ISOLATION AND CHARACTERIZATION OF BACTERIA ASSOCIATED WITH GASTROENTERITIS IN WEANED PIGLETS

ATULYA M

Thesis submitted in partial fulfillment of the requirement for the degree of

Master of Veterinary Science

Faculty of Veterinary and Animal Sciences

Kerala Agricultural University, Thrissur

2009

Department of Veterinary Microbiology

COLLEGE OF VETERINARY AND ANIMAL SCIENCES

Mannuthy, Thrissur

DECLARATION

I hereby declare that the thesis entitled "**ISOLATION AND CHARACTERIZATION OF BACTERIA ASSOCIATED WITH GASTROENTERITIS IN WEANED PIGLETS"** is a bonafide record of research work done by me during the course of research and that this thesis has not previously formed the basis for the award to me of any degree, diploma, associateship, fellowship or other similar title, of any other University or Society.

Mannuthy,
13 -05- 2009.

Atulya M

CERTIFICATE

Certified that this thesis entitled **" ISOLATION AND CHARACTERIZATION OF BACTERIA ASSOCIATED WITH GASTROENTERITIS IN WEANED PIGLETS "** is a record of research work done independently by **Atulya M** under my guidance and supervision and that it has not previously formed the basis for the award of any degree, diploma, fellowship or associateship to her.

Mannutthy,
13 -05- 2009.

Dr. Koshy John
(Chairman, Advisory committee)
Associate Professor,
Department of Veterinary Microbiology,
College Of Veterinary and Animal Sciences, Mannuthy.

CERTIFICATE

We, the undersigned members of the Advisory Committee of **Atulya M.,** a candidate for the degree of **Master of Veterinary Science** in Veterinary Microbiology**,** agree that the thesis entitled **"ISOLATION AND CHARACTERIZATION OF BACTERIA ASSOCIATED WITH GASTROENTERITIS IN WEANED PIGLETS"** may be submitted by **Atulya M.,** in partial fulfillment of the requirement for the degree.

Dr. Koshy John,
(Chairman, Advisory Committee)
Associate Professor,
Department of Veterinary Microbiology,
College of Veterinary and Animal Sciences,
Mannuthy.

Dr. G. Krishnan Nair,
Professor and Head,
Department of Veterinary Microbiology,
College of Veterinary and Animal Sciences,
Mannuthy .
(Member)

Dr. A. Kannan
Associate Professor,
Department of Livestock Production and Management,
College of Veterinary and Animal Sciences, Mannuthy.
(Member)

Dr. N. Divakaran Nair
Associate Professor,
Centre of Excellence in Pathology,
College of Veterinary and Animal Sciences, Mannuthy.
(Member)

External Examiner

ACKNOWLEDGEMENTS

Words are seldom sufficient to express, but given an opportunity, I would like to thank ***Almighty God*** *in the very first place who gave me the strength and courage to complete this work.*

It is greatest pleasure for me to convey my deepest sense of gratitude, indebtness and whole hearted thanks to my revered mentor ***Dr. Koshy John,*** *Associate Professor, Department of Veterinary Microbiology for his guidance, critical assessment and everlasting help. I am very much privileged to work under him.*

I wish to express my heartfelt gratitude and sincere thanks to ***Dr. G. Krishnan Nair,*** *Professor and Head, Department of Veterinary Microbiology. I will remain indebted forever to him for his guidance, keen interest, constant support, supervision, inspiration, infinite patience, criticisms and affection throughout the period of the study.*

Dr. N. Divakaran Nair, *Professor, Centre of Excellence in Pathology and one of the advisory members has always been a source of great help and encouragement. I am thankful to him for the support given to me.*

My sincere thanks to ***Dr. A. Kannan,*** *Associate Professor, Department of Livestock Production and Management for providing the facilities, valuable comments and generous help whenever I approached him.*

I am grateful to the ***Dr. E. Nanu,*** *Dean, College of Veterinary and Animal Sciences, for providing the facilities for the study. I am indebted to Kerala Agricultural University for awarding me the fellowship needed for postgraduate study.*

*Grateful acknowledgement is made to the **Director,** NSEC, Central Research Institute, Kasauli for their valuable help for the elucidation of serotype identity of cultures.*

*The guidance and suggestions extended by **Dr. V. K. Lalitha,** Professor and Head, Centre of Excellence in Pathology; **Dr. Priya P. M., Dr. Siju Joseph,** and **Dr. Binu K. Mani,** Assistant Professors, Department of Veterinary Microbiology are gratefully acknowledged.*

*I am extremely thankful to **Dr. Jesto George**, PhD Scholar, Department of Veterinary Microbiology for his guidance and generous help. I warmly remember and acknowledge **Dr. Jini George, Dr. Surya Sankar and Dr. Thulasi Pillai** for their company and love.*

*I treasure the generous help rendered by **Mr. Prajay Gandhi** and **Ms. Ann Mary Joy**, Dept. Pharmaceutical Biotechnology, M.C.O.P.S, M.U., Manipal for conducting plasmid profile studies.*

*I am thankful to supporting staffmembers of the Department of Veterinary Microbiology, **Mrs. Bhavana, Mrs. Nicy Sebastian, Mrs. Nicy, Mr. Balu, Mrs. Leela, Mr. Anto, Mrs. Kamalam, Mrs. Sheeja, Mrs. Bindhu** and **Mrs. Sarala** for their co-operation and assistance.*

I would like to express my gratitude to my beloved husband for his guidance, help and encouragement through out my studies and thesis work. . I would like to mention a word of thanks to my Father, Mother, Mother in law and Daddy. It is their support and best wishes that has helped me throughout.

Atulya M

CONTENTS

LIST OF TABLES

LIST OF FIGURES

Introduction

1. INTRODUCTION

Antimicrobial resistance in pathogenic bacteria is a global threat and therefore increasing attention is being paid to the prudent use of antibiotics in food producing animals (WHO, 2001). Gastrointestinal diseases of growing pigs cause huge economical loss for pig production worldwide (Thomson, 2006) and enteric bacterial infections are often treated with antimicrobials. The emergence, propagation, accumulation and maintenance of strains of antimicrobial resistant (AR) pathogenic bacteria have become a worldwide health concern in human and veterinary medicine (Anderson, 1999; WHO, 2001; Levy and Marshall, 2004). The intensive therapeutic uses and misuses of antimicrobial agents in humans and companion animals, as well as their therapeutic, prophylactic and subtherapeutic uses for growth promotion in food animals have substantially increased selective pressures on both pathogenic and commensal bacteria, thus favoring the propagation, accumulation and maintenance of AR bacteria (Levy, 2002).

Pork is one of the most commonly consumed meat worldwide, with evidence of pig husbandry dating back to 5000 BC. Global pork production in last year was 85 million tons, a third position to beef and poultry production (FAOSTAT, 2008).

Diarrhoea in piglets is a complex problem resulting from interaction between infective agents, host immunity and management procedures. It causes considerable economic loss to the pig production.

The digestive system is responsible for the intake and digestion of food, absorption of nutrients and the excretion of waste material. Pathogenic strains of certain bacteria (those capable of causing disease under stressed condition of host or opportunistic pathogen) and non pathogenic bacteria are normal inhabitants of specific sections of the gut. Some organisms assist in the breakdown of feed and the production of nutrients. Changes to the gastrointestinal environment may upset the

secretion of fluids and electrolytes into the stomach and gut, the rate of passage of food through the system and the ability to digest and resorb nutrients, water and electrolytes.

Gastroenteritis is a major cause of diarrhoea in piglets. The words ‘scour’ or ‘diarrhoea’ describes the excretion of faeces containing excess fluid. Depending on the cause of the scours, there may also be blood, undigested food, mucus and pieces of membrane from the intestinal lining. The effects of scours are dehydration, electrolyte loss, poor nutrient absorption, septicaemia, toxaemia and predispose to other diseases and cause intestinal damage. Various types of scours are dietary or nutritional, bacterial, viral and parasitic (Taylor, 1986).

Therefore management of a sustainable swine production system entails control of enteric pathogens and it involves isolation and identification of the causative agent and preventing multiplication and spread of the infection.

Considering the importance of the problem, this study was undertaken with the following objectives.

a) Isolation and identification of bacterial organisms responsible for gastroenteritis in piglets
b) Antibiogram of isolates
c) Pathogenicity test of the isolates in mice
d) Attempts to isolate plasmids from the pathogens.

Review of Literature

2. REVIEW OF LITERATURE

Diarrhoea is the clinical manifestation of one of the most common disease complexes in piglets worldwide. Diarrhoea can be defined as malabsorption of water and electrolytes (Cowart and Casteel, 2001; Jacobson, 2003), the frequent passage of soft or watery faeces (Liebler-Tenorio EM, 1999), a condition with a water content in faeces exceeding 80 per cent (Makinde *et al.*, 1996). Enteric diseases show a wide spectrum of clinical signs, ranging from a soft stool for a few days in a seemingly healthy animal to profuse watery faeces with dehydration and a rapid decrease in body condition (Svendsen *et al.*, 1974; Morin *et al.*, 1983; Johnston *et al.*, 2001). The intestinal content may be mucous, hemorrhagic or necrotic but the disease may also appear so rapidly that death occurs without any preceding clinical signs (Alexander and Taylor, 1969; Svendsen *et al.*, 1974; Kwon *et al.*, 1999; Fratamico *et al.*, 2004). Thus, the general condition of the pig may be unaltered or severely depressed, causing anything from no obvious signs to severe suffering in the individual animal.

2.1.POST-WEANING DIARRHOEA

Post-weaning diarrhoea (PWD) is considered to be a multifactorial disease (Hampson, 1994; Madec *et al.*, 2000). Weaned piglets are variously predisposed to enteric disorders. Newly weaned piglets are stressed by nutritional, psychological, environmental and physiological factors (Hampson, 1994). At weaning the feed is changed from milk to a weaner diet, piglets are separated from their sow and often moved from the farrowing pen and mixed with unfamiliar piglets. Weaned piglets also lose passive intestinal immunity provided by antibodies in sow's milk (Porter *et al.*, 1970). After weaning there are alterations in the structure (Hampson, 1986) and function (Hampson and Smith, 1986; Kidder and Manners, 2008) of the piglet small intestine, changes in intestinal *Escherichia coli (E. coli)* flora of piglets (Melin *et al.*, 1997; Katouli *et al.*, 1999) and impairment of immune functions in early weaned

piglets (Blecha *et al.*, 1983; Wattrang *et al.*, 1998; Boerlin *et al.*, 1999; Brunder *et al.*, 1999).

Several factors have been reported to influence the occurrence of diarrhoea in weaned piglets. The susceptibility for diarrhoea after weaning had been associated with management related factors such as low feed intake during the first week after weaning (Madec *et al.*, 1998), excessive feed intake (Hampson and Smith, 1986), the hygiene and management level (Madec *et al.*, 1998), low weaning weight and age (Svensmark *et al.*, 1989; Skirrow *et al.*, 1997), moderate cold stress (Wathes *et al.*, 1989), texture of feed and number of feeder spaces per pen (Amezcua *et al.*, 2002) Cleaning pens between batches of weaned piglets had been associated with a decreased risk of PWD (Lofstedt *et al.*, 2002; Ngeleka *et al.*, 2003).

2.2. INFECTIOUS DIARRHOEA

Diarrhoea caused by infectious agents is responsible for large economic losses in pig production farms, especially in suckling and weaned piglets. Coronavirus and rotavirus as well as enterotoxigenic *Escherichia coli* (ETEC) are described as the most frequent viral and bacterial enteropathogens, respectively (Hill and Sainsbury, 1995; Potter, 1998; Quinn *et al.*, 2002). Of the metazoic parasites of piglets, *Isospora suis* and *Cryptosporidium parvum* are considered the most prevalent. Investigations determining the prevalence of these pathogens have been conducted world-wide (Blanco *et al.*, 1997; Bertschinger *et al.*, 1999; Blanco *et al.*, 2004; Bhat *et al.*, 2008).

In a study on the prevalence of enteropathogens in suckling and weaned piglets with diarrhoea in southern Germany, *Isospora suis* was diagnosed in 26.9 per cent and *Cryptosporidium parvum* in 1.4 per cent of the piglets investigated. The occurrence of the pathogens was significantly associated with the age of the animals examined. *Isospora suis* was predominantly isolated from suckling piglets (in the second and third week of life); while in weaned piglets entero toxigenic *E. coli* were

most prevalent(Farmer *et al.*, 1985; Garabal *et al.*, 1996; Dunlop *et al.*, 1998; Frydendahl, 2002) .

Most of the bacterial pathogens associated with post-weaning enteric disorders were found to be strains of enterotoxin producing *E. coli*, (Bertschinger, 1999). Same strains of *E. coli* were also found in the digestive tract of healthy pigs (Celemin *et al.*, 1995) and hence experimental reproduction of the disease through *E. coli* inoculation was found to be difficult (Wathes *et al.*, 1989; Melin *et al.*, 2000).

2.3. BACTERIAL AGENTS

2.3.1. *Escherichia coli*

Postweaning diarrhoea are serious infectious diseases of piglets and responsible for huge economic losses worldwide. Postweaning diarrhoea (PWD) is mainly caused by certain groups of *Escherichia coli* strains including enterotoxigenic (ETEC) and Shiga toxin-producing *E. coli* (STEC) (Mainil, 1999; Nagy and Fekete, 1999).

During the first two weeks after weaning, pathogenic *Escherichia coli* plays a significant role in the etiology of PWD (Svendsen *et al.*, 1974; Tzipori *et al.*, 1980; Madec *et al.*, 2000), pathogenic *E. coli* does not unequivocally lead to the development of diarrhoea in weaned piglets (Hampson and Smith, 1986; Wathes *et al.*, 1989; Madec *et al.*, 2000). Pens contaminated with pathogenic *E. coli* strains are likely sources of infection for weaned piglets, but the infection can also be acquired before weaning (Hampson *et al.*, 1987). On farm outbreaks of PWD the morbidity was over 50per cent among weaned piglets (Svendsen *et al.*, 1974; Tzipori *et al.*, 1980). An acute infection may be produced in severely affected piglet (Svendsen *et al.*, 1974; Sarmiento *et al.*, 1988). In surviving piglets diarrhoea could be transient (Madec *et al.*, 2000) or it can last for up to four days (Sarmiento *et al.*, 1988). The case fatality rate seldom exceeded 10 per cent in uncomplicated cases (Taylor, 1999).

Post Weaning Diarrhoea associated with enterotoxigenic *E. coli* typically affected piglet during the immediate post weaning period.

Enteric infections caused by *Escherichia coli* in piglets are ubiquitous in the swine industry. Among the clinical manifestations, diarrhoea, particularly in suckling piglets is the most frequent (WHO, 1998; Mini *et al.*, 2005; Jesto, 2007). Postweaning gastroenteritis (PWGE) caused by *E. coli* also occurs sporadically (Barker and Van Dreumel, 1985). The manifestations of PWGE may range from peracute and fatal to chronic with delayed growth (Richards and Fraser, 1961). In peracute and acute cases of PWGE, there is a shock like syndrome in which marked gastric and enteric congestion occur with occasional hemorrhages in the intestinal lumen.

Hemorrhagic gastroenteritis (HGE) is a descriptive term referring to severe form of PWGE (Thomlinson and Buxton, 1963). Hemorrhagic gastroenteritis is generally described as a postweaning condition and is associated with the serogroups O138, O139, O141 and O149 of *E. coli* (Barker and Van Dreumel, 1985).

The clinical, pathological and bacteriological findings in 55 cases of hemorrhagic gastroenteritis (HGE) caused by *Escherichia coli* in weaned and suckling piglets were associated with several serogroups of *E. coli*. Most of the isolates of *E. coli* were hemolytic (Faubert and Drolet, 1992).

In HGE, serogroup O149 was the most common isolate followed by isolates of serogroups O8 and O157 and a few cases involved O138 and O45. Hemorrhagic gastroenteritis was not commonly associated with the edema disease serogroups (O138, O139 and O141), but rather with the classical enterotoxigenic F4-positive serogroups (O149, O8 and O157). The latter were found in piglets of all ages. Serogroups of *E. coli* that have been previously associated with HGE are O138, O139 (Richards and Fraser, 1961; Svendsen *et al.*, 1974; Moxley *et al.*, 1988), O141

((Richards and Fraser, 1961; Svendsen *et al.*, 1974), O8 (Nielsen, 1986; Moxley *et al.*, 1988), O157 (Moxley *et al.*, 1988) and O149 (Leman *et al.*, 1986; Moxley *et al.*, 1988).

2.3.2. *Serratia fonticola*

Presence of pathogens like *Serratia fonticola* and *Staphylococcus aureus* were detected in pig meat. *Serratia fonticola* is a known pathogen in fishes and it may be getting into the intestine of swine through fish meal. It is also reported in other plants and animals (Gavini *et al.*, 1979; Muller *et al.*, 1986; Bollet *et al.*, 1991; Müller *et al.*, 1995; Anahory *et al.*, 1998). It was proved beyond doubt that animal feed might serve as a carrier for a wide variety of microorganisms (Smith *et al.*, 1994; Schmidt *et al.*, 2000; Stock *et al.*, 2003; Maciorowski *et al.*, 2007).

2.3.3. *Salmonella* sp.

One of the first enteric diseases described in swine was salmonellosis (Salmon and Smith, 1886). Isolation from piglets which had enteritis and diarrhoea was found to be higher. This probably points to the possible association of *Salmonella* in clinical outbreaks of enteritis either singly or in association with other enteropathogens. The prevalence rate had been found to be higher in young population and that too among animals in which enteritis and diarrhoea were major clinical symptoms. These facts suggested that young ones were more prone to infection by *Salmonella* (Benson et al., 1985; Farrington et al., 1999).

Pigs and fowls were considered as major sources of Salmonellae for human infections (Wilson and Miles, 1964). They isolated *S. cholerae-suis* var. *kunzendorf*, *S. Colombo*, *S. paratyphi* and *S. hyittingfoss* from pigs with enteritis(Murray, 1991; Seyfarth *et al.*, 1997).

A number of workers have isolated various serotypes of *Salmonella* from young pigs suffering from diarrhoea like syndrome (Murthy and Kausik, 1964; Sweeney, 1966; Mc Erlean, 1968; Sulochana *et al.*, 1973; Both *et al.*, 1982). A few reports on isolation of *Salmonella typhimurium* and *Salmonella weltevreden* from pigs in Kerala are also available (Sulochana *et al.*, 1973; Rajasenan, 1983).

Out of the seventy specimens from various disease conditions in piglets of one to four month of age belonging to the University Pig Breeding Farm, Mannuthy, eighteen strains of *Salmonella*, twelve from mesenteric lymph nodes and six from rectal swabs were isolated (Pillai *et al.*, 1974).

Salmonella typhimurium, which is a non-host specific and ubiquitous serotype, attacks a wide variety of animal hosts and man causing much morbidity and mortality in infected population. In pigs, it is the most important pathogen next to *Salmonella choleraesuis*. A study was carried out to establish the carrier status and the topographic distribution of *Salmonella* in the carcass and organs of healthy pigs and calves (Ionova *et al.*, 1981). It was found that 4.29 per cent of the slaughtered healthy pigs were carriers of *Salmonella* organisms. Most frequently isolated serotypes in a descending order were *S. anatus, S. cholerae suis, S. derby, S. agona, S. thompson, S. dublin, S. essen, S. lille, S. kottbus and S. enteritidis*.. The highest per cent of *Salmonellae* in the slaughtered normal pigs was found in the caecal content.

Reports by various workers indicated that environmental contamination was an important contributor of *Salmonella* infection (Nietfeld *et al.*, 1998; Davies *et al.*, 1999a). The genus *Salmonella* encompasses a large taxonomic group with over 2500 recognized serovars (Popoff *et al.*, 2004). Almost 60 per cent of all *Salmonella* strains identified and 99 per cent of the serovars associated with disease in warm blooded animals are members of subspecies *S. enterica* (Chan *et al.*, 2003). Some serovars of subspecies *S. enterica* show restricted host specificity, however, most can

colonize and cause disease in a wide range of animal species (Davies *et al.*, 2000; Davies and Breslin, 2004b). The most common clinical manifestation of salmonellosis in animals is enteric disease but numerous other conditions may be observed including acute septicemia, abortion, arthritis and respiratory disease (Ionova *et al.*, 1981; Davies *et al.*, 1999b; Davies *et al.*, 2000; Davies *et al.*, 2001; Davison *et al.*, 2001; Jones *et al.*, 2002; Wang *et al.*, 2002; Threlfall *et al.*, 2003; Davies and Breslin, 2004a). *Salmonella* are difficult to control in food animal environments, since animals may be asymptomatic fecal shedders. These carrier animals likely play an important role in the spread of infection between herds and flocks and consequently serve as sources of food contamination and human infection (Guerin *et al.*, 2005; Hegde *et al.*, 2005; Bahnson *et al.*, 2006; Cobbold *et al.*, 2006).

Three hundred and eighty *Salmonella* isolates recovered from animal diagnostic samples obtained from four state veterinary diagnostic laboratories in USA, between 2002 and 2003 were tested for antimicrobial susceptibilities. Forty-seven serovars were identified, the most common being *S. typhimurium* (26 per cent), *S. heidelberg* (9 per cent), *S, dublin* (8 per cent), *S. newport* (8 per cent), *S. derby* (7 per cent) and *S. choleraesuis* (7 per cent). Three hundred and thirteen (82 per cent) isolates were resistant to at least one antimicrobial and 265 (70 per cent) to three or more antimicrobials. Resistance was most often observed to tetracycline (78 per cent), followed by streptomycin (73 per cent), sulfamethoxazole (68 per cent), ampicillin (54 per cent) and to a lesser extent chloramphenicol (37 per cent), kanamycin (37 per cent), amoxycillin clavulanic acid (20 per cent) and ceftiofur (17 per cent). With regards to animal of origin, *Salmonella* isolates from swine displayed the highest rate of resistance, being resistant to at least one antimicrobial (92 per cent), followed by those recovered from turkey (91 per cent), cattle (77 per cent), chicken (68 per cent) and equine (20 per cent). Serovars commonly showing multidrug resistance (MDR) to 9 antimicrobials were *S. uganda* (100 per cent), *S. agona* (79 per cent) and *S.*

newport (62 per cent), compared to *S. heidelberg* (11 per cent) and *S. typhimurium* (7 per cent) (Zhao *et al.*, 2005; Zhao *et al.*, 2007).

2.3.4. *Pseudomonas* sp.

The presence of *Pseudomonas aeruginosa* in piglet diarrhoea was reported. Two outbreaks of diarrhoea in suckling piglets were investigated (Choudhary *et al.*, 1983). Pure infection with either *Pseudomonas aeruginosa* or *E. coli* and mixed infection with both were observed(Lambert, 2002). Isolation of *P. aeruginosa* from internal organs and/or intestine of fatal cases, from rectal swabs of diarrhoeic cases and diarrhoeagenicity of these isolates proved that *P. aeruginosa* was the etiologic agent at least in cases showing pure infection (Giwercman *et al.*, 1990; Hancock, 1998). Aeruginocine typing and drug-sensitivity pattern of isolates of *P. aeruginosa* suggested that mother sows were the source of infection in the first outbreak and environment in the second (Brinkman *et al.*, 2000).

2.3.5. *Aeromonas* sp.

