sterilized by autoclaving at 121°C for 15 minutes. The media was distributed into sterile test tubes and allow to settle at a slant. The culture is inoculated gently over the agar surface and kept for incubation at 37°C for 24 hours. Based on color changes, gram-negative and gram-positive rods are differentially identified.

Indole Test

The fecal coliform species tryptone broth is used for detection of indole production. The culture media is prepared by boiling 1.5 grams of tryptone broth in 100ml distilled water and poured into individual 5ml test tubes and allowed to solidify. A single colony is inoculated and incubated at 37°C for 24 hours. Indole production by the bacteria is tested by adding few drops of Kovac's reagent over the cultured broth and a cherry red coloration for positive reaction is noted.

Methyl Red Test

The isolated colonies were inoculated into MR-VP broth prepared in sterile 5ml test tubes by stabbing directly into the media till bottom using a sterilized inoculation loop and incubated at 37°C for 24 hours. 1-2 drops of methyl red solution were added. Color change is noted for detection of gram's positive or negative strains.

Simmons Citrate Test

The utilization of citrate by bacteria is tested by Simmons citrate test. The 2.42 grams of Simmons citrate agar is added in 100ml distilled water and mix until completely dissolved by heating. The prepared agar is poured into sterile test tubes and solidified in slanting position. An isolated single colony of bacteria is gently streaked over the surface of agar and incubated at 37°C for 24 hours. Based on the retainment of deep forest green or color change in the agar, the isolates can be biochemically confirmed.

Voges Proskauer Test

MR-VP broth is prepared aseptically in 5ml sterile test tubes and an isolated bacterial colony was inoculated directly by stabbing into the broth using an inoculation loop. The 5-6 drops of Barritt's reagent A were added and mixed by gentle shaking which was followed by addition of 2-3 drops of Barritt's reagent B and mixed gently. Red-pink coloration will be observed at the surface of the medium after 30 minutes for positive reaction.

Oxidase Test

A drop of deionised water was placed over the oxidase disc to wet it prior testing and a single isolated colony was taken using an inoculation loop and spread gently over oxidase discs. A dark violet colour over the area within 10 seconds indicates positive reaction. No colour change indicates negative reaction.

Catalase Test

Few drops of hydrogen peroxide were placed over a clean glass slide. A thick bacterial colony was picked with an inoculation loop and smeared with hydrogen peroxide to check for effervescence.

Motility Indole Lysine (MIL) Test

Motility test is performed to check for motile or non-motile bacterial isolates. Suspend 3.65 grams of motility indole lysine medium in 100ml distilled water and boil until dissolved. The media was dispensed into 5ml sterile test tubes and sterilized by autoclaving at 121 °C for 15mins. A bacterial colony was inoculated by introducing it directly into the broth using a sterile inoculation loop. Based on the turbidity of the broth, motile or non-motile species is visually confirmed.

Antibiotic Sensitivity Test (ABST)

The antibiotic sensitivity tests for the Enterobacter isolates were performed by disc diffusion method according to the standards recommended by World Health Organization (WHO). The pure isolates were cultured in Muller Hinton agar through spread plate technique for testing their antibiotic sensitivity patterns. Gentamicin (10 µg), chloramphenicol (30 µg), co-trimazole (25 µg), sulphadiazine (300 µg), cefotaxime (10 µg), ofloxacin (5 µg), streptomycin (10 µg), amoxiclav (30 µg), moxifloxacin (10 µg) and levofloxacin (10 µg) were placed in MH agar and the plates were incubated at 37 °C overnight. Based on their zone of inhibition, the susceptibility of the isolates to these antibiotics were studied. The sensitive, intermediate sensitive or resistance patterns of antibiotic were interpreted using the interpretative chart of the zone sizes of the Kirby – Bauer sensitivity test method (fig. 14 and fig. 15)

DNA extraction from bacterial isolates

The genetic materials of *E. coli* were isolated for molecular study. The individual colony is grown in Nutrient agar were used for DNA extraction. A sufficient amount of grown bacterial colony taken using an inoculation loop was transferred into a 1.5 ml Eppendorf tube containing 180 µL deionised water, 20 µL sodium hydroxide (0.5mM) and kept in boiling water bath for 10 mins to facilitate the release of DNA by cell lysis. The DNA extracted was used as a template for the following PCR reactions.

