BIOCHEMICAL AND MOLECULAR CHARACTERIZATION OF COMMON FOOD BORNE ORGANISMS IN PORK AND CHICKEN

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In partial fulfillment of the requirements for the Degree of

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IN

VETERINARY PUBLIC HEALTH

BY

BIDGAR GANESH JIBHAU

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DECLARATION OF STUDENT

I hereby declare that the experimental research work and interpretation of the thesis entitled**“BIOCHEMICAL AND MOLECULAR CHARACTERIZATION OF COMMON FOOD BORNE ORGANISMS IN PORK AND CHICKEN”**or part thereof has not been submitted for any other degree or diploma of any university, nor the data have been derived from any thesis/publication of any University or Scientificorganization. The sources of materials used and all assistance received during the course of investigation have been duly acknowledged.

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LIST OF ABBREVIATIONS

%	:	Per- cent
@	:	At the rate
µg	:	Microgram
µl	:	Microlitre
µM	:	Micro mole
ATCC	:	American Type Culture Collection
BHI	:	Brain Heart Infusion broth
Bp	:	Base Pair
CAMP	:	Christie, Atkins, Munch-Petersen
CHO	:	Carbohydrate
Cm	:	Centimeter
DNA	:	Deoxyribose nucleic acid
dNTP	:	Deoxynucleotide triphosphate
DRIA	:	Dominguez Rodriguez Isolation agar
DW	:	Distilled water
EDTA	:	Ethylenediaminetetraacetic acid
EMB	:	Eosine Methylene Blue
EtBr	:	Ethidium bromide
Fig	:	Figure
Gm	:	Gram
Hr	:	Hour
KTT-20	:	KCl-Tris-Tween -20
lit.	:	Litre
M	:	Molar
mA	:	Milli ampere
Mg	:	Milligram
Min	:	Minute
Ml	:	Millilitre
mM	:	Millimolar
mPCR	:	Multiplex Polymerase Chain Reaction
mCCDA	:	modified Charcoal Cefoperazone Deoxycholate Agar
MR	:	Methyl Red

MW	:	Molecular weight
NA	:	Nutrient Agar
NaCl	:	Sodium Chloride
NaOH	:	Sodium Hydroxide
NSS	:	Normal saline solution
^{o}C	:	Degree Celsius
OD	:	Optical density
OIE	:	World Organisation for Animal Health
PALCAM	:	Polymixin Acriflavin Lithium chloride Ceftazidime Aesculin Mannitol
PBS	:	Phosphate buffer saline
PCR	:	Polymerase Chain Reaction
PI-PLC	:	Phosphatidylinositol-specific phospholipase – C
Rpm	:	revolution per minute
SBA	:	Sheep Blood Agar
TAE	:	Tris-glacial acetic acid –EDTA
UV	:	Ultra violet
UVM	:	University of Vermont
V	:	Volts
v/v	:	Volume by volume
VP	:	Voges Prausker
w/v	:	Weight by volume
XLD	:	Xylose Lysine Deoxycholate
A	:	Alpha
B	:	Beta

INTRODUCTION

Food has remained one of the major concerns of mankind and food safety is one of the most important priorities of all countries. Food safety needs to be ensured throughout the process from production, processing, and distribution to consumers. The major threat to food safety is the emergence and re-emergence of food borne pathogens. Foodborne pathogens continue to cause major public health problems worldwide. Zoonotic agents including those with food origin have gained more importance and food chain has been recognized as one of the main route of transmission of microbial agents between animal and humans. Food borne pathogens are a major contributor to human illnesses, hospitalizations, and deaths every year. Chicken and Pork are emerging as an important source of protein for non-vegetarians due to decrease in livestock population in country. The Govt. of India has also started National Mission of Protein Supplementation programme under which the pig has been considered as an important source of protein.

The WHO South-East Asia Region has the second highest burden of 150 million cases of food borne diseases and 175000 deaths a year. Some 60 million children under the age of five fell ill and 50000 die from food borne diseases in the South-East Asia Region every year (WHO, 2014). The Centers for Disease Control and Prevention (CDC) estimated that 47.8 million illnesses and 3000 deaths are caused by food-borne pathogens each year (CDC 2011). These organisms are leading cause of illness and death in most of the developed countries, killing approximately 1.8 millions of cases of infectious gastroenteritis diseases each year, costing billions of dollars in medical care (Pinna *et al.* 2005).

Many bacteria responsible for bacterial zoonosis have been isolated from chicken and pork and are of major public health significance *viz*. *Salmonella* species, *Campylobacter* species, *Escherichia coli* and *Listeria* species (Zhao *et al.* 2001, Katre, 2008).

The faecal contamination of meat (especially poultry meat) during processing is considered to be a major source of food-borne disease. In mammals and birds, detection of intestinal colonization is based on the isolation of the organism from faeces, rectal swabs and/or caecal contents.

Campylobacter jejuni and *C. coli* are thermophilic, Gram-negative, highly motile bacteria that, for optimal growth, require microaerobic environment and incubation temperatures of 37–42°C. Large numbers of *Campylobacter* have been isolated from young livestock, including piglets, lambs andcalves, with enteritis, but the organisms are also found in healthy animals. Outbreaks of avian hepatitis have been reported, but the pathogenic role of *Campylobacter*spp. is unclear. The control of *Campylobacter* in the food chain has now become a major target of agencies responsible for food safety world-wide (OIE, 2008).

Campylobacter infection is the leading cause of bacterial enteritis worldwide. Most *Campylobacter* infection causes acute self-limiting diarrheal disease. Prevalence of *Campylobacter* species in chicken, chevon and milk samples were observed 17.33%, 6%, 0% respectively from Uttar Pradesh, India (Pallavi and Kumar, 2014). Aradwad (2005) reported 78.33% of chicken, 70% of mutton, 60% of pork, 33.33% of chevon and 6.66% of beef contaminated with *Campylobacter* species from India.

Escherichia coli are normal inhabitants of the gastrointestinal tract of animals and humans. Some strains have become highly adapted to cause diarrhoea and a range of extra-intestinal diseases (OIE, 2008). Meat samples and diarrhoeagenic stool samples were screened for STEC, using conventional culture methods and PCR in Manglore, India. Among the 103 meat samples, 90 were positive for *eae* gene and one among them positive for Shiga toxin producing *E. coli* (STEC) (Van *et al.* 2008). Shiga toxin producing *E. coli* (STEC) of different serotype are important human pathogens of animal origin and can cause severe diseases such as haemorrhagic colitis, haemolytic uraemic syndrome. STEC are strains of *E. coli* that produce potent cytotoxins referred to as shiga toxin (*stx)* which are further characterized as stx 1 and stx 2. Retail meats derived from animals could potentially act as a transmission vehicle for STEC and other diarrheagenic *E. coli* strains. The morbidity and mortality associated with several out breaks of gastrointestinal diseases caused by shiga toxin producing *E. coli* (STEC) indicating the threat of these organisms to public health (Satpute, 2014).

In foreign countries such as Saudi Arabia; incidence of *E. coli* serotype O157:H7 from raw chicken, ground chicken and chicken hamburgers were 2.5%, 5%, 10% respectively in Riyadh, (Hessain *et al.,* 2015). Likewise in United States, *E. coli* contaminated food reported approximately 1,000 disease outbreaks and an estimated 48 million illnesses, 128,000 hospitalizations and 3,000 deaths annually (www.acceleratingscience.com).

Escherichia coli are a group of bacteria, which inhabit the intestines of all the humans and most animals. The *E. coli* O157:H7 serotype belongs to the enterohemorrhagic *E. coli* (EHEC) group. EHEC bacteria contain one or more virulence attributes: the ability to produce shiga-like toxin(s) (SLT also known as verotoxins or VT), adherence factor(s) and enterohemolysin.

Listeriosis, as an important cause of severe illness accounts to 3.8% of food borne disease hospitalization and 27.6% of food borne deaths (www.cdc.gov.listeria). *L. monocytogenes* is a cause of food-borne disease; it is linked to disproportionately high levels of morbidity and mortality (Shanta *et al.,* 2013) Due to its wide spread occurrence in nature and ability to survive in diverse environmental conditions, listeria are gradually becoming part of microbial ecosystem in foods of animal origin. In humans, food borne transmission is the prominent source of infection. Previous studies in the same laboratory reported 1.98% to 20% prevalence of *Listeria monocytogenes* from Nagpur region among different food animals.

Salmonellosis is an infectious disease of humans and animals caused by the two species of *Salmonella; S. enterica*, and *S. bongori*. Although, *Salmonellae* are primarily intestinal bacteria, they are widespread in the environment and common in farm effluents, human sewage and can be found in any material as a result of faecal contamination. *Salmonella* organisms may also be present in animal feed stuffs, causing subclinical gastro-intestinal carriage or infectious disease in animals, particularly poultry and pigs. Salmonellosis has been recognized in all countries, but appears to be most prevalent in areas of intensive animal husbandry, especially in pigs and calves and some types of poultry reared in confinement. Many animals, especially pigs and poultry, may also be infected but show no clinical illness. Such animals play an important role in transmission of infection between flocks and herds and remain as sources of food contamination and human infection (OIE, 2010).

In India, 78 personnel was reported to be affected by food borne disease in 1998 by the armed forces at high altitude, wherein *Salmonella enteritidis* was identified as the etiological agent and frozen fowl was implicated as source of infection. Prevalence of Salmonellae in chicken and pork meat was 67.78% and 32.21% respectively collected from Romania (Mihaiu *et al.* 2014). Prevalence of Salmonellae species in chicken meat from local markets of Patna, India was found to be 18.42% (Kaushik *et al.* 2014). Two separate food poisoning outbreaks were also reported due to *Salmonella weltevreden* and *Salmonella wein* affecting 34 and 10 people respectively, from Mangalore in 2008-09 due to non-vegetarian food (chicken and fish) (www.ncdc.gov.in).

The emergence of antibiotic resistant strain and their transfer to humans through food chain is major public health consequence (Perreten, 2005). Antimicrobial resistant bacteria are biologically hazardous, associated with increased human morbidity and mortality and a have a serious public health concern. The use of antimicrobial at sub therapeutic level in food-producing animals has long been viewed as matter of concern.

The widespread use of antibiotics in food animal production systems has resulted in the emergence of antibiotic resistant zoonotic bacteria that can be transmitted to humans through the food chain. Infection with antibiotic resistant bacteria negatively impacts public health, due to an increased incidence of treatment failure and severity of disease. Development of resistant bacteria in food animals can result from chromosomal mutations but is more commonly associated with the horizontal transfer of resistance determinants borne on mobile genetic elements (Walsh and Fanning, 2008).

Keeping in mind the public health significance of the food borne pathogens and their presence in ecosystem/food chain the present study was planned with following objectives.

Objectives:

1) To study prevalence of *Campylobacter spp.,E.coli*, *Listeria monocytogenes* and *Salmonella* pathogens in pork and chicken in and around Nagpur region of Maharashtra.

2) To study *in-vitro* pathogenicity and molecular characterization of the isolates.

3) To study antibiogram and Minimum Inhibitory Concentration (MIC) determination of the isolates.

REVIEW OF LITERATURE

2.1 Prevalence of Common Food Borne Pathogens in Pork and Chicken

2.1.1 *Campylobacter* spp.

Zhao *et al.* (2001) analyzed 719 samples of retail raw meats (chicken, turkey, pork and beef) for the presence of *Campylobacter* spp. obtained from super market chains in Washington DC. The majority of chicken samples (70.7%) followed by turkey (14%), pork (1.7%) and beef (0.5%) were positive for *Campylobacter* spp. A total of 722 *Campylobacter* isolates were obtained from meat samples; among these 53.6% were *Campylobacter jejuni*, 41.3% *Campylobacter coli* and 5.1% were other species.

Aradwad (2005) carried out isolation and identification of *Campylobacter* spp. from raw meat and poultry obtained from retail sales outlets in Mumbai. The prevalence of *Campylobacter* species in chicken was (78.33%), mutton (70%), pork (60%), chevon (33.33%) and beef (6.66%).

Singh *et al.* (2011) analyzed 238 samples, including human diarrhoeic stools, poultry fecal swabs, cheese, milk and beef for presence of *Campylobacter* spp. The prevalence of *C. jejuni* from 143 faecal/stool samples was reported as 10% by cultural and biochemical examination and only one beef sample turned positive for *Campylobacter jejuni*.

Dabiri *et al.* (2014) studied prevalence and antibiotic susceptibility of *Campylobacter* species in chicken (n=250) and beef (n=200). Out of 450 samples, 121 (26.8%) isolates were confirmed as *Campylobacter* spp. Of these isolates, 93 (76.8%) were identified as *C. jejuni* and 28(23.1%) were confirmed as *C. coli*. The *Campylobacter* spp. could be isolated from a significantly larger number of chickens 110(44%) compared to beef 11 (5.5 %). Among the isolates from chicken, 87(79%) and 23(21%) were identified as *C. jejuni* and *C. coli* respectively. In case of isolates from beef, the prevalence of *C. jejuni* and *C. coli* were 54.5% and 45.5%, respectively.