Enterotoxigenic *Aeromonas hydrophila* was reported as a causative agent of piglet diarrhoea (Dobrescu, 1978). Later, it was shown that bacteria of *Aeromonas hydrophila* subsp. biotype 1 and *Aeromonas anaerogenes*, biotype 2 (Aoki, 1992), as well as *Proteolitica* of the *Vibrio parahaemoliticus* biotype were isolated from the intestine of pigs with diarrhoea (Andrusenko *et al.*, 1983). Close antibiotic sensitivity of the isolates was shown. The differences in their sensitivity were not sufficient for defining the taxonomic features. There are several reports of *Aeromonas hydrophila* as an intestinal pathogen in pigs, piglets and human beings (Burke and Gracey, 1986; Khardori and Fainstein, 1988; Altwegg *et al.*, 1989; Gray and Stickler, 1989; Ceylan *et al.*, 2009). The entry of this organism to the intestine of pigs may be through the fish meal fed to the animal. Existence of these bacteria as a pathogen in fish is reported by many workers (Hazen *et al.*, 1978; Allan and Stevenson, 1981; Dooley

and Trust, 1988; Son *et al.*, 1997; Zhang *et al.*, 2000). The antibiotic sensitivity pattern and multidrug resistance in these organisms were reported by several workers (Janda *et al.*, 1987; Bakken *et al.*, 1988; Khardori and Fainstein, 1988; Paniagua *et al.*, 1990; Cascon *et al.*, 1996; Overman and Janda, 1999; Rhodes *et al.*, 2000; Ko *et al.*, 2005).

2.3.6. *Campylobacter* sp.

Campylobacter fetus subspecies *coli* was isolated from the small intestines of 17 piglets less than six weeks of age (Taylor and Olubunmi, 1981). Sixteen of these animals had enteritis and in five of them no other probable bacterial cause of the enteric lesions was identified. *Campylobacter fetus* subsp. *coli* were also isolated from the large intestinal mucosa of all the infected piglets.

Campylobacter jejuni were isolated from pigs and man of Thrissur district (Raju, 1994). Fourteen isolates of *Campylobacter jejuni* of different serotypes and one *Campylobacter coli* isolate, from various human and animal sources, were tested for potential pathogenic mechanisms (Manninen *et al.*, 1982).

A total of 191 *Campylobacter jejuni* and 125 *Campylobacter coli* were isolated from the intestinal content of 398 chickens, 421 cattle and 203 pigs (Munroe *et al.*, 1983). All 108 chicken isolates and 73 of 80 cattle isolates were *C. jejuni*, but 115 of the 118 pig isolates were *C. coli*. A total of 84 per cent of the *C. jejuni* and 64 per cent of the *C. coli* isolates were typed on the basis of thermostable antigens with 20 antisera prepared against frequently occurring serotypes in *Campylobacter* enteritis in man (15 *C. jejuni*, 6 *C. coli* serotypes). A total of 96 per cent of the chicken isolates and 67 per cent of the cattle isolates belonged to 11 *C. jejuni* serotypes that occur most frequently in human cases of enteritis (serotypes 1, 2, 3, 4, 5, 13/16, 18, 21, 23, 31 and 36). Serotype 8, a relatively common human isolate, was not recovered. The *C. coli* isolates from pigs belonged to serotypes uncommon among human isolates.

A survey of *Campylobacter* species in the faeces or rectal contents of domestic animals was carried out using direct or enrichment culture methods and 259 (31 per cent) campylobacters were isolated from 846 faecal specimens (Manser and Dalziel, 1985). The highest isolation rate was found in pigs (66 per cent); lower rates were found in cattle (24 per cent) and sheep (22 per cent). In pigs all the isolates were *C. coli,* in sheep and cattle about 75 per cent were *C. jejuni*. Only five isolations of *C. fetus* subsp. *fetus* were made, all from cattle. More pigs with diarrhoea had *C. coli* in their faeces than healthy pigs (77 per cent vs. 47 per cent), but such a clear difference in isolation rate between sick and healthy animals was not seen in cattle or sheep. The enrichment method increased the total isolation rate of *C. jejuni* and *C. coli* by 33 per cent, but for cattle specimens it increased by 69 per cent.

A study was carried out to investigate the occurrence and antimicrobial resistance of *Campylobacter* isolated from French fattening pigs (Payot *et al.*, 2004). From March 1998 to June 1999, stomach samples were collected at slaughter from 240 fattening pigs originating from 24 different farms. Half of the pigs were found to be positive for *Campylobacter* but considerable variation was observed between farms. All the isolates were belonging to the *Campylobacter coli* species. Susceptibilities of the strains were determined. More than one third of the strains were resistant to at least three antimicrobial drugs. Results indicated a high prevalence of *C. coli* in the stomach of the French pigs examined. In addition, high proportions of the strains were resistant to antimicrobial drugs, particularly to tetracycline and erythromycin, or were multiresistant (Rowe-Magnus *et al.*, 2002).

2.3.7. Gram positive organisms

The BP classification system of staphylococcus was introduced by Baired Prker in 1965(Baird-Parker, 1965). The presence of Micrococci and staphylococci BP

subgroups in the intestines of pigs and piglets were reported (Wilssens and Vande Casteele, 1967). The other Gram positive organisms found in pig and piglet intestinal micro flora included streptococci and lactobacilli (Fuller *et al.*, 1960; Schwarz and Blobel, 1990; Wegener *et al.*, 1993). Among the streptococcal population, *Streptococcus faecium* and *S. faecalis* and its varieties were the most frequent, *S. bovis* and *S. equines* were also found to be very numerous(Schulz, 1970; Muller, 1974; Sato *et al.*, 1990; Chen *et al.*, 2007). In addition, many closely related strains were having been recognized (Fewins *et al.*, 1957; Fuller *et al.*, 1960; Raibaud *et al.*, 1961; Barrow *et al.*, 1980; Barlow *et al.*, 2004).

Systematic identification of unknown bacteria was discussed by several workers (Cowan *et al.*, 1993a; Bergey and Holt, 1994). The simplest way to determine antibiotic sensitivity and resistance pattern in bacteria using agar diffusion assay was discussed by several workers (Takahashi *et al.*, 1990; Bauer *et al.*, 1999). It is known that drug resistance genes are generally coded in plasmids and plasmid isolation is necessary for further characterization of these genes.

2.4.VIRAL AGENTS.

Rotavirus and corona virus are the most commonly identified viral causes of diarrhoea in neonatal food animals (Torres-Medina *et al.*, 1985). These viruses have also been associated with diarrhoea in adult food animals, but their disease incidence in adults is low compared with that in neonates. However, adults infected both clinically and subclinically shed the viruses and provide sources of infection for the young (Bulgin *et al.*, 1989; Collins *et al.*, 1989). In 1980, Bridger and Saif described characteristics of viruses that were morphologically identical to conventional rotaviruses but expressed a different genome profile and lacked the group-specific antigen. These atypical rotaviruses were isolated from diarrhoeic pigs (Bridger, 1980;

Saif *et al.*, 1980) and subsequently from calves (Pedley *et al.*, 1983; Chasey and Davies, 1984; Snodgrass *et al.*, 1984; Pedley *et al.*, 1986), lambs (Snodgrass *et al.*, 1984; Theil *et al.*, 1995; Wani *et al.*, 2004) and humans (Snodgrass *et al.*, 1984). The viruses were assigned the names Pararotavirus or group C rotaviruses (Bohl *et al.*, 1982; Saif *et al.*, 1988), atypical rotavirus (Bridger and Brown, 1985; Pedley *et al.*, 1986), antigenically distinct rotaviruses (Eiden *et al.*, 1986), rotaviruslike viruses or group B rotaviruses (Pedley *et al.*, 1983) and novel rotaviruses (Bridger, 1987). Other viruses that have been implicated in diarrhoea of young farm animals included togavirus (bovine viral diarrhoea virus) (Werdin *et al.*, 1989), parvovirus (Storz *et al.*, 1978; Yasuhara *et al.*, 1989), calicivirus (Woode and Bridger, 1978; Bridger, 1980; Saif *et al.*, 1980; Bridger *et al.*, 1984), adenoviruses (Coussement *et al.*, 1981; Ducatelle *et al.*, 1982; Orr, 1984), bredaviruses (Woode *et al.*, 1982; Woode *et al.*, 1985) and astroviruses (Snodgrass and Gray, 1977; Woode and Bridger, 1978; Bridger, 1980; Gray *et al.*, 1980).

2.5.THE ROLE OF ANIMAL HYGIENE IN POSTWEANING DIARRHOEA

The numerous and abrupt changes that occur at weaning make this period a real challenge to the piglet, although the ability of the pig to adapt to different environments is well known. In particular the adaptative capacity of the digestive tract had been investigated and was found to be remarkable (Aumaitre *et al.*, 1995). Physical changes in the size and shape of the organs have been observed during this period. Profound modifications were detected in enzyme production and release (Pluske *et al.*, 1997; Hedemann and Jensen, 2004). Unfortunately, despite the natural ability of piglets to adapt, the conditions they experience on commercial farms sometimes can result in the challenge exceeding this capacity. The consequences can vary considerably but enteric disorders, (diarrhoea and related growth checks) are by far the most common signs of disruption.

Matterials and Methods

3. MATERIALS AND METHODS

All the chemicals and reagents used in the study were of analytical grade unless otherwise specified and were procured from M/S Hi-media Laboratories Private Limited, Mumbai. The glassware used in the study were from M/S Borosil Glass works Limited. All ready made media and antibiotic discs were procured from M/S Hi-media Laboratories Private Limited, Mumbai, unless otherwise specified. Nucleospin Plasmid Isolation Kit used for isolation and purification of plasmids was from M/S Macherey Nagel GmbH and Co. Germany.

3.1.ISOLATION OF BACTERIA FROM GASTROENTERITIS CASES

3.1.1. Collection of Samples

Samples were collected from different cases of gastroenteritis from Centre for Pig Production and Research, KAU, Mannuthy, cases brought to Centre of Excellence in Pathology and to the Department of Veterinary Microbiology, College of Veterinary and Animal Sciences, Mannuthy as well as from commercial farms.

Rectal swabs were collected from live diarrhoeic piglets and intestinal contents, pieces of jejunum, ileum, colon, mesenteric lymph nodes, liver, spleen and stomach were collected during post mortem examination after taking all sterile precautions. A total of 82 samples were collected from piglets showing clinical signs and 53 bacterial isolates were obtained from them.

3.1.2. Method of Collection of Sample

3.1.2.1. *Rectal swabs*

Piglets with diarrhoea or enteritis in the Centre for Pig Production and Research were identified. Samples were taken only from piglets with diarrhoea that had not been previously treated with antibiotics. Using sterile cotton swabs rectal

samples from these piglets were collected with maximum aseptic precaution. Immediately after collection, before drying, the swabs were streaked on the media.

3.1.2.2. *Postmortem Samples*

Samples were collected from those characterized grossly by marked congestion of the gastrointestinal tract. Clinical information for each case was obtained from the owner; it included the age and the clinical signs observed for other pigs in the herd as well as those submitted. Intestinal contents, pieces of jejunum, ileum, colon, mesenteric lymph nodes, liver, spleen and stomach were collected.

The organs were collected and placed in a sterile Petri dish. The dorsal surface was cleaned using sterile cotton dipped in alcohol. The surface was then seared using a hot spatula and a cut was made superficially on the surface using a sterile scissors. A sterile loop was inserted through the cut, rotated inside the tissue, taken back and streaked the media.

3.2.MEDIA USED FOR ISOLATION

3.2.1. Materials

Isolation of causative bacteria was made by culturing on Brain Heart Infusion Agar (BHIA), Mac Conkey Agar, Mannitol Salt Agar, Blood Agar, *Brucella* Agar and Cooked Meat medium.

3.2.1.1. *Brain Heart Infusion Agar*

Brain heart infusion agar base (Hi-media) was prepared as per the manufacturer's instructions.

3.2.1.2. *Mac Conkey Agar.*

Mac Conkey Agar base (Hi-media) was used and agar plates were prepared as per the instructions of the manufacturer.

3.2.1.3. *Blood Agar*

Defibrinated bovine blood was collected under aseptic conditions. After autoclaving, the melted nutrient agar was cooled to a temperature just above the temperature at which solidification of agar takes place (around 50-55^{0}C). Sterile bovine blood was then added at a concentration of five to ten per cent. Mixed well and transferred melted agar to Petri dish up to a depth of four millimeter under sterile conditions. The agar plates were incubated for 24 hours to check for any contamination, packed in polythene bags and stored under refrigeration.

3.2.1.4. *Mannitol Salt Agar*

Mannitol Salt Agar base (Hi-media) was used and agar plates were prepared as per the instructions of the manufacturer.

3.2.1.5. *Brucella Agar*

Brucella agar base (Hi-media) was used and agar plates were prepared as per the instructions of the manufacturer.

3.2.1.6. *Cooked meat medium*

Cooked meat medium (Hi-media) was used and tubes were prepared as per the instructions of the manufacturer.

3.3.IDENTIFICATION OF BACTERIA

3.3.1. Morphology , Staining and Colony Characters

The identification of bacteria was done as per standard protocols prescribed by Barrow and Feltham (Cowan *et al.*, 1993b) and Bergy's manual. (Bergey and Holt, 1994). For *Staphylococcus aureus* classification of Baired Parker was considered (Baird-Parker, 1965).

3.3.2. Biochemical Identification of Isolates

Unless otherwise indicated, cultures were incubated at 37°C and biochemical identification methods were followed as described by Barrow and Feltham (Cowan *et al.*, 1993b).

3.3.2.1. *Catalase test*

To one ml of 3 to 6 per cent hydrogen peroxide added a small amount of a 24 hour nutrient agar culture with a clean sterile platinum loop, examined immediately and after five minutes for evolution of gas, which indicate catalase activity.

3.3.2.2. *Oxidase test*

An Oxidase disc taken on a glass slide was moistened with a drop of distilled water and smeared with a colony using a platinum loop. A positive reaction was indicated by an intense deep-purple hue, appearing within 5-10 seconds, a 'delayed positive' reaction by purple colouration in 10-60 seconds and a negative reaction by absence of colouration or by colouration later than 60 seconds.

3.3.2.3. *Motility*

Young broth culture of the organism was incubated at 37°C and examined in hanging drop preparations using a high-power dry objective with reduced illumination and observed for motility.

3.3.2.4. *Coagulase Test*

Mixed 0.5 ml. of undiluted rabbit plasma with an equal volume of an 18-24 hour broth culture of organism and incubated at 37°C for four hours. Examined after one hour and four hours for coagulation. If negative, the tubes were left at room temperature overnight and then re-examined. A positive result was indicated by definite clot formation; granular or ropy growth was regarded as doubtful and the organism was retested.

3.3.2.5. *Oxidation or Fermentation of Glucose*

Steamed the OF medium to remove dissolved air and quickly cooled just before use and then stab-inoculated duplicate tubes with a straight wire. To one of the tubes added a layer of melted soft paraffin (petrolatum) to a depth of about three centimeters above the medium to seal it from air. Incubated at 37 ^{0}C and examined daily for up to 14 days and read the results according to the following table.

Table 1. Format for determination of oxidation or fermentation property of isolate.

Reaction	**Open tube**	**Sealed tube**
Oxidation	Yellow	green
Fermentation	Yellow	yellow
No action on Carbohydrate	blue or green	green

3.3.2.6. *Growth on MacConkey Agar*

The test organism was inoculated on MacConkey agar and incubated at 37^0C for 24 hours and observed for growth. Lactose fermenters will produce pink colonies and non lactose fermenters will produce pale colonies in MacConkey agar. The indicator used was neutral red, which turned pink in acidic pH produced by organic acids generated by fermentation of lactose incorporated in agar**.**

3.3.2.7. *Growth on Mannitol Salt Agar*

The test organism was inoculated on Mannitol salt agar and incubated at 37^0C for 24 hours and observed for the growth. It contains a high concentration (7.5 per cent to 10 per cent) of salt (NaCl), making it selective and is a differential medium, containing mannitol and the indicator phenol red. Acid production as a result of mannitol fermentation will result in the agar's normal red color changing to yellow. Mannitol fermenters produce a yellow colony while non-mannitol fermenters will produce a reddish or purple colony.

3.3.2.8. *Growth on Brucella Agar*

The samples were streaked on *Brucella* agar plate supplemented with Difco *Campylobacter* antimicrobial supplements (FD006 and FD009) and 5-7 per cent sheep blood. The inoculated plates were incubated at 42^0C under microaerophilic condition (candle jar system) for 48 hours. The plates were examined for non hemolytic wet, glossy, spreading or discrete, convex colonies.

3.3.2.9. *Growth on Cooked meat medium*

The samples were inoculated on Cooked meat medium and incubated at 37^0C for 24 hours and observed for the growth. Growth was indicated by turbidity.

3.3.2.10. *Methyl red (MR) reaction*

Glucose Phosphate peptone water was inoculated with the culture and incubated at 37ºC for 48 hours. About five drops of the methyl red reagent, was added, shaken well and examined. In positive tests a bright red and in negative a yellow colour is developed.

3.3.2.11. *Voges-Proskauer Reaction (acetoin production) test*

After completion of the MR test added 0.6 milliliter of five per cent α-naphthol solution and 0.2 ml 40 per cent KOH aqueous solution; shaken well, kept the tube in slanting position to increase the area of the air-liquid interface and examined after 15 minutes and one hour. A positive reaction is indicated by a cherry red colour.

3.3.2.12. *Citrate Utilization Test*

The organism was inoculated as a single streak over the surface of slope of the Simmons' citrate medium slant. It was incubated for 96 hrs at 37ºC, examined daily for growth and colour change.

Blue colour and streak of growth - citrate utilized
Original green colour and no growth - citrate not utilized

3.3.2.13. *Indole Production*

Inoculated peptone water and incubated at 37ºC for 48 hrs. Added 0.5 ml Kovac's reagent, shaken gently and examined after about one minute. A red colour in the alcohol layer indicated a positive reaction.

3.3.2.14. *Urease Activity*

The organism was inoculated heavily over the entire slope surface of Christensen's urea agar slant. Incubated at 37ºC and examined after four hours and

after overnight incubation, no tube being reported negative until after four days of incubation. Red colour indicated positive reaction.

3.3.2.15. *Nitrate Reduction*

Inoculated Nitrate Broth lightly and incubated at 37ºC for 96 hrs. Added one ml of 'Nitrate reagent A', followed by one ml of 'Nitrate reagent B'. A red colour developing within a few minutes indicated the presence of nitrite and hence the ability of the organism to reduce nitrate.

To tubes not showing a red colour within five minutes added powdered zinc (up to five milligram per milliliter of culture) and allowed to stand. Formation of red colour indicated nitrate was present in the medium (*i.e.*, not reduced by the organism). Absence of red colour indicated absence of nitrate in the medium (*i.e.*, reduced by the organisms to nitrite, which in turn was itself reduced).

3.3.2.16. *Phosphatase Test*

Lightly inoculated phenolphthalein phosphate agar to obtain discrete colonies and incubated at 37ºC for 18 hrs. Placed 0.1 ml. ammonia solution (specific gravity 0.880) in the lid of the Petri dish and inverted the medium above it. Free phenolphthalein liberated by phosphatase reacts with the ammonia and phosphatase positive colonies become bright pink.

3.3.2.17. *Decarboxylase Reactions*

From a plate culture, heavily inoculated, with a straight wire, tubes of the three media (arginine, lysine and ornithine) through the paraffin layer, incubated at 37ºC and read daily for four days for any colour change. Uninoculated controls were also kept. The media first become yellow due to acid production during glucose fermentation; later, if decarboxylation occurs, the medium becomes violet. The control should remain yellow. With non-fermentative organisms,

no acid (or insufficient acid) is produced from glucose and there is no change in colour of the media to yellow.

3.3.2.18. *Acid and Gas Production from Carbohydrates*

To five milliliters of Andrade's peptone water taken in a test tube, added disc of the sugar to be tested. A small inverted tube (Durham tube) completely filled with liquid and containing no air bubbles was usually included in each culture tube to detect gas. Inoculated medium with test organism and incubated at 37ºC for five days. Acid production was indicated by the change in the colour of the medium to red and rising of Durham tube indicated gas production.

3.3.2.19. *Triple Sugar Iron agar (TSI) test for H_2S production*

Streaked a heavy inoculum over the surface of the TSI agar slope and stabbed into the butt. Incubated aerobically at 37ºC for 24 hrs. Blackening of butt indicated H_2S production.

Table 2. TSI test for H_2S production

Slant / butt	Colour	Utilization
Alkaline / acid	Red / yellow	Glucose only fermented
Acid / acid	Yellow/yellow	Glucose, lactose and sucrose fermented
Alkaline/alkaline	Red / red	No fermentation of glucose, lactose or sucrose

3.3.2.20. *ONPG (β-galactosidase) test*

Placed one ONPG disc into a sterile test tube. Added 0.1ml of sterile 0.85 per cent w/v sodium chloride solution (physiological saline). Inoculated and incubated for 24 hrs at 37ºC. If the test is positive a yellow colour will develop in the fluid due to lactose fermentation.

3.4.ANTIBIOGRAM

3.4.1. Materials

Mueller Hinton agar was used to study the antibiotic sensitivity pattern of the isolates. The discs supplied by M/S Himedia laboratories, with known concentrations of antibiotics as noted in micrograms (µg) or international unit (IU), were used.

The antibiotics and its concentration per disc were given below.

Amoxycillin (Am)-10µg
Ampicillin (A)-10 µg
Cephotaxim (Ce)-10 µg
Chloramphenicol (C)-30 µg
Ciprofloxacin (Cf)-10µg
Clindamycin (Cd)-2 µg
Co-trimoxazole (Co)-25 µg
Enrofloxacin (Ex) - 5µg
Erythromycin (E)-15 µg
Gentamicin (G) - 10 µg
Sulphadiazine (Sz) - 300 µg
Penicillin G (P) - 10 IU
Oxytetracycline (O) -30 µg

3.4.2. Method

Antibiotic sensitivity test was done as per the standard single disc diffusion method (Bauer *et al.*, 1999). Sterile Mueller Hinton agar plates were prepared. A single colony of organism was selected from the agar plate and transferred into about five milliliters of sterile tryptone soya broth. It was incubated at 37°C for six to eight hours till light to moderate turbidity was developed. Turbidity of the culture was adjusted to yield a uniform suspension containing 10^5 – 10^6 cells / ml. A sterile non-toxic cotton swab on a

wooden applicator was dipped into the standardized inoculum and rotated the soaked swab firmly against the upper inside wall of the tube to express excess fluid. Streaked the entire agar surface of the plate with the swab three times, turning the plate at 60° angle between each streaking. Allowed the inoculum to dry for 5-15 min. with lid in place. Applied the discs using aseptic technique. Deposited the discs with centre at least 24 mm apart. Incubated immediately at 37°C and examined after 14-19 hrs or later if necessary. Measured the zones showing complete inhibition and recorded the diameters of the zones to the nearest millimeter.

3.5. *IN VIVO* PATHOGENICITY IN MICE

The pathogenicity of all bacterial isolates was checked in murine models. (Janda *et al.*, 1985; Janda *et al.*, 1987; Blanco *et al.*, 1997).

Four BALB/C mice (25 to 30 g each) per isolate were inoculated intraperitoneally with 0.2 ml of a 24 hrs Mueller Hinton broth culture containing 10^7 CFU of organisms. Mortality of mice over a period of one week was scored. The degree of pathogenicity of isolates was recorded as follows. Those isolates produced a rate of 50 per cent or more mortality in mice within a week was considered as highly pathogenic (a mortality of three to four mice within a week). If the mortality rate was 50 per cent of the mice within a week (a mortality of two mice within a week), the isolate was considered as moderately pathogenic. An isolate was considered to have low pathogenicity if the mortality rate was less than 50 per cent within a week of injection of the isolate (a mortality of one or no animal).

3.6. PLASMID PROFILE OF THE ISOLATES

3.6.1. Isolation of Plasmid DNA

3.6.1.1. *Materials*

Luria Bertani Broth: Luria Bertani Broth base (Hi-media) was used and agar plates were prepared as per the instructions of the manufacturer.