Molecular Analysis by Polymerase Chain Reaction (PCR)

PCR reactions were performed in a total volume of 10µL, constituting 5 µL 2X Taq polymerase master mix, 0.5 µL forward primer, 0.5 µL reverse primer, 2 µL of template DNA, 2 µL nuclease free water. The virulence factor F 18 specific primers (F-GTGAAAAGACTAGTGTATTTC, R-CTTGTAAGTAACCGCGTAAGC) used for amplification of template DNA (Kim et al., 2019) The amplification conditions are as follows: 95°C denaturation (15 min), 94°C denaturation for 1 min, 58°C annealing for 1 min, 72°C extension for 1 min (30 cycles) and a final extension for 7 min at 72°C. Afterward, electrophoresis was performed to analyse amplicons in 1.5% agarose gel stained with ethidium bromide. A molecular weight marker with 100 bp increments (100 bp DNA ladder) was used as a standard. (Fig. 16)

Results

The clinical examination and the post mortem were performed with suspected piglets and the clinical examination of suspected piglet shows severe emaciation and swelling of head (fig. 1). The post mortem lesions show severe congestion and haemorrhages of intestinal area and ballooning of small intestine filled with unclotted blood and severe haemorrhages and severe congestion of other visceral organs (fig 1,2,3,3a,4,5). The suspected samples were subjected into culture isolation and identification. The organisms as identified based on colony morphology and biochemical character (fig 17). Some of the sample showed metallic shine colony in EMP agar and Pink colour colony in Mac Conkey agar and SDA agar, XLD agar and Hichrome agar used for isolation of other pathogens. The gram staining showed gram negative rods, *E. coli* (n=48) and some sample showed Staphylococcus (n=2) few of them showed clostridium (n=3), Pseudomonas (n=2), Klebsiella (n=10), Candida species (n=5), Salmonella (n=3) and Aspergillus (n=3) (table.1 and Fig 6,7,8,9)

The histopathological evaluation of stomach shows diffused mucosal oedema, degeneration and necrosis of epithelium and Diffused moderate degeneration, necrosis, sloughing of epithelium with infiltration of lymphocytes in the lamina propria in small intestine. Degeneration and necrosis of epithelial cells with congestion of blood vessels in the lamina propria and submucosa in caecum and Diffused sinusoidal haemorrhages with vascular degeneration of hepatocytes in liver (fig 10,11,12,13)

A total of 48 *Escherichia coli* isolates obtained from the collected samples were all subjected to antibiotics by disc diffusion method to understand their sensitivity and resistance patterns against the drug and the *E. coli* isolates showed resistance to co-trimazole (25 µg), sulphadiazine (300 µg), cefotaxime (10 µg),

ofloxacin (5 µg), streptomycin (10 µg), amoxiclav (30 µg) oxytetracycline (30 µg) some of the were showed intermediately resistant to Gentamicin (10 µg) and some of the E.coli isolates were sensitive to moxifloxacin, levofloxacin, and chloramphenicol. (table 2 and fig 14,15). The PCR amplification of the isolates was done for F18 *Escherichia coli* gene. The PCR product was run on 1.5% agarose gel stained with Ethidium bromide and the amplified DNA bands could be observed at a size of 510bp.(fig 16)

In this present study along with E.coli, other microbes like Klebsiella, Salmonella, Staphylococcus, Candida, Clostridium also isolated. A total of 87 samples *Escherichia coli* (n= 48), *Klebsiella* (n=10), *Salmonella* (n=3), *Pseudomonas* (n=2), *Candida* (n=5), *Aspergillus* (n=3), *Clostridium* (n=3) isolates obtained from the collected faecal samples (table 1 and fig. 6,7,8,9).