Ghimire *et al.* (2014) studied the prevalence of *Campylobacter* species in 139 dressed porcine carcasses in Chitwan, Nepal and reported the prevalence of *Campylobacter spp.* as 38.84% (*C. coli* 76% and *C. jejuni* 24%).

Pallavi and Kumar, (2014) studied the prevalence of *Campylobacter* species in chevon, chicken and milk samples by using Preston Enrichment Broth with Preston Selective Supplement under microaerobic conditions. The researchers reported 17.33% and 6%, prevalence of *Campylobacter* from chicken and chevon, respectively whereas none of the milk sample turned positive for *Campylobacter* spp.

Sivasankari *et al.* (2015) processed 89 samples from ducks including intestine, feathers, skin, nails, anus, liver, beak, feces and feed collected from different poultry farms and slaughter houses in and around Erode District. Out of these 89 samples, 40(44.94%) were found positive for *Campylobacter jejuni* based on morphology, biochemical and Hippurate hydrolysis.

Vaishnavi *et al.* (2015) analyzed 127 samples of poultry meat from Chandigarh for presence of *Campylobacter* spp. and reported 44% (57/127) prevalence of *Camplyobacter* spp.

2.1.2 ***E. coli*.**

Zhao *et al.* (2001) studied prevalence of *Escherichia coli*, in 825 retail chicken, turkey, pork and beef raw meat samples from the greater Washington, D.C., area. Of the 212 chicken samples, 82 (38.7%) yielded *E. coli*, while 19.0% of the beef samples, 16.3% of the pork samples and 11.9% of the turkey samples were positive for *E. coli*.

Trotz-Williams LA *et al.* (2012) investigated a food borne outbreak in Canada. Analysis of laboratory samples revealed Leftover pork served the day after the pig roast was the item most significantly associated with an increased risk of illness. STEC O157:H7 was isolated from 11 of the 29 ill attendees and also from the pork.

Boonyasiri *et al.* (2014) studied prevalence of antibiotic resistant bacteria in healthy adults, foods, food animals and the environment from selected areas in Thailand. Among 544 healthy adult food factory workers, 75.5% were positive for ESBL producing *E. coli* while 77.3% of *E. coli* were isolated from 30 healthy animal farm workers. Amongst healthy food animals, ESBL producing status among *E. coli* isolates were more commonly detected in pigs (76.7%) than broilers (40%). Extended-spectrum beta-lactamase producing *E. coli* seemed to be more prevalent in fresh meat samples than in fresh vegetables, in fresh foods than in cooked foods and in water samples collected from the animal farms than those from canals and fish and shrimp ponds.

Magwedere *et al.* (2016) analyzed samples from ground beef (*n* = 51), pork (*n* = 16), chicken (*n* = 16) and game meat (deer, wild boar, bison, and rabbit; *n* = 55) collected from retail vendors for the detection of 7 STEC O-groups (O26, O45, O103, O111, O121, O145, and O157). Meat samples were processed employing a multiplex polymerase chain reaction assay targeting the *wzx* gene of O antigen gene clusters of the 7 STEC O-groups. Out of a total of 83 ground beef, pork, and chicken samples, 17 (20%) carried O121, 9 (10%) carried O45, 8 (9%) carried O157, 3 (3%) carried O103, and 1 (1%) carried O145 gene. None of the samples were positive for O26, O111, or the *stx* gene.

2.1.3 *Listeria monocytogenes*

Uyttendaele *et al.* (1999) reported 38.2% prevalence of *Listeria monocytogenes* from 772 samples of poultry carcasses and poultry products available for sale at the retail markets in Belgium.

Kanuganti *et al.* (2002) examined 1849 pork tissues besides 300 small intestines, and 340 ground pork samples collected from Ames, USA for prevalence of *Listeria* spp. The workers processed these samples by employing combination of (UVM -1 and UVM-2) as selective enrichment and PALCAM as selective plating media and reported prevalence of *L. monocytogenes* as 0.87%, 8.3%, 45% as in pork tissue, small intestines, and ground pork, respectively.

Krzysztof Kwiatek (2004) examined 1118 fish and fish product samples, 478 pork, 317 beef, 84 cooked sausages and 1293 pasteurized pork ham samples in Poland for prevalence of *Listeria monocytogenes*. Out of 478 pork samples examined 45 contained *L. monocytogenes* with a prevalence of 9.41%. Further, out of 84 cooked sausage samples *L. monocytogenes* was isolated from 2 (2.38%) samples. None of the 1293 pasteurized canned pork ham was positive for *L. monocytogenes*.

Zhou and Jiao (2006) examined 290 samples of pork meat collected from retail market and slaughterhouse in Yangzhou city, China. They reported 0.95% prevalence of *L. monocytogenes* in slaughtered hogs by using combination of

UVM-I and UVM-II as double enrichment medium and PALCAM as selective plating media.

Kanarat *et al.* (2011) studied the prevalence *of Listeria monocytogenes* in the chicken production chain from primary production stages to processing plants in Thailand. A total of 14,670 samples were taken from 43 breeder farms, 32 hatcheries, 1331 broiler farms, 22 slaughterhouses and 22 ready-to-eat (RTE) chicken products from processing plants. A total of 59 (2.5%) samples of frozen chicken meat and 2 (0.2%) samples of RTE chicken products were found to be contaminated with *L. monocytogenes*. Whereas; none of the sample taken from farms and hatcheries and 1888 samples of cloaca swabs collected from chickens entering slaughterhouses were positive for *Listeria monocytogenes*.

Dudhe (2012) screened pork and blood samples (50 each) collected from the pigs slaughtered at Nagpur. The worker reported 8% prevalence from pork and 4% from blood samples using combination of UVM-1 and UVM-2 as selective enrichment and PALCAM as selective plating media.

Gamboa-Marin *et al.* (2012) analyzed a total of 1519 samples including 566 carcasses, 472 meat cuts and 481 processed meat-products from Columbia for presence of *L. monocytogenes*. The study revealed highest prevalence of *Listeria monocytogenes* in deboned meat (76%) followed by carcasses (10%), meat products (9%) and ham (5%).

2.1.4 ***Salmonella* spp**.

Zhao *et al.* (2001) studied a total of 212 Chicken and 209 of pork samples and reported the prevalence of *Salmonella* as 9% and 7% in Chicken and Pork respectively in Washington D.C.

Davis *et al.* (2004) studied the faecal carriage of *Salmonella* in healthy pigs and contamination of pig carcass with *Salmonella* at slaughter. Carriages of *Salmonella* in pig was identified in 578 (23%) faecal swabs, but only 134(5.3%) carcass were found contaminated with *Salmonella*.

Ejeta *et al.* (2004) undertook a cross-sectional study to determine the prevalence and distribution of *Salmonella* serotypes in minced beef (160), mutton (85) and pork (55) from retail supermarkets in Addis Ababa, Ethiopia. An overall prevalence of *Salmonella* was recorded as 29.3% from 300 samples. Out of total meat samples examined *Salmonella* was detected in 14.7% samples followed by minced beef 14.4%, mutton14.1% and pork 16.4%.

Kalambe *et al.* (2016) analyzed a total of 400 samples comprising 50 each of blood and meat from each slaughtered male cattle, buffaloes, pigs and goats for presence of *Salmonella* from Nagpur. Isolation was done by pre-enrichment in buffered peptone water and enrichment in Rappaport-Vassiliadis broth with subsequent selective plating onto xylose lysine deoxycholate agar. A total of 10(5%) isolates of *Salmonella* spp. were isolated from meat samples of cattle (3), buffaloes (1) and pork (6).

2.2 Molecular Characterization of food borne pathogens:

2.2.1 *Campylobacter* species

Linton *et al.* (1997) processed diarrheic samples by PCR for identification of *Campylobacter jejuni* and *Campylobacter coli* up to species level and fingerprinting. Of the 20 clinically positive samples for *Campylobacter* spp., workers detected *C. jejuni* in 17, *C. coli* in 2 samples and co-infection of *C. jejuni* and *C. coli* in one sample. These results were in concurrence with culture and phenotypic identification to species level by employing genus (*16S rRNA*) and species specific (*hip O* and *asp K*) PCR.

Stoyanchev (2004) detected *Campylobacter* spp. in fresh chilled poultry and poultry products employing PCR targeting 16S rRNA gene and reported 40.8% prevalence.

Kolackova & Karpiskora (2005) compared phenotypic and genotypic methods for confirmation of thermo tolerant *Campylobacter* of human and food origin (chicken & pork) from the Czech Republic. The 911 (800 humans & 111 of food origin) *Campylobacter* strains were tested. Based on PCR techniques 85.1% *C. jejuni*, 12.5% *C. coli* and 2.3% mixed cultures were confirmed whereas; through biochemical tests 28.5% of the isolates could not be confirmed correctly. Therefore researchers concluded that phenotypic methods are time-consuming and sometimes lead to intermediate results. Further, the results are affected by different parameters, for example by the quantity of inoculums or the growth phase. In case of mixed cultures further sub culturing is required which is again a time consuming method therefore replacement by more specific and rapid methods are needed.

Yamazaki-Matsune *et al.* (2007) developed multiplex PCR assay using a combination of newly designed and published primers for identification of *Campylobacter* spp. associated with human gastroenteritis and/or septicaemia. On evaluation of efficacy with 142 *Campylobacter* strains, the assay correctly identified all strains as one of the 6 *Campylobacter* taxa. The authors concluded that multiplex PCR assay is a rapid, simple and practical tool for identification of the six *Campylobacter* taxa commonly associated with gastroenteritis and/or septicaemia in humans and offers an effective alternative to conventional biochemical-based assays.

Modi *et al. (*2015) studied prevalence of *Campylobacter* species from milk and milk products and their virulent genes. A total of 240 samples of buffalo milk (85), cow milk (milk), cheese (30), ice-cream and paneer 30 each Anand city Gujarat. The samples were processed by standard microbiological culture method and presumptive isolates were further confirmed by genus and species-specific polymerase chain reaction using previously reported primers. *Campylobacter* species were detected in 7(2.91%) raw milk samples whereas none of the milk product turned positive. All the isolates identified were designated as *Campylobacter jejuni* by targeting *16S rRNA (Campylobacter* spp.*, hipO (C. jejuni) and aspK gene (C. coli*).

Vaishanvi *et al.* (2015) attempted the isolation of *Campylobacter* from intestinal tract samples of poultry in northern region of India. *Campylobacter* was isolated from 44.9% (57/127) of the samples. All cultured isolates were reconfirmed as *Campylobacter* by 16S rRNA polymerase chain reaction assay. Molecular identification of isolates revealed the presence of *C. jejuni* in 45 (79.0%), *C. coli* in 1 (1.8%) and co-infection of *C. coli* and *C. jejuni* in 11 (19.3%).

Vaishanvi *et al.* (2015) studied the prevalence of *Campylobacter* species in faecal samples of the diarrhoeal patients employing genus and species specific PCR targeting *16S rRNA* gene *(Campylobacter* spp.*)*, *hipO* gene (*C. jejuni)* and *aspK* gene (*C. coli*). The study revealed 27 *C. jejuni* and 3 *C. coli* among 1145 patients through genus and species specific PCR.

Ushijima *et al.* (2014) detected *Campylobacter* spp. in 5% (20/380) of diarrheal stool samples collected at an outpatient clinic in Kyoto using a commercial loop-mediated isothermal amplification (LAMP) kit with a fluorescent detection reagent after DNA extraction. Fourteen of the 20 samples were already determined as *C. jejuni* by the culture method. All 20 samples were also positive for *C. jejuni* by the semi nested PCR method targeting hipO gene.

2.2.2 *E. coli*.

Bouvet *et al.* (2002) analyzed (546) pig carcasses, (1600) pork and (876) slaughter house environment samples by PCR for prevalence of VETCH. Among the 2146 carcass and pork samples and 876 environmental samples (swabs of surfaces or materials), 328 (15%) and 170 (19%) were PCR-positive for *stx* genes respectively

Tome *et al.* (2003) developed a multiplex PCR assay for the identification of human diarrheagenic *Escherichia coli.* The targets selected for each category were *eae* for entero-pathogenic *E. coli, stx* for Shiga toxin producing *E coli*, *let* and *est.* for entero-toxigenic *E. coli, Ipoh* for entero-invasive *E. coli*, and *age* for entero-aggregative *E. coli.* Thirteen previously characterized strains were used in the study. Polymerase Chain Reaction products of the expected sizes *eae* (881 bp), stx (518 bp), *let* (322 bp), *est.* (147 bp), *age* (254 bp), and *Ipoh* (619 bp) were targeted. PCR products were obtained for all six genes, and no PCR product was detected in the negative control.

Dhanashree and Mallya (2008) analyzed diarrhoeagenic stool samples (n=192) and meat samples (n = 103) for STEC by PCR. Of the 40 *eae* positive *E. coli* isolates from meat sample, one was positive for all the STEC genes *viz. stx1, stx2, rfb* O157 and EHEC *hlyA*. Of the110 *eae* positive *E. coli* isolated from stool samples, two were positive for EHEC *hlyA* and one was positive for bfp gene. Among the 192 stool enrichment broths tested, 160 were positive for *eae* gene, of which two were EHEC *hlyA* positive and one was *bfp* gene positive. Among the 103 meat enrichment cultures, 90 were positive for *eae* gene and one among them was positive for all the STEC genes.