NucleoSpin Plasmid Isolation Kit (Macherey Nagel GmbH and Co., Germany)

Kit contents: Buffers (A1, A2, A3, AW, A4, AE), RNase A (lyophilized), NucleoSpin Plasmid columns, NucleoSpin collecting tubes (2 ml) and protocol.

3.6.1.2. ***Method***

Plasmid isolation was performed as per the protocols provided with the kit. Briefly, pure culture of the organism was inoculated in 5 ml of Luria Bertani broth (LB broth) and incubated at 37ºC with constant shaking (200 to 250 rpm) for eight hrs. The saturated LB culture was centrifuged at 11,000 xg for 30 sec and the supernatant was discarded. 250 µl of buffer A1 was added and the cell pellet was resuspended by vigorous vortexing. To this, 250 µl of buffer A2 was added, mixed gently by inverting the tube 6 to 8 times and then incubated at room temperature for a maximum of 5 mins. This was followed by addition of 300 µl of buffer A3 and gentle mixing by inverting the tube 6 to 8 times. The tube was centrifuged for 5 to 10 min at 11,000 xg at room temperature.

A NucleoSpin Plasmid column was placed in a two ml collecting tube provided in the kit and the supernatant from the previous step was loaded into the column. The assembly was centrifuged for one minute at 11,000 xg and the flow through was discarded. This step was repeated with 600 µl of buffer A4. The silica membrane was completely dried by reinserting the NucleoSpin Plasmid column into an empty two ml collecting tube and centrifuging for one minute at 11,000 xg.

The NucleoSpin Plasmid column was then placed in a 1.5 ml micro centrifuge tube and 50 µl of buffer AE was added. It was incubated for one minute at room temperature and centrifuged for one minute at 11,000 xg to elute the plasmids. The eluted buffer AE containing plasmid DNA was used for electrophoresis.

3.6.2. Electrophoresis

3.6.2.1. *Materials*

Agarose

Molecular Weight standards: 2 Kb ladder

TE buffer (Tris EDTA buffer):

10 mM Tris Hydrochloride

1 mM EDTA (pH: 8.0)

TAE buffer (Tris-Acetate Buffer):

40 mM Tris Hydrochloride

20 mM Acetic Acid

2 mM disodium EDTA (pH: 8.0)

Loading buffer

0.25 per cent Bromophenol blue

0.25 per cent Xylene cyanol

15 per cent ficoll

40 per cent (w/v) sucrose

Ethidium bromide staining solution (10 mg / ml)

Distilled water

Electrophoresis apparatus and power supply (Consort, Belgium)

3.6.2.2. *Agarose Gel Electrophoresis*

The isolated plasmids were all visualized by agarose gel electrophoresis. Agarose gel was casted by melting 0.8 per cent agarose in 1x TAE buffer in a

microwave oven until a clear, transparent solution was achieved. The flask containing molten agarose was then transferred into a water bath at 55°C. When the molten gel had cooled, ethidium bromide was added to a final concentration of 0.5µg/ml. The gel solution was mixed thoroughly by gentle swirling. The melted solution was then poured into a gel casting unit, keeping the desired comb in the appropriate position. The agarose gel was allowed to harden. The comb was removed gently after adding a small amount of electrophoresis buffer on the top of the gel. The gel was mounted in the electrophoresis tank. Enough electrophoresis buffer to cover the gel to a depth of approximately one mm was added to the electrophoresis unit.

Plasmid DNA samples were mixed with two µl 6x gel loading buffer. This was then slowly loaded into the slots of the submerged gel using a disposable micropipette. A 2k DNA ladder was loaded into the wells in a similar manner to ascertain the size of the plasmids. Electrophoresis was carried out at 100 V till the tracking dye reached one third of the gel. The DNA fragments were viewed and photographed using a gel documentation system (Alpha imager).

3.6.2.3. *Determination of size of plasmids*

The bands obtained for the DNA ladder was used as standard to ascertain the size of the plasmids. Size of the plasmids was determined using the software Alpha imager.

Results

4. RESULTS

4.1.ISOLATION OF ORGANISM

A total of 82 samples were collected from weaned piglets. Samples comprised of rectal swabs and postmortem specimens. Postmortem samples were collected from those characterized grossly by marked congestion of the gastrointestinal tract (Fig.1-4). Intestinal contents, pieces of jejunum, ileum, colon, mesenteric lymph nodes, liver, spleen and stomach were collected during post mortem examination after taking all sterile precautions. All the samples were collected under sterile precautions and 53 bacterial isolates were obtained from them. Details are given in Table 3.

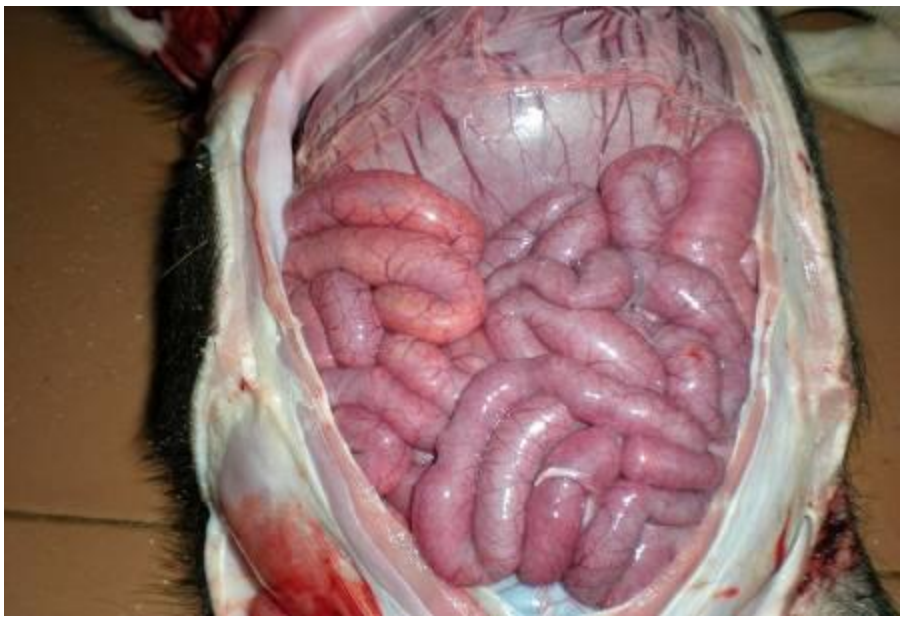

Figure 1. Piglet with gastroenteritis – showing marked serosal congestion.

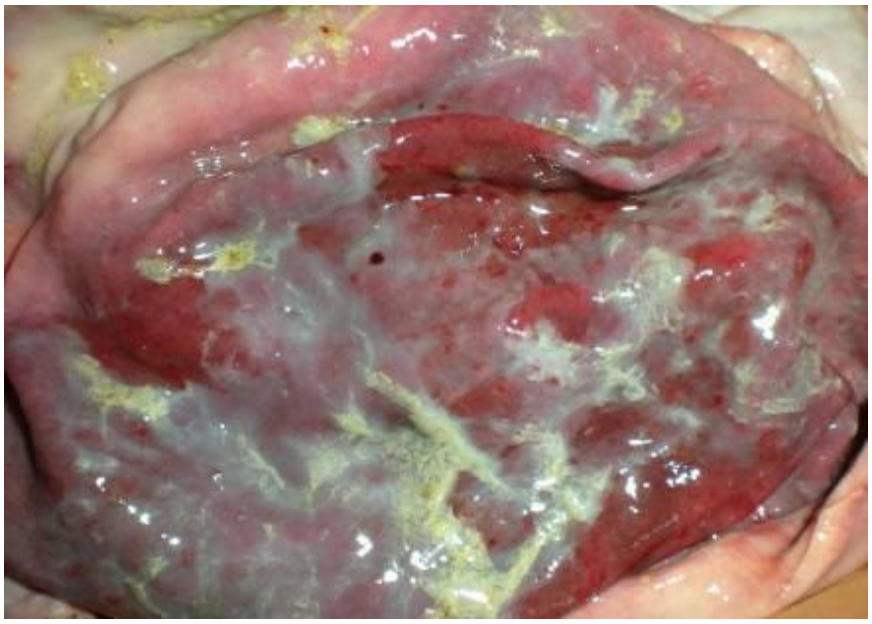

Figure 2. Piglet with gastritis- mucosa with congestion, haemorrhage and excess mucus.

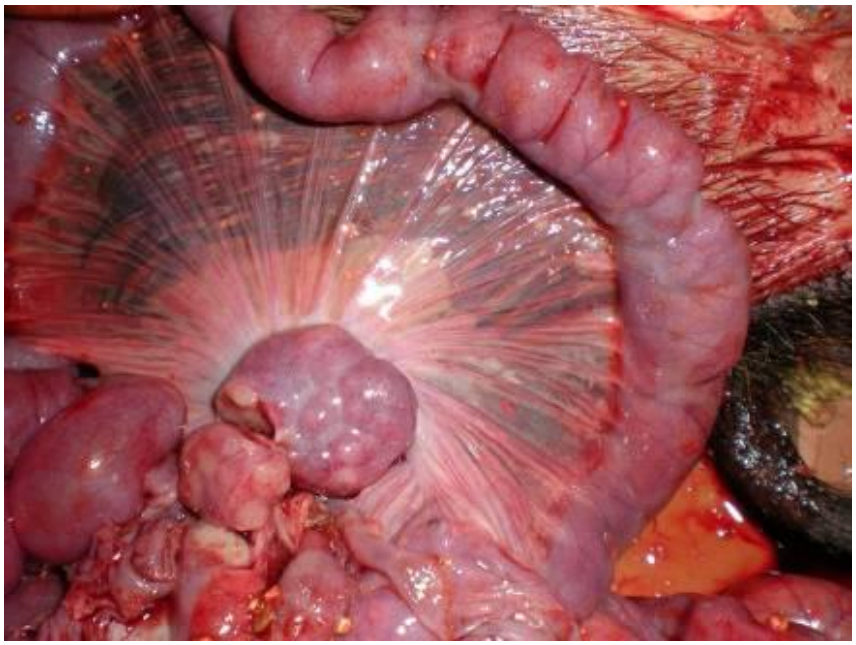

Figure 3. Enteritis with enlarged mesenteric lymph node.

Figure 4. Exposed intestinal mucosa - haemorrhagic enteritis.

4.2.IDENTIFICATION OF ORGANISM

The identification of the isolates was done as described by Barrow and Feltham (1993) unless otherwise mentioned.

Table 3. Details of sample collection

Isolate number	Species	Clinical signs / symptoms reported	Isolated from
Ec 1 to Ec24, Ec 27, Ec 28, Ec 31	Swine	Diarrhoea	Rectal swab
Ec25, Ec 29, Ec 30, Ec 32	Swine	Diarrhoea	Intestine, liver
Ec26, Ec 33	Swine	Diarrhoea	Intestine
Ec 34	Swine	Diarrhoea	Intestine, stomach
S1 to S3	Swine	Diarrhoea	Rectal swab
S4 to S6	Swine	Diarrhoea	Intestine
P1 to P3	Swine	Diarrhoea	Intestine, stomach, liver, kidney
P4 to P7	Swine	Diarrhoea	Rectal swab
Sf1 to Sf4	Swine	Diarrhoea	Rectal swab

Ah 1	Swine	Diarrhoea	Rectal swab
St1	Swine	Diarrhoea	Rectal swab

4.2.1. Identification of Gram negative bacteria

First stage identification of Gram negative bacteria was done based on the following characters of the organism like shape, motility, growth in air, growth anaerobically, catalase, oxidase, O/F reaction and the isolates were identified up to the family level, the details of which are given in table 4.

Table 4. First stage identification of Gram negative bacteria

Test done	Isolates																
	Ec 1	Ec 2	Ec 3	Ec 4	Ec 5	Ec 6	Ec 7	Ec 8	Ec 9	Ec 10	Ec 11	Ec 12	Ec 13	Ec 14	Ec 15	Ec 16	Ec 17
Shape	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R
Motility	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Growth in air	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Growth anaerobically	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Catalase	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Oxidase	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Acid from glucose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
O/F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F
Family	Enterobacteriaceae	Enterobacteriaceae	Enterobacteriaceae	Enterobacteriaceae	Enterobacteriaceae	Enterobacteriaceae	Enterobacteriaceae	Enterobacteriaceae	Enterobacteriaceae	Enterobacteriaceae	Enterobacteriaceae	Enterobacteriaceae	Enterobacteriaceae	Enterobacteriaceae	Enterobacteriaceae	Enterobacteriaceae	Enterobacteriaceae

Test done	Ec18	Ec 19	Ec 20	Ec 21	Ec 22	Ec 23	Ec 24	Ec 25	Ec 26	Ec 27	Ec 28	Ec 29	Ec 30	Ec 31	Ec 32	Ec 33	Ec 34
Shape	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R
Motility	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Growth in air	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Growth anaerobically	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Catalase	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Oxidase	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Acid from glucose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
O/F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F
Family	Enterobacteriaceae	Enterobacteriaceae	Enterobacteriaceae	Enterobacteriaceae	Enterobacteriaceae	Enterobacteriaceae	Enterobacteriaceae	Enterobacteriaceae	Enterobacteriaceae	Enterobacteriaceae	Enterobacteriaceae	Enterobacteriaceae	Enterobacteriaceae	Enterobacteriaceae	Enterobacteriaceae	Enterobacteriaceae	Enterobacteriaceae

Test done	P 1	P 2	P 3	P 4	P 5	P 6	P 7	Sf1	Sf2	Sf3	Sf4	Ah1	St1
Shape	R	R	R	R	R	R	R	R	R	R	R	R	R
Motility	+	+	+	+	+	+	+	-	-	-	-	+	+
Growth in air	+	+	+	+	+	+	+	+	+	+	+	+	+
Growth anaerobically	-	-	-	-	-	-	-	-	-	-	-	+	+
Catalase	+	+	+	+	+	+	+	+	+	+	+	+	+
Oxidase	+	+	+	+	+	+	+	+	+	+	+	+	-
Acid from glucose	-	-	-	-	-	-	-	-	-	-	-	+	+
O/F	-	-	-	-	-	-	-	F	F	F	F	F	F
Family	Pseudomonadaceae	Pseudomonadacea	Pseudomonadaceae	Pseudomonadaceae	Pseudomonadaceae	Pseudomonadaceae	Pseudomonadaceae	Enterobacteriaceae	Enterobacteriaceae	Enterobacteriaceae	Enterobacteriaceae	Aeromonadaceae	Enterobacteriaceae

R – Rod, F – Fermentative

4.2.1.1. *Identification of Enterobacteriae*

4.2.1.1.1. *Escherichia* sp.

A total of 34 *E. coli* isolates were obtained. All the isolates gave lactose fermenting rosy pink colonies on Mac Conkey agar. They gave a characteristic metallic sheen on EMB agar (Fig.5). The isolates were identified mainly based on the IMViC test for which they gave a result of Indole (+), Methyl red (+), VP (-) and Citrate (-). The other characters used for confirmatory identification were motility at 37^0C and biochemical tests like urease production, H_2S production from TSI, ONPG, arginine dihydrolase (ADH), lysine decarboxylase (LDB), ornithine decarboxylase (ODB) and nitrate reduction. Details of characterization of *E. coli* isolates are given in table 5. Carbohydrate fermentation reactions were carried out for 21 different sugars and are given in table 6. Serotyping results of *E. coli* are given in table 7. Percentages of various *E. coli* serotypes obtained are given in table 8 and figure 6.

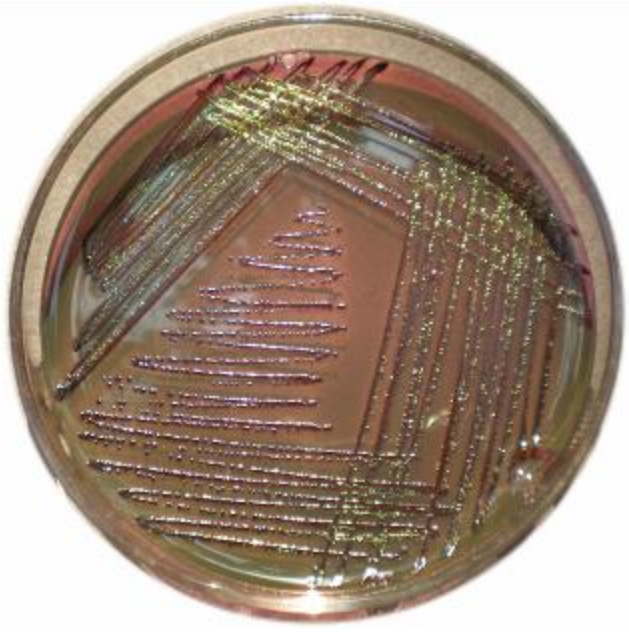

Figure 5. Colonies of *E.coli* with characteristic metallic sheen on EMB agar

4.2.1.1.2. *Salmonella* sp.

Only one isolate of *Salmonella typhimurium* was obtained. The isolate gave non lactose fermenting colourless colonies on Mac Conkey agar and red colonies on Brilliant Green agar. The isolate was identified mainly based on IMViC test for which they gave a result of Indole (-), Methyl red (+), VP (-) and Citrate (+). The other characters used for confirmatory identification were motility at 37^0C and biochemical tests like urease production, H_2S production from TSI, ONPG, arginine dihydrolase (ADH), lysine decarboxylase (LDB), ornithine decarboxylase (ODB) and nitrate reduction. Details of characterization and carbohydrate fermentation reactions of *Salmonella typhimurium* are given in table 5 and 6 respectively.

Table 5. Identification of *Escherichia coli* and *Salmonella typhimurium*

Test done	Isolates																																		
	Ec 1	Ec 2	Ec 3	Ec 4	Ec 5	Ec 6	Ec 7	Ec 8	Ec 9	Ec 10	Ec 11	Ec 12	Ec 13	Ec 14	Ec 15	Ec 16	Ec 17	Ec18	Ec 19	Ec 20	Ec 21	Ec 22	Ec 23	Ec 24	Ec 25	Ec 26	Ec 27	Ec 28	Ec 29	Ec 30	Ec 31	Ec 32	Ec 33	Ec 34	St1
Motility	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Colony colour in Mac Conkey agar	Pink	Pink	Pink	Pink	Pink	Pink	Pink	Pink	Pink	Pink	Pink	Pink	Pink	Pink	Pink	Pink	Pink	Pink	Pink	Pink	Pink	Pink	Pink	Pink	Pink	Pink	Pink	Pink	Pink	Pink	Pink	Pink	Pink	Pink	colourless
Urease	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
H_2S from TSI	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+
ONPG	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-
ADH	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
LDB	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
ODB	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-
Indole	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-
MR	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
VP	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Citrate Utilization	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+
Nitrate reduction	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Organism	*E. coli*	*E. coli*	*E. coli*	*E. coli*	*E. coli*	*E. coli*	*E. coli*	*E. coli*	*E. coli*	*E. coli*	*E. coli*	*E. coli*	*E. coli*	*E. coli*	*E. coli*	*E. coli*	*E. coli*	*E. coli*	*E. coli*	*E. coli*	*E. coli*	*E. coli*	*E. coli*	*E. coli*	*E. coli*	*E. coli*	*E. coli*	*E. coli*	*E. coli*	*E. coli*	*E. coli*	*E. coli*	*E. coli*	*E. coli*	*Salmonella typhimurium*

Table 6. Carbohydrate fermentation reactions of *E. coli* and *Salmonella typhimurium*.

Carbohydrate utilized	**Isolates**																																		
	Ec 1	Ec 2	Ec 3	Ec 4	Ec 5	Ec 6	Ec 7	Ec 8	Ec 9	Ec 10	Ec 11	Ec 12	Ec 13	Ec 14	Ec 15	Ec 16	Ec 17	Ec18	Ec 19	Ec 20	Ec 21	Ec 22	Ec 23	Ec 24	Ec 25	Ec 26	Ec 27	Ec 28	Ec 29	Ec 30	Ec 31	Ec 32	Ec 33	Ec 34	St1
Adonitol	-	+	-	-	+	-	-	+	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	+	+	+	+	-	-	-	-
Arabinose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+	+	+	+	+	+	-
Cellobiose	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Dextrose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Dulcitol	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	-	-	-	-	-
Fructose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+	+	+	+	-	+
Galactose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-
Inositol	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	+	-	-	-
Inulin	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Lactose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-
Maltose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Mannitol	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Mannose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-
Melibiose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-
Raffinose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+	-	-	+	-	-
Rhamnose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-
Salicin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+	-	+	+	-	+	+	-	-	-
Sorbitol	+	+	+	+	-	+	+	-	-	-	-	-	+	+	+	+	+	-	-	-	+	-	+	+	+	-	-	-	-	-	-	+	+	+	+
Sucrose	+	+	+	+	+	+	+	+	+	+	+	+	-	-	-	-	+	+	+	+	+	+	+	+	-	+	+	+	+	+	+	+	+	+	-
Trehalose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Xylose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+

Table 7. Serotyping results of *E. coli*

Isolate	Antigenic structure	Isolate	Antigenic structure	Isolate	Antigenic structure	Isolate	Antigenic structure
Ec 1	O38	Ec 10	O69	Ec 19	O41	Ec 28	O69
Ec 2	O5	Ec 11	O69	Ec 20	O79	Ec 29	O69
Ec 3	O84	Ec 12	O69	Ec 21	O25	Ec 30	O69
Ec 4	O132	Ec 13	O69	Ec 22	O109	Ec 31	O69
Ec 5	O69	Ec 14	O69	Ec23	O103	Ec 32	O69
Ec 6	O80	Ec 15	O69	Ec24	UT	Ec 33	O69
Ec 7	O79	Ec 16	O69	Ec25	O69	Ec 34	O69
Ec 8	O69	Ec 17	O56	Ec26	O69	-	-
Ec 9	O69	Ec 18	UT	Ec 27	O69	-	-

UT- un transcribed (O antigen not typeable)

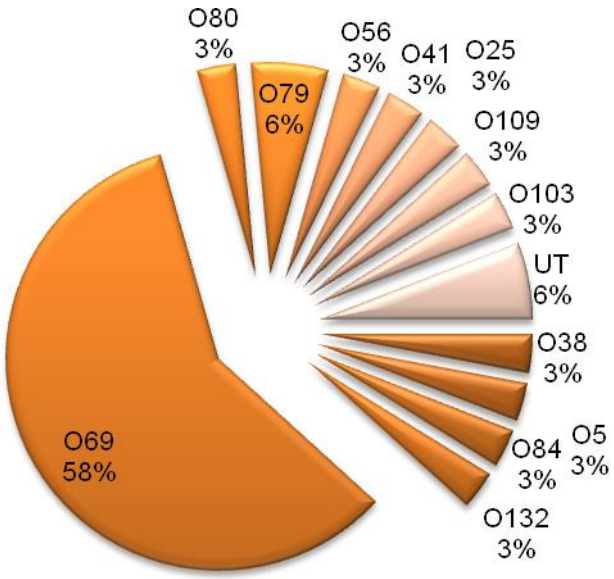

Figure 6. Percentage fraction of various serotypes of *E. coli* isolated from different samples during the study

Table 8. Percentage of various serotypes of *E. coli*

Serotype	Number of isolates	Percentage of serotypes	Serotype	Number of isolates	Percentage of serotypes
O38	1	2.94	O56	1	2.94
O5	1	2.94	O41	1	2.94
O84	1	2.94	O25	1	2.94
O132	1	2.94	O109	1	2.94
O69	20	58.82	O103	1	2.94
O80	1	2.94	UT	2	5.88
O79	2	5.88	-	-	-

4.2.1.2. *Identification of Pseudomonads*

Identification of *Pseudomonas* up to species level was done by conducting a set of biochemical reactions, the details of which are given in table 9.