All the *E.coli* isolates were subjected to antibiotics by disc diffusion method to understand their sensitivity and resistance patterns against the drug. The ABST results shows sensitivity towards chloramphenicol and levofloxacin and moxifloxacin resistance to most of the antibiotics (table 2 & fig 14, 15)



Fig 1: E.coli infected piglet shows swollen head and emaciated bodies



Fig 2: PM examination of abdominal cavity of affected piglet shows ascites and white fibrinous deposition



Fig 3: Balloning of intestine with unclotted blood seen in intestine



Fig 3a: severe intestinal ulceration in affected piglets

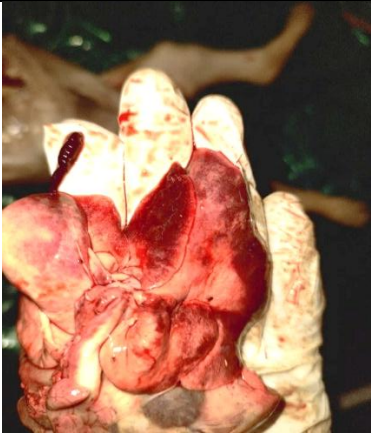


Fig 4: Severe haemorrhages on the lung of affected piglets



Fig 5: Severe congestion of small and large intestine of affected piglets



Fig 6: Culture isolation and identification



Fig 7: Isolation of E. coli in Mac Conkey agar

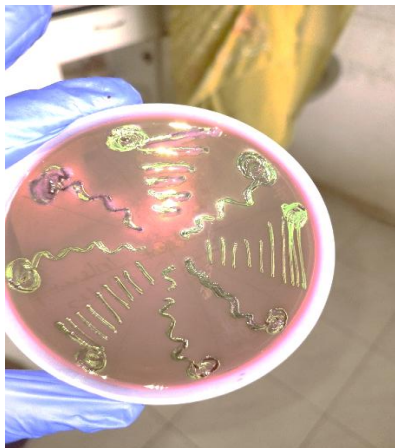


Fig 8: Pure E. coli isolate shows metallic shine colony in EMP agar



Fig 9: Pure E. coli isolate shows metallic shine colony in EMP agar

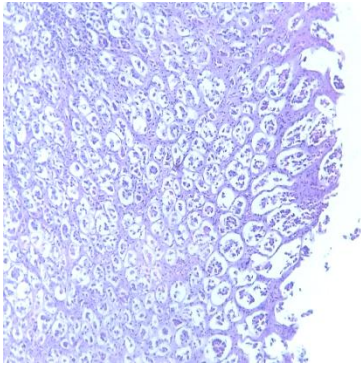


Fig 10: Diffused mucosal oedema, degeneration and necrosis of epithelium seen in stomach in HP examination

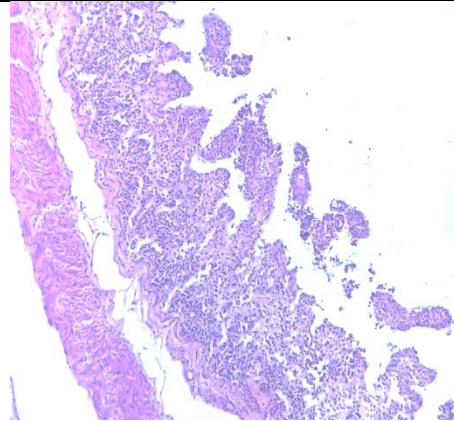


Fig 11: Diffuse moderate degeneration, necrosis, sloughing of epithelium with infiltration of lymphocytes in the lamina propria seen in small intestine in HP examination

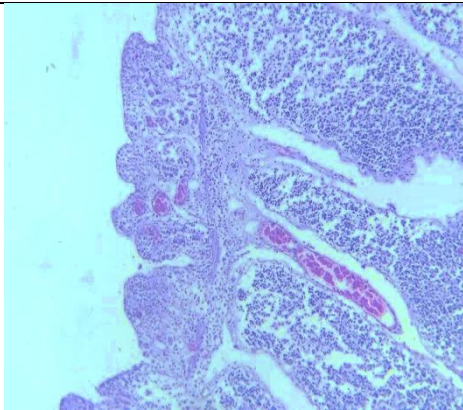


Fig 12: Degeneration and necrosis of epithelial cells with congestion of blood vessels in the lamina propria and submucosa seen in caecum in HP examination