Azeem *et al.* (2013) carried out a study to detect *E. coli* O157:H7 in 200 samples including raw milk (100), rectal swabs from apparently health and diarrheic calves (70) and stools samples from children (30). Molecular identification of the species was done using *uid*A gene, *SLT*1, and *fliCH7* gene by PCR. The overall occurrence of *E. coli* O157:H7 and *E. coli* O157:H7 were 4/200 & 5/200 respectively. *E. coli* O157:H7 was confirmed from two raw milk and two children stool samples with percentage of 1% and 6.7% respectively by PCR.

2.2.3 ***Listeria monocytogenes***

Yadav *et al.* (2011) screened 56 faecal samples collected from mammals (9) and birds (47) from Baroda Zoo, Gujarat. The researchers found three samples positive for *Listeria spp.*, of which one was identified as *L. monocytogenes* and two as *L. innocua.* All three isolates showed amplification of 370 bp product corresponding to *prs* gene.

Dudhe (2012) reported 12 *Listeria* isolates (2 from goats, 4 from cattle and 6 from pigs). The workers targeted *prs* gene and *isp* gene by PCR to confirm the *Listeria monocytogens*.

Kuan CH *et al.* (2013) examined a total of 216 chicken offal samples (chicken liver = 72; chicken heart = 72; chicken gizzard = 72) from wet markets and hypermarkets in Selangor, Malaysia for the presence and density of *Listeria monocytogenes* by using a combination of most probable number and PCR assay. The prevalence of *L. monocytogenes* in 216 chicken offal samples examined was recorded to the tune of 26.39% and among the positive samples the chicken gizzard showed the highest percentage of 33.33% compared with chicken liver (25.00%) and chicken heart (20.83%).

Sakhare (2014) confirmed 12 *L. monocytogenes* isolates from food animals (goats n=5 and pigs n=7) slaughtered in Nagpur region by using multiplex PCR.

Khawase (2015) studied the prevalence of *Listeria monocytogenes* in clinical cases of small ruminants by targeting *prs* gene and *isp* species specific primers. Worker reported overall prevalence of *L. monocytogenes* in goats as 8.7% whereas in sheep it was 14.28%.

2.2.4 ***Salmonella* species:**

Ruban *et al.* (2010) carried out isolation and identification of *Salmonella* spp. from retail chicken meat by PCR. Of 450 samples (225 of breast and 250 of thigh muscles) tested, the prevalence of *Salmonella* spp. was higher in thigh meat (31.99%) as compared to breast muscle (24.88%) targeting 16S rRNA gene (572bp).

Shanmugasamy *et al.* (2011) collected 60 samples of poultry carcasses from commercial broiler slaughtering facility in Namakkal, Tamil Nadu. Samples processed by cultural isolation, followed by *inv*A gene specific PCR for the detection of *Salmonella* spp. The study revealed 8.3% of poultry carcasses contaminated with *Salmonella* spp.

Kaushik *et al*. (2014) studied isolation and prevalence of *Salmonella* in chicken meat and cow milk samples collected from local markets of Patna, India. Prevalence of *Salmonella* was found to be 18.42% by PCR for *inv*A gene (284bp) in chicken and milk samples.

Mihaiu *et al*. (2014) collected total of 650 chicken and pork samples from all regions of Romania and processed for detection of *Salmonella*, a total of 149 isolates were positive (22.92%) that were also confirmed by PCR.

Kalambe *et al.* (2016) reported 10 isolates of *Salmonella* spp. from meat (3 from cattle, 1 from buffaloes and 6 from pigs) with an overall prevalence of 5% among food animals by employing PCR of *invA* gene (284 bp).

2.3. ***In-vitro*** **pathogenicity test:**

2.3.1 Campylobacter species:

In-vitro pathogenic characters were studied by assessment of haemolysis on 7% sheep blood agar and targeting virulent genes *viz*; *hcp+* and *gltA.*

2.3.1.1Haemolysin Production:

Misawa *et al.* (1994) reported the alpha and beta hemolysis like activity of *Campylobacter jejuni*. A total of 40 strains of *C. jejuni* (29 human and 11 nonhuman strains) were included in the study. The researchers reported the effect of pH, temperature and duration of incubation on α and β haemolysis production by *Campylobacter* spp. The organisms produced α haemolysis when pH of the medium ranged from 6.0-6.5 within 24 hrs of incubation at 42°C. After incubation for 3 days at 42°C or 6 days at 37°C, α-HLA was replaced by a transparent zone immediately beneath the bacterial growth but not around it

2.3.1.2 Determination of Virulent gene:

Biswas *et al.* (2011) processed 49 *Campylobacter jejuni* fecal isolates collected from Alberta feedlots and 50 clinical *C. jejuni* isolates from people in Alberta for the presence of 14 genes encoding putative virulence factors by PCR. These genes included those implicated in adherence and colonization (*flaC, cadF, docC, racR, jlpA, peb1* and *dnaJ*) invasion (*virB11, ciaB, pldA,* and *iamA)* and protection against harsh conditions (*htrA, cbrA,* and *sodB*). Out of 102 isolates tested 67% contained all of the genes except *virB11*. The *cadF* gene was found in 100% of the isolates tested.

Corcionivoschi *et al.* (2015) studied the virulence of *Campylobacter* isolates by PCR targeting *hcp+* gene and *gltA* gene. A higher prevalence was found in *C. coli* isolates (56.1%) than in *C. jejuni* (28.8%). The *gltA* housekeeping gene in the multiplex PCR served as a positive control and confirmed that the isolates were all *Campylobacter* spp.

Sivasankari *et al.* (2015) processed five high multidrug resistant *Campylobacter jejuni* isolates obtained from duck for PCR analysis for presence of virulence gene. The entire 5 isolates had *cad*F gene which is responsible for expression of adherence and colonization, with band range of 400bp and 3 isolates had *Vir*B11 as pathogenic gene with band range of 494 bp.

2.3.2 *E. coli*

2.3.2.1 Haemolysis on sheep blood agar (SBA):

Deshmukh (2005) reported 29 out of 53 *E. coli* isolates from diarrheic cases producing heamolysis on 5% washed sheep blood agar.

Jaulkar (2009) screened 15 *E. coli* isolates for haemolytic study. The study revealed 13(86.66%) isolates with haemolytic among which 10(66.66%) isolates were α-haemolytic and three (20%) were β-haemolytic whereas two (13.33) isolates were negative for haemolysin production.

Gupta *et al*. (2013) reported an overall incidence of *E. coli* as 29.3% from 184 samples comprising 96 raw fish and 88 ready-to-eat fish products and all the isolate were heamolytic on Sheep Blood Agar.

Shekh *et al*. (2013) reported 22 (8.8%) *E. coli* isolates from 250 buffalo meat samples. Out of 22 only 9 isolates were found haemolytic on sheep blood agar and were also positive for congo red dye binding assay.

2.3.2.2 Congo red binding test:

Chausalkar *et al.(*2004) reported positivity of 22 *E. coli* isolates from healthy pigeon & only one was congo red positive.

Chaudhari *et al.*(2007)studied pathogenic nature of *E. coli* from smoked meat by congo red adsorption (CRDA) test. The study reported positivity of all isolates for congo red binding**.**

Jaulkar (2009) screened 15 isolates of *E. coli* from foods of animal origin out of which five isolates showed positivity to Congo red binding assay.

Gupta *et al.* (2013) reported 11.11% Congo red positive *E. coli* isolates from fish and fish products whereas majority (88.89%) of the isolates failed to uptake the dye.

2.2.3.3 Determination of virulence gene:

Dhanashree *et al.* (2008) detected shiga-toxigenic *Escherichia coli* from stool and meat samples in Manglore (India). Among 110 *eae* positive isolates from 192 stool samples, two were positive for all STEC genes and of the 40 *eae* positive isolates from 103 meat samples, only one showed positive for all STEC genes like *stx*1 gene (614 bp), *stx*2 gene (779 bp), *eae* gene (384 bp), *rfb*O157 gene (497 bp), *hly*A gene (166 bp).

Zhao *et al.*(2001) studied prevalence of *Campylobacter* spp., *Escherichia coli* and *Salmonella* serovars in retail chicken, turkey, pork and beef from the greater Washington, D.C., area. A total of 825 samples of retail raw meats (chicken, turkey, pork, and beef) were examined for the presence of *Escherichia coli* and *Salmonella* serovars and 719 of these samples were also tested for *Campylobacter* spp. Of the 212 chicken samples, 82 (38.7%) yielded *E. coli*, while 19.0% of the beef samples, 16.3% of the pork samples and 11.9% of the turkey samples were positive for *E. coli* by employing virulent gene *stx1* and *stx2* for *E coli.* Chicken also had the highest rate of *E. coli* contamination (38.7%).

Kiranmayi *et al.* (2010) processed a total of 250 samples (50 each of beef, mutton and chicken and 50 samples each of beef swabs and mutton swabs) collected from various sources for detection of virulence genes of *E. coli* O157:H7 by PCR and cultural methods. Primers for *hly*A, *stx*1 & 2 genes were used for the detection of *Escherichia coli* O157:H7 and shiga toxins, respectively. Out of 250 samples, 27 showed presence of *Escherichia coli* O157:H7 by PCR. Of the 27 *Escherichia coli* O157:H7 positive samples by PCR, 12 showed *stx*1, 7 showed stx2 and 5 showed both *stx*1 and *stx*2.

Gupta *et al.* (2013) isolated 54 isolates from raw fish and ready to eat fish products. Of the 54 *E. coli* isolates, 72.22% possessed *stx1* gene and 51.85% isolates carried *stx2* gene with raw fish samples being the major source for virulence genes.

2.3.3 *Listeria monocytogenes*:

Pathogenicity of *Listeria monocytogenes* is studied by haemolysin production on Sheep Blood Agar (SBA); Christie-Atkins-Munch-Peterson test (CAMP), phosphatidylinositol-phospholipase-C (PI-PLC) assay, PCR targeting virulent gene *hly A*.

2.3.3.1Haemolysis on sheep blood agar (SBA)

Gebretsadik*et al.* (2011) reported 5.4% haemolytic *L. monocytogenes* and 1% *L. seeligeri* from food samples of animal origin at Addis Ababa, Ethiopia.

Chaudhari *et al.* (2004) reported 2.4% *L. monocytogenes* from buffalo beef samples collected at Bareilly, India. All the isolates revealed characteristic haemolysis on SBA and were designated as pathogenic.

Bhanu Rekha*et al.* (2006) processed 113 meat samples collected from goats slaughtered at Bareilly, India and reported 7.08% *L. monocytogenes*as haemolytic.

Nikas *et al.* (2013) obtained 13% *L. monocytogenes*from 100 samples of raw chicken (muscle and viscera 50 each) in Karnataka. All the isolates were positive for haemolysin assay.

Cetinkaya *et al.* (2014) confirmed 3.9% *L. monocytogenes*from 512 food samples including raw milk, dairy products, meat and meat products, chicken meat, sea food and raw vegetables collected at Turkey. All the isolates produced haemolysis on 5% sheep blood agar.

Daskalov *et al.* (2014) analyzed 148 vacuum packaged sausages in Bulgaria. The worker reported 10 *L. monocytogenes*isolates and all were positive for haemolysin assay on sheep blood agar.

2.3.3.2 Christie, Atkins, Munch-Petersen (CAMP) test

Chaudhari *et al.* (2004) tested 5 *Listeria monocytogenes* isolates obtained from buffalo for pathogenicity by CAMP test. All the isolates were CAMP positive.

Molla *et al.* (2004) screened 316 food samples and reported 5.1% prevalence of CAMP testpositive*L. monocytogenes.*

Gebretsadik *et al.* (2011) attempted isolation of *Listeria* spp. from animal origin food samples and reported 5.4 per cent *L. monocytogenes* as confirmed by CAMP test at Addis Ababa, Ethiopia.

Mathakiya *et al.* (2012) obtained a total of 28 *L. monocytogenes* field isolates from different animal species which were characterized phenotypically by CAMP test. Characterization by CAMP test of all the 28 field isolates revealed positive reaction, of which twenty-three isolates showed characteristics enhancement of haemolytic zone with *S. aureus* on 5% Sheep Blood Agar (SBA) and five isolates showed weak haemolytic zone.

Shelke (2011) studied pathogenic profile of four isolates of *Listeria* from beef samples by CAMP test. Three isolates were confirmed as pathogenic *L. monocytogenes* by CAMP test.

Dudhe (2012) confirmed 12 *List*eria isolates (2 from goats, 4 from cattle and 6 from pigs). The isolates revealed enhanced zone of haemolysis against *S. aureus* and accordingly were classified as pathogenic *L. monocytogenes.*

Vaidya (2013) employed CAMP test to check degree of pathogenicity of 17 isolates of *Listeria* obtained from goats and pigs. All the isolates were CAMP positive and were identified as *Listeria monocytogenes.*

Nikas *et al.* (2013) processed 13 *Listeria monocytogenes* isolates obtained from chicken samples by using CAMP test and reported all the isolates as positive for CAMP test.