4.2.1.2.1. Pseudomonas sp.

A total of 7 *Pseudomonas aeruginosa* isolates were obtained. All the isolates gave spreading non lactose fermenting colonies on Mac Conkey agar. They gave lactose non fermenting pink colonies on EMB agar. All the isolates were having green pigmentation in Brain Heart Infusion agar. All the isolates produced beta haemolysis on blood agar. The characters used for identification were motility at 37^0C and biochemical tests like citrate utilization, indole, methyl red, Voges Proskauer reaction ONPG, arginine dihydrolase (ADH), lysine decarboxylase (LDB), ornithine decarboxylase (ODB) and nitrate reduction. Details of characterization of *P. aeruginosa* isolates are given in table 9. Carbohydrate fermentation reactions were carried out for 21 different sugars and are given in table 10.

Table 9. Identification of Pseudomonads

Test done	**Isolates**						
	P 1	**P 2**	**P 3**	**P 4**	**P 5**	**P 6**	**P 7**
Motility	+	+	+	+	+	+	+
Green pigment	+	+	+	+	+	+	+
Growth in Mac Conkey agar	+	+	+	+	+	+	+
Urease	-	-	-	-	-	-	-
H2S from TSI	-	-	-	-	-	-	-
ONPG	-	-	-	-	-	-	-
ADH	+	+	+	+	+	+	+
LDB	-	-	-	-	-	-	-
ODB	-	-	-	-	-	-	-
Indole	-	-	-	-	-	-	-
MR	+	+	+	+	+	+	+
VP	-	-	-	-	-	-	-
Citrate Utilization	+	+	+	+	+	+	+
Nitrate reduction	+	+	+	+	+	+	+
Organism	*P. aeruginosa*	*P. aeruginosa*	*P. aeruginosa*	*P. aeruginosa*	*P. aeruginosa*	*P. aeruginosa*	*P. aeruginosa*

Table 10. Carbohydrate fermentation reactions of *Pseudomonas aeruginosa*

Carbohydrate utilized	Isolates						
	P 1	P 2	P 3	P 4	P 5	P 6	P 7
Adonitol	-	-	-	-	-	-	-
Arabinose	+	+	+	+	+	+	+
Cellobiose	-	-	-	-	-	-	-
Dextrose	+	+	+	+	+	+	+
Dulcitol	-	-	-	-	-	-	-
Fructose	+	+	+	+	+	+	+
Galactose	+	+	+	+	+	+	+
Inositol	-	-	-	-	-	-	-
Inulin	-	-	-	-	-	-	-
Lactose	-	-	-	-	-	-	-
Maltose	-	-	-	+	+	+	+
Mannitol	+	+	+	+	+	+	+
Mannose	-	-	-	-	-	-	-
Melibiose	-	-	-	-	-	-	-
Raffinose	-	-	-	-	-	-	-
Rhamnose	-	-	-	-	-	-	-
Salicin	-	-	-	-	-	-	-
Sorbitol	-	-	-	-	-	-	-
Sucrose	-	-	-	-	-	-	-
Trehalose	-	-	-	+	-	+	-
Xylose	+	+	+	+	+	+	+

4.2.1.3. *Identification of Serratia* sp.

Identification of *Serratia* up to species level was done by conducting a set of biochemical reactions, the details of which are given in table 11.

4.2.1.3.1. Serratia fonticola.

A total of four *Serratia fonticola* isolates were obtained. The characters used for identification were motility at 37^0C, Mac Conkey growth and biochemical tests like citrate utilization, indole, methyl red, Voges Proskauer reaction (VP), ONPG,

arginine dihydrolase (ADH), lysine decarboxylase (LDB), ornithine decarboxylase (ODB) and nitrate reduction. Details of characterization of *Serratia fonticola* isolates are given in table 11. Carbohydrate fermentation reactions were carried out for 21 different sugars and are given in table 12.

Table 11. Identification of *Serratia*

Test done	**Isolates**			
	Sf 1	**Sf 2**	**Sf 3**	**Sf 4**
Motility	+	+	+	+
Growth in Mac Conkey agar	+	+	+	+
Urease	-	-	-	-
H_2S from TSI	-	-	-	-
ONPG	+	+	+	+
ADH	-	-	-	-
LDB	+	+	+	+
ODB	+	+	+	+
Indole	-	-	-	-
MR	+	+	+	+
VP	-	-	-	-
Citrate utilization	+	+	+	+
Nitrate reduction	+	+	+	+
Organism	*Serratia fonticola*	*Serratia fonticola*	*Serratia fonticola*	*Serratia fonticola*

Table 12. Carbohydrate fermentation reactions of *Serratia fonticola*

Carbohydrate utilized	Isolates			
	Sf 1	**Sf 2**	**Sf 3**	**Sf 4**
Adonitol	+	+	+	+
Arabinose	+	+	+	+
Cellobiose	+	+	+	-
Dextrose	+	+	+	+
Dulcitol	+	+	+	+
Fructose	+	+	+	+
Galactose	+	+	+	+
Inositol	+	+	+	+
Inulin	+	+	+	+
Lactose	+	+	+	+
Maltose	+	+	+	+
Mannitol	+	+	+	+
Mannose	+	+	+	+
Melibiose	+	+	+	+
Raffinose	+	+	+	+
Rhamnose	+	+	+	+
Salicin	+	+	+	+
Sorbitol	+	+	+	+
Sucrose	-	+	+	-
Trehalose	-	-	+	+
Xylose	-	-	+	+

4.2.1.4. *Identification of Aeromonas* sp.

Identification of *Aeromonas* up to species level was done by conducting a set of biochemical reactions, the details of which were given in table 13.

4.2.1.4.1. *Aeromonas* sp.

Only one *Aeromonas hydrophila* isolate was obtained. The isolate gave non lactose fermenting yellow colonies on Mac Conkey agar. The characters used for identification were motility at 37^0C and biochemical tests like citrate utilization, indole, methyl red, Voges-Proskauer reaction, ONPG, arginine dihydrolase (ADH), lysine decarboxylase (LDB), ornithine decarboxylase (ODB) and nitrate reduction. Characterization of *Aeromonas* sp. and its carbohydrate fermentation reactions for 21 different sugars are given in table 13.

Table 13. Characterization of *Aeromonas* sp. and its carbohydrate fermentation reactions

Test done	Ah 1	Carbohydrate utilized	Reaction
Motility	+	Adonitol	-
Growth in Mac Conkey agar	+	Arabinose	+
Urease	-	Cellobiose	-
H_2S from TSI	-	Dextrose	+
ONPG	+	Dulcitol	_
ADH	+	Fructose	+
LDB	-	Galactose	+
ODB	-	Inositol	-
Indole	+	Inulin	+
MR	+	Lactose	+
VP	-	Maltose	+
Citrate utilization	-	Mannitol	+
Nitrate reduction	+	Mannose	+
Organism	*Aeromonas hydrophila*	Melibiose	+
		Raffinose	-
		Rhamnose	-
		Salicin	-
		Sorbitol	-
		Sucrose	+
		Trehalose	-
		Xylose	-

4.2.2. Identification of Gram positive bacteria

First stage identification of Gram positive bacteria was done based on the following characters of the organism like shape, acid fast staining, spore staining, motility, growth in air, growth anaerobically, catalase, oxidase, acid production from glucose and O/F reaction. The isolates were identified up to the family level. A total

of six *Staphylococcus* sp. were isolated and identified, the details of which are given in table 14.

Table 14. First stage identification of Gram positive bacteria

Test done	Isolates					
	S1	**S2**	**S3**	**S4**	**S5**	**S6**
Shape	C	C	C	C	C	C
Acid fast	-	-	-	-	-	-
Spores	-	-	-	-	-	-
Motility	-	-	-	-	-	-
Growth in air	+	+	+	+	+	+
Growth anaerobically	+	+	+	+	+	+
Catalase	+	+	+	+	+	+
Oxidase	-	-	-	-	-	-
Acid from glucose	+	+	+	+	+	+
O/F	F	F	F	F	F	F
Family	*Staphylococcaceae*	*Staphylococcaceae*	*Staphylococcaceae*	*Staphylococcaceae*	*Staphylococcaceae*	*Staphylococcaceae*

C - Cocci, F - Fermentative

4.2.2.1.1. ***Staphylococcus* sp.**

A total of five *Staphylococcus* sp. *BP V* isolates and one *Staphylococcus* sp. *BP III* isolate were obtained. Identification of *Staphylococcus* sp. was made based on cultural characteristics and biochemical tests like growth Voges-Proskauer reaction (VP), coagulase, phosphatase, nitrate, arginine dihydrolase (ADH), lysine decarboxylase (LDB), ornithine decarboxylase (ODB), ONPG, urease, citrate, hydrogen sulphide production, indole, nitrate reduction tests, haemolysis on blood agar, colony colour on BHIA, mannitol fermentation and carbohydrate fermentation

tests, the details of which are given in table15 and 16. All the *Staphylococcus* sp. *BP V* isolates produced mannitol fermenting colonies in Mannitol Salt Agar (Fig. 7)

Table 15. Second stage identification of *Staphylococcus* sp.

Test done	**Isolates**					
	S1	**S2**	**S3**	**S4**	**S5**	**S6**
White-cream colony	+	+	+	+	+	-
Yellow-orange colony	-	-	-	-	-	+
VP	-	-	-	-	-	-
Coagulase	-	-	-	-	-	-
Phosphatase	-	-	-	-	-	+
Nitrate	+	+	+	+	+	+
ADH	+	+	+	+	+	+
LDB	-	-	-	-	-	-
ODB	-	-	-	-	-	-
Urease	-	-	-	-	-	+
ONPG	+	+	+	+	+	-
Haemolysis	-	-	-	-	-	-
Citrate	-	-	-	-	-	-
H_2Sproduction	-	-	-	-	-	-
Urease	-	-	-	-	-	+
Indole	-	-	-	-	-	-
Growth in MSA (colony colour)	Yellow	Yellow	Yellow	Yellow	Yellow	Red
Organism	*Staphylococcus* sp. *BP V*	*Staphylococcus* sp. *BP V*	*Staphylococcus* sp. *BP V*	*Staphylococcus* sp. *BP V*	*Staphylococcus* sp. *BP V*	*Staphylococcus* sp. *BP III*

Table 16. Carbohydrate fermentation reactions of *Staphylococcus* sp.

Test done	Isolates					
	S1	S2	S3	S4	S5	S6
Adonitol	-	-	-	-	-	-
Arabinose	+	+	+	+	+	-
Cellobiose	-	-	-	-	-	-
Dextrose	+	+	+	+	+	+
Dulcitol	-	-	-	-	-	-
Fructose	+	+	+	+	+	+
Galactose	+	+	+	+	+	+
Inositol	-	-	-	-	-	-
Inulin	-	-	-	-	-	-
Lactose	+	+	+	+	+	+
Maltose	+	+	+	+	+	-
Mannitol	+	+	+	+	+	-
Mannose	-	-	-	-	-	+
Mellibiose	-	-	-	-	-	-
Raffinose	-	-	-	-	-	-
Rhamnose	-	-	-	-	-	-
Salicin	-	-	-	-	-	-
Sorbitol	+	+	+	+	+	+
Sucrose	+	+	+	+	+	+
Trehalose	+	+	+	+	+	+
Xylose	+	+	-	+	+	-

Figure 7. *Staphylococcus* sp. *BP V* colonies on Mannitol Salt Agar.

4.3.ANTIBIOGRAM

4.3.1. Antibiogram of *Escherichia coli*

Antibiogram of *Escherichia coli* isolates are given in table 17. Of the *E. coli* isolates obtained, 91.18 per cent were sensitive to ciprofloxacin, gentamicin and chloramphenicol. The sensitivity shown to other antibiotics were enrofloxacin (88.24 per cent), cephotaxim (76.47 per cent), erythromycin (64.71 per cent), amoxycillin (58.82 per cent), oxytetracycline (35.29 per cent), co-trimoxazole (26.47 per cent) and clindamycin (23.53 per cent). The *E. coli* isolates were completely resistant to sulphadiazine and ampicillin. Multi drug resistance (resistance to at least three antimicrobials) was found among all the *E. coli* isolates obtained in the study (Fig.10). Antibiotic sensitivity pattern and Percentage sensitivity of *Escherichia coli* isolates against selected antibiotics are shown in figure 8 and 9 respectively.

Table 17. Antibiogram of *E. coli* isolates

Isolates	Antibiotics												
	Am	A	Ce	C	Cf	Cd	Co	E	Ex	G	Sz	O	T.R.A
Ec 1	S	R	S	S	S	R	R	R	S	S	R	R	6
Ec 2	R	R	S	R	R	I	S	R	R	S	R	S	7
Ec 3	R	R	S	S	S	R	R	R	S	S	R	R	7
Ec 4	R	R	I	R	S	R	R	R	R	S	R	R	9
Ec 5	I	R	I	S	S	I	S	I	S	S	R	R	3
Ec 6	R	R	R	S	S	R	R	R	S	I	R	R	8
Ec 7	R	R	R	S	S	R	R	R	R	I	R	R	9
Ec 8	I	R	I	S	S	R	R	I	S	S	R	R	5
Ec 9	S	R	S	I	S	R	R	I	S	S	R	R	5
Ec 10	S	R	S	S	S	I	S	I	S	S	R	R	3
Ec 11	S	R	S	S	S	R	R	I	S	S	R	R	5
Ec 12	S	R	S	S	S	R	R	S	S	S	R	S	4
Ec 13	I	R	I	S	S	R	R	R	S	S	R	R	6
Ec 14	I	R	I	S	S	I	R	I	S	S	R	R	4
Ec 15	I	R	I	S	S	R	S	I	S	S	R	R	4
Ec 16	S	R	I	S	S	R	R	I	S	S	R	R	5
Ec 17	S	R	S	S	S	R	S	R	S	S	R	I	4
Ec 18	I	R	I	S	S	R	R	R	I	R	R	I	6
Ec 19	I	R	I	S	S	R	R	I	S	R	R	I	5
Ec 20	R	R	I	S	S	I	R	R	S	S	R	R	6
Ec 21	S	R	I	S	S	I	R	S	S	I	R	R	4
Ec 22	R	R	S	S	S	R	R	R	S	S	R	I	6
Ec 23	R	R	S	I	S	R	S	I	S	S	R	S	4
Ec 24	R	R	R	I	I	R	R	S	I	R	R	I	7
Ec 25	R	R	R	S	S	R	R	I	I	I	R	R	7
Ec 26	R	R	R	R	R	I	S	I	R	I	R	R	8
Ec 27	R	R	R	I	S	R	R	I	S	S	R	I	6
Ec 28	R	R	R	I	S	R	R	I	S	S	R	I	6
Ec 29	S	R	I	S	S	R	R	S	S	S	R	S	4
Ec 30	S	R	R	S	S	R	R	I	S	S	R	R	6
Ec 31	S	R	S	S	S	I	R	R	S	S	R	R	5
Ec 32	S	R	S	S	R	R	S	I	S	S	R	R	5
Ec 33	R	R	S	S	I	R	S	I	S	S	R	S	4
Ec 34	S	R	I	S	S	R	R	I	S	S	R	R	5
Total no: of sensitive isolates	20	0	26	31	31	8	9	22	30	31	0	12	-
Percentage sensitivity	58.82	0	76.47	91.18	91.18	23.53	26.47	64.71	88.24	91.18	0	35.29	-
Percentage Resistance	41.18	100	23.53	8.82	8.82	76.47	73.53	35.29	11.76	8.82	100	64.71	-

R - Resistant, I - Intermediate, S - Sensitive, T.R.A - Total resistant antibiotics

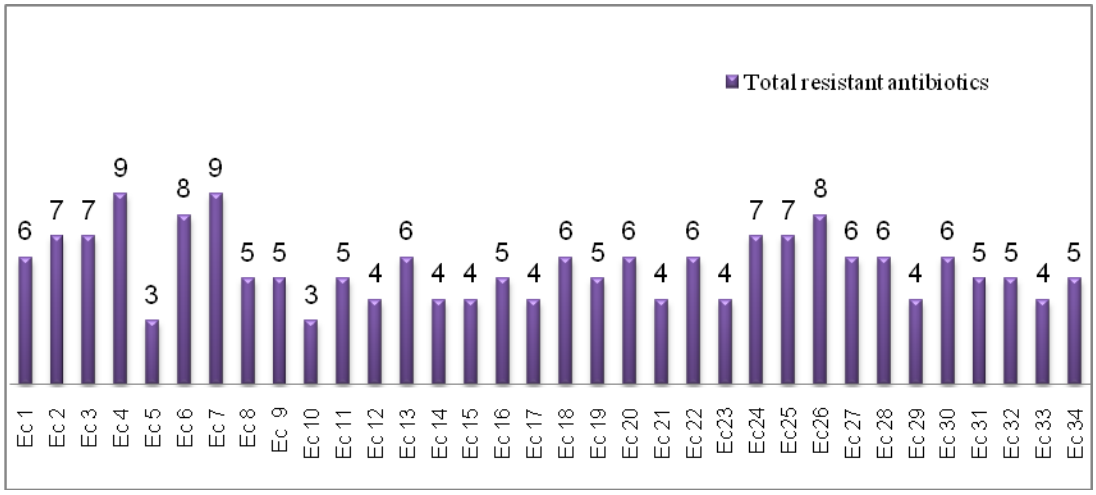

Figure 8. Antibiotic sensitivity pattern of *E. coli* isolates.

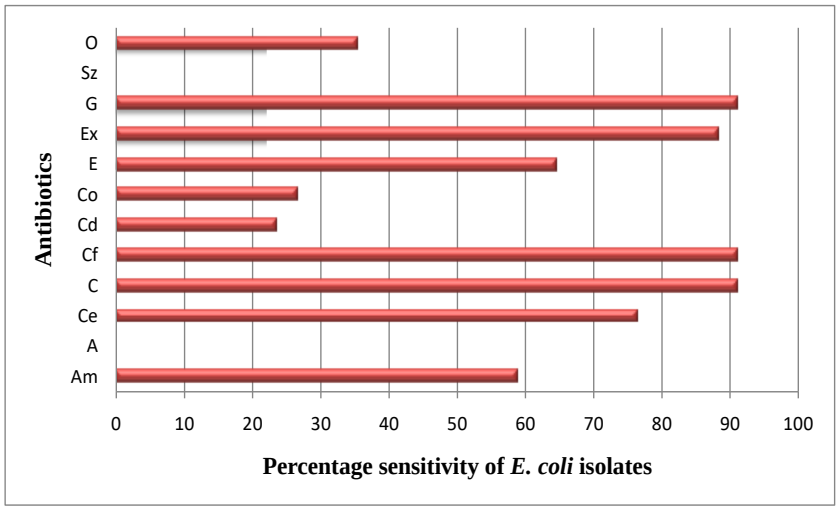

Figure 9. Percentage sensitivity of *Escherichia coli* isolates against selected antibiotics.

Figure 10. Antibiogram of an *E. coli* isolate against selected antibiotics.

4.3.2. Antibiogram of *Pseudomonas aeruginosa* isolates

Antibiogram of *P. aeruginosa* isolates are given in table 17. All the *P. aeruginosa* isolates were sensitive to gentamicin and 57.14 per cent of the isolates were sensitive to ciprofloxacin, cephotaxim, chloramphenicol and enrofloxacin. A sensitivity per cent of 28.57 was given by amoxycillin, ampicillin, erythromycin, oxytetracycline and 14.29 per cent sensitivity was given by clindamycin and co-trimoxazole. The isolates were completely resistant to sulphadiazine. Antibiotic sensitivity pattern and percentage sensitivity of *Pseudomonas aeruginosa* isolates against selected antibiotics are given in figure 11 and 12 respectively.

Table 18. Antibiogram of *Pseudomonas aeruginosa* isolates

Isolates	Antibiotics												
	Am	A	Ce	C	Cf	Cd	Co	E	Ex	G	Sz	O	Total resistant antibiotics
P 1	R	R	S	S	S	R	R	I	S	S	R	R	6
P 2	R	R	R	R	R	R	R	R	R	S	R	R	11
P 3	R	R	S	R	R	R	R	R	R	S	R	R	10
P 4	S	R	I	S	S	R	R	R	S	I	R	I	5
P 5	R	I	R	S	R	R	I	R	S	S	R	I	6
P 6	S	I	I	R	S	I	R	I	S	S	R	R	4
P 7	R	R	R	S	S	R	R	R	R	S	R	R	9
Total no: of sensitive isolates	2	2	4	4	4	1	1	2	4	7	0	2	-
Percentage sensitivity	28.57	28.57	57.14	57.14	57.14	14.29	14.29	28.57	57.14	100	0	28.57	-
Percentage Resistance	71.43	71.43	42.86	42.86	42.86	85.71	85.71	71.43	42.86	0	100	71.43	-

R - Resistant, I - Intermediate, S - sensitive

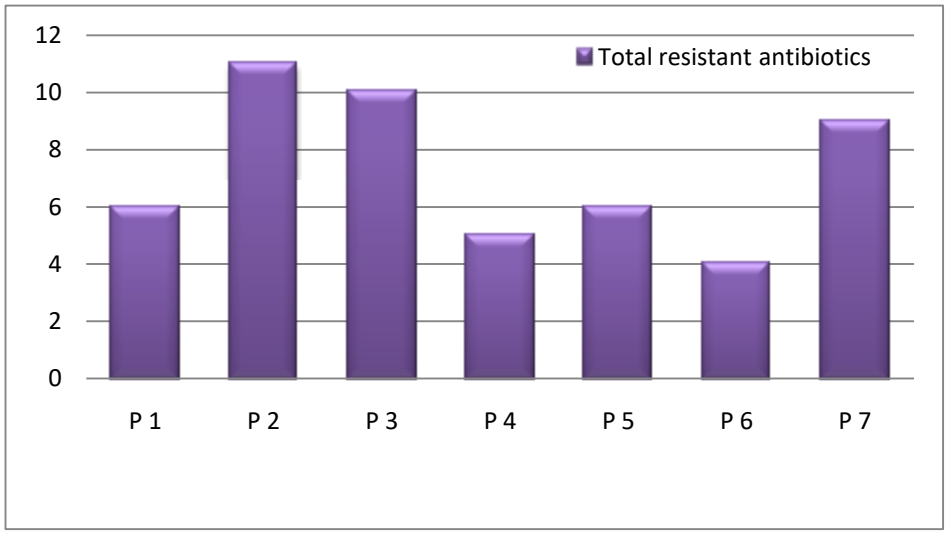

Figure 11. Antibiotic sensitivity pattern of *Pseudomonas aeruginosa* isolates.