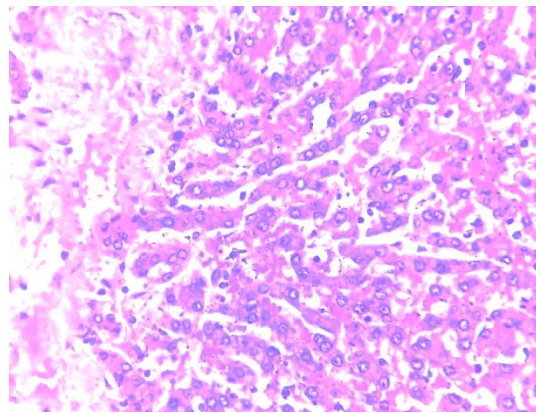


Fig 13: Diffuse sinusoidal hemorrhages with vascular degeneration of hepatocytes seen in liver in HP examination



Fig 14: antibiotic sensitivity patterns analysis of isolates

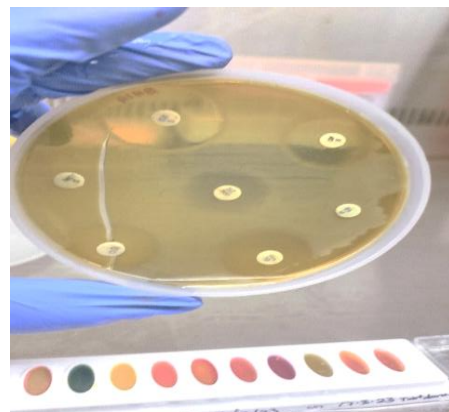


Fig 15: antibiotic sensitivity pattern analysis of isolates

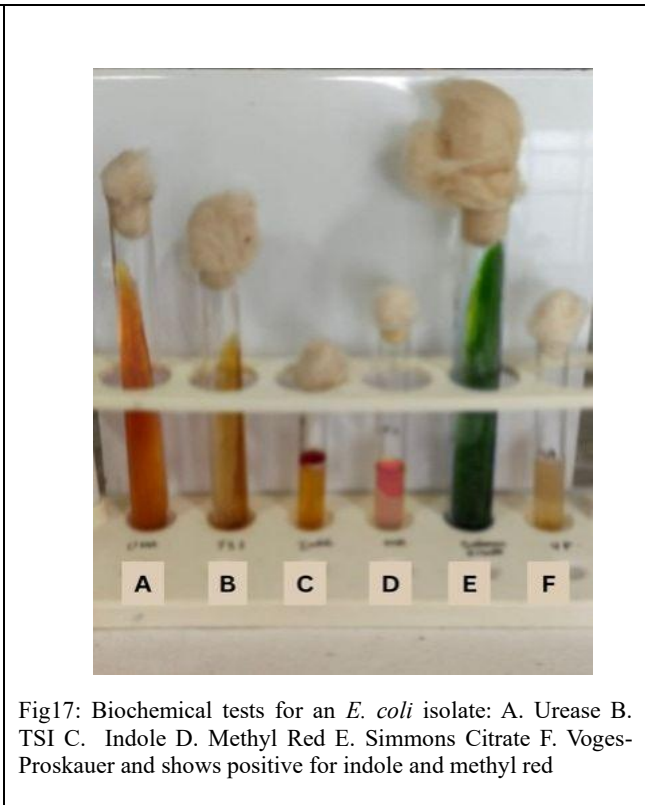
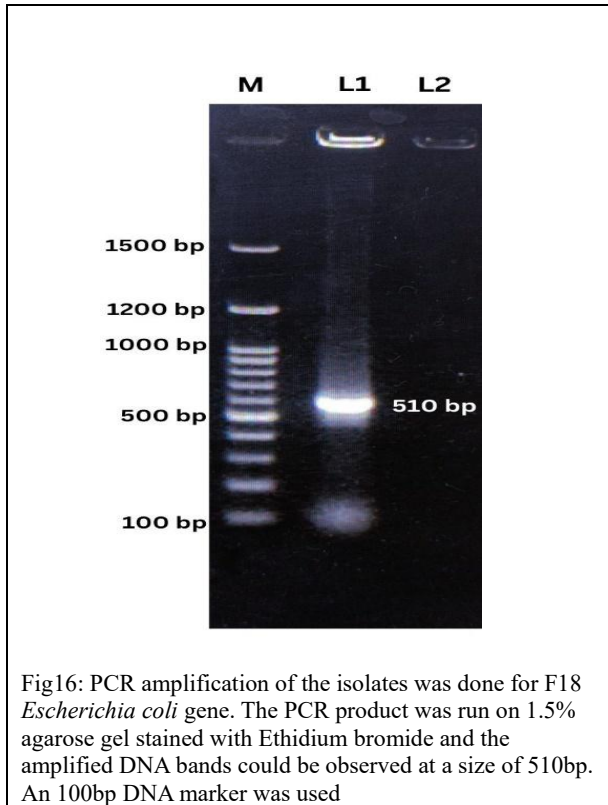