Gusman *et al.* (2014) reported 18 (1.67%) of 912 ready to eat food samples positive for *L. monocytogenes*by using CAMP test collected at Serbia.

Lotfollahi *et al.* (2014) attempted isolation of *Listeria* spp. from dairy and meat product in Iran and reported 3.1% *L. monocytogenes* confirmed by CAMP test as pathogenic.

2.3.3.3 Phosphatidylinositol-specific phospholipase C (PI-PLC) Assay:

Paziak-Domaneska *et al.* (1999) screened 46 isolates of *L. monocytogenes* for pathogenicity on the basis of production of phosphatidylinositol-specific phospholipase C (PI-PLC). The study revealed all *L. monocytogenes* as PI-PLC positive.

Yadav *et al.* (2010) studied biochemically characterized *Listeria* isolates for PI-PLC activity employing L. mono differential agar. The *Listeria* isolates overgrown on 5% SBA were streaked on L. mono differential agar and plates were incubated at 37^0 C in a humidified chamber for 24 hr. The worker reported development of light blue colonies indicative of positive PI-PLC assay.

Waskar *et al.* (2013) examined pathogenicity of 53 isolates of *L. monocytogenes* obtained from 736 processed meat samples. Of those 46 isolates were positive while 7 isolates were negative for PI-PLC test.

Nikas*et al.* (2013) screened 13 isolates of *L. monocytogenes* from raw chicken samples and reported all isolates positive for PI-PLC test.

Dudhe (2012) isolated 12 *Listeria* strains from different food animals and confirmed the pathogenicity on the basis of PI-PLC production. The worker reported all isolates positive for PI-PLC test.

Vaidya (2013) screened 17 isolates of *L. monocytogenes* for pathogenicity by PI-PLC test employing L. mono differential agar. The worker reported all isolates positive for PI-PLC test.

2.3.3.4 Determination and characterization targeting *hly*-gene by PCR

Paziak-Domaneska *et al.* (1999) studied pathogenicity of 46 isolates of *L. monocytogenes* obtained from meat and sausage and confirmed all as pathogenic by PCR demonstrating *hlyA* at 456 bp.

Aurora *et al.* (2008) tested 471 bovine raw milk samples from unorganized sector Agra, India. The workers reported all 18 isolates positive for *L. monocytogenes* by PCR amplification of the *hly* gene (456 bp).

Swetha *et al.* (2013) processed 150 samples (25 each of chicken, swab of chicken, pork, swab of pork, fish and swab of fish). The workers revealed 17 samples positive for *hly*A gene.

Waskar *et al.* (2013) screened 108 isolates of *L. monocytogenes* from 736 processed meat product by targeting 4 genes namely *hly*A, *act, iap* and *plc* genes. The overall distribution of various genes among isolates in the decreasing order was reported as 3.66%, 2.30%, 2.17% and 0.95% for *hly*A, *act, iap* and *plc* respectively.

Nikas *et al.* (2013) isolated 35 listerial strains from raw chicken at Karnataka and confirmed those as pathogenic by PCR using primer against *hly*A gene.

Shakuntala *et al.* (2013) tested 256 pork samples collected from various places of North eastern states. A total of 20 *L. monocytogenes* were isolated and confirmed through BD phoenix 100.The amplification of virulence gene *hly*A was observed among 9 isolates and reported as pathogenic.

Vaidya (2013) studied the pathogenicity of 17 isolates of *L. monocytogenes* (7 from goats and 10 from pigs) in Nagpur region. The Researcher reported all 17 isolates positive for the *hly*A gene at 456 bp.

Thomas (2013) reported all 18 isolates comprising 9 each from meat and faecal samples obtained from the cattle slaughtered in Nagpur region bearing *hly*A gene at 456 bp. accordingly positive isolates were considered as pathogenic.

2.3.4 *Salmonella* species:

2.3.4.1 Haemolysin production:

Production of haemolysin on blood agar has been demonstrated as an indication of pathogenicity among various bacterial populations in general.

Jaulkar(2009) demonstrated production of haemolysis by 36.36% *Salmonella* isolates. Among the total isolates, 36.36% produced α- haemolysis whereas none of the isolates showed β- haemolysis.

Agrawal, (2005) determined haemolytic potential of *Salmonella gallinarum* strains through demonstration of haemolysis on blood agar. Their study on 94 strains of *S. gallinarum* revealed production of two types of haemolysis *viz*., beneath the colony haemolysis (BCH) or contact haemolysis and clear zone haemolysis (CZH). The workers further reported clear zone of haemolysis on blood agar prepared with washed erythrocytes of goat and a total of 12 per cent (11 of 94) different haemolytic patterns of strains, revealing multiplicity of haemolysins in *S. gallinarum*.

2.3.4.2Congo Red Dye Binding Assay:

Tiwari*et al.* (2002) studied differences in haemolysin expression in a strain of *Salmonella enteric* serovar *Typhimurium* definitive phage type (DT) 98 cultured under various conditions and observed colonies as smaller, with dark centers and wider zones of haemolysis on Congo red blood agar. The workers directly correlated Haemolysin production with Congo red binding in nutrient broth. However, they also reported culture-cell-free haemolysin activity as higher, but cell-bound haemolysin activity was very low in growth medium supplemented with Congo red.

Kalambhe *et al.* (2016) screened10 isolates of *Salmonella* obtained from beef and pork for haemolysin production and Congo Red Dye binding assay. Cent percent beef and buffalo beef isolates (4) turned positive for hemolysin production and Congo Red binding assay, whereas 66.66% and 100% pork samples revealed positivity for hemolysin and Congo Red binding assay respectively.

2.3.4.3 Detection of *invA* gene among *Salmonella* isolates

Jamshidi *et al.* (2009) analyzed 60 samples randomly collected from chilled broiler carcasses at Iran. The workers showed that 5 (8.3%) and 1(1.6%) samples were contaminated with *Salmonella* spp. and *Salmonella typhimurium*, respectively by using S139 and S141 primers that amplifies a 284 bp sequence of the *inv*A gene and Fli15 and Tym primers that amplifies a 559 bp sequence of the *fli*C gene.

Karmi (2013) conducted a study in which a total of 100 meat and poultry product samples were collected from shops and supermarkets, at Egypt. *Salmonella* was detected by conventional isolation methods and then PCR was carried out to detect *invA* gene in isolates. It was observed that 16% of samples were positive for *Salmonella* isolation; 26% (13/50) in meat samples and 6% (3/50) in poultry samples. All *Salmonella* isolates were positive for the *invA* gene exhibiting 284 bp DNA fragment.

Ezzat *et al.* (2014) carried out PCR assay for the detection of the *invA* gene from six isolated strains (*S. enteritidis, S. macclesfield, S. rissen, S. derby, S. magherafelt* and *S. enterica*sub-spp. *salamae*) obtained from broiler chicken. The study revealed the presence of *inv*A gene in all of the isolates bearing 284 bp PCR amplified fragment.

2.4 Antibiogram study

Antibiotic sensitivity is the susceptibility of bacteria to antibiotics. Antibiotic susceptibility testing (AST) is usually carried out to determine which antibiotic will be most successful in treating a bacterial infection *in vivo*. Antibiotic susceptibility testing determines the susceptibility of a bacterial strain to a specific antibiotic or a panel of antibiotics.

2.4.1 *Campylobacter species*:

Dallal *et al. (*2010) studied the prevalence and antimicrobial resistance profile of *Campylobacter*, *Salmonella* and *Yersinia* serotypes from retail chicken and beef in Iran. Antibiotic sensitivity test was done for 92 isolates comprising *C.*

jejuni(70) and *C. coli* (22).Of the 92 isolates,71% were resistant to nalidixic acid followed by ciprofloxacillin (47%), tetracycline (28%), ampicillin (22.6%), colistin (27%) and amoxicillin (11%). Of the 92 isolates 12(13%) were susceptible to all of the antibiotics evaluated.

Baserisalehi *et al.* (2007) carried out a comparative study on antimicrobial susceptibility of *Campylobacter*spp. isolated from faecal sample of domestic animals and poultry in India and Iran. The researchers reported cent percent sensitivity of *Campylobacter* isolates towards ciprofloxacin. All *Campylobacter* strains from India were resistant to cephalexin and cefotaxim whereas *Campylobacter* isolates in Iran were sensitive.Rate of existence of ampicillin resistant strain of *Campylobacter*in India was relatively high.

Rahimi *et al.* (2011) studied antibiotic resistance pattern of *Campylobacter* spp. from raw duck and goose meat in Iran. The resistance pattern of *Campylobacter* isolates againsta panel of 10 antimicrobial agents was studied. Overall,43 of 52 *Campylobacter* isolates (82.7%) were resistant to one or more antimicrobial agent. Resistance to ciprofloxacin was the most common finding (40.44%), followed by tetracycline (32.7%), and nalidixic acid (30.8%). All *Campylobacter* isolates were susceptible to amoxicillin, chloramphenicol, erythromycin and gentamicin.

Sivasankari *et al.* (2015) studied antibiotic resistance pattern of *Campylobacter* isolates from raw duck meat in Erode district of Tamilnadu (INDIA).All 40 positive isolates were resistantto tetracycline and amoxicillin followed by erythromycin (85%), nalidixic acid(68%), norfloxacin (63%), doxycycline (45%) and gentamicin (43%).Highest sensitivity was observed towards chloramphenicol (35%) followed by cephalexin (30%) and ciprofloxacillin(18%).

Dabiri *et al. (*2013) studied antibiotic susceptibility of *Campylobacter species* isolated from chicken and beef meat. Susceptibilities of 121 strains including *C. jejuni* (93) and *C. coli* (28) were determined against 12 antimicrobial drugs using the agar disk diffusion method. Resistance to nalidixic acid (75%) and ciprofloxacin (50%) was an alarming finding, followed by tetracycline(32.6%), ampicillin(10.8%), colistin (29.3%) and amoxicillin (26.1%). Highest sensitivity was seen towards gentamicin (96%) and erythromycin (95%).

Ghimire *et al.* (2014) studied the antibiogram pattern of thermophilic Campylobacter spp. in dressed porcine carcass of chitwan, Nepal. The study revealed highest degree of resistance by Campylobacter isolates against ampicillin and erythromycin (92.59% each) followed by colistin (72.2%), tetracycline (61.1%), nalidixic acid and cotrimoxozole (44.4% each), ciprofloxacin (31.5%) and gentamicin (5.56%). Moreover 77.8% of the isolates were resistant to more than two antimicrobials. As far as the calculation of MAR index is concerned the study revealed 22% of the isolates having MAR index between 0.1 and 0.2 and 77.8% of the isolates had MAR index greater than 0.2.

Pallavi *et al. (*2014) screened total of 29 *Campylobacter* isolates including *C. jejun i*(23) and *C. coli* (6) recovered from 280 samples of chicken, chevon, and milk for antibiogram pattern. None of the isolate was found resistant to erythromycin. Whereas; varying pattern of resistance was observed for other antibiotics *viz;* 26.67%, 16.67%, 6.67%, 10%, 3.33% and 33.33% resistance was observed for ciprofloxacin, ampicillin, levofloxacin, gentamicin, norfloxacin and ceftriaxone, respectively. Cen percent isolates were resistant to co-trimoxazole.

2.4.2 *E. coli*

Goswami *et al.*(2004) studied antibiogram of 51 strains of *E.coli* isolated from poultry and noticed highest degree of resistance to penicillin (100%) followed by tetracycline (98.3%), ampicillin (62.74%), cloxacillin (15.68%), erythromycin (7.84%) and nalidixic acid (1.96%).

Bhong and Brambhatt (2006) studied antimicrobial resistance pattern of *E.coli* isolates obtained from mutton samples. The study reported 93.40% isolates sensitive to perfloxacin followed by norfloxacin and nalidixic acid (91.40% each). Most of isolates were resistant to amikacin (12.9%), followed by cefuroxime (6.45%), cefotaxime (5.38) and ceftazidime (4.30%).

Van *et al.* (2008) analyzed the *Escherichia coli* isolates for antibiotic resistance and virulence genes from raw meat and shellfish in Vietnam. About 80% of the isolates were resistant to one or more antibiotics. The rates of multi-resistant were up to 89.5% in chicken, 95% in chicken faeces and 75% in pork isolates. Resistance was frequently observed against tetracycline (77.8%), sulfafurazole (60.6%), ampicillin (50.5%) and amoxicillin(50.5%).

Jaulkar *et al.* (2009) studied the antibiogram of *E coli* isolates from foods of animal origin by employing agar discs diffusion method using 15 antibiotic discs. Cent percent organisms were resistant against penicillin and tetracycline followed by doxycycline hydrochloride (73.33%), ceftazidim (53.33%), ampicillin (46.66%), erythromycin and streptomycin (40% each). Maximum sensitivity was observed towards chloramphenicol (43.4%) followed by streptomycin (39.4%), nalidixic acid (34.3 %) and gentamicin (24.2%).