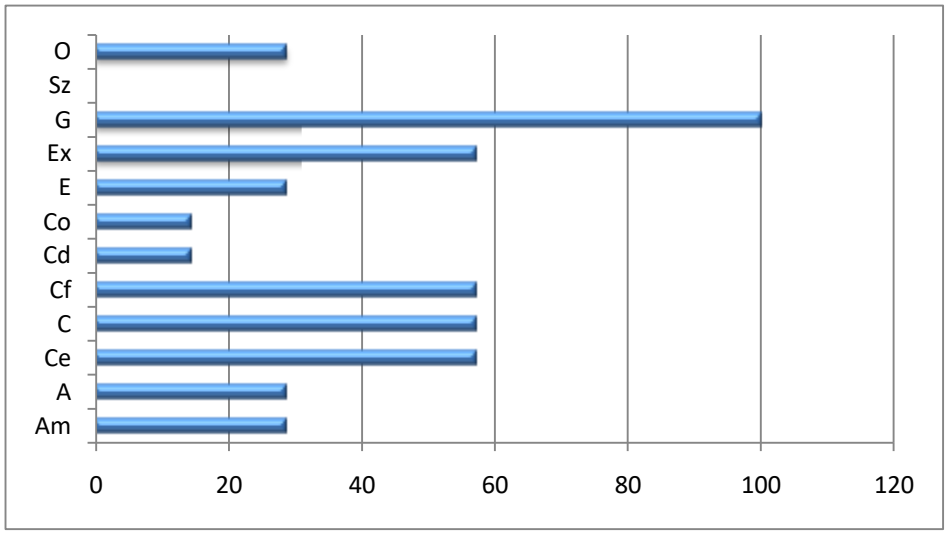

Figure 12. Percentage sensitivity of *Pseudomonas aeruginosa* isolates against selected antibiotics

4.3.3. Antibiogram of *Salmonella typhimurium* isolate

Antibiogram of *Salmonella typhimurium* isolate is given in table 19. The isolate was sensitive to enrofloxacin, ciprofloxacin, intermediate sensitive to gentamicin, cephotaxim and resistant to amoxycillin, ampicillin, chloramphenicol, clindamycin, co-trimoxazole, erythromycin, sulphadiazine and oxytetracycline.

Table 19. Antibiogram of *Salmonella typhimurium* isolate

Isolates	Antibiotics												
	Am	A	Ce	C	Cf	Cd	Co	E	Ex	G	Sz	O	Total resistant antibiotics
St1	R	R	I	R	S	R	R	R	S	I	R	R	8

R - Resistant, I - Intermediate, S - Sensitive

4.3.4. Antibiogram of *Serratia fonticola* isolates

Antibiogram of *S. fonticola* isolates are given in table 20. Of the *S. fonticola* isolates obtained 75 per cent were sensitive to cephotaxim, chloramphenicol, ciprofloxacin, enrofloxacin, gentamicin and 25 per cent were sensitive to erythromycin and clindamycin. The isolates were completely resistant to co-trimoxazole, amoxycillin, ampicillin, sulphadiazine and oxytetracycline. Percentage sensitivity of *S. fonticola* isolates against selected antibiotics is shown in figure 13.

Table 20. Antibiogram of *Serratia fonticola* isolates

Isolates	Antibiotics												
	Am	A	Ce	C	Cf	Cd	Co	E	Ex	G	Sz	O	Total resistant antibiotics
S1	R	R	S	S	R	R	R	R	R	R	R	R	10
S 2	R	R	R	R	S	R	R	I	S	S	R	R	8
S 3	R	R	S	S	S	I	R	R	S	S	R	R	6
S 4	R	R	S	S	S	R	R	R	S	S	R	R	7
Total number of sensitive isolates	0	0	3	3	3	1	0	1	3	3	0	0	-
Percentage sensitivity	0	0	75	75	75	25	0	25	75	75	0	0	-
Percentage Resistance	100	100	25	25	25	75	100	75	25	25	100	100	-

R - Resistant, I - Intermediate, S - Sensitive, T.R.A - Total resistant antibiotics

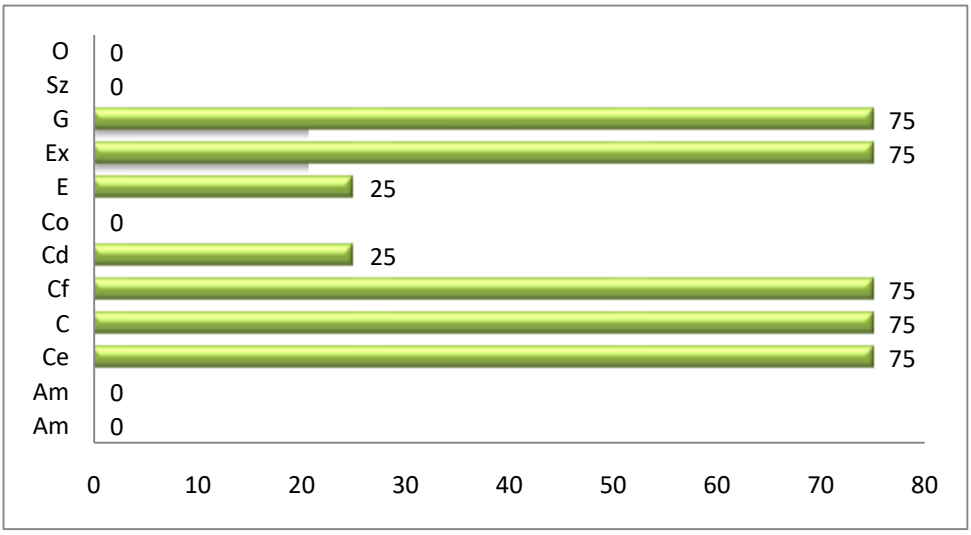

Figure 13. Percentage sensitivity of *S. fonticola* isolates against selected antibiotics

4.3.5. Antibiogram of *Aeromonas hydrophila* isolate

Antibiogram of *Aeromonas hydrophila* isolate is given in table 21. *Aeromonas hydrophila* isolate was found to be sensitive to amoxycillin, ampicillin, cephotaxim, chloramphenicol, ciprofloxacin, clindamycin, co-trimoxazole, erythromycin , enrofloxacin and gentamicin and resistant to sulphadiazine and oxytetracycline.

Table 21. Antibiogram of *Aeromonas hydrophila* isolate

Isolate	Antibiotics												
	Am	A	Ce	C	Cf	Cd	Co	E	Ex	G	Sz	O	Total resistant antibiotics
Ah1	S	S	S	S	S	I	I	I	S	S	R	R	2
Total no: of sensitive isolates	1	1	1	1	1	1	1	1	1	1	0	0	-
Percentage sensitivity	100	100	100	100	100	100	100	100	100	100	0	0	-
Percentage Resistance	0	0	0	0	0	0	0	0	0	0	100	100	-

R - Resistant, I – Intermediate, S - sensitive, T.R.A - Total resistant antibiotics

4.3.6. Antibiogram of *Staphylococcus* sp.

Antibiogram of *Staphylococcus* isolates are given in table 22. All the *Staphylococcus* isolates were sensitive to cephotaxim, chloramphenicol, ciprofloxacin, clindamycin, co-trimoxazole, erythromycin, enrofloxacin, penicillin G and 66.67 per cer sensitive to amoxycillin, ampicillin, gentamicin. A sensitivity per cent of 33.33 was given by oxytetracycline and all the isolates were resistant to sulphadiazine. Percentage sensitivity of *Staphylococcus* subgroups against selected antibiotics is shown in figure 14. Antibiotic sensitivity pattern of *S. typhimurium*, *Staphylococcus* subgroups and *A. hydrophila* against a panel of eight antibiotics are given in figure 15.

Table 22. Antibiogram of *Staphylococcus* sp.

Isolate no	Antibiotics												
	Am	**A**	**Ce**	**C**	**Cf**	**Cd**	**Co**	**E**	**Ex**	**G**	**O**	**P**	**Total resistant antibiotics**
S1	S	S	S	S	S	S	S	S	S	R	R	R	3
S2	S	S	S	S	S	S	S	S	S	S	S	R	1
S3	R	R	I	S	S	S	S	S	S	S	R	R	4
S4	S	S	S	S	S	S	S	S	S	R	R	R	3
S5	S	S	S	S	S	S	S	S	S	S	S	R	1
S6	R	R	I	S	S	S	S	S	S	S	R	R	4
Total sensitive isolates	4	4	6	6	6	6	6	6	6	4	2	6	'
Percentage sensitivity	66.67	66.67	100	100	100	100	100	100	100	66.67	33.33	100	-
Percentage resistance	33.33	33.33	0	0	0	0	0	0	0	33.33	66.67	0	-

R - Resistant, I – Intermediate, S - Sensitive, T.R.A - Total resistant antibiotics

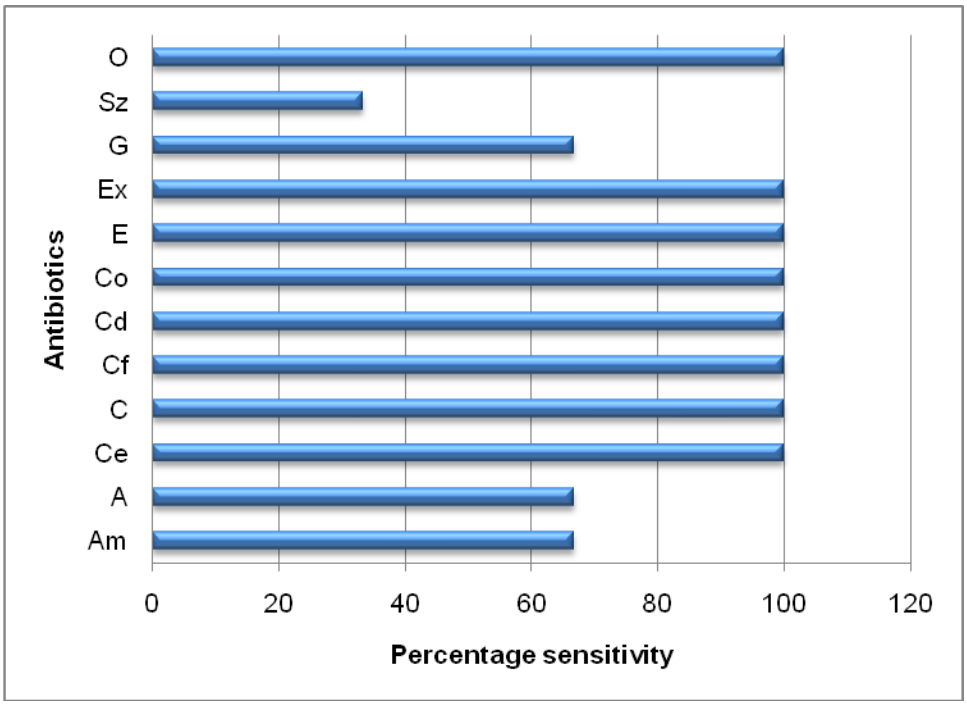

Figure 14. Percentage sensitivity of *Staphylococcus* subgroups against selected antibiotics

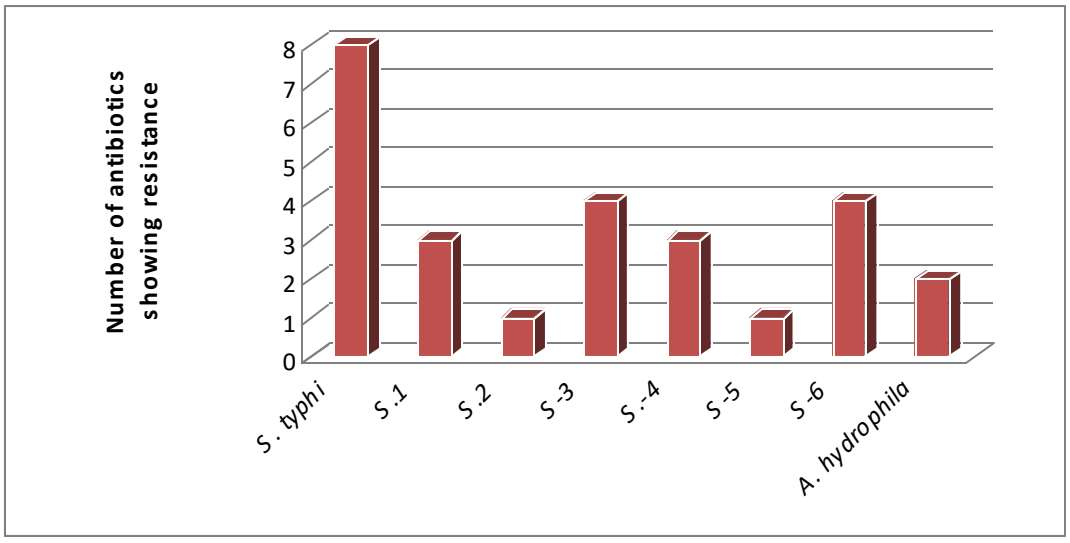

Figure 15. Antibiotic sensitivity pattern of *S. typhimurium*, *Staphylococcus* subgroups and *A. hydrophila* against a panel of eight antibiotics.

4.4. PLASMID PROFILE

The plasmid DNA content of the Gram negative isolates was extracted by alkaline lysis method and analysed on agarose gel electrophoresis. Most of these isolates harboured plasmids.

Among the isolates *E. coli* (79.41 per cent), *Pseudomonas aeruginosa* (100 per cent) and *Serratia fonticola* (75 per cent) were found to harbor plasmids. *Salmonella* isolate did not harbor any plasmid. The only isolate of *Aeromonas hydrophilla* was found to have five plasmids in it. The number of plasmids varied from zero to five and size varied from 2.82 k Da to 20.8 k Da. Electropherogram of plasmids isolated from *E. coli* isolates is given in Figure 16 and the details of plasmids isolated are given in Table 23.

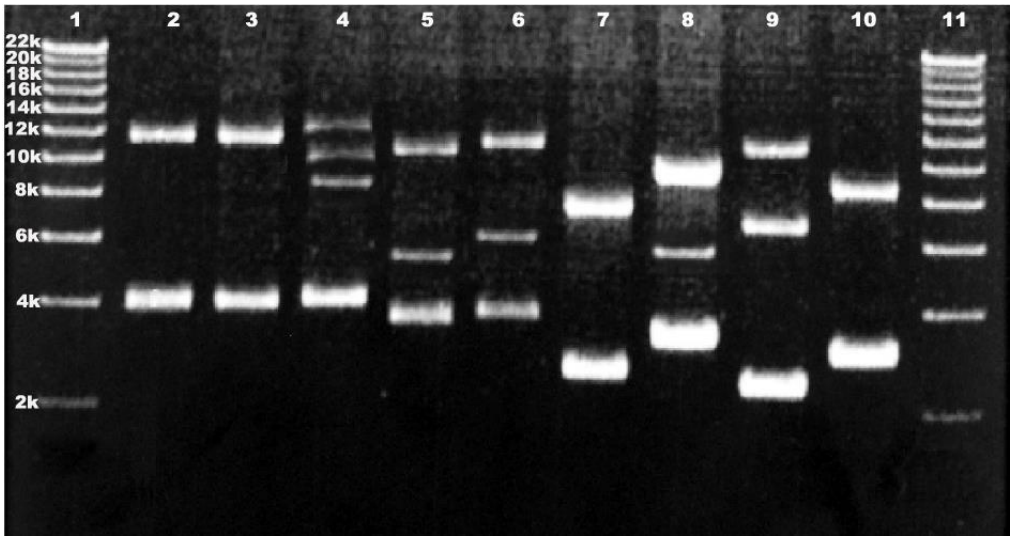

Figure 16. Electropherogram of plasmids from *E. coli* isolates

Lane 1 and Lane 11: 2k molecular weight marker
Lane 2 to Lane 10: Plasmids isolated from various isolates (Ec10 – Ec18 respectively)

Table 23. Plasmid profile of isolates

Isolate	Serotype	No. of plasmids	Molecular weight (k Da)	Isolate	Serotype	No. of plasmids	Mol. weight (k Da)
Ec 1	O38	5	18.22	Ec 19	O41	1	6.79
			15.89	Ec 20	O79	1	10.89
			10.87	Ec 21	O25	2	15.18
			9.06				12.09
			6.59	Ec 22	O109	1	5.09
Ec 2	O5	2	16.63	Ec 23	O103	0	-
			10.87	Ec 24	UT	0	-
Ec 3	O84	0	-	Ec 25	O69	1	7.66
Ec 4	O132	0	-	Ec26	O69	1	3.45
Ec 5	O69	4	20.89	Ec 27	O69	2	15.18
			15.18				12.09
			12.09	Ec28	O69	2	8.32
			5.09				5.67
Ec 6	O80	1	18.45	Ec 29	O69	0	-
Ec 7	O79	1	3.36	Ec30	O69	1	4.98
Ec 8	O69	0	-	Ec 31	O69	3	18.22
Ec 9	O69	4	20.89				9.06
			15.18				6.59
			12.09	Ec 32	O69	2	8.18
			5.09				4.29
Ec 10	O69	2	1.19	Ec 33	O69	0	-
			4.29	Ec 34	O69	1	14.73
Ec 11	O69	2	11.87	St1	-	0	-
			4.29	Sf 1	-	2	19.96
Ec 12	069	4	12.30				7.90
			10.32	Sf 2	-	3	9.82
			8.18				7.85
			4.29				6.54
Ec 13	O69	3	11.04	Sf 3	-	0	-
			5.09				
			3.82	Sf 4	-	1	19.96
Ec 14	069	3	11.90	P 1	-	2	4.58
			6.22				5.42
			3.92	P 2	-	1	7.89
Ec 15	O69	2	7.55	P 3	-	1	5.63
			3.67	P 4	-	1	9.85
Ec 16	O69	3	9.86	P 5	-	2	14.70
			5.90				7.69
			3.72	P 6		1	6.75
Ec 17	O56	3	11.62	Ah1	-	5	19.36
			7.09				13.65
			2.82				9.34
Ec 18	UT	2	8.12				6.59
			3.69				3.54

4.5.*IN VIVO* PATHOGENICITY IN MICE

Mice pathogenicity test revealed that five isolates were highly pathogenic to mice, whereas four isolates showed moderate pathogenicity. Remaining 42 isolates tested were found to be less pathogenic or non pathogenic to mice. Out of five pathogenic strains four were found to be *E. coli* and the only strain of *Salmonella typhimurium* was found to be highly pathogenic. Details of *in vivo* pathogenicity test are given in Table 24.

Table 24. *In vivo* pathogenicity test in mice

Serial number	Isolate	Serotype	Highly pathogenic	Intermediate-pathogenic	Low-pathogenic
1.	Ec 1	O38			✔
2.	Ec 2	O5	✔		
3.	Ec 3	O84			✔
4.	Ec 4	O132	✔		
5.	Ec 5	O69			✔
6.	Ec 6	O80		✔	
7.	Ec 7	O79		✔	
8.	Ec 8	O69			✔
9.	Ec 9	O69			✔
10.	Ec10	O69			✔
11.	Ec11	O69			✔
12.	Ec12	O69			✔
13.	Ec13	O69			✔
14.	Ec14	O69			✔
15.	Ec15	O69			✔
16.	Ec16	O69			✔
17.	Ec17	O56	✔		
18.	Ec18	UT			✔
19.	Ec19	O41		✔	
20.	Ec20	O79		✔	
21.	Ec21	O25	✔		
22.	Ec22	O109			✔
23.	Ec23	O103			✔
24.	Ec24	UT			✔
25.	Ec25	O69			✔
26.	Ec26	O69			✔
27.	Ec27	O69			✔
28.	Ec28	O69			✔
29.	Ec29	O69			✔
30.	Ec30	O69			✔
31.	Ec31	O69			✔
32.	Ec32	O69			✔
33.	Ec33	O69			✔
34.	Ec34	O69			✔
35.	P1	-			✔
36.	P2	-			✔
37.	P3	-			✔
38.	P4	-			✔
39.	P5	-			✔
40.	P6	-			✔
41.	St1	-	✔		
42.	Ah1	-			✔

4.6. GASTROENTERITIS RELATED MORTALITY OF PIGLETS DURING THE PERIOD OF STUDY.

Data regarding the death of piglets due to gastroenteritis during the period of research work were collected from Centre for Pig Production and Research and Centre of Excellence in Pathology (Table 25). Mortality of piglets due to gastroenteritis reported in Centre for Pig Production and Research and Centre of Excellence in Pathology during the year 2007, 2008 and 2009 are shown in figure 17, 18 and 19 respectively.

Proportional mortality ratio = Death from a specific cause / Total death from all causes.

Proportional mortality ratio of gastroenteritis in weaned piglets per month during Summer (April - June) 2008

= Death due to gastroenteritis in weaned piglets per month during Summer 2008 / Total death of weaned piglets from all causes per month during Summer 2008

= (5/6 + 3/3 + 4/4) / 3

= 0.94

Proportional mortality ratio of gastroenteritis in weaned piglets per month during Monsoon (July - October) 2008

= Death due to gastroenteritis in weaned piglets per month during Monsoon 2008 / Total death of weaned piglets from all causes per month during Monsoon 2008

= (8/10 + 13/13 + 8/9 + 11/11) / 4

= 0.92

Proportional mortality ratio of gastroenteritis in weaned piglets per month during Winter (November - March) 2008

= Death due to gastroenteritis in weaned piglets per month during Winter 2008 / Total death of weaned piglets from all causes per month during Winter 2008

= (0/2 + 15/16 + 0/0 +2/2 + 17/18) / 5

= 0.58

Table 25. Data collected from Centre for Pig Production and Research and Centre of Excellence in Pathology

	2007				2008				2009			
Month	No. of preweaned piglets died	preweaned piglets with gastroenteritis	No. of Weaned piglets died	Weaned piglets with gastroenteritis	No. of preweaned piglets died	preweaned piglets with gastroenteritis	No. of Weaned piglets died	Weaned piglets with gastroenteritis	No. of preweaned piglets died	preweaned piglets with gastroenteritis	No. of Weaned piglets died	Weaned piglets with gastroenteritis
January	0	0	2	2	14	11	0	0	9	9	21	15
February	0	0	0	0	17	12	2	2	14	13	24	21
March	0	0	0	0	7	7	18	17				
April	0	0	0	0	2	2	6	5				
May	0	0	3	1	3	3	3	3				
June	0	0	3	1	1	1	4	4				
July	0	0	3	0	12	9	10	8				
August	8	7	1	1	14	12	13	13				
September	0	0	2	2	7	7	9	8				
October	0	0	1	1	5	5	11	11				
November	1	1	0	0	3	2	2	0				
December	0	0	0	0	13	11	16	15				
Total	**9**	**8**	**15**	**8**	**98**	**82**	**94**	**86**	**23**	**22**	**45**	**36**

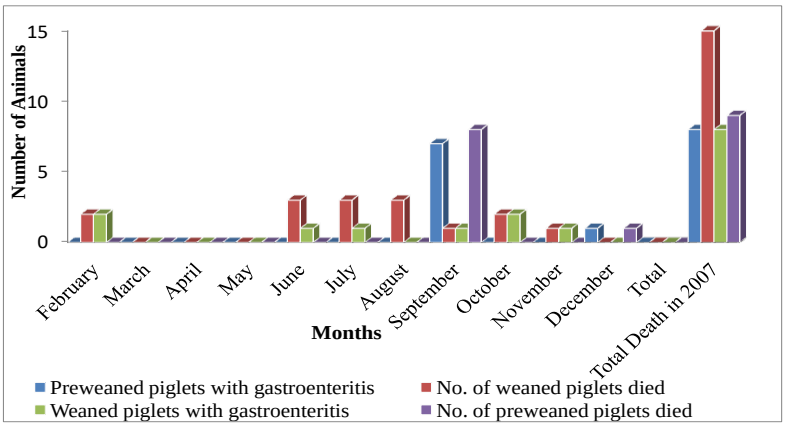

Figure 17. Mortality of piglets due to gastroenteritis reported in Centre for Pig Production and Research and Centre of Excellence in Pathology during the year 2007

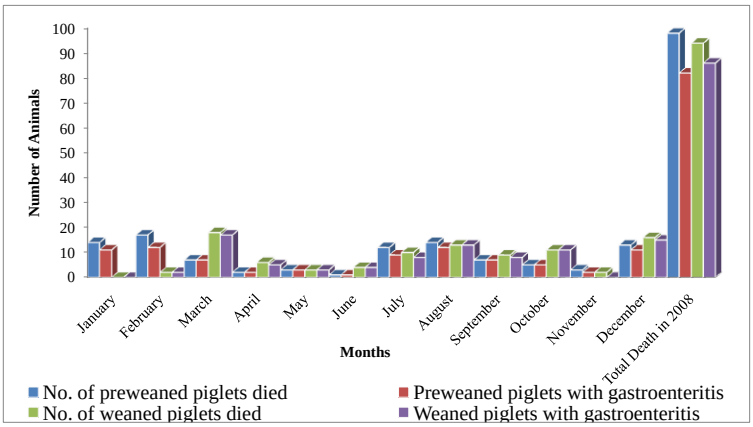

Figure 18. Mortality of piglets due to gastroenteritis reported in Centre for Pig Production and Research and Centre of Excellence in Pathology during the year 2008

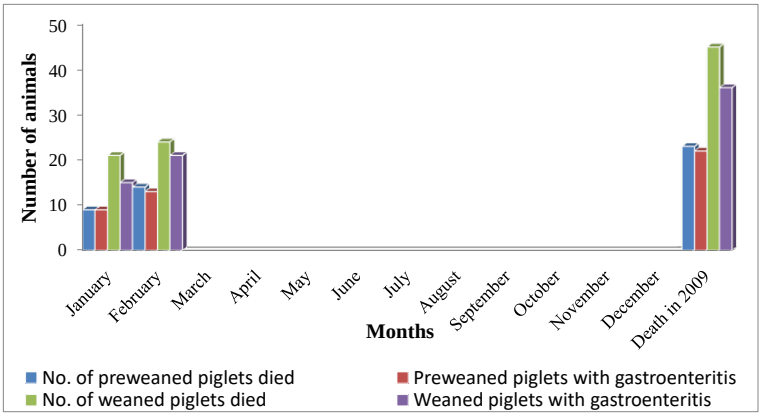

Figure 19. Mortality of piglets due to gastroenteritis reported in Centre for Pig Production and Research and Centre of Excellence in Pathology during the year 2009

Discussion

5. DISCUSSION

5.1.ISOLATION OF ORGANISM

A total of 82 samples were collected from different cases of gastroenteritis, from Centre for Pig Production and Research, cases brought to Centre of Excellence in Pathology and to the Department of Veterinary Microbiology, College of Veterinary and Animal Sciences, Mannuthy as well as from commercial farms and 53 bacterial isolates were obtained from them. The isolates include *Escherichia coli, Salmonella typhymurium, Pseudomonas aeruginosa, Serratia fonticola, Aeromonas hydrophila* and *Staphylococcus* sp.