Table 1: Bacteria isolated from intestinal / faecal sample of piglets and their positive percentage

S.No	Organism isolated	Sheep		Percent positivity
		No. of positive	Total number of sample	%
1	<i>E.coli</i>	48	87	55.17
2	<i>Staphylococcus</i>	2	87	1.74
3	<i>Salmonella</i>	3	87	3.44
4	<i>Pseudomonas</i>	2	87	1.74
5	<i>Klebisella</i>	10	87	11.49
6	<i>Candida</i>	5	87	5.747
7	<i>Aspergillus</i>	3	87	3.44
8	<i>Clostridium</i>	3	87	3.44
9	Total	76	87	-

Table 2: Antibiotic sensitivity patterns for different isolates *E. coli*

Antibiotics	Sensitive (%)	Moderate sensitive (%)	Resistant (%)
Ampicillin/Sulbactam	12/48 (25 %)	8/48 (16%)	28/48 (58.3%)**
Amoxicillin clavulanate	10/48 (20.83%)	3/48 (6.25%)	35/48 (72.9%)**
Cefoperazone	14 /48 (29 %)	4/48 (8.33%)	30/48 (62.5%)**
Cefotaxime	3/48 (6%)	5/48 (10%)	40/48 (83%)**
Chloramphenicol	35/48 (72.9 %)*	3/48 (6.2)	10/48 (20.83%)
Ciprofloxacin	19 /48 (39.5%)	7/48 (14.5%)	22/48 (46.5%)
Metronidazole	15/48 (31%)	8/48 (16%)	25/48 (52%)**
Gentamicin	19/48 (39.5%)	12/48 (25%)	17/48 (35%)
Sulphadiazine	9/48 (18%)	3/48 (6.25%)	36/48 (75%)**
Levofloxacin	25/48 (52%)*	5/48 (10%)	25/48 (41.6%)
Moxifloxacin	18 /48 (53.33%)*	6/48 (12%)	24/48 (50%)
Tetracycline	3 /48 (6%)	10/48 (20.83%)	35/48 (72.9%)**
Enrofloxacin	23/48 (47.9)	8/48 (16.6%)	17/48 (47%)