Adentunji and Isola (2011) screened a total of 30 isolates comprising of 10 each of *E coli*, *Listeria* and S*almonella* from retail meat table in Ibandan municipal abattoir in Nigeria for antibiotic sensitivity by agar disc diffusion assay. Antibiotic sensitivity profile expressed in zone of inhibition showed that all isolates were resistant to 3 or more antibiotics. All the isolates were resistant to tetracycline. The incidence of antibiotic resistance observed was *E. coli* (60%) and *Salmonella* (60%).

Thaker*et al.* (2012) processed 38 *E. coli* isolates obtained from raw milk for antibiogram study. Antibiogram pattern revealed cent percent isolate were resistant to ampicillin, whereas; moderate resistance was observed for streptomycin (57.89%), oxytetracycline (47.37%) and amoxy-clav (42.11%). Also lesser percentage of resistance was observed for co-trimoxazole (13.16%) and chloramphenicol (5.26 %).

Dutta *et al.* (2013) analyzed 91 isolates of *E.coli* obtained from pigeons for antibiotic sensitivity study. Cent percent isolates exhibited resistance against ampicillin followed by nitro-furantoin (73.62%), tetracycline (65.93%), oxytetracycline (62.63 %) and streptomycin (61.54).

Shekh *et al.*(2013) subjected 9 pathogenic isolates of *E. coli* obtained from buffalo meat to antibiogram study. The researchers reported all 9 isolates sensitive to gentamycin (20 ± 1.49 mm) while 7 isolate were resistant to enrofloxacin (18.22 ± 3.58 mm) and tetracycline (11.44 ± 2.04 mm).

2.4.3*Listeria monocytogenes*

Katre *et al.*(2009) performed the antibiotic sensitivity assay of nine *Listeria* isolates obtained from foods of animal origin against 15 antimicrobials and reported found highest degree of sensitivity towards ciprofloxacin (88.8%) followed by amoxyclave and teteracycline (77.7%) and penicillin-G (33.3%).The worker reported 66.66 per cent resistance against azythromycin, cloxacillin and penicillin-G followed by 44.44, 22.22 and 11.11 per cent against ceftazidime, amoxyclav and amikacin, ciprofloxacin and tetracycline, respectively.

Shelke (2011) studied antimicrobial susceptibility of five isolates of *Listeria* spp. obtained from male cattle slaughtered in the Nagpur region using 12 antibiotic agents. The isolates were cent per cent resistant to nalidixic acid and trimethoprim. The isolates were cent percent sensitive towards ampicillin and gentamicin followed by cloxacillin, kanamycin, norfloxacin, penicillin-G, rifampicin and tetracycline (80% each), cefotaxime and erythromycin (60% each). The isolates were moderately sensitive towards cefotaxime (40%) followed by kanamycin, erythromycin, norfloxacin and rifampicin (20% each).

Suryawanshi (2011) assessed antimicrobial susceptibility of five isolates of *Listeria* spp. obtained from goats slaughtered in the Nagpur region. Cent percent isolates were found resistant against penicillin-G and nalidixicacid, followed by kanamycin and trimethoprim (80% each), cloxacillin, erythromycin and rifampicin (40% each) and ampicillin and tetracycline (20% each). Cent percent organisms were moderately sensitive towards norfloxacin followed by cefotaxime(80%), cloxacillin and rifampicin (40% each) and gentamycin and kanamycin (20% each). Highest sensitivity was recorded towards ampicillin, gentamycin and tetracycline (80% each), followed by erythromycin (60%) and cefotaxime, cloxacillin, rifampicin and trimethoprim (20% each).

Dudhe (2012) conducted antibiogram study of *Listeria monocytogenes* isolates obtained from food animals slaughtered in the Nagpur region using a panel of 12 antibiotics by disc diffusion assay and reported cent per cent drug resistance against nalidixic acid, penicillin-G and cloxacillin, followed by kanamycin (91.66%), rifampicin (66.66%) and ceftriaxone (50%). The isolates were sensitive to gentamicin (91.66%) and all the isolates moderately sensitive towards the cefotaxime followed by ampicillin and erythromycin (50% each).

Nayak *et al.*(2013) studied sensitivity of *Listeria monocytogenes* isolated from raw meat (buffalo meat, chevon and mutton) in Anand, India. They noted cent per cent sensitivity towards penicillin-G followed by ampicillin, chloramphenicol, ciprofloxacin, teteracycline (92.3%), erythromycin (84.7%), and rifampicin (76.9%) whereas maximum resistance was recorded against ceftriaxone, cefotaxime, and ceftazidime.

Vaidya (2013) reported antimicrobial susceptibility of 17 isolates of *Listeria monocytogenes* obtained from goats and pig slaughtered in the Nagpur region towards 12 antimicrobial agents by agar disc diffusion method. The isolates were resistant to Kanamycin (52.94%), followed by Rifampicin (47.05%), and cefotaxim (35.30%). Cent percent isolates revealed moderate sensitivity towards vancomycin, followed by gentamicin and norfloxacin (94.11%); erythromycin (82.35%); rifampicin (52.94%), ceftriaxone and oxytetracycline(47.05% each) and cloxacillin (23.53%). The highest degree of sensitivity was observed towards penicillin and cloxacillin (76.47% each) followed by oxytetracycline and ceftriaxone (52.94% each).

Raorane *et al.* (2014) processed 28 isolates of *Listeria* spp. obtained from clinical and environmental sources for antibiotic susceptibility study using disc diffusion method. All isolates were sensitive towards, erythromycin, oxytetracycline, ampicillin, doxycycline and ciprofloxacin and showed intermediate resistances towards the chloramphenicol, penicillin, gentamicin and vancomycin.

Sakhare (2014) tested 12 isolates of *Listeria monocytogenes* for antibiogram study with agar disc diffusion method against 12 most commonly used antibiotics. Cent per cent isolates were resistant to Kanamycin, whereas the highest degree of sensitivity was observed towards Penicillin-G (75%) followed by cloxacillin (25%) and ceftriaxone (25%).

Khawase (2015) studied antibiogram of 3 *L. monocytogenes* isolatesby agar disc diffusion method using 12 antibiotics. Cent per cent resistance was observed towards kanamycin and rifampicin followed by ceftriaxone (66.66%), whereas cent per cent sensitivity was observed towards ampicillin, cloxacillin and oxytetracycline followed by cefotaxime and vancomycin (66.66% each), erythromycin, norfloxacin and penicillin-G (33.33% each). Cent per cent isolates exhibited moderate sensitivity towards gentamycin followed by erythromycin, norfloxacin and penicillin G (66.66% each), ceftriaxone, cefotaxime and vancomycin (33.33% each).

2.4.4*Salmonella* spp.

Jaulkar *et al.*(2009) reported the antibiotic resistance of *Salmonella* strains obtained from various foods of animal origin at Nagpur Veterinary College Nagpur, Maharashtra. The isolates were resistant to penicillin-G, ampicillin, amoxyclave and trimethoprim (81.81% each) followed by erythromycin and tetracycline (63.63% each); doxycycline hydrochloride (54.54%); ceftazidime (45.45%); streptomycin (18.18%); azithromycin, cephotaxim and nalidixic acid (9.09% each).

Singh *et al.* (2009) studied the antibiotic resistance pattern among non typhoidal*Salmonella spp*. isolated from human, animal and meat at National *Salmonella*Center (Vet), Bacteriology and Mycology Division, Indian Veterinary research institute, Izzatnagar, UP, India. The researcher reported cent percent resistance of *Salmonella* isolates against erythromycin and metronidazole followed by clindamicin (94.59%), ampicillin(86.49%), co-trimoxazole(48.65%), colistin (45.94%), nalidixic acid (35.10%), amoxyclave (18.90%), cephalexin, meropenem, tobramycin, nitrofurantoin, teracycline, amoxicillin (8.10% each), perfloxacilin and streptomycin (5.40% each).

Selvaraj *et al. (*2010) subjected 8 isolates from poultry carcasses to antibiogram study against a panel of 7 antibiotics. All the isolates were sensitivetoamikacin, kanamycin and ciprofloxacin followed by chloramphenicol (87.50%), oxytetracycline and ceftriaxone (62.60% each) and all the organisms were resistant against cefotaxime.

Kim *et al.*(2011) performed antimicrobial susceptibility tests for *Salmonella* against eight antibiotics and noted highest degree of resistance against tetracycline (76.19%) followed by nitorfurantoin (38.10%), kanamycin (33.33%), chloramphenicol, sulfamethoxazole/trimethoprim and cephalothin (28.57% each); polymyxin B (9.52%); and ampicillin/sulbactam (4.76%).

Moon (2011) isolated *Salmonella* spp. from chicken samples and studied the antibiotic resistance pattern. Maximum sensitivity was recorded towards ampicillin, colistin, piperacillin, netillin and norfloxacin. Highest resistance was recorded against ciprofloxacin and ofloxacin.

Kalambhe *et al.* (2016) processed 10 isolates of *Salmonella* obtained from food animals for antibiotic sensitivity pattern. The overall resistance of 50% was noted for trimethoprim followed by ampicillin (20%). A maximum sensitivity (80%) was reported towards gentamycin followed by ampicillin and trimethoprim (40% each), amikacin (30%) and kanamycin (10%).

2.5 Estimation of Minimum Inhibitory Concentration (MIC)

2.5.1 *Campylobacter* spp.

Guevremonte *et al.*(2006) studied antimicrobial susceptibilities of thermophilic*Campylobacter*isolated from humans, swine, and broilers in Quebec. Breakpoint values of the MIC for resistance were as follows: for ciprofloxacin, clindamycin and enrofloxacin less than4 µg/mL; for erythromycin less than 8 µg/mL; for gentamicin and tetracycline, µ16 ug/mL; for ampicillin and chloramphenicol, less than 32 µg/mL; for streptomycin, less than 64 µg/mL; and for sulfamethoxazole, less than 512 µg/ml.

Baserisalehi *et al. (*2007) conducted a comparative study on antimicrobial susceptibility of *Campylobacter* spp. isolated from faecal sample of domestic animals and poultry in India and Iran. Minimum Inhibitory Concentration was performed for ampicillin, ciprofloxacin, erythromycin, gentamycin, tetracycline, chloramphenicol and was observed varied range of MIC values against *Campylobacter* isolates. Lowest MIC values were found for ciprofloxacin (2 µg /ml) and highest MIC values were found for ampicillin and chloramphenicol with 256 µg/ml in case of all isolates in India and 64 µg/ ml in case of isolates in Iran.

Pallavi and Kumar (2014) studied the antibiotic resistance pattern of *Campylobacter* species from foods of animal origin. Worker reported the MICs of erythromycin sensitive isolates ranged between 0.01 and 0.1 µg/ml, MIC level of 0.01µg/ml was recorded in 10 isolates and 0.1 µg/ml in 19 isolates. MICs of norfloxacin sensitive isolates ranged between 0.1 and 1 µg/ml, MIC level of 0.1 µg/ml, 0.5 µg/ml and 1 µg/ml were recorded in 10, 6 and 13 isolates, respectively. MICs of levofloxacin sensitive isolates ranged between 0.05 and 0.25 µg/ml, MIC level of 0.05 µg/ml was recorded in 6 isolates, 0.1 µg/ml in 16 isolates and 0.25

μg/ml in 7 isolates. MICs of gentamicin sensitive isolates ranged between 0.064 and 8 μg/ml, MIC level of 0.064 μg/ml, 0.128 μg/ml and 0.512 μg/ml were recorded in 18, 7 and 4 isolates, respectively. MICs of ceftriaxone sensitive isolates ranged between 0.512 and 32 μg/ml, MIC level of 4 μg/ml was recorded in 8 isolates, 8 μg/ml in 11 isolates, 16 μg/ml in 6 isolates and 32 μg/ml in 4 isolates. MIC results revealed that all the isolates were within prescribed concentrations for sensitivity for the antibiotics tested *viz*., erythromycin (=0.5 μg/ml), gentamicin (=4 μg/ml), levofloxacin (=2 μg/ml), norfloxacin (=4 μg/ml) except for ceftriaxone (=8 μg/ml) where 10 isolates revealed MIC value of more than 8 μg/ml.

Miflin *et al.*(2007) carried out antibiotic sensitivity study in *Campylobacter jejuni* and *Campylobacter coli* isolated from poultry in the south-east Queensland region.The level of resistance to any of the antibiotic examined in this study never exceeded 20% for either *C. jejuni* or *C. coli.* Among the 125 *C. jejuni* isolates, the highest level of resistance was observedagainst tetracycline (19.2% by MIC and 18.4% by disc) and ampicillin (19.2% by MIC and 17.6% by disc). A similar level of resistance to these same two antibiotics was found in the 27 *C. coli* isolates. A low level of resistance to nalidixic acid (2.4%) was found in the *C. jejuni* isolates by discdiffusion, whereas all the *C. jejuni* isolates were susceptible by MIC. All *C. coli* isolates were susceptible to this antibiotic by MIC and disc methods. A low level of resistance (11.1% by MIC and disc) was found to erythromycin among the *C. coli* isolates, whereas resistance to more than one antibiotic was detected by disc diffusion in 9 *C. jejuni* isolates (7.2%) and by MIC in 11 *C. jejuni* isolates (8.8%). All of these isolates were resistant to both tetracycline and ampicillin. By disc diffusion and MIC methods, none of the 27 *C. coli* isolates showed resistance to more than one antibiotic.