5.1.1. *Escherichia* sp.

Escherichia coli is one of the most important causes of postweaning diarrhoea in piglets. A total of 34 *E. coli* isolates were obtained and identified. All the *E. coli* isolates had the first and second stage biochemical identification tests and cultural characters consistent with as described by Barrow and Feltham (1993). Twenty seven isolates were collected from rectal swabs whereas four of the isolates were obtained from liver and intestine of the piglets. Another two isolates were of intestinal origin and one was isolated from the intestine and stomach of a piglet.

The most commonly used methods of typing today include serotyping to detect heat-labile (HL) antigens and O antigens (formerly called heat-stable antigens). In the present study serotyping was performed by using O typing systems. The O serotyping of various *Escherichia coli* isolates were done and majority of the *E.coli* isolates were found to be O69 serotype (58 per cent). The existence of these serotypes in animal pathogens are documented (WHO, 1998). The O69 serotype was found in Shiga toxin-producing *Escherichia coli* strains (STEC) (Boerlin *et al.*, 1999; Brunder *et al.*, 1999; Fratamico *et al.*, 2004). Only limited information is available

on the existence of this serotype in piglets (Ngeleka *et al.*, 2003). The first report of existence of O69 serotypes in abundance among diarrhoegenic *E.coli* isolates in India was from Kashmir Valley (Bhat *et al.*, 2008) . The reason for the dominance of serogroup O69 is not known. A possible explanation could be that the antigen composition of these strains makes them specially adapted to propagation in swine populations and in their environment.

The other serotypes found were O79 (6 per cent), O38, O5, O84, O132, O80 (3 per cent each), O56, O41, O25, O109, O103 (1 per cent each). Another 6 per cent of the isolates were found to be untranscribed. The O5 and O79 serotypes were common in animals (Bhat *et al.*, 2008). Many workers have tried to analyse the serotypes prevalent in piglets. In a similar study, Kai Frydendahl of Danish Veterinary institute, Denmark, attempted to identify the serotypes of *E.coli* associated with post weaning diarrhoea in piglets (Frydendahl, 2002). The seroprevalence pattern of *E coli* found in his study was different from another study carried out in Spain (Garabal *et al.*, 1996). Even though Kai could isolate a few organisms with O79 serotype, their dominance was too less and O149 was more abundant. But in a similar study in Korea (Kwon *et al.*, 1999) it was found that seroprevalence of O101 was most common. The excistance of O80 and O32 were reported in post weaning piglet diarrhoea in Spain, but they could isolate O5 and O132 from healthy pigs. It can be concluded that the existence of specific serotypes and its dominance is purely dependent upon their adaptation to propagate in swine populations and their environment.

All the *E. coli* isolates gave lactose fermenting rosy pink coloured colonies on Mac Conkey agar. They gave a characteristic metallic sheen on EMB agar. These isolates were identified mainly based on the IMViC test for which it gave a result of Indole (+), Methyl red (+), Voges-Proskauer (-) and Citrate (-). The other biochemical tests used for confirmatory identification were motility, colony colour

in Mac Conkey agar, urease, H_2S from TSI, ONPG, arginine dihydrolase, lysine decarboxylase, ornithine decarboxylase and nitrate reduction.

Carbohydrate fermentation reactions were carried out for 21 different sugars. The isolates showed mild variation in the sugar utilization tests. Barrow and Feltham (1993) explained that an indication of '+' sign in the identification table means 85 to 100 per cent are positive to that test and an indication of '-' sign means 0 to 15 per cent are positive to that test and rest negative.

A low percentage of sensitivity was observed against the antibiotics like ampicillin, sulphadiazine, clindamycin, co-trimoxazole and oxytetracyclin and a high percentage of sensitivity was observed against ciprofloxacin, chloramphenicol and gentamicin. Multi drug resistance (resistance to at least three antimicrobials) was found among all the *E. coli* isolates obtained in the study. The results are comparable with the antibiotic sensitivity pattern reported in *E.coli* (Mora *et al.*, 2005). Pathogenic typing of the strains in to six most prevalent *E. coli* strains are required to compare the antimicrobial susceptibility pattern. It is clear that multidrug resistance is common among the population of *E.coli* and the situation needs immediate attention.

5.1.2. *Salmonella* sp.

Salmonellae were first identified by D. Salmon, a veterinarian at the Bureau of Animal Industry in 1886 (Salmon and Smith, 1886). Consistently high frequency of antimicrobial resistance among *Salmonella* serovars isolated from swine has been reported mainly for tetracycline (Benson *et al.*, 1985; Takahashi *et al.*, 1990; Murray, 1991; Lee *et al.*, 1993; Seyfarth *et al.*, 1997; Farrington *et al.*, 1999) and β -lactam agents (Benson *et al.*, 1985; Lee *et al.*, 1993; Seyfarth *et al.*, 1997; Farrington *et al.*, 1999). A study conducted in Canada among related pathogens found that 71 per cent of *Salmonella* isolated from swine were resistant to tetracycline, while 29 per cent

were resistant to β-lactams. This result is consistent with most findings in studies done in North America (Dunlop *et al.*, 1998). *Salmonella* isolated from rectal swabs from piglets suffering from diarrhoea, kept at University Pig Breeding Farm, Mannuthy were sensitive to chloramphenicol, septran, gentamicin, neomycin, kanamycin, nalidixic acid and furazolidone. Resistance to streptomycin (80 per cent) followed by erythromycin (70 per cent) and ampicillin (40 per cent) were the most common resistances (Rajasenan, 1983). The *Salmonella* isolate of our study was found to be sensitive to enrofloxacin, ciprofloxacin, intermediate sensitive to gentamicin, cephotaxim and resistant to amoxycillin, ampicillin, chloramphenicol, clindamycin, co-trimoxazole, erythromycin, sulphadiazine and oxytetracycline. The results are agreeing with the findings of earlier workers cited above.

5.1.3. *Pseudomonas aeruginosa*

Pseudomonas aeruginosa is a notoriously difficult organism to control with antibiotics or disinfectants (Hancock, 1998). A low percentage of sensitivity was observed against the antibiotics like sulphadiazine, clindamycin, co-trimoxazole, oxytetracyclin, erythromycin, amoxycillin and ampicillin and a high Percentage of sensitivity was observed against gentamicin. Multi drug resistance (resistance to at least three antimicrobials) was found among all *P. aeruginosa* isolates obtained in the study.

In general resistance exhibited by *Pseudomonas aeruginosa* is due to a combination of factors like low permeability of its cell wall, the genetic capacity to express a wide repertoire of resistance mechanisms such as mutation in chromosomal genes, procurement of resistance genes from other organisms via plasmids, transposons and bacteriophages (Lambert, 2002). All of the major classes of antibiotics used to treat *P. aeruginosa* infection have to cross the cell wall to reach their targets. The aminoglycosides (gentamicin, tobramycin, amikacin) inhibit

protein synthesis by binding to the 30S subunit of the ribosome. Quinolones (ciprofloxacin) bind to the A subunit of DNA gyrase, which maintains the ordered structure of the chromosome 22 inside the cells. Small hydrophilic antibiotics such as the β -lactams and quinolones can only cross the outer membrane by passing through the aqueous channels provided by porin proteins. These are barrel-shaped molecules which span the outer membrane, usually associated as trimers. *P. aeruginosa* produces several different porins, *oprF* being the major porins present in all strains (Brinkman *et al.*, 2000).

All *P. aeruginosa* strains possess the *ampC* gene for the inducible chromosomal β-lactamase. However, induction alone probably does not account for resistance in *Pseudomonas* strains. Instead, over-expression of the enzyme results from spontaneous mutation in the regulatory gene, *ampR*. This has occurred particularly where heavy reliance has been placed on ceftazidime therapy (Giwercman *et al.*, 1990).

5.1.4. *Serratia fonticola*

Apart from *Serratia marcescens* and the *Serratia liquefaciens* complex (*S. liquefaciens*, *Serratia proteamaculans* and *Serratia grimesii*) which are regarded as causing the majority of human *Serratia* infections, there is little information about the remaining *Serratia* species ('unusual' serratiae), including their susceptibility patterns to antimicrobial agents or underlying mechanisms of resistance. Identification of 'unusual' serratiae is of particular interest because a reliable species assignment of these strains requires carbon source utilization tests that are not included in common commercial identification systems (Anahory *et al.*, 1998; Stock *et al.*, 2003).'Unusual' *Serratia* species include six species, namely, *Serratia ficaria*, *Serratia fonticola*, *Serratia odorifera*, *Serratia plymuthica*, *Serratia rubidaea* and *Serratia entomophila*. *S. fonticola* is widely distributed in nature and has been

predominantly isolated from water (Gavini *et al.*, 1979; Stock *et al.*, 2003), soil, sewage (Gavini *et al.*, 1979), mollusks (Müller *et al.*, 1995), birds (Muller *et al.*, 1986) and clinical samples, mainly from wounds and the respiratory tract (Farmer *et al.*, 1985; Bollet *et al.*, 1991).

In the present study, a low Percentage of sensitivity was observed against the antibiotics like amoxycillin, ampicillin, co-trimoxazole, sulphadiazine, oxytetracycline, clindamycin and erythromycin and a high Percentage of sensitivity was observed against cephotaxim, chloramphenicol, ciprofloxacin, enrofloxacin and gentamicin. Multi drug resistance (resistance to at least three antimicrobials) was found among all *S. fonticola* isolates obtained in the study. This is the first report on the sensitivity pattern of *S. fonticola* isolates from diarrhoeic piglets. The sensitivity pattern of the isolates are agreeing with the findings of Stock *et al* (2003).

5.1.5. *Aeromonas hydrophila*

The genus *Aeromonas* comprises a group of Gram-negative, facultatively anaerobic bacteria that are pathogenic for aquatic and terrestrial animals and have also been associated with a wide spectrum of infectious diseases in humans (Altwegg *et al.*, 1989; Paniagua *et al.*, 1990; Cascon *et al.*, 1996). Enterotoxigenic *Aeromonas hydrophila* as a causative of piglet diarrhoea was reported (Dobrescu, 1978). There are several reports of *Aeromonas hydrophila* as an intestinal pathogen in pigs, piglets and human beings. (Burke and Gracey, 1986; Khardori and Fainstein, 1988; Gray and Stickler, 1989; Ceylan *et al.*, 2009). The entry of this organism to the intestine of pigs might be through the fish meal fed to the animal. Existence of these bacteria as a pathogen in fish was reported by many workers (Hazen *et al.*, 1978; Allan and Stevenson, 1981; Dooley and Trust, 1988; Son *et al.*, 1997; Zhang *et al.*, 2000). The prevention and treatment of both human and fish diseases by the extensive use of antimicrobial agents have undoubtedly contributed to an increase in the frequency of resistant strains (Rhodes *et al.*, 2000). Additionally, since antimicrobial agents are

released into the surrounding water during treatment of bacterial fish diseases, there is a direct negative impact on the aquaculture environment (Aoki, 1992; Smith *et al.*, 1994).

An increase in resistance levels of the genus *Aeromonas*, particularly to β-lactam antibiotics has been reported (Bakken *et al.*, 1988; Overman and Janda, 1999; Schmidt *et al.*, 2000; Rowe-Magnus *et al.*, 2002). This evolution towards increasing levels of resistance is, in part, attributed to the production of different β -lactamases, for instance inducible β -lactamases active against penicillins, cephalosporins and carbapenems (Overman and Janda, 1999). Although most studies tested clinical isolates, β -lactamases from the environmental microbiota have recently been described. The genus *Aeromonas* has been the subject of various antimicrobial susceptibility studies over the last 30 years (Overman and Janda, 1999; Barlow *et al.*, 2004). Although *Aeromonas* species are distributed throughout the world, there are geographic differences in the frequency of diseases caused by these bacteria (Ko *et al.*, 2005). The access of fish pathogen to the piglets may be through the feed made of fish materials.

5.1.6. *Staphylococcus* sp.

A low percentage of sensitivity was observed against the antibiotics sulphadiazine, oxytetracyclin and a high percentage of sensitivity was observed against cephotaxim, chloramphenicol, ciprofloxacin, clindamycin, co-trimoxazole, erythromycin, enrofloxacin and penicillin G.

The *Staphylococcus* BP subgroups obtained in this study were reported earlier from the intestines of pigs and piglets (Wilssens and Vande Casteele, 1967). Several workers have reported the presence of multidrug resistant *Staphylococcus* in piglets (Schulz, 1970; Muller, 1974; Sato *et al.*, 1990; Schwarz and Blobel, 1990; Wegener *et al.*, 1993; Chen *et al.*, 2007). To the best of our knowledge, no work on antibiotic

resistance of *Staphylococcus* sp. causing diarrhoea in piglets could be found. Coagulase production by *Staphylococcus* sp. acts as an important indicator of pathogenicity (Quinn *et al.*, 2002).Since all the isolates were coagulase negative it could be concluded that none are pathogenic. Hence we believe that the isolates are not enteropahogens, but may be a part of normal microbiota of piglets.

No single antibiotic was found to be effective against all the isolates. Hence antibiotic for treatment can be chosen only after sensitivity testing of isolates causing gastroenteritis.

5.2.*IN VIVO* PATHOGENICITY TEST IN MICE

Mice pathogenicity test revealed that five isolates were highly pathogenic to mice, whereas four isolates showed moderate pathogenicity. Remaining 33 isolates were shown to be less pathogenic or non pathogenic to mice. Out of five pathogenic strains four were found to be *E. coli* and the only strain of *Salmonella* that could be isolated from rectal swab of a diarrhoeic piglet was found to be highly pathogenic. The antibiotic resistance shown by the pathogenic *E. coli* and *S. typhimurium* isolates might be an indication of their virulence.

5.3.PLASMID PROFILE

The plasmid DNA content of the Gram negative isolates was extracted by alkaline lysis method and analysed on agarose gel electrophoresis. Most of these isolates harboured plasmids; 79.41 per cent of *E.coli* isolates, 100 per cent of *Pseudomonas aeruginosa* isolates and 75 per cent of *Serratia fonticola* isolates were found to harbor plasmids. *Salmonella typhimurium* isolate did not harbor any plasmid. The only isolate of *Aeromonas hydrophilla* was found to have five plasmids in it. The number of plasmids varied from zero to five and size varied from 2.82 k Da

to 20.8 k Da. No apparent correlation was found between the plasmid profiles of the strains and their resistance patterns to the antimicrobial agents.

A study involving isolation of plasmids from *E coil* causing bovine mastitis, conducted in Kerala Agricultural University, reported isolation of a size range of plasmids from 1.86 to 48.06 k Da (Mini *et al.*, 2005). In another study the number of plasmids from *E. coli* isolates obtained from respiratory infections in poultry varied from one to three and size varied from 1.54 k Da to 31.8 k Da (Jesto, 2007). The molecular weight of plasmids obtained in our study is comparable with these earlier reports. A correlation between the number of plasmids and antibiotic resistance could not be ascertained in this study.

The proportional mortality ratio of gastroenteritis in weaned piglets per month during various seasons was determined. Three major seasons *viz.*, Summer (April to June), Monsoon (July to October) and Winter (November to March) were taken into consideration. Due to Swine fever outbreak in Centre for Pig Production and Research no stock was maintained from January 2007 to July 2007. During the year 2008 the proportional mortality ratio of gastroenteritis in weaned piglets per month was maximum during Summer season (0.94) followed by Monsoon (0.92) and Winter (0.58).

In conclusion, the results of this study provide evidence for significant antimicrobial resistance among bacterial isolates from piglets. Long term prospective studies involving isolation, identification and antibiogram from more samples are required to identify novel pathogens causing gastroenteritis in piglets. Molecular analysis of plasmids such as Amplified Fragment Length Polymorphism (AFLP), Restriction Fragment Length Polymorphism (RFLP), Random Amplification of Polymorphic DNA (RAPD) are required to correlate the antibiotic resistance with the plasmid profiles of these organisms.

Summary

6. SUMMARY

Diarrhoea among post weaned piglets is a significant problem in many herds. The weaning process increases the stress on piglets and thus their susceptibility to viral and bacterial infections. Diarrhoea in these older piglets tends to be less severe and mortality rates lower. Potential sources of diarrhoea at this stage include *Escherichia coli*, Rotavirus, *Salmonella*, *Campylobacter* and *Brachyspira hyodysenteriae*. Colibacillosis or *E. coli* infection is a bacterial disease affecting many herds, both chronically and sporadically (Potter, 1998). *Escherichia coli* infections are common in the first week after farrowing and again in the first week after weaning (Hill and Sainsbury, 1995; Bertschinger *et al.*, 1999). *Escherichia coli* infections can also be linked with other infections including rotavirus, edema disease and urinary tract infections (Bertschinger *et al.*, 1999; Cowart and Casteel, 2001). At weaning time the loss of sows milk and IgA allow the *E. coli* to attach to the villi of the small intestines, the toxins cause acute enteritis and diarrhoea. Post-weaning diarrhoea is a common cause of mortality and morbidity.

Considering the importance of these problems, this study was undertaken to isolate and identify bacteria causing enteritis in weaned piglets and to study the sensitivity of these organisms against common antibiotics used in the management of diarrhoeal conditions. An attempt was also made to isolate the plasmids from these isolates.

Samples were collected from different cases of gastroenteritis, from Centre for Pig Production and Research, cases brought to Centre of Excellence in Pathology and to the Department of Veterinary Microbiology, College of Veterinary and Animal Sciences, Mannuthy as well as from commercial farms.

Rectal swabs were collected from live diarrhoeic piglets and intestinal contents, pieces of jejunum, ileum, colon, mesenteric lymph nodes, liver, spleen and stomach were collected during post mortem examination after taking all sterile precautions. Isolation of causative bacteria was made by culturing on Brain Heart Infusion Agar, Mac Conkey Agar, Mannitol Salt Agar, Blood Agar, *Brucella* Agar and Cooked Meat Medium. A total of 82 samples were collected from piglets showing clinical signs and 53 bacterial isolates were obtained from them.

A total of 34 *E. coli* isolates were obtained and identified. 27 isolates were collected from rectal swab whereas 4 of the isolates were obtained from liver and intestine of the piglets another two isolates were of intestinal origin and one was isolated from the intestine and stomach of a piglet. Another isolate obtained was identified as *Salmonella typhymurium*, which was found in a rectal swab culture.

Out of seven isolates of *Pseudomonas aeruginosa*, three were obtained from internal organs such as intestine, stomach, liver and kidney during postmortem. Another three samples were obtained from rectal swabs.

A total of four *Serratia fonticola* isolates were also obtained and all of them were of rectal swab origin. One isolate was found to be *Aeromonas hydrophila*, which is a known pathogen in fish.

Six *Staphylococcus* subgroups were obtained and none of them were found to be haemolytic. Three of them were obtained from rectal swab and remaing three were from intestine of the piglets.

The O serotyping of various isolates were done and majority of the *E.coli* isolates were found to be O69 serotype (58 per cent). The other serotypes found were, O79 (6 per cent), O38, O5, O84, O132, O80 (3 per cent each). Another six per cent of the isolates were found to be untranscribed.

The effectiveness of various antibiotics against these isolates was tested using standard antibiotic discs using Kirby Bauer method.

In case of *E. coli* a low percentage of sensitivity was observed against the antibiotics like ampicillin, sulphadiazine, clindamycin, co-trimoxazole and oxytetracyclin and a high percentage of sensitivity was observed against ciprofloxacin, chloramphenicol and gentamicin. Multi drug resistance (resistance to at least three antimicrobials) was found among all the *E. coli* isolates obtained in the study.

Salmonella typhimurium isolate was found to be sensitive to enrofloxacin , ciprofloxacin, intermediate sensitive to gentamicin, cephotaxim and resistant to amoxycillin, ampicillin, chloramphenicol, clindamycin, co-trimoxazole, erythromycin, , sulphadiazine and oxytetracycline.

Pseudomonas isolates gave a low percentage of sensitivity against the antibiotics like sulphadiazine, clindamycin, co-trimoxazole, oxytetracyclin, erythromycin, amoxycillin and ampicillin and a high percentage of sensitivity was observed against gentamicin. Multi drug resistance (resistance to at least three antimicrobials) was found among all *P. aeruginosa* isolates obtained in the study.

Serratia fonticola isolates gave a low percentage of sensitivity against the antibiotics like amoxycillin, ampicillin, co-trimoxazole, sulphadiazine, oxytetracyclin, clindamycin and erythromycin and a high Percentage of sensitivity was observed against cephotaxim, chloramphenicol, ciprofloxacin, enrofloxacin and gentamicin. Multi drug resistance (resistance to at least three antimicrobials) was found among all *S. fonticola* isolates obtained in the study.

Aeromonas hydrophila isolate was found to be sensitive to amoxycillin, ampicillin, cephotaxim, chloramphenicol, ciprofloxacin, clindamycin, co-

trimoxazole, erythromycin, enrofloxacin and gentamicin and resistant to sulphadiazine and oxytetracycline.

In case of *Staphylococcus* isolates a low percentage of sensitivity was observed against the antibiotics sulphadiazine, oxytetracyclin and a high percentage of sensitivity was observed against cephotaxim, chloramphenicol, ciprofloxacin, clindamycin, co-trimoxazole, erythromycin, enrofloxacin and penicillin G.