*-sensitive

** resistance

Discussion

The diarrhoea in pigs is one of the major problems worldwide leading to huge economic losses to swine industry. Neonatal and post-weaning diarrhea (PWD) is responsible for high morbidity and mortality where mortality may reach up to 20-30% during acute outbreaks of PWD. Many viral, bacterial, and parasitic agents are involved in the cause of neonatal diarrhea such as transmissible gastroenteritis (TGE) virus, swine enteric coronavirus disease (SECD) including porcine epidemic disease virus (PEDV) and swine delta corona virus (SDCV), rotavirus, *E. coli*, *Salmonella*, *Clostridium perfringens* type C, *Strongyloides ransomi* and *Coccidia*. The PWD is a multifactorial condition and it involves interaction between sow, piglet, environment, ETEC bacteria and managemental practices (Hong *et al.*, 2021). The major pathotypes of *E. coli* consist enteropathogenic *E. coli* (EPEC), enterohaemorrhagic *E. coli* (EHEC), enteroaggregative *E. coli* (EAEC), enteroinvasive *E. coli* (EIEC), diffusely adherent *E. coli* (DAEC), Vero- or Shiga-like toxin-producing *E. coli* (VTEC or STEC) and enterotoxigenic *E. coli* (ETEC). Among these, the most common etiological agents responsible for PWD in pigs are ETEC displaying the fimbriae F4 (K88) and F18 strains (Makvana *et al.*, 2015). The fimbriae are hair-like appendages in *E. coli* outer surface of the outer membrane of the bacterial cells, which is facilitate the adhesion of ETEC to the small intestinal mucosa of pig (Dubreuil *et al.*, 1916). In pigs, the F4 and F18 fimbriae are mostly associated with PWD and these two fimbrial genes were found in 92.7% of all ETEC-induced PWD in piglets. After adhesion in the intestinal epithelium, the ETEC rapidly proliferates to produce one or more types of heat labile and heat stable enterotoxins which stimulate water and electrolyte secretion and reduce fluid absorption in the small intestine leads to severe diarrhoea (Silvia *et al.*, 2014).

The intestinal epithelium forms as a single layer lining the gastrointestinal tract and is responsible for the uptake of nutrients and water and serves as a physical barrier to exclude potential antigens, pathogens and toxins from the external environment. The ETEC infection could damage the intestinal epithelial barrier functions, resulting in electrolytes and water imbalance and watery diarrhoea and induce intestinal inflammation in piglets (McLamb *et al.*, 2013). The inner mucus layer of intestine contains antimicrobial peptides, immunoglobulin-A (IgA), and other molecules that are essential in the innate immune response and the maintenance of intestinal homeostasis. The ETEC infection could alter the expression of MUC2 gene in the small intestine (Kim *et al.*, 2019).

The innate immune responses are observed by the elevations of inflammatory mediators, including IL-1b, IL-6, and IL-8, which further promote leukocyte accumulation and survival in the inflamed sites. The immune recruited neutrophils and activated macrophages are responsible for the elimination of pathogens and stimulating systemic inflammation of intestine particularly ETEC F18. The heat labile and heat stable toxin and Shiga-like toxins remarkably induced the recruitment of neutrophils and macrophages in the ileum of weaned pigs during the peak of infection and the up-regulated expression of genes encoding inflammatory mediators like COX2, IL1B, IL6, IL7, and TNF were also observed in the ileal mucosa of ETEC F18 infected piglets (Fournier *et al.*, 2012, Kolaczowska *et al.*, 2013, Sugimoto *et al.*, 2013).

The incidence of PWD in piglets causes higher economic loss to the swine industry and most of the studies showed that the enterotoxigenic *E. coli* is the most important agent of enteric colibacillosis in pig and the fimbrial adhesins are necessary in the pathogenetic mechanism. The *E. coli* with fimbrial adhesins can be also detected in pigs without any diarrhoea. Moredo *et al.* (2015) demonstrated that the percentage of ETEC infection in non-diarrhoeic pigs was 16.6% during the lactation period, 66% during lactation phase and 17.3% during the finisher population. In our study the percentage of *E. coli* induced post weaning diarrhoea in piglet is 55.17% in symptomatic animals.

The F4 and F18 fimbriae have been frequently detected in weaned piglets in several countries such as Korea, Japan, Europe and United States (Lee, 2016). The frequency of different virulence types is mainly mediated by the different specific receptors on porcine intestinal brush border epithelial cells (enterocytes). These receptors change from pigs to pigs depending on the age of piglet (Luppi, 2016) and infection with ETEC carrying the F5, F6 or F41 fimbriae which occurs in pigs less than 2 weeks of age. The two non-fimbrial adhesin genes were also frequently identified in China and other countries which is causing diarrhoea in piglets.