2.5.2 *E.coli*.

Oie *et al.*(1997) studied In vitro susceptibility of *Escherichia coli* O157 to several antimicrobial agents. Worker determined the minimum inhibitory concentration (MIC) and the minimum bactericidal concentration (MBC) of ciprofloxacin, polymyxin B, cefoperazone and kanamycin for each strain as 6.25μg/ml. However, the MIC of fosfomycin was 3.13-100 μg/ml, and its MBC was 100μg/ml. The MIC of ampicillin and tetracycline was less than 100μg/ml in some strains.

Boonyasiri *et al.* (2014) studied antibiotic resistant pattern of *E.coli* isolated from healthy adults, foods, food animals and the environment in selected areas in Thailand. The MIC_{50}, MIC_{90}, and MIC range for ceftriaxone against isolated *E. coli* were 8, 64, and 0.06–512 mg/l, respectively. The MIC50, MIC90, and MIC range for ciprofloxacin against isolated *E. coli* were 8, 32, and 0.06–128 mg/l, respectively. Extended-spectrum beta-lactamase-producing *E. coli* were detected in 75.5% of food factory workers and accounted for 47.4% of isolates. The prevalence of colistin-resistant *E. coli* was 8.8%.

2.5.3 *Listeria monocytogenes*

Safdar and Armstrong (2003) subjected 84 isolates of *Listeria monocytogenes* obtained from systemic cases of human listeriosis to antibiogram study. The organism were resistant to clindamycin (96.2%) followed by cefuroxime (80.8%), cefotaxime (66.6%) and ceftriaxone (76.1%) ampicillin (9.2%), erythromycin (1.9%), gentamicin (2%), penicillin (2.3%), tetracycline (3%) and ticarcillin-calvulanate (3.8%). The organism were susceptible to chloramphenicol (87%) followed by amikacin, cefazolin, cephalothin, rifampin, trimethoprim-sulfamethoxazole (TMP-SMX) and vancomycin. The MIC_{90} was estimated for amikacin (6.0µg/ml), ampicillin (1.0µg/ml), erythromycin (0.25µg/ml), gentamicin (1.0µg/ml), penicillin (0.5µg/ml), tetracycline (0.25µg/ml) and TMP-SMX (2.0 µg/ml for TMP and 38.0 µg/ml for SMX).

Shrinivasan*et al.* (2005) performed antibiotic susceptibility testing of 38 *Listeria monocytogenes* isolates obtained from dairy farms. Minimal inhibitory concentrations (MICs) of 15 antimicrobials for *L. monocytogenes* isolates were determined by micro dilution method with a range of MIC of 0.25 - 512 µg/ml for all the antibiotics. All the isolates were resistant to cephalosporin C (MIC ≥ 512 µg/ml), streptomycin (MIC ≥ 32 µg/ml) and trimethoprim (MIC ≥ 512 µg/ml). Most *L. monocytogenes* isolates (92%) were resistant to ampicillin (MIC ≥ 2 µg/ml), rifampicin (84%, MIC ≥ 4 µg/ml), rifamycin (84%, MIC ≥ 4 µg/ml), and florfenicol (66%, MIC ≥ 32 µg/ml) and some were resistant to tetracycline (45%, MIC ≥ 16 µg/ml), penicillin G (40%, MIC ≥ 2 µg/ml), and chloramphenicol (32%, MIC ≥ 32 µg/ml). All *L. monocytogenes* were susceptible to amoxicillin, erythromycin, gentamicin, kanamycin and vancomycin.

Korsak*et al.* (2012) carried out the antimicrobial susceptibility testing and estimation of Minimum Inhibitory Concentration (MIC) of 471 isolates of *Listeria monocytogenes* obtained from different foods and food related sources. Almost all the organisms were susceptible to all the antibiotics tested thus no resistance against any of the antibiotic tested was observed. Of all the antimicrobial agents tested, rifampicin and trimethoprim had the lowest MIC_{90} of 0.094μg/ml, indicating their strong activity against *L. monocytogenes*. In contrast, the analyzed isolates were least susceptible to sulfamethoxazole 48μg/ml, Norfloxacin and chloramphenicol had a slightly higher MIC_{90} value of 4 and 6μg/ml, respectively.

Moreno *et al.* (2014) conducted antibiotic sensitivity assay of 46 *Listeria* spp. isolates using 28 antibiotics. The workers reported cent percent sensitive isolates towards penicillin (MIC_{90} 0.5μg/ml), ampicillin (MIC_{90} 0.5μg/ml)), tetracycline (MIC_{90} 2μg/ml), erythromycin (MIC_{90} 0.25 μg/ml) and Meropenems (MIC_{90} 1 μg/ml). Cent percent isolates were resistant against ceftazidime (MIC_{90} 256μg/ml). Furthermore; all studied *Listeria* species revealed intermediate resistance towards clindamycin, ciprofloxacin (MIC_{90} 4μg/ml) each, levofloxacin (MIC_{90} 2 μg/ml) and moxifloxacin,MIC_{90} 1 μg/ml.

2.5.4 *Salmonella* spp.

Tankhiwale*et al.*(2003) processed twenty three isolates of *S. typhi* isolated from blood cultures in suspected cases of enteric fever. Of the 23 isolates 13 (56.52%) were chloramphenicol sensitive with minimum inhibitory concentration (MIC) < 8μg/mL while 8 had MIC of > 32 μg/mL indicating resistance; 2 isolates were intermediate sensitive with MIC value between 8 and 32 μg/ml.

Saha *et al.* (2006) processed three ciprofloxacin-resistant *S. enterica* typhi strains isolated from the blood of three patients from different parts of Dhaka, Bangladesh for antibiogram and estimation of MIC by Etest for various antibiotics. Isolates were highly resistant to ampicillin (>256 μg/ml), cotrimoxazole(>32μg/ml), chloramphenicol (>256μg/ml), ciprofloxacin (512μg/ml) and nalidixic acid (>256μg/ml) and were susceptible to ceftriaxone (0.094μg/ml) and azithromycin (zone, 20 to 22 mm).

Capoor *et al.* (2009) carried out estimation of Minimum Inhibitory Concentration of azithromycin, ciprofloxacin, cefixime, cefepime, ceftriaxone, gatifloxacin, imipenem, levofloxacin, meropenem and ofloxacin (E-test strip) and tigecycline and faropenem (agar dilution) against 210 *Salmonella* spp. recovered from enteric fever and septicemia cases. MIC_{90} of the carbapenems (imipenem and meropenem) for *Salmonella* typhi and *Salmonella Paratyphi* A was 0.064 mg $ml^{-}1$. MIC_{90} of faropenem was 0.25 mg $ml^{-}1$ for *S.* Typhi, *S.* Paratyphi *A* and *Salmonella* Typhimurium. The MIC_{90} of azithromycin for all *Salmonella* spp. ranged from 8 to 16 mg $ml^{-}1$. Tigecycline showed MIC_{90} of 2 mg $ml^{-}1$ for *S. Typhi*, 1 mg $ml^{-}1$ for *S. Paratyphi A* and 4 mg $ml^{-}1$ for *S.* Typhimurium.

Akhtar *et al.* (2010) processed 14 *S. enteritidis* isolates obtained from poultry for estimation of minimum inhibitory concentration against different antimicrobial drugs. The isolates were totally resistant to all concentrations of bacitracin, erythromycin and novobiocin. More than 60% strains showed resistance at higher concentration (100µg/10µl) to spectinomycin and trimethoprim. Resistance to ampicillin and chloramphenicol was also observed by the isolates at higher concentration (100 µg/10µl).

MATERIALS AND METHODS

3.1 Bacteriological Media / Reagents / Chemicals / Biologicals / Equipments

3.1.1 Media / Chemicals / Reagents:

The bacteriological media used in the study for isolation, identification and characterisation of food borne pathogenes *viz. Campylobacter species, E. coli, Listeria monocytogenes and Salmonella species* were obtained from Hi-Media Laboratories Limited, Mumbai & Oxoid (BD).For molecular studies pertaining to polymerase chain reaction the chemicals and reagents of analytical grade from Promega (USA), Sigma Aldrich (US), E Merck (India), SRL Chemicals (Mumbai) and S.d.fine Chem (Mumbai) were used.

3.1.2 Biologicals

The standard strain of *Campylobacter jejuni* (ATCC BAA 1153), *Escherichia coli* (ATCC 25922), *Salmonella enteridis (ATCC 13076)*,*Salmonella* Typhimurium (MTCC 98),*L. monocytogenes* (ATCC 19118), *Rhodococcus equi* (MTCC 1135) and *Staphylococcus aureus* (MTCC 3160) included in the study were procured from Microbial Type Culture Collection and Gene Bank, Institute of Microbial Technology, Chandigarh, India.

3.1.3 Equipments and instruments

Details of the various equipments and instruments used during the study are stated accordingly.

3.2 Collection and transportation of the samples

A total of 300 samples, comprising 50 each of blood, faecal & meat from pig (50) and poultry (50) were included in the study. The samples from pigs were collected from slaughter shops in Immamwada while of poultry origin were from retail shops in Nagpur city.

Table 1. Details of samples collected

Source	Type of sample	No. of sample	Labelling of sample	Place	Total
Pigs (50)	Pork	50	SP1 to SP50	Imamwada Nagpur	150
	Faecal	50	SF1 to SF 50		
	Blood	50	SB1 to PB50		
Poultry (50)	Chicken	50	PC1 to PC50	Chicken retail shops, Nagpur	150
	Faecal	50	PF1 to PC50		
	Blood	50	PB1 to PB50		
	Grand total				**300**

The meat samples from food animals were collected aseptically in sterile zip lock pouches and blood samples (2 ml) were collected in sterile EDTA vacutainer. While faecal samples were collected in sterile clinicol /sterile swab sticks procured from Hi-media, Mumbai. The samples were labelled properly as mentioned in Table No. 1 and immediately transported on ice to laboratory. Meat and faecal samples were immediately processed for isolation and identification of *Campylobacter* spp, *Escherichia coli, Listeria monocytogenes, Salmonella* spp. as stated below. The blood samples were processed for DNA extraction.

3.3 Processing of samples

Meat sample of approximately of 10 gm was aseptically homogenized using blender/stomacher paddle (Lab Med, UK) in 90 ml of sterile normal saline solution (NSS) for 60 sec at medium speed.The homogenised samples were inoculated in respective enrichment medium for isolation of *Campylobacter* species, *E. coli*, *Listeria monocytogenes* and *Salmonella* species as stated below.Faecal samples were directly inoculated in enrichment media.

3.4 Preparation of media:

The isolation and identification of the *Campylobacter spp., Escherichia coli, Listeria monocytogenes and Salmonella species* were carried out as per standard protocol (OIE, 2008; Pallavi *et al.* 2014; Curtis & Le, 1995).The details of the composition of the media are stated in Appendix-I.

3.5 Isolation of foodborne pathogens:

3.5.1 *Campylobacter* species:

3.5.1.1 Enrichment:

For isolation of *Campylobacter* species one ml of the homogenized solution was added to 9 ml of the Preston enrichment broth and faecal samples were directly enriched with preston media and were kept for incubation at 42^0C for 24 to 48 hrs under microaerophilic condition provided by Campy Gen gas generating pack (Oxoid).

3.5.1.2 Selective plating:

After complete incubation in enrichment medium a loopful of inoculum was streaked onto sterile modified Charcoal Cefoperazone Deoxycholate agar (mCCDA) plates and further kept for incubation at 42^0C for 24-48 hrs under microaerophilic condition provided by CampyGen gas generating pack (Oxoid). Samples which showed typical grey, flat, moist type colonies with a tendency to form metallic sheen (seramic type) were identified as *Campylobacter spp*. These colonies were then subjected to Gram staining, motility, biochemical characterization, *in-vitro* pathogenicity and antibiotic sensitivity tests and minimum inhibitoty concentration (MIC) as mentioned below.

3.5.1.3 Characterization of isolates:

The presumptive *Campylobacter spp* colonies were subjected to Gram's staining. The isolates which were Gram negative and having morphology of curved, spiral shaped rod appearance were subjected for motility (OIE, 2008). Suspected colonies were inoculated in Brain Heart Infusion broth (BHI) and kept for incubation at 42^0C for 24 to 48 hrs of incubation under microaerophilic condition using CampyGen gas generating pack. The isolates were then checked for typical corkscrew, darting type motility by hanging drop in concavity slide technique under 40 X magnification of the microscope (Olympus, CX-31).

The isolates with darting motility were transfered on BHI slants for further biochemical characterization, as per the method described by Pallavi *et al.* (2014), Cruikshank *et al.* (1975), Bergey's Manual of Systematic Bacteriology (1984) and Agarwal *et al.* (2003). The isolates were also characterized using conventional PCR technique employing genus and species specific primers (Modi *et al.* 2015 and Vaishnavi *et al.* 2015).

3.5.2 *Escherichia coli*:

3.5.2.1 Enrichment:

One ml of homogenized solution was added to 9 ml of the MacConkey broth and kept for incubation at 37^0C for 18 to 24 hrs. Faecal samples were added directly with the enrichment media.