Mice pathogenicity test revealed five isolates were highly pathogenic to mice, whereas four isolates showed moderate pathogenicity. Remaining 33 isolates were shown to be less pathogenic or non pathogenic to mice. Out of five pathogenic strains four were found to be *E. coli* and the only strain of *Salmonella* that could be isolated from piglet was found to be highly pathogenic. On isolation of plasmids a total of 77 plasmids could be isolated from the *E. coli*, *S. fonticola*, *P. aeruginosa* and *A. hydrophila* samples. The antibiotic resistance shown by the pathogenic *E. coli* and *S. typhimurium* isolates might be an indication of their virulence.

The plasmid DNA content of the Gram negative isolates was extracted by alkaline lysis method and analysed on agarose gel electrophoresis. Most of these isolates harboured plasmids; 79.41 per cent of *E.coli* isolates, 100 per cent of *Pseudomonas aeruginosa* isolates and 75 per cent of *Serratia fonticola* isolates were found to harbor plasmids. *Salmonella typhimurium* isolate did not harbor any plasmid. The only isolate of *Aeromonas hydrophilla* was found to have five plasmids in it. The number of plasmids varied from zero to five and size varied from 2.82 k Da to 20.8 k Da. No apparent correlation was found between the plasmid profiles of the strains and their resistance patterns to the antimicrobial agents.

The proportional mortality ratio of gastroenteritis in weaned piglets per month during various seasons was determined. Three major seasons *viz.*, Summer (April to June), Monsoon (July to October) and Winter (November to March) were

taken into consideration. Due to Swine fever outbreak in Centre for Pig Production and Research no stock was maintained from January 2007 to July 2007. During the year 2008 the proportional mortality ratio of gastroenteritis in weaned piglets per month was maximum during Summer season (0.94) followed by Monsoon (0.92) and Winter (0.58).

In conclusion, the results of this study provide evidence for significant antimicrobial resistance among bacterial isolates from piglets. Long term prospective studies involving isolation, identification and antibiogram from more samples are required to identify novel pathogens causing gastroenteritis in piglets. Molecular analysis of plasmids such as Amplified Fragment Length Polymorphism (AFLP), Restriction Fragment Length Polymorphism (RFLP), Random Amplification of Polymorphic DNA (RAPD), are required to correlate the antibiotic resistance with the plasmid profiles of these organisms. Such studies provide data on temporal and spatial difference in antibiotic resistance patterns, which in turn helps the scientific community to design better disease control strategies.

References

7. REFERENCES

Alexander, T.J. and Taylor, D.J. 1969. The clinical signs, diagnosis and control of swine dysentery. *Vet. Rec.* 85: 59-63.

Allan, B.J. and Stevenson, R.M.W. 1981. Extracellular virulence factors of *Aeromonas hydrophila* in fish infections. *Can. J. Microbiol.* 27: 1114-1122.

Altwegg, M., Geiss, H.K. and Freij, B.J. 1989. Aeromonas as a human pathogen. *Critical Rev. Microbiol.* 16: 253-286.

Amezcua, R., Friendship, R., Dewey, C. and Gyles, C. 2002. A case-control study investigating risk factors associated with postweaning *Escherichia coli* diarrhoea in southern ontario. *J. Swine Health and Prod.* 10: 245-250.

Anahory, T., Darbas, H., Ongaro, O., Jean-Pierre, H. and Mion, P. 1998. *Serratia ficaria*: A misidentified or unidentified rare cause of human infections in fig tree culture zones. *J. Clin. Microbiol.* 36: 3266-3272.

Andrusenko, I.T., Aleksandrova, I.K., Zaviriukha, A.I., Kharchuk, A.I. and Gorodisskaia Iu, I. 1983. Antibiotic sensitivity of bacteria of the family Vibrionaceae isolated from the intestines of swine. *Antibiotiki.* 28: 592-595.

Aoki, T. 1992. Present and future problems concerning the development of resistance in aquaculture. *Chemotherapy in aquaculture: from theory to reality. Office International des Epizooties, Paris, France.* 254-262.

Aumaitre, A., Peiniau, J. and Madec, F. 1995. Digestive adaptation after weaning and nutritional consequences in the piglet. *Pig News and Inf.* 16: 73N-79N.

Bahnson, P.B., Fedorka-Cray, P.J., Ladely, S.R. and Mateus-Pinilla, N.E. 2006. Herd-level risk factors for *Salmonella enterica* subsp. Enterica in U.S. Market pigs. *Prev. Vet. Med.* 76: 249-262.

Baird-Parker, A.C. 1965. Recommendations of the subcommittee on taxonomy of staphylococci and micrococci. *Int. Bull. bact. Nomencl. Taxson.* 15: 109.

Bakken, J.S., Sanders, C.C., Clark, R.B. and Hori, M. 1988. Beta-lactam resistance in *Aeromonas* spp. Caused by inducible beta-lactamases active against penicillins, cephalosporins, and carbapenems. *Antimicrob. Agents Chemother.* 32: 1314-1319.

Barker, I.K. and Van Dreumel, A.A. 1985. The alimentary system. In Pathology of domestic animals, Jubb K.V.F, Kennedy P.C and Palmer N, eds. (Orlando, Academic Press), pp. 128-135.

Barlow, R.S., Pemberton, J.M., Desmarchelier, P.M. and Gobius, K.S. 2004. Isolation and characterization of integron-containing bacteria without antibiotic selection. *Antimicrob. Agents Chemother.* 48: 838-842.

Barrow, P.A., Brooker, B.E., Fuller, R. and Newport, M.J. 1980. The attachment of bacteria to the gastric epithelium of the pig and its importance in the microecology of the intestine. *J. Appl. Microbiol.* 48: 147-154.

Barrow GI, Feltham RKA (eds). (1993). Cowan and Steel's Manual for the identification of medical bacteria, 3rd edition, University Press, Cambridge.

Bauer, A.W., Kirby, W.M.M., Sherris, J.C. and Turck, M. 1999. Antibiotic susceptibility testing by a standardized single disk method. *Microbiology: A Centenary Perspective.*

Benson, C.E., Palmer, J.E. and Bannister, M.F. 1985. Antibiotic susceptibilities of salmonella species isolated at a large animal veterinary medical center: A three year study. *Can. J. Comp. Med.* 49: 125-128.

Bergey, D.H. and Holt, J.G. 1994. Bergey's manual of determinative bacteriology. Vol, Edition. Lippincott Williams & Wilkins.

Bertschinger, H.J. 1999. Postweaning *E.coli* diarrhoea and edema disease. In "diseases of swine", be straw ed (Iowa State Univ. Press).

Bertschinger, H.U., Fairbrother, J.M., Nielsen, N.O. and Pohlenz, J.F. 1999. *Escherichia coli* infections. *Diseases of swine.* 431–468.

Bhat, M.A., Nishikawa, Y. and Wani, S.A. 2008. Prevalence and virulence gene profiles of shiga toxin-producing *Escherichia coli* and enteropathogenic *Escherichia coli*. *Small Ruminant Research.* 75: 65-70.

Blanco, J.E., Blanco, M., Mora, A. and Blanco, J. 1997. Production of toxins (enterotoxins, verotoxins, and necrotoxins) and colicins by *Escherichia coli* strains isolated from septicemic and healthy chickens: Relationship with *in vivo* pathogenicity [in process citation]. *J. Clin. Microbiol.* 35: 2953-2957.

Blecha, F., Pollman, D.S. and Nichols, D.A. 1983. Weaning pigs at an early age decreases cellular immunity. *J. Anim. Sci.* 56: 396.

Boerlin, P., McEwen, S.A., Boerlin-Petzold, F., Wilson, J.B., Johnson, R.P. and Gyles, C.L. 1999. Associations between virulence factors of shiga toxin-producing *Escherichia coli* and disease in humans. *J. Clin. Microbiol.* 37: 497-503.

Bohl, E.H., Saif, L.J., Theil, K.W., Agnes, A.G. and Cross, R.F. 1982. Porcine pararotavirus: Detection, differentiation from rotavirus, and pathogenesis in gnotobiotic pigs. *J. Clin. Microbiol.* 15: 312-319.

Bollet, C., Gainnier, M., Sainty, J.M., Orhesser, P. and De Micco, P. 1991. *Serratia fonticola* isolated from a leg abscess. *J. Clin. Microbiol.* 29: 834-835.

Both, G., Möller, K., Busse, F.W., Nitzschke, E. and Jonas, D. 1982. Investigations on the occurrence of salmonella in clinically unsuspected pig breeding farms (author's transl). *DTW. Deutsche tierärztliche Wochenschrift*. 89: 3.

Bridger, J.C. 1980. Detection by electron microscopy of caliciviruses, astroviruses and rotavirus-like particles in the faeces of piglets with diarrhoea. *Vet. Rec.* 107: 532-533.

Bridger, J.C. 1987. Novel rotaviruses in animals and man. *Ciba Found. Symp.* 128: 5-23.

Bridger, J.C. and Brown, J.F. 1985. Prevalence of antibody to typical and atypical rotaviruses in pigs. *Vet. Rec.* 116: 50.

Bridger, J.C., Hall, G.A. and Brown, J.F. 1984. Characterization of a calici-like virus (newbury agent) found in association with astrovirus in bovine diarrhoea. *Infect. Immun.* 43: 133-138.

Brinkman, F.S., Bains, M. and Hancock, R.E. 2000. The amino terminus of *Pseudomonas aeruginosa* outer membrane protein oprf forms channels in lipid bilayer membranes: Correlation with a three-dimensional model. *J. Bacteriol.* 182: 5251-5255.

Brunder, W., Schmidt, H., Frosch, M. and Karch, H. 1999. The large plasmids of shiga-toxin-producing *Escherichia coli* (STEC) are highly variable genetic elements. *Microbiol.* 145: 1005-1014.

Bulgin, M.S., Ward, A.C., Barrett, D.P. and Lane, V.M. 1989. Detection of rotavirus and coronavirus shedding in two beef cow herds in Idaho. *Can. Vet. J.* 30: 235-239.

Burke, V. and Gracey, M. 1986. Aeromonas species in human diarrhoeal disease. *J. Gastroenterol. Hepatol.* 1: 237-249.

Cascon, A., Anguita, J., Hernanz, C., Sanchez, M., Fernandez, M. and Naharro, G. 1996. Identification of *Aeromonas hydrophila* hybridization group 1 by PCR assays. *Appl. Environ. Microbiol.* 62: 1167.

Celemin, C., Rubio, P., Echeverria, P. and Suarez, S. 1995. Gene toxin patterns of *Escherichia coli* isolated from diseased and healthy piglets. *Vet. Microbiol.* 45: 121-127.

Ceylan, E., Berktas, M. and Agaoglu, Z. 2009. The occurrence and antibiotic resistance of motile Aeromonas in livestock. *Trop. Anim. Health Prod.* 41: 199-204.

Chan, K., Baker, S., Kim, C.C., Detweiler, C.S., Dougan, G. and Falkow, S. 2003. Genomic comparison of *Salmonella enterica* serovars and *Salmonella bongori* by use of an *S. enterica* serovar typhimurium DNA microarray. *J. Bacteriol.* 185: 553-563.

Chasey, D. and Davies, P. 1984. Atypical rotaviruses in pigs and cattle. *Vet. Rec.* 114: 16-17.

Chen, S., Wang, Y., Chen, F., Yang, H., Gan, M. and Zheng, S.J. 2007. A highly pathogenic strain of *Staphylococcus sciuri* caused fatal exudative epidermitis in piglets. *PLoS ONE.* 2: e147.

Choudhary, S.P., Singh, S.N. and Narayan, K.G. 1983. *Pseudomonas aeruginosa*, an aetiologic agent of piglet diarrhoea. *Indian J. Anim. Sci.* 53: 629-634.

Cobbold, R.N., Rice, D.H., Davis, M.A., Besser, T.E. and Hancock, D.D. 2006. Long-term persistence of multi-drug-resistant *Salmonella enterica* serovar newport in two dairy herds. *J. Am. Vet. Med. Assoc.* 228: 585-591.

Collins, J.E., Benfield, D.A. and Duimstra, J.R. 1989. Comparative virulence of two porcine group-a rotavirus isolates in gnotobiotic pigs. *Am. J. Vet. Res.* 50: 827-835.

Coussement, W., Ducatelle, R., Charlier, G. and Hoorens, J. 1981. Adenovirus enteritis in pigs. *Am. J. Vet. Res.* 42: 1905-1911.

Cowan, S.T., Steel, K.J., Barrow, G.I. and Feltham, R.K.A., 1993a. Cowan and steel's manual for the identification of medical bacteria. Vol.2 Edn.2. Cambridge University Press. p.243.

Cowan, S.T., Steel, K.J., Barrow, G.I. and Feltham, R.K.A., 1993b. Cowan and steel's manual for the identification of medical bacteria. Vol.1, Edn.3. Cambridge University Press. p. 331.

Cowart, R.P. and Casteel, S.W., 2001. An outline of swine diseases: A handbook. Vol 3, Edn.2. Lowa State Press.

Davies, P., Funk, J. and Morrow, M. 1999a. Fecal shedding of salmonella by a cohort of finishing pigs in North Carolina. *Swine Health Prod.* 7: 231-236.

Davies, R. and Breslin, M. 2004a. Observations on salmonella contamination of eggs from infected commercial laying flocks where vaccination for *Salmonella enterica* serovar enteritidis had been used. *Avian. Pathol.* 33: 133-144.

Davies, R., Paiba, G., Evans, S. and Dalziel, B. 2000. Surveys for salmonella in pigs, cattle and sheep at slaughter in Great Britain. *Vet. Rec.* 147: 695.

Davies, R.H., Bedford, S. and Shankster, S. 2001. Enhanced culture techniques for the detection of salmonella. *Vet. Rec.* 148: 539-540.

Davies, R.H. and Breslin, M.F. 2004b. Observations on the distribution and control of salmonella contamination in poultry hatcheries. *Br. Poult. Sci.* 45 Suppl. 1: S12-14.

Davies, R.H., Teale, C.J., Wray, C., McLaren, I.M., Jones, Y.E., Chappell, S. and Kidd, S. 1999. Nalidixic acid resistance in salmonellae isolated from turkeys and other livestock in Great Britain. *Vet. Rec.* 144: 320-322.

Davison, H., Evans, S., Kidd, S., Smith, R., Cook, A., Paiba, G., Davies, R., O'Brien, S. and Ward, L. 2001. *Salmonella typhimurium* dt170 in cattle. *Vet. Rec.* 149: 751-752.

Dobrescu, L. 1978. Enterotoxigenic *Aeromonas hydrophila* from a case of piglet diarrhoea. *Zentralbl Veterinarmed B.* 25: 713-718.

Dooley, J.S. and Trust, T.J. 1988. Surface protein composition of *Aeromonas hydrophila* strains virulent for fish: Identification of a surface array protein. *J. Bacteriol.* 170: 499-506.

Ducatelle, R., Coussement, W. and Hoorens, J. 1982. Sequential pathological study of experimental porcine adenovirus enteritis. *Vet. Pathol.* 19: 179-189.

Dunlop, R.H., McEwen, S.A., Meek, A.H., Clarke, R.C., Black, W.D. and Friendship, R.M. 1998. Associations among antimicrobial drug treatments and antimicrobial resistance of fecal *Escherichia coli* of swine on 34 farrow-to-finish farms in Ontario, Canada. *Prev. Vet. Med.* 34: 283-305.

Eiden, J., Vonderfecht, S., Theil, K., Torres-Medina, A. and Yolken, R.H. 1986. Genetic and antigenic relatedness of human and animal strains of antigenically distinct rotaviruses. *J. Infect. Dis.* 154: 972-982.

FAOSTAT 2008. Faostat home page [on-line]. Available:Http://faostat.Fao.Org. [18 march 2009].

Farmer, J.J., Davis, B.R., Hickman-Brenner, F.W., McWhorter, A., Huntley-Carter, G.P., Asbury, M.A., Riddle, C., Wathen-Grady, H.G., Elias, C. and Fanning, G.R. 1985. Biochemical identification of new species and biogroups of enterobacteriaceae isolated from clinical specimens. *J. Clin. Microbiol.* 21: 46-76.

Farrington, L.A., Harvey, R.B., Buckley, S.A., Stanker, L.H. and Inskip, P.D. 1999. A preliminary survey of antibiotic resistance of salmonella in market-age swine. *Adv. Exp. Med. Biol.* 473: 291-297.

Faubert, C. and Drolet, R. 1992. Hemorrhagic gastroenteritis caused by *Escherichia coli* in piglets: Clinical, pathological and microbiological findings. *Can. Vet. J.* 33: 251-256.

Fewins, B.G., Newland, L.G.M. and Briggs, C.A.E. 1957. The normal intestinal flora of the pig. Iii. Qualitative studies of lactobacilli and streptococci. *J. Appl. Microbiol.* 20: 234-242.

Fratamico, P.M., Bagi, L.K., Bush, E.J. and Solow, B.T. 2004. Prevalence and characterization of shiga toxin-producing *Escherichia coli* in swine feces recovered in the national animal health monitoring system's swine 2000 study. *Appl. Environ. Microbiol.* 70: 7173-7178.

Frydendahl, K. 2002. Prevalence of serogroups and virulence genes in *Escherichia coli* associated with postweaning diarrhoea and edema disease in pigs and a comparison of diagnostic approaches. *Vet. Microbiol.* 85: 169-182.

Fuller, R., Newland, L.G.M., Briggs, C.A.E., Braude, R. and Mitchell, K.G. 1960. The normal intestinal flora of the pig. The effect of dietary supplements of

penicillin, chlortetracycline or copper sulphate on the faecal flora. *J. Appl. Microbiol.* 23: 195-205.

Garabal, J.I., Gonzalez, E.A., Vazquez, F., Blanco, J., Blanco, M. and Blanco, J.E. 1996. Serogroups of *Escherichia coli* isolated from piglets in spain. *Vet. Microbiol.* 48: 113-123.

Gavini, F., Ferragut, C., Izard, D., Trinel, P.A., Leclerc, H., Lefebvre, B. and Mossel, D.A.A. 1979. *Serratia fonticola*, a new species from water. *Int. J. Syst. Evol. Microbiol.* 29: 92.

Giwercman, B., Lambert, P.A., Rosdahl, V.T., Shand, G.H. and Hoiby, N. 1990. Rapid emergence of resistance in *Pseudomonas aeruginosa* in cystic fibrosis patients due to in-vivo selection of stable partially derepressed beta-lactamase producing strains. *J. Antimicrob. Chemother.* 26: 247-259.

Gray, E.W., Angus, K.W. and Snodgrass, D.R. 1980. Ultrastructure of the small intestine in astrovirus-infected lambs. *J. Gen. Virol.* 49: 71-82.

Gray, S.J. and Stickler, D.J. 1989. Some observations on the faecal carriage of mesophilic Aeromonas species in cows and pigs. *Epidemiol. infect*. 523-537.

Guerin, M.T., Martin, S.W., Darlington, G.A. and Rajic, A. 2005. A temporal study of salmonella serovars in animals in Alberta between 1990 and 2001. *Can. J. Vet. Res.* 69: 88-99.

Hampson, D.J. 1986. Alterations in piglet small intestinal structure at weaning. *Res. Vet. Sci.* 40: 32-40.

Hampson, D.J. 1994. Postweaning *Escherichia coli* diarrhoea in pigs. Vet. Rec. 43:16-30.

Hampson, D.J., Fu, Z.F. and Robertson, I.D. 1987. Investigation of the source of haemolytic *Escherichia coli* infecting weaned pigs. *Epidemiol. Infect*. 99: 149-153.

Hampson, D.J. and Smith, W.C. 1986. Influence of creep feeding and dietary intake after weaning on malabsorption and occurrence of diarrhoea in the newly weaned pig. *Res. Vet. Sci.* 41: 63-69.

Hancock, R.E. 1998. Resistance mechanisms in *Pseudomonas aeruginosa* and other nonfermentative gram-negative bacteria. *Clin. Infect. Dis.* 27 Suppl 1: S93-99.

Hazen, T.C., Fliermans, C.B., Hirsch, R.P. and Esch, G.W. 1978. Prevalence and distribution of *Aeromonas hydrophila* in the united states. *Appl. Environ. Microbiol.* 36: 731-738.

Hedemann, M.S. and Jensen, B.B. 2004. Variations in enzyme activity in stomach and pancreatic tissue and digesta in piglets around weaning. *Archives Anim. Nutr.* 58: 47-59.

Hegde, N.V., Cook, M.L., Wolfgang, D.R., Love, B.C., Maddox, C.C. and Jayarao, B.M. 2005. Dissemination of *Salmonella enterica* subsp. Enterica serovar typhimurium var. Copenhagen clonal types through a contract heifer-raising operation. *J. Clin. Microbiol.* 43: 4208-4211.

Hill, J.R. and Sainsbury, D.W.B. 1995. The health of pigs: Nutrition, housing and disease prevention. 65-70.

Ionova, I., Monov, G. and Kunev, Z. 1981. [carrier state and body distribution of salmonella bacteria in healthy piglets and calves]. *Vet. Med. Nauki.* 18: 98-104.

Jacobson, M., 2003. Enteric diseases in pigs from weaning to slaughter. Vol, Edition. Dept. of Large Animal Clinical Sciences, Swedish Univ. of Agricultural Sciences.

Janda, J.M., Clark, R.B. and Brenden, R. 1985. Virulence ofaeromonas species as assessed through mouse lethality studies. *Curr. Microbiol.* 12: 163-167.

Janda, J.M., Oshiro, L.S., Abbott, S.L. and Duffey, P.S. 1987. Virulence markers of mesophilic aeromonads: Association of the autoagglutination phenomenon with mouse pathogenicity and the presence of a peripheral cell-associated layer. *Infection and Immun.* 55: 3070-3077.

Jesto, G. 2007. Bacteria associated with respiratory infections in poultry. M.V.Sc. Thesis. Kerala Agricultural university, Thrissur.

Johnston, W.T., Dewey, C.E., Friendship, R.M., Smart, N., McEwen, B.J., Stalker, M. and de Lange, C.F. 2001. An investigation of the etiology of a mild diarrhoea observed in a group of grower/finisher pigs. *Can. Vet. J.* 42: 33-37.

Jones, Y.E., Chappell, S., McLaren, I.M., Davies, R.H. and Wray, C. 2002. Antimicrobial resistance in salmonella isolated from animals and their environment in england and wales from 1988 to 1999. *Vet. Rec.* 150: 649-654.

Katouli, M., Melin, L., Jensen-Waern, M., Wallgren, P. and Mollby, R. 1999. The effect of zinc oxide supplementation on the stability of the intestinal flora with special reference to composition of coliforms in weaned pigs. *J. Appl. Microbiol.* 87: 564-573.

Khardori, N. and Fainstein, V. 1988. Aeromonas and plesiomonas as etiological agents. *A. Rev. in Microbiol.* 42: 395-419.

Kidder, D.E. and Manners, M.J. 2008. The level and distribution of carbohydrases in the small intestine mucosa of pigs from 3 weeks of age to maturity. *Br. J. Nutr.* 43: 141-153.

Ko, W.C., Chiang, S.R., Yan, J.J. and Chuang, Y.C. 2005. Comparative pathogenicity of bacteraemic isolates of *Aeromonas hydrophila* and *Klebsiella pneumoniae*. *Clin. Microbiol. Infect*. 11: 553-558.

Kwon, D., Kim, O. and Chae, C. 1999. Prevalence of genotypes for fimbriae and enterotoxins and of o serogroups in *Escherichia coli* isolated from diarrheic piglets in korea. *J. Vet. Diag. Investig*. 11: 146.

Lambert, P.A. 2002. Mechanisms of antibiotic resistance in *Pseudomonas aeruginosa*. *J. R. Soc. Med.* 95 Suppl 41: 22-26.