In India, the most common cause of morbidity and mortality in piglets is diarrhoea (Sinha *et al.*, 2018) and *E. coli* is primarily associated with these diarrheic pigs. The antimicrobials are widely used in piggeries for prophylactic and therapeutic purposes to control post weaned diarrhoea. The most commonly used antimicrobials to treat affected piglets are amoxicillin/clavulanate, trimethoprim/ sulphonamide, enrofloxacin, colistin, apramycin, ceftiofur, neomycin and gentamicin (Fairbrother *et al.*, 2012). Frequent and long-term use of a particular antibiotic in a specific region may result in the development of resistance in the bacteria to the particular antibiotic (Aslani *et al.*, 2008). The multiple antibiotic resistance strains of *E. coli* emerged and causes severe threat to piggery industry and may spill over to other livestock as well as human population. The increased antimicrobial resistance to several antibiotics such as apramycin, neomycin, trimethoprim-sulphamethoxazole and colistin has been observed in ETEC strains causing post-weaned diarrhoea (Zhang *et al.*, 2014). Multidrug resistance among ETEC isolates has been isolated and recently there has been an increasing tendency for porcine ETEC to express a multidrug-resistant phenotype. The continuous need to update the

antibiotic sensitivity profile of *E. coli* isolated from piggeries, particularly from diarrheic pigs is important in selection of effective antibiotics for therapeutic purposes to reduce treatment cost and to reduce antibiotic resistance. In this study the antibiotic sensitivity as well as the resistance patterns also analysed. The prevention of post-weaning *E. coli* infection is extremely important to maintain growth performance, prevention of piglet mortality and welfare of pigs during the entire lifespan however further research needed for analysis of multi drug resistance ETEC strains among the pig population.

Conclusion

In this research the *E. coli* was isolated from piglet, molecularly confirmed and antibiotic resistance patterns were analysed. The therapeutic use of antimicrobials is widely practiced to control both neonatal and post-weaning colibacillosis. The increase of antimicrobial resistance among pathogenic *E. coli* strains with an increased prevalence of multi-resistant *E. coli* strains from diarrhoeic pigs is leading problem to more attention on the alternatives to antibiotics such as vaccines, probiotics, prebiotics, additives and management practices also needed.