3.5.2.2 Selective plating:

After completion of the incubation a loopful of inoculum from the MacConkey broth was streaked onto sterile Eosin Methylene Blue (EMB) agar plates and further kept for incubation at 37^0C. Samples with typical round colonies & greenish metallic sheen were presumed as *Escherichia coli*.These colonies were then subcultured on BHI slants and were used for further identification.

3.5.2.3 Characterization of isolates:

The presumptive *Escherichia coli* colonies were subjected to Gram's staining. The isolates which were Gram negative and having morphology of coccobacillary appearance were then checked for motility & further biochemical characterization, as per the method described by Cruikshank *et al.* (1975), Bergey's Manual of Systematic Bacteriology (1984) and Agarwal *et al*. (2003) (Appendix-I). The isolates were also characterized using Multiplex PCR technique employing genus and species specific primers (Azeem *et al.*2013).

3.5.3 *Listeriamonocytogenes* :

3.5.3.1Enrichment:

The one ml of the homogenized solution was added to 9 ml of the University of Vermont Medium (UVM-I) and kept for incubation at 37^0C for 18 to

24 hrs. Faecal samples were inoculated with enrichment media. After 18 to 24 hrs of incubation, one ml of the inoculum was transferred to 9 ml of the UVM-II and further incubated for 18 to 24 hrs at 37^0C.

3.5.3.2 Selective plating

From UVM II, a loopful of inoculum from was streaked onto sterile Polymixin Acriflavin Lithium chloride Ceftazidime Aesculin (PALCAM) agar plates and further kept for incubation at 37^0C. Samples which showed typical diffuse black zones of aesculin hydrolysis with greenish yellow colonies of about 0.5mm diameter were identified as listeriae. These suspected colonies of listriae were then inoculated on BHI agar slants for further confirmation.

3.5.3.3 Characterization of isolates:

The presumptive *Listeria* colonies were subjected to Gram's staining. The isolates which were Gram positive and showed morphology of coccobacillary appearance were subjected for motility (Low and Donachie,1997). Suspected colonies were inoculated in Brain Heart Infusion broth (BHI) and incubated at 25^0C for 12 to 18 hrs and observed for tumbling motility (hanging drop technique) under 40 X magnification of the microscope (Olympus, CX-31).

The isolates with tumbling motility were then further biochemically characterized, as per the method described by Cruikshank *et al.* (1975), Bergey's Manual of Systematic Bacteriology (1984) and Agarwal *et al.* (2003) (Appendix-I). The isolates were also identified using Multiplex PCR technique employing genus and species specific primers (Rawool, 2013).

3.5.4 *Salmonella* species.

3.5.4.1 Enrichment:

One ml of the homogenized solution was added to 9 ml of Buffered Peptone Water (BPW) and incubated at 37^0C for 18-24hrs. After pre-enrichment incubation in BPW, the inoculum was transferred to enrichment media i.e. Rappaport-Vassiliadis enrichment (RV) broth and further incubated at 42^0C for 24-48hrs.

3.5.4.2 Selective plating:

The enriched inoculums from RV were streaked onto Xylose Lysin Deoxycholate (XLD) agar and incubated at 37°C for 18-24hrs. The translucent colonies with typical black centre in case of H_2S producers and translucent red colonies in non H_2S producers on XLD agar were considered to be Salmonellae (OIE, 2010). The presumptive Salmonella colonies were further characterized biochemically as mentioned below.

3.5.4.3 Characterization of the isolates

The presumptive *Salmonella* colonies were subjected to Gram staining which revealed the organisms as Gram negative bacilli. Further, the Gram negative organisms were subjected to biochemical characterization, as suggested by Cruikshank *et al.* (1975), Bergey's Manual of Systemic Bacteriology (1984) and Agarwal *et al.* (2003), OIE (2010).

3.6 Biochemical Characterization of foodborne pathogenes:

3.6.1 Catalase test

The presumptive colonies of all four pathogenes viz. *Campyobacter*, *E. coli*, *Listeria* and Salmonella species were taken onto a clean glass slide separately with a sterile tooth pick and on each a drop of 3 per cent hydrogen peroxide (H_2O_2) was put. The prompt effervescence within a second was considered catalase positive.

3.6.2 Oxidase test

This reaction is due to cytochrome oxidase which catalyses oxidation of reduced cytochrome by oxygen. The suspected colonies of all the isolates (*Campylobacter* species, *E. coli*, *Salmonella* species and *Listeria monocytogenes*) were rubbed on the strip of filter paper soaked in freshly prepared solution of tetramethyl-p-phenylene-diamine (Hi-Media, Mumbai). Development of deep purple colour within 5-10 seconds were considered as oxidase positive reaction, while no coloration even after 60 seconds were considered as oxidase negative test.

3.6.3 Indole test

The test isolates were cultured in a medium containing tryptophan and incubated at 37^0C for 24-48hrs for *E. coli*, *Listeria*, *Salmonella* and for *Campylobacter* species at 42^0c for 48 hrs. The positive reaction was detected by addition of 0.5 ml Kovac's reagent containing p-dimethylaminobenzaldehyde which on gentle shaking produced a red colour ring in the surface layer within 10 min.

3.6.4 Methyl Red and Voges-Proskauer (MR-VP) tests

A sterilized solution of glucose phosphate peptone water (Hi Media, Mumbai) was prepared (Appendix-I) and transfer into separate sterile test tubes @ 5 ml/tubes. Loopful fresh growth of test culture suspected *E.coli*, *Listeria*, *Salmonella* and for *Campylobacter* species were added to this broth and incubated for 36-48 hrs at 37^0C& 42^0c as per the requirement of the organism.

For MR test, a drop of methyl red indicator was added into broth and observed for development of bright red colour which was considered as positive.

For VP test, to the overnight grown cultures, 0.2 ml of potassium hydroxide (40 per cent) and 0.6 ml of α-naphthol solution (5 per cent) were added and tube was shaken vigorously. The cotton plugs were then removed and left for an hour at room temperature. The development of pink red colour was considered as positive test.

3.6.5 Citrate utilization test

Citrate utilization test was conducted on the test organisms (*Salmonella* species, *Campylobacter* species and *E.coli*) by streaking and stabbing the slope and butt of the slants. Inoculated slants were incubated at 37^0C for 24-48hrs. Slants which turned from green to bright blue were recorded as positive isolates.

3.6.6 Nitrate Reduction Test

A loopful of the test culture was inoculated in sterile nitrate broth (Appendix-I) and incubated for 48 hrs at 37^0C. To the broth culture; 0.8 per cent sulphanilic acid and 0.6 per cent α-napthalamine (ratio 1:1) were added (0.1ml each) and observed for development of pink red colour due to the reduction of nitrate to nitrite and was considered as positive. All the presumptive isolates of *E.coli*, *Listeria*, *Salmonella* and for *Campylobacter* species were screened for nitrate reduction test.

3.6.7 Urease test

The suspected isolates of *E. coli*, *Listeria*, *Salmonella* and *Campylobacter* species were inoculated on to the urease agar slants by first streaking the slope and then stabing the butt and incubating at 37°C for 3-12hrs. The change in colour from yellow to bright pink was treated as positive.

3.6.8 Triple sugar iron (TSI) test

All the isolates of *Salmonella*, *Campylobacter* and *E.coli* were subjected to TSI test for confirmation of genus.TSI slants were first streaked on slope and then stabbed in butt. Inoculated slants were then incubated at 37°C for 24 hrs (42°C for 5-7 days). The slants showing pink slope, yellow butt and blackening in slant and the slants with pink slope, yellow butt and no blackening were considered as H_2S producer and non H_2S producer.

3.6.9 Malonate utilization test

The test isolates of Salmonella were cultured in a malonate-phenylalanine broth and incubated at 37°C for 24 hrs. The isolates which turned the green colour of the medium to deep blue were considered as positive for the test.

3.6.10 Hippurate Hydrolysis test

A loopful of the culture of *Campylobacter* was suspended in 1 ml of 1% hippuric acid (1% hippurate, pH 8.0). The suspension was mixed and incubated at 42°C for 4 hrs. Further, 0.5 ml of freshly prepared ninhydrin solution was added from side of the test tube and incubated further for 10 min at 42°C. Appearance of a deep purple blue colour ring at the top or colouration of the entire tube indicated the positive result.

3.6.11 Indoxyl Acetate hydrolysis

An absorbent filter paper disc was soaked with indoxyl acetate (10% w/v) and air dried Loopful growth of *Campylobacter* colonies from agar medium was applied on test disc and drop of sterile distilled water was added. Apperance of blue green colour within 5-10 min indicated positive result.

3.6.12 Rapid H_2S test

A large ball-like inoculum of *Campylobacter* colonies, enough to fill 5 mm diameter loop was gently suspended without mixing in the upper third of medium .The inoculated tubes were incubated at 37°C in a water bath for 4 hrs.Positive result was indicated by blackening reaction around the mass of inoculums.

3.6.13 Sugar Fermentation Pattern

All the presumptive isolates of *Campylobacter* species, *E. coli*, *Salmonella* and *Listeria monocytogenes* were subjected to standard carbohydrate fermentation tests. The isolates were grown on five ml sterile peptone solution containing 0.2 per cent bromocresol purple indicator and one per cent solution of the sugars separately and incubated at 37^0C for 36-48 hrs(42°C for 36-48 hrs under microaerophilic condition). The change in colour of broth from purple to yellow was considered as a positive test as a result of carbohydrate fermentation. However variation if any was also recorded.

The standard biochemical and sugar fermentation pattern exhibited by all four organisms are mentioned in Table 2,3,4,5 & 6

Table 2. Standard biochemical characterization of *Campylobacter spp*

Sr. No	Characteristics	Reaction
1	Gram’s staining	-ve
2	Motility	+ve
3	Catalase	+ ve
4	Oxidase	+ ve
4	Indole	-ve
5	Methyl red	+ve
6	Voges Proskauer test	-ve
7	Citrate	+ve

8	Nitrate	+ve
9	Urease	+ ve
10	Glucose utilization	–ve
11	Indoxyl acetate hydrolysis	+ve
12	TSI	+ve (pink slope, yellow butt and H2S production indicated by blackening). Non-H2S producers have also been documented.

Table 3. Differentiation of *Campylobacter* upto species level

Sr.No	**Species Characteristics**	***C. jejuni***	***C. coli***	***C. lari***	***C. fetus***
1	Growth @ 25°C	–ve	–ve	–ve	+ve
2	Growth @ 42°C	+ve	–ve	–ve	–ve
3	Hippurate hydrolysis	+ve	–ve	–ve	–ve
4	Sensitivity to Nalidixic Acid (30 ug disc)	S	S	R	NR
5	Sensitivity to Cephalothin (30 ug disc)	R	R	R	S
6	Rapid H_2S in test	+ve	–ve	+ve	V

(R: Resistant, S: Sensitivity, NR: Non-Resistant)

Table 4. Standard biochemical characterization of *Escherichia coli* species

Sr.No	**Characteristics**	**Reaction**
1	Gram's staining	–ve
2	Catalase	+ ve
3	Oxidase	–Ve
4	Acid and gas from carbohydrates	–ve
	Lactose	–ve
	Sucrose	–ve
	Salicin	–ve
	Adonitol	+ve
	Glucose	+ve
	Dulcitol	±ve

	Inositol Sorbitol	+ve
5	Indole	+ve
6	Methyl red	+ve
7	Voges Proskauer test	–ve
8	Citrate	–ve
9	Nitrate	+ve
10	Urease	–ve
11	TSI	+ve (pink slope, yellow butt, Acid and gas production).

Table 5. Standard biochemical characterization of *Listeria* species

Sr. No.	**Test**	***Listeria monocytogenes***	***Listeria ivanovii***	***Listeria innocua***	***Listeria seeligeri***
1	Catalase	+	+	+	+
2	Oxidase	-	-	-	-
3	Methyl Red	+	+	+	+
4	Voges-Proskuer	+	+	+	+
5	Nitrate Reduction	-	-	-	-
6	Acid from Sugars a) L-Rhamnose	+	-	-	-
	b) D-xylose	-	+	-	+
	c) α-Methyl-d-Mannoside	+	-	+	-

Table 6. Standard biochemical characterization of *Salmonella* species

Sr.No	Characteristics	Reaction
1	Gram's staining	-ve
2	Catalase	+ve
3	Oxidase	–ve
4	Acid and gas from carbohydrates	
	Lactose	-ve
	Sucrose	–ve
	Salicin	–ve
	Adonitol	–ve
	Glucose	+ve
	Dulcitol	+ve
	Inositol	±ve
	Sorbitol	+ve
5	Methyl red	+ve
6	Voges Proskauer test	-ve
7	Indole	-ve
8	Citrate	+ve
9	Nitrate	+ve
10	Urease	-ve
11	Malonate utilization	-ve
12	TSI	+ve (pink slope, yellow butt and H2S production indicated by blackening). Non-H2S producers have also been documented.