Lee, C., Langlois, B.E. and Dawson, K.A. 1993. Detection of tetracycline resistance determinants in pig isolates from three herds with different histories of antimicrobial agent exposure. *Appl. Environ. Microbiol.* 59: 1467-1472.

Leman, A.D., Straw, B.E., Glock, R.D. and Mengeling, W.L., 1986. Diseases of swine. II Vol, I Edition. Iowa.

Levy, S.B.(2002). Antibiotics, animals and the resistance gene pool. In: The antibiotic paradox: How the misuse of antibiotics destroys their curative powers. pp. 149 –180.

Levy, S.B. and Marshall, B. 2004. Antibacterial resistance worldwide: Causes, challenges and responses. *Nat. Med.* 10: S122-129.

Liebler-Tenorio, E.M. and Whipp, S.C. 1999. Diseases of the digestive system. In Diseases of swine, BE Straw, S.D.A., WL Mengeling, DJ Taylor, ed. (Iowa, USA, Iowa State University Press, Ames,), pp. 821-831.

Lofstedt, M., Holmgren, N. and Lundeheim, N. 2002. Risk factors for post-weaning diarrhoea in pigs. *Svensk Veterinartidning*. 54: 457-461.

Maciorowski, K.G., Herrera, P., Jones, F.T., Pillai, S.D. and Ricke, S.C. 2007. Effects on poultry and livestock of feed contamination with bacteria and fungi. *Anim. Feed Sci. Tech.* 133: 109-136.

Madec, F., Bridoux, N., Bounaix, S., Cariolet, R., Duval-Iflah, Y., Hampson, D.J. and Jestin, A. 2000. Experimental models of porcine post-weaning colibacillosis and their relationship to post-weaning diarrhoea and digestive disorders as encountered in the field. *Vet. Microbiol.* 72: 295-310.

Madec, F., Bridoux, N., Bounaix, S. and Jestin, A. 1998. Measurement of digestive disorders in the piglet at weaning and related risk factors. *Preventive Vet. Med.* 35: 53-72.

Mainil, J. 1999. Shiga/verocytotoxins and shiga/verotoxigenic *Escherichia coli* in animals. *Vet. Res.* 30: 235-257.

Makinde, M.O., Umapthy, E., Akingbemi, B.T., Mandisodza, K.T. and Skadhauge, E. 1996. Effects of dietary soybean and cowpea on gut morphology and faecal composition in creep and noncreep-fed pigs. *Zentralbl Veterinarmed A.* 43: 75-85.

Manninen, K.I., Prescott, J.F. and Dohoo, I.R. 1982. Pathogenicity of Campylobacter jejuni isolates from animals and humans. *Infect. Immun.* 38: 46-52.

Manser, P.A. and Dalziel, R.W. 1985. A survey of campylobacter in animals. *J. Hyg (Lond).* 95: 15-21.

Mc Erlean, B.A. 1968. *Salmonella dublin* meningitis in piglets. *Vet. Rec.*, 82: 257-258.

Melin, L., Jensen-Waern, M., Johannisson, A., Ederoth, M., Katouli, M. and Wallgren, P. 1997. Development of selected faecal microfloras and of phagocytic and killing capacity of neutrophils in young pigs. *Vet. Microbiol.* 54: 287-300.

Melin, L., Katouli, M., Lindberg, A., Fossum, C. and Wallgren, P. 2000. Weaning of piglets. Effects of an exposure to a pathogenic strain of *Escherichia coli. J. Vet. Med. B. Infect. Dis. Vet. Public Health.* 47: 663-675.

Mini, M., Venkatesan, R.A. and and Purushothaman, V. 2005. Plasmid profile and curing of plasmids of *Escherichia coli* of bovine mastitis origin. *Indian Vet. J.*, 82: 587-590.

Mora, A., Blanco, J.E., Blanco, M., Alonso, M.P., Dhabi, G., Echeita, A., Gonzalez, E.A., Bernardez, M.I. and Blanco, J. 2005. Antimicrobial resistance of shiga toxin (verotoxin)-producing *Escherichia coli* O157:H7 and non-O157 strains isolated from humans, cattle, sheep and food in spain. *Res. Microbiol.* 156: 793-806.

Morin, M., Turgeon, D., Jolette, J., Robinson, Y., Phaneuf, J.B., Sauvageau, R., Beauregard, M., Teuscher, E., Higgins, R. and Lariviere, S. 1983. Neonatal diarrhoea of pigs in quebec: Infectious causes of significant outbreaks. *Can. J. Comp. Med.* 47: 11-17.

Moxley, R.A., Erickson, E.D. and Breisch, S. 1988. Shock associated with enteric colibacillosis in suckling and weaned swine. In, pp. 33–38.

Müller, H.E., Fanning, G.R. and Brenner, D.J. 1995. Isolation of *Serratia fonticola* from mollusks. *Syst. Appl. Microbiol.* 18: 279-284.

Muller, H.E., Steigerwalt, A.G. and Brenner, D.J. 1986. Isolation of *Serratia fonticola* from birds. *Zentralblatt für Bakteriologie, Mikrobiologie und Hygiene. Series A,*

Medical microbiology, infectious diseases, virology, parasitology. 261: 212-218.

Muller, W. 1974. Staphylococcal flora of air in a broiler house and a piglet battery system. *Berl Munch Tierarztl Wochenschr*. 87: 298-301.

Munroe, D.L., Prescott, J.F. and Penner, J.L. 1983. *Campylobacter jejuni* and *Campylobacter coli* serotypes isolated from chickens, cattle, and pigs. *J. Clin. Microbiol.* 18: 877-881.

Murray, C.J. 1991. Salmonellae in the environment. *Rev. Sci. Tech.* 10: 765-785.

Murthy, D.K. and Kausik, R.K. 1964. Occurance of *Salmonella choleraesuis. Var kunzendorf* in pigs in India. *Indian Vet. J.* . 41: 569.

Nagy, B. and Fekete, P.Z. 1999. Enterotoxigenic *Escherichia coli* (ETEC) in farm animals. *Vet. Res.* 30: 259-284.

Ngeleka, M., Pritchard, J., Appleyard, G., Middleton, D.M. and Fairbrother, J.M. 2003. Isolation and association of *Escherichia coli* aida-i/stb, rather than east1 pathotype, with diarrhoea in piglets and antibiotic sensitivity of isolates. *J. Vet. Diagn. Investig.* 15: 242.

Nielsen, N.O.(1986). Edema disease. In: Leman, A.D. (Ed.) *Diseases of swine*. Iowa State University Press, Ames, Iowa (USA), pp. 528-540.

Nietfeld, J.G., Feder, I., Kramer, T.T., Schoneweis, D. and Chengappa, M.M. 1998. Preventing salmonella infection in pigs with offsite weaning. *Swine Health and Production.* 6: 27-32.

Orr, J.P. 1984. Necrotizing enteritis in a calf infected with adenovirus. *Can. Vet. J.* 25: 72-74.

Overman, T.L. and Janda, J.M. 1999. Antimicrobial susceptibility patterns of *Aeromonas jandaei, A. Schubertii, A. Trota, and A. Veronii* biotype veronii. *J. Clin. Microbiol.* 37: 706-708.

Paniagua, C., Rivero, O., Anguita, J. and Naharro, G. 1990. Pathogenicity factors and virulence for rainbow trout (salmo gairdneri) of motile Aeromonas spp. isolated from a river. *J. Clin. Microbiol.* 28: 350-355.

Payot, S., Dridi, S., Laroche, M., Federighi, M. and Magras, C. 2004. Prevalence and antimicrobial resistance of *Campylobacter coli* isolated from fattening pigs in france. *Vet. Microbiol.* 101: 91-99.

Pedley, S., Bridger, J.C., Brown, J.F. and McCrae, M.A. 1983. Molecular characterization of rotaviruses with distinct group antigens. *J. Gen. Virol.* 64 (Pt 10): 2093-2101.

Pedley, S., Bridger, J.C., Chasey, D. and McCrae, M.A. 1986. Definition of two new groups of atypical rotaviruses. *J. Gen. Virol.* 67 (Pt 1): 131-137.

Pillai, K.P.B., Punnoose, K.T.A. and Abdulla, P.K. 1974. A preliminary note on the isolation of salmonella species from piglets. *Kerala J. Vet. Sci.* 5.

Pluske, J.R., Hampson, D.J. and Williams, I.H. 1997. Factors influencing the structure and function of the small intestine in the weaned pig: A review. *Livestock Prod. Sci.* 51: 215-236.

Popoff, M.Y., Bockemühl, J. and Gheesling, L.L. 2004. Supplement 2002 (no. 46) to the kauffmann–white scheme. *Research in Microbiology.* 155: 568-570.

Porter, P., Noakes, D.E. and Allen, W.D. 1970. Secretory IgA and antibodies to *Escherichia coli* in porcine colostrum and milk and their significance in the alimentary tract of the young pig. *Immunol.* 18: 245.

Potter, R. 1998. Clinical conditions of pigs in outdoor breeding herds (Br Veterinary Assoc), pp. 3-14.

Quinn, P.J., Markey, B.K., Carter, M.E., Donnelly, W.J. and Leonard, F.C., 2002. Veterinary microbiology and microbial disease. Vol, Edition. Iowa (EUA). Blackwell Science.

Raibaud, P., Caulet, M., Galpin, J.V. and Mocquot, G. 1961. Studies on the bacterial flora of the alimentary tract of pigs. Streptococci: Selective enumeration and differentiation of the dominant group. *J. Appl. Microbiol.* 24: 285-306.

Rajasenan, K. 1983. Antibiogram of salmonellae isolated from young pigs Kerala Agricultural University, Thrissur.

Raju, P.V. 1994. Characterization of *campylobacter jejuni* isolated from pigs and man. Kerala agricultural university, Thrissur. 113p.

Rhodes, G., Huys, G., Swings, J., McGann, P., Hiney, M., Smith, P. and Pickup, R.W. 2000. Distribution of oxytetracycline resistance plasmids between aeromonads in hospital and aquaculture environments: Implication of tn 1721 in dissemination of the tetracycline resistance determinant tet a. *Appl. Environ. Microbiol.* 66: 3883-3890.

Richards, W.P. and Fraser, C.M. 1961. Coliform enteritis of weaned pigs. A description of the disease and its association with hemolytic *Escherichia coli*. *Cornell Vet*. 51: 245-257.

Rowe-Magnus, D.A., Guerout, A.M. and Mazel, D. 2002. Bacterial resistance evolution by recruitment of super-integron gene cassettes. *Mol. Microbiol.* 43: 1657-1669.

Saif, L.J., Bohl, E.H., Theil, K.W., Cross, R.F. and House, J.A. 1980. Rotavirus-like, calicivirus-like, and 23-nm virus-like particles associated with diarrhoea in young pigs. *J. Clin. Microbiol.* 12: 105-111.

Saif, L.J., Terrett, L.A., Miller, K.L. and Cross, R.F. 1988. Serial propagation of porcine group C rotavirus (pararotavirus) in a continuous cell line and characterization of the passaged virus. *J. Clin. Microbiol.* 26: 1277-1282.

Salmon, D.E. and Smith, T. 1886. The bacterium of swine-plague. *Am. Monthly Microscopical J.* 7: 204-205.

Sarmiento, J.I., Casey, T.A. and Moon, H.W. 1988. Postweaning diarrhoea in swine: Experimental model of enterotoxigenic *Escherichia coli* infection. *Am. J. Vet. Res.* 49: 1154-1159.

Sato, H., Tanabe, T., Nakanowatari, M., Oyama, J., Yamazaki, N., Yoshikawa, H., Yoshikawa, T., Koyama, H. and Saito, H. 1990. Isolation of *Staphylococcus hyicus* subsp. Hyicus from pigs affected with exudative epidermitis and experimental infection of piglets with isolates. *Kitasato. Arch. Exp. Med.* 63: 119-130.

Schmidt, A.S., Bruun, M.S., Dalsgaard, I., Pedersen, K. and Larsen, J.L. 2000. Occurrence of antimicrobial resistance in fish-pathogenic and environmental bacteria associated with four danish rainbow trout farms. *Appl. Environ. Microbiol.* 66: 4908-4915.

Schulz, W. 1970. Exudative epidermitis of the piglet (piglet eczema)--etiology and pathogenesis with special reference to *Staphylococcus hyicus* . I. Literature review. *Monatsh Veterinarmed.* 25: 428-435.

Schwarz, S. and Blobel, H. 1990. A new streptomycin-resistance plasmid from *Staphylococcus hyicus* and its structural relationship to other staphylococcal resistance plasmids. *J. Med. Microbiol.* 32: 201-205.

Seyfarth, A.M., Wegener, H.C. and Frimodt-Moller, N. 1997. Antimicrobial resistance in *Salmonella enterica* subsp. Enterica serovar typhimurium from humans and production animals. *J. Antimicrob. Chemother.* 40: 67-75.

Skirrow, S.Z., Buddle, J.R., Mercy, A.R., Madec, F. and Nicholls, R.R. 1997. Epidemiological studies of pig diseases: 2. Post-weaning diarrhoea and performance in western Australian pigs. *Aust. Vet. J.* 75: 282-288.

Smith, P., Hiney, M.P. and Samuelsen, O.B. 1994. Bacterial resistance to antimicrobial agents used in fish farming: A critical evaluation of method and meaning. *Annual review of fish diseases.* 4: 273-313.

Snodgrass, D.R. and Gray, E.W. 1977. Detection and transmission of 30 nm virus particles (astroviruses) in faeces of lambs with diarrhoea. *Arch. Virol.* 55: 287-291.

Snodgrass, D.R., Herring, A.J., Campbell, I., Inglis, J.M. and Hargreaves, F.D. 1984. Comparison of atypical rotaviruses from calves, piglets, lambs and man. *J. Gen. Virol.* 65 (Pt 5): 909-914.

Son, R., Rusul, G., Sahilah, A.M., Zainuri, A., Raha, A.R. and Salmah, I. 1997. Antibiotic resistance and plasmid profile of *Aeromonas hydrophila* isolates from cultured fish, telapia (telapia mossambica). *Letters in Appl. Microbiol.*. 24: 479-482.

Stock, I., Burak, S., Sherwood, K.J., Gruger, T. and Wiedemann, B. 2003. Natural antimicrobial susceptibilities of strains of 'unusual' serratia species: *S. ficaria, S.*

fonticola, S. odorifera, S. plymuthica and S. rubidaea. J. Antimicrob. Chemother. 51: 865-885.

Storz, J., Leary, J.J., Carlson, J.H. and Bates, R.C. 1978. Parvoviruses associated with diarrhoea in calves. *J. Am. Vet. Med. Assoc.* 173: 624-627.

Sulochana, S., Punnoose, K.T.A. and Abdulla, P.K. 1973. Experimental infection study on *Salmonella weltevreden* isolated from piglets. *Kerala J. Vet. Sci.,* 47.i: 12-18.

Svendsen, J., Larsen, J.L. and Bille, N. 1974. Outbreaks of post weaning *Escherichia coli* diarrhoea in pigs. *Nord. Vet. Med.* 26: 314-322.

Svensmark, B., Jorsal, S.E., Nielsen, K. and Willeberg, P. 1989. Epidemiological studies of piglet diarrhoea in intensively managed danish sow herds. I. Pre-weaning diarrhoea. *Acta veterinaria Scandinavica.* 30: 43-53.

Sweeney, E.J. 1966. The etiology of dysentery of swine. *Vet.Rec.*, 78: 872-875.

Takahashi, I., Yoshida, T., Higashide, Y. and Sakano, T. 1990. Susceptibilities of *Escherichia coli,* Salmonella and *Staphylococcus aureus* isolated from animals to ofloxacin and commonly used antimicrobial agents. *Jpn. J. Antibiot.* 43: 89-99.

Taylor, D. 1986. Scours in pigs. Br. Vet. Ass., pp. 40-48.

Taylor, D.J. 1999. Pig diseases. 7th edition, Suffolk, B.S.E., St Edmundsbury Press Ltd.

Taylor, D.J. and Olubunmi, P.A. 1981. A re-examination of the role of *Campylobacter fetus* subspecies *coli* in enteric disease of the pig. *Vet. Rec.* 109: 112-115.

Theil, K.W., Grooms, D.L., McCloskey, C.M. and Redman, D.R. 1995. Group b rotavirus associated with an outbreak of neonatal lamb diarrhoea. *J. Vet. Diagn. Invest.* 7: 148-150.

Thomlinson, J.R. and Buxton, A. 1963. Anaphylaxis in pigs and its relationship to the pathogenesis of oedema disease and gastro-enteritis associated with *Escherichia coli*. *Immunol.* 6: 126-139.

Thomson, J.R. 2006. Diseases of the digestive system. In Diseases of swine, Straw BE, Z.J., D'Allaire S, Taylor DJ. Ames, ed., pp. 37-55.

Threlfall, E.J., Teale, C.J., Davies, R.H., Ward, L.R., Skinner, J.A., Graham, A., Cassar, C. and Speed, K. 2003. A comparison of antimicrobial susceptibilities in nontyphoidal salmonellas from humans and food animals in England and Wales in 2000. *Microb Drug Resist.* 9: 183-189.

Torres-Medina, A., Schlafer, D.H. and Mebus, C.A. 1985. Rotaviral and coronaviral diarrhoea. *Vet. Clin. North Am. Food Anim. Pract.* 1: 471-493.

Tzipori, S., Chandler, D., Smith, M., Makin, T. and Hennessy, D. 1980. Factors contributing to postweaning diarrhoea in a large intensive piggery. *Aust. Vet. J.* 56: 274-278.

Wang, L., Andrianopoulos, K., Liu, D., Popoff, M.Y. and Reeves, P.R. 2002. Extensive variation in the O-antigen gene cluster within one *Salmonella enterica* serogroup reveals an unexpected complex history. *J. Bacteriol.* 184: 1669-1677.

Wani, S.A., Bhat, M.A., Nawchoo, R., Munshi, Z.H. and Bach, A.S. 2004. Evidence of rotavirus associated with neonatal lamb diarrhoea in India. *Trop. Anim. Health Prod.* 36: 27-32.

Wathes, C.M., Miller, B.G. and Bourne, F.J. 1989. Cold stress and post-weaning diarrhoea in piglets inoculated orally or by aerosol. *Anim. Prod.* 49: 483–496.

Wegener, H.C., Andresen, L.O. and Bille-Hansen, V. 1993. *Staphylococcus hyicus* virulence in relation to exudative epidermitis in pigs. *Can. J. Vet. Res.* 57: 119-125.

Werdin, R.E., Ames, T.R., Goyal, S.M. and DeVries, G.P. 1989. Diagnostic investigation of bovine viral diarrhoea infection in a Minnesota dairy herd. *J. Vet. Diagn. Invest.* 1: 57-61.

WHO 1998. Zoonotic non-O157 shiga toxin-producing *Escherichia coli* (STEC). In Report of a WHO Scientific Working Group Meeting (Berlin), p. 1.

WHO 2001. Global strategy for containment of antimicrobial resistance. Who/cds/csr/drs/ 2001.2 original:English. 2001:1-96 p. http://www.who.int/drugresistance/WHO_Global_Strategy_English.pdf

Wilson, G.S. and Miles, A.A., 1964. Topley and wilson's principles of bacteriology and immunity. Vol-1, 3rd Edition. Edward Arnold London.

Wilssens, A.T.E. and Vande Casteele, J.C. 1967. Occurrence of micrococci and staphylococci in the intestines of piglets and pigs. *J. Appl. Microbiol.* 30: 336-339.

Woode, G.N. and Bridger, J.C. 1978. Isolation of small viruses resembling astroviruses and caliciviruses from acute enteritis of calves. *J. Med. Microbiol.* 11: 441-452.

Woode, G.N., Reed, D.E., Runnels, P.L., Herrig, M.A. and Hill, H.T. 1982. Studies with an unclassified virus isolated from diarrhoeic calves. *Vet. Microbiol.* 7: 221-240.

Woode, G.N., Saif, L.J., Quesada, M., Winand, N.J., Pohlenz, J.F. and Gourley, N.K. 1985. Comparative studies on three isolates of breda virus of calves. *Am. J. Vet. Res.* 46: 1003-1010.

Yasuhara, H., Matsui, O., Hirahara, T., Ohgitani, T., Tanaka, M., Kodama, K., Nakai, M. and Sasaki, N. 1989. Characterization of a parvovirus isolated from the diarrheic feces of a pig. *Nippon Juigaku Zasshi.* 51: 337-344.

Zhang, Y.L., Ong, C.T. and Leung, K.Y. 2000. Molecular analysis of genetic differences between virulent and avirulent strains of *Aeromonas hydrophila* isolated from diseased fish the genbank accession numbers for the sequences determined in this work are given in table 5 (Soc General Microbiol), pp. 999-1009.

Zhao, S., Fedorka-Cray, P.J., Friedman, S., McDermott, P.F., Walker, R.D., Qaiyumi, S., Foley, S.L., Hubert, S.K., Ayers, S. and English, L. 2005. Characterization of *Salmonella typhimurium* of animal origin obtained from the national antimicrobial resistance monitoring system. *Food borne Pathogens & Disease.* 2: 169-181.

Zhao, S., McDermott, P.F., White, D.G., Qaiyumi, S., Friedman, S.L., Abbott, J.W., Glenn, A., Ayers, S.L., Post, K.W., Fales, W.H., Wilson, R.B., Reggiardo, C. and Walker, R.D. 2007. Characterization of multidrug resistant salmonella recovered from diseased animals. *Vet. Microbiol.* 123: 122-132.

ISOLATION AND CHARACTERIZATION OF BACTERIA ASSOCIATED WITH GASTROENTERITIS IN WEANED PIGLETS

ATULYA M

Thesis submitted in partial fulfillment of the requirement for the degree of

Master of Veterinary Science

Faculty of Veterinary and Animal Sciences

Kerala Agricultural University, Thrissur

2009

Department of Veterinary Microbiology

COLLEGE OF VETERINARY AND ANIMAL SCIENCES

Mannuthy, Thrissur

8. ABSTRACT

Gastroenteritis is a leading cause of morbidity and mortality in piglets. A number of factors are involved in post weaning diarrhoea in piglets. A comprehensive study was performed to examine the incidence, bacterial etiology, drug sensitivity, plasmid profile and pathogenicity of the bacteria isolated from weaned piglets with gastroenteritis prevalent in and around Kerala Agricultural university.

Samples were taken only from piglets with diarrhoea that had not been previously treated with antibiotics. Rectal swabs were collected from live diarrhoeic piglets and intestinal contents, pieces of jejunum, ileum, colon, mesenteric lymph nodes, liver, spleen and stomach were collected during post mortem examination after taking all sterile precautions.

Isolation of causative bacteria was made by culturing on Brain Heart Infusion Agar, Mac Conkey agar, Mannitol Salt agar, Blood agar, *Brucella* agar and Cooked Meat medium. The identification of isolates was carried out as per standard protocols. All the procedures of biochemical testing were followed as described by Barrow and Feltham (1993). For classification of Staphylococcus isolates, a system suggested by Baird Parker (1965) was considered. A total of 53 bacterial isolates were identified to species level from 82 samples tested for pathogens.

Six different microorganisms were encountered in this study, with *Escherichia coli* being dominant, followed by *Pseudomonas aeruginosa*, *Staphylococcus* subgroups *BPIII* and *BPV, Serratia fonticola, Salmonella typhimurium* and *Aeromonas hydrophila*. Thirteen different serotypes of *E. coli* were encountered, with O69 being dominant and the others were O38, O5, O84, O132, O80, O79, O56, O41, O25, O109 and O103 and two were untypable. Majority of the isolates exhibited multidrug resistance. Plasmid profile of the Gram negative

isolates were determined and 80.43 per cent were found to bear plasmids. Pathogenicity of the isolates was determined by performing *in vivo* mice pathogenicity test.