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References

- 1) Begum, J., Dutta, T.K., Chandra, R., Choudhary, P.R., Varte, Z. and Bitew, M., (2014). Molecular and phenotypic characterization of shigatoxigenic *Escherichia coli* (STEC) and enteropathogenic *E. coli* (EPEC) from piglets and infants associated with diarrhoea in Mizoram, India. *African Journal of Biotechnology*, 13(13).pp1453-1461
- 2) Burow, E., Rostalski, A., Harlizius, J., Gangl, A., Simoneit, C., Grobbel, M., Kollas, C., Tenhagen, B.A. and Käsbohrer, A., 2019. Antibiotic resistance in *Escherichia coli* from pigs from birth to slaughter and its association with antibiotic treatment. *Preventive Veterinary Medicine*, 165, pp.52-62.
- 3) Dubreuil JD, Isaacson RE, Schifferli DM. (2016). Animal enterotoxigenic *Escherichia coli*. *EcoSal. Plus* 7:1–80. doi: 10.1128/ecosalplus.ESP-0006-2016
- 4) Franco-Duarte R, Cernakova L, Kadam, S. et al. 2019. Advances in chemical and biological methods to identify microorganisms—from past to present. *Microorganisms*; 7: 130. 10.3390/microorganisms7050130.
- 5) Fairbrother, J.M., Nadeau, É. and Gyles, C.L., (2012). *Escherichia coli* in postweaning diarrhoea in pigs: an update on bacterial types, pathogenesis, and prevention strategies. *Animal Health Research Reviews*, 6(1), pp.17-39.
- 6) Fournier B, Parkos C. (2012), The role of neutrophils during intestinal inflammation. *Mucosal Immunology* 5:354–66. doi: 10.1038/mi.2012.24
- 7) Hong J, Ariyibi S, Antony L, Scaria J, Dilberger-Lawson S, Francis D, et al. (2021) Growth performance and gut health of *Escherichia coli*-challenged weaned pigs fed canola meal-containing diet. *Journal of Animal Science* 99:skab196. doi: 10.1093/jas/skab196
- 8) Kim K, He Y, Xiong X, Ehrlich A, Li X, Raybould H, et al. (2019). Dietary supplementation of *Bacillus subtilis* influenced intestinal health of weaned pigs experimentally infected with a pathogenic *e. coli*. *Journal of Animal Science & Biotechnology* 10:1–12. doi: 10.1186/s40104-019-0364-3
- 9) Kolaczowska E, Kubes P. (2013). Neutrophil recruitment and function in health and inflammation. *Nature Review of Immunology* 13:159–75. doi: 10.1038/nri3399
- 10) Lee IK, Kye YC, Kim G, Kim HW, Gu MJ, Umboh J, et al. (2016). Stress, nutrition, and intestinal immune responses in pigs — a review. *Asian-Australasian Journal of Animal Sciences* 29:1075–82. doi: 10.5713/ajas.16.0118
- 11) Luppi A, Gibellini MV, Gin T, Vangroenweghe F, Vandenbroucke V, Bauerfeind R, et al. (2016). Prevalence of virulence factors in enterotoxigenic *Escherichia coli* isolated from pigs with post-weaning diarrhoea in Europe. *Porcine Health Management*; 2:20. doi:10.1186/s40813-016-0039-9.
- 12) Makvana S, Krilov LR. (2015). *Escherichia coli* infections. *Pediatric Review* 36:167–70. doi: 10.1542/pir.36-4-167
- 13) Mazurek, J., Bok, E. and Baldy-Chudzik, K., (2018). Complexity of antibiotic resistance in commensal *Escherichia coli* derived from pigs from an intensive-production farm. *Microbes and environments*, 33(3), pp.242-248.
- 14) McLamb BL, Gibson AJ, Overman EL, Stahl C, Moeser AJ. (2013). Early weaning stress in pigs impairs innate mucosal immune responses to enterotoxigenic *e. coli* challenge and exacerbates intestinal injury and clinical disease. *PLoS One* 8: e59838. doi: 10.1371/journal.pone.0059838

- 15) Moredo FA, Piñeyro PE, Márquez GC, Sanz M, Colello R, Etcheverría A, et al. (2015). Enterotoxigenic *Escherichia coli* Subclinical Infection in Pigs: Bacteriological and Genotypic Characterization and Antimicrobial Resistance Profiles. *Foodborne Pathogenic Disease.*;12(8):704–11.
- 16) Ovcharova, A., Ostrenko, K., Pyankova, E. 2022. Effect of the probiotic *Lactobacillus reuteri* on the immune status of piglets after weaning. *Journal of Livestock Science* 13: 33-37 doi. 10.33259/JLivestSci.2022.33-37
- 17) Silva, L.E., Souza, T.B., Silva, N.P. and Scaletsky, I.C., (2014). Detection and genetic analysis of the enteroaggregative *Escherichia coli* heat-stable enterotoxin (EAST1) gene in clinical isolates of enteropathogenic *Escherichia coli* (EPEC) strains. *BMC Microbiology*, 14, pp.1-6.
- 18) Sugimoto MA, Sousa LP, Pinho V, Perretti M, Teixeira MM. (2016). Resolution of inflammation: What controls its onset? *Frontiers of Immunology* 7:160. doi: 10.3389/ fimmu.2016.00160
- 19) Sun, Y. and Kim, S.W., (2017). Intestinal challenge with enterotoxigenic *Escherichia coli* in pigs, and nutritional intervention to prevent postweaning diarrhea. *Animal Nutrition*, 3(4), pp.322-330.
- 20) Zhang W. (2014). Progress and Challenges in Vaccine development against enterotoxigenic *Escherichia coli* (ETEC) – Associated porcine Post-weaning Diarrhoea (PWD). *Journal of Veterinary Medical Research*1(2):1006.a