3.7 Molecular characterization of Foodborne pathogenes:

3.7.1 Preparation of DNA template from cultural positive isolates

Each organism was grown in BHI broth overnight at 37^0C

↓

Checked for typical motility and purity by Gram staining

↓

Centrifuged at 2000 rpm for 15 minutes

↓

Discarded the supernatant and collected pellet

↓

Suspended the pellet in sterile mili-Q water and centrifuged at 2000 rpm for 15 min (twice)

↓

Discarded supernatant and collected milky white pellet

↓

Added 100 µl of mili-Q water to get DNA template

↓

Processed for PCR as per targeted gene.

3.7.2 Preparation of DNA template from blood samples

400ul of blood sample was taken in 1.5 ml eppendorf tube

↓

1ml RBC buffer was mixed in each tube

↓

Contents were properly mixed and centrifuged at 10000 rpm for 3 minutes

↓

The supernatant was discarded

↓

The pellet was suspended in 750 µl of RBC lysis buffer

↓

Contents were properly mixed and centrifuged at 10000 rpm for 3 minutes

↓

The supernatant was discarded and the procedure was repeated until the pellet was clear of haemoglobin

↓

A clear pellet was resuspended in 100 µl of PCR buffer (KTT-20) containing 100 µg proteinase-K per ml

↓

The tube was then incubated in a water bath at 56^{o}C for 2 hours and then stored at -20^{0}c till further use

3.7.3 *Campylobacter* species:

3.7.3.1 Primers:

The details of the primers employed for the characterization of the genus level *16S rRNA*and species level (*hipO* gene and *aspK* gene) are given in Table 7.

Table 7. Details of Primers for PCR

Target Gene	Primer	Sequence (5' to 3')	Base Pair
16S rRNA (*Campylobacter* genus specific)	*C412F*	GGATGACACTTTTCGGAGC	816
	C1228R	CATTGTAGCACGTGTGTC	
hipO (*C. jejuni*)	*hipO F*	GGAGAGGGTTTGGGTGGT	735
	hipO R	AGCTAGCCTCGCATAATAACTTG	
aspK (*C. coli*)	*aspK F*	GGTATGATTTCTACAAAGCGAG	500
	aspK R	ATAAAAGACTATCGTCGCGTG	

The PCR was set for 25 µL reaction volume for the detection of *16s rRNA* gene with following contents and conditions are described in Table no 8.

Table 8. Contents for *16S rRNACampylobacter* genus specific PCR

Contents		Volume (25 µl)
Mili-Q water sterile		10.5
2X Go Taq master mix		12.5
16S rRNA	Forward Primer (10 pmol/ µl)	0.5
	Reverse Primer (10pmol/ µl)	0.5
DNA Template		1
	Total	**25 µl**

The PCR was set for 25 µL reaction volume for the detection of *hipO* gene and conditions are described in Table 9.

Table 9. Ingredients for PCR confirmation of *hipO* gene of *Campylobacter jejunii*

Contents		Volume (25 µl)
Mili-Q water sterile		10.5
2X Go Taq master mix		12.5
hipO (C. jejuni)	Forward Primer (10 pmol/ µl)	0.5
	Reverse Primer (10pmol/ µl)	0.5
DNA Template		1
	Total	**25**

The PCR was set for 25 µL reaction volume for the detection of *aspK* gene The PCR conditions are illustrated in Table 10.

Table 10. Contents for PCR confirmation of *aspK* gene of *Campylobacter coli*

Contents		Volume (25 µl)
Mili-Q water sterile		10.5
2X Go Taq master mix		12.5
aspK (C. coli)	Forward Primer (10 pmol/ µl)	0.5
	Reverse Primer (10pmol/ µl)	0.5
DNA Template		1
	Total	**25**

3.7.3.2: Conditions for optimization:

PCR tube (0.2 ml) containing the reaction mixture was flash spun in a spinner (Labnet, Korea).The reaction was performed in a thermal cycler (ABS, USA) with a pre-heated lid.

Table11. Cycling conditions for genus specific *16s rRNA* PCR

Stage		**Temperature (^{0}C)**	**Time (min)**	
Stage 1	Initial denaturation	95	5:00	1 cycle
Stage 2	Denaturation	95	0:30	35 cycles
	Annealing	53	0:30	1 cycle
	Extension	72	0:30	
Stage 3	Final extension	72	10:00	
Stage 4	Holding	4	∞	

Table 12. Cycling conditions for species specific (*hipO*) PCR

Stage		**Temperature (^{0}C)**	**Time (min)**	
Stage 1	Initial denaturation	95	5:00	1 cycle
Stage 2	Denaturation	95	0:45	35 cycles
	Annealing	51	0:45	1 cycle
	Extension	72	0:45	
Stage 3	Final extension	72	7:00	
Stage 4	Holding	4	∞	

Table 13. Cycling conditions for species specific (*aspK*) PCR

Stage		**Temperature (^{0}C)**	**Time (min)**	
Stage 1	Initial denaturation	95	5:00	1 cycle
Stage 2	Denaturation	95	0:30	35 cycles
	Annealing	53	0:30	
	Extension	72	0:30	1 cycle
Stage 3	Final extension	72	7:00	
Stage 4	Holding	4	∞	

3.7.4 *Escherichia coli:*

3.7.4.1 Primers:

The details of the primers employed for the characterization of the species specific *uidA* gene are given in Table 14.

Table 14. Details of Primers for Conventional PCR for *uidA* gene of *E.coli*

Target Gene	**Primer**	**Sequence (5' to 3')**	**Base Pair**
uidA (Species specific *Escherichia coli*)	*F*	CCAAAAGCCAGACAGAGT	623
	R	GCACAGCACATCAAAGAG	

The PCR was set for 25 µL reaction volume for the detection of *uidA* gene with following contents given in table 15.

Table 15: Contents forPCR confirmation of *uidA* gene of *E.coli*

Contents		Volume (25 µl)
Mili-Q water sterile		3.5
2X Go Taq master mix		12.5
uidA (species specific *E .coli)*	Forward Primer (10 pmol/ µl)	1
	Reverse Primer (10pmol/ µl)	1
DNA Template		7
	Total	**25**

PCR tube (0.2 ml) containing the reaction mixture was flash spun in a spinner (Labnet, Korea).The reaction was performed in a thermal cycler (ABS, USA) with a pre-heated lid.

3.7.4.2 Conditions for optimization:

Table 16. Cycling conditions for Species specific (*uidA* gene) PCR

Stage		Temperature (^{0}C)	Time (min)	
Stage 1	Initial denaturation	94	3:00	1 cycle
Stage 2	Denaturation	94	0:40	30 cycles
	Annealing	52	0:30	
	Extension	72	1:00	1 cycle
Stage 3	Final extension	72	10:00	
Stage 4	Holding	4	∞	

3.7.5 *Listeria monocytogenes*:

All isolates of *Listeria* were further processed for molecular identification employing genus and species specific primers as per the method of Rawool (2013) with slight modifications.

3.7.5.1 Primers

The details of the primers employed for the characterization of the species and genus level are given in Table 17.

Table 17. Details of Primers for Conventional PCR for identification of *Listeria monocytogenes*

Target Gene	Primer	Sequence (5' to 3')	Base Pair
prs A (Genus specific)	*prs F*	CTAGCTGAAGAGATTGCGAAAGA	370
	prs R	CCAAGAAGAGCTGCAACAGATA	370
Isp (Species specific)	*isp F*	TGCAGCGAATGCTCTTAGT	711
	isp R	CCAAGCACGGCTACTTTAATC	711

The PCR was set for 25 µL reaction volume for the detection of *prs A* gene of *Listeria* and following contents were used for optimization with detailed conditions given in Table 18 and 20.

Table 18. Details of contents for PCR confirmation of *prs* gene of *Listeria*

Contents		Volume (25 µl)
Mili-Q water sterile		10.5
2X Go Taq master mix		12.5
prs	Forward Primer (10 pmol/ µl)	0.5
	Reverse Primer (10pmol/ µl)	0.5
	DNA Template	1
	Total	**25**

The PCR was set for 25 µL reaction volume for the detection of *Isp* gene of *Listeria* and following contents were used for optimization with detailed conditions given in Table 19 and 21.

Table 19. Details of contents for PCR confirmation of *Isp* gene of *Listeria monocytogenes*

contents		Volume (25 µl)
Mili-Q water sterile		10.5
2X Go Taq master mix		12.5
Isp	Forward Primer (10 pmol/ µl)	0.5
	Reverse Primer (10pmol/ µl)	0.5
	DNA Template	1
	Total	**25**

Table 20. Cycling conditions for PCR confirmation of *prs* gene of *Listeria*

Stage		**Temperature (^0C)**	**Time (min)**	
Stage 1	Initial denaturation	95	5:00	1 cycle
Stage 2	Denaturation	95	0:30	40 cycles
	Annealing	53	1:00	
	Extension	72	2:00	1 cycle
Stage 3	Final extension	72	7:00	
Stage 4	Holding	4	∞	

Table 21. Cycling conditions for PCR confirmation of *lsp* gene of *Listeria monocytogenes*

Stage		**Temperature (^0C)**	**Time (min)**	
Stage 1	Initial denaturation	95	5:00	1 cycle
Stage 2	Denaturation	95	0:30	40 cycles
	Annealing	53	1:00	
	Extension	72	2:00	1 cycle
Stage 3	Final extension	72	7:00	
Stage 4	Holding	4	∞	

PCR tube (0.2 ml) containing the reaction mixture was flash spun in a spinner (Labnet, Korea).The reaction was performed in a thermal cycler (ABS, USA) with a pre-heated lid.

3.7.6*Salmonella species*

3.7.6.1 Primers:

The details of the primers employed for the characterization of the species specific *invA* gene are given in Table 22.

Table 22. Details of Primers for Conventional PCRfor *invA* gene of *Salmonella* species

Target Gene	Primer	Sequence (5' to 3')	Base Pair
invA (Salmonella genus and virulent gene)	*F*	GTG AAA TTA TCG CCA CGT TCG GGC AA	284
	R	TCA TCG CAC CGT CAA AGG AAC C	

The PCR was set for 25 µL reaction volume for the detection of *invA* gene of *Salmonella* following contents were used for optimization with detailed conditions given in Table 23 and 24.

Table 23 Details of contents for PCR confirmation of *invA* gene of *Salmonella* species

Contents		Volume (25 µl)
Mili-Q water sterile		10.5
2X Go Taq master mix		12.5
invA (Salmonella genus and virulent gene*)*	Forward Primer (10 pmol/ µl)	0.5
	Reverse Primer (10pmol/ µl)	0.5
DNA Template		1
	Total	**25**

Table 24. Cycling conditions for PCR confirmation of *invA* gene of *Salmonella* species

Stage		**Temperature (^{0}C)**	**Time (min)**	
Stage 1	Initial denaturation	94	2:00	1 cycle
Stage 2	Denaturation	94	0:30	30 cycles
	Annealing	65	1:00	
	Extension	72	2:00	1 cycle
Stage 3	Final extension	72	5:00	
Stage 4	Holding	4	∞	

PCR tube (0.2 ml) containing the reaction mixture was flash spun in spinner (Labnet, Korea).The reaction was performed in a thermal cycler (ABS, USA) with a pre-heated lid.

3.7.7 Agarose Gel Electrophoresis

In order to visualize the PCR product of each isolate, horizontal gel electrophoresis (BIORAD, USA) was used. The gel casting tray was placed on a levelled surface and gel comb was placed across the gel casting tray, so that the tooth of the comb remained 1 mm above the base of the tray. Agarose gel (1%) was prepared by boiling molecular biology grade Agarose (SRL) in 0.5X Tris-Borate-EDTA (TBE) buffer to dissolve it completely. After cooling to about 500C, ethidium bromide was added to the agarose solution to a final concentration of 0.5 µg/mL. The molten agarose was then poured onto the gel-casting tray and was kept undisturbed for about an hour to solidify. After the gel solidification, the comb was removed. The set gel with the gel casting tray was then merged in the electrophoresis tank with the wells at the cathode end of the tank with sufficient quantity (about 1 mm level) of electrophoresis buffer (TBE, 0.5 X) above the surface of the gel. About 10 µl of PCR product was mixed with 2 µl of Bromophenol blue gel loading dye (6X) and loaded into the well. Simultaneously

100 bp DNA ladder (Bangalore GeNei, India) as molecular weight marker was also loaded in one of the wells. Electrophoresis was performed at 60 volts and the progress of mobility was monitored by the migration of dye front. At the end of electrophoresis the gel was visualized under Gel Documentation System (BIORAD), for the amplicons of base pairs.

3.8 *In-vitro* pathogenicity tests

For pathogenecity assessment of foodborne pathogenes (*Campylobacter* species, *E.coli*, *Listeria monocytogenes*&*Salmonella* species) different *in-vitro* pathogenicity test *viz*; haemolysin production test, congo red dye binding assay, CAMP test ,demonstration of PI-PLC activity, were performed as per the standerd protocols suggested by Cruickshank *et al*. (1975). Similarly all the biochemically and molecurlarly confirmed isolates were also screened for specific virulent genes of the respective organisms.

3.8.1 Haemolysis on Sheep blood agar (SBA)

Detection of Haemolysin production was conducted on seven per cent sheep blood agar (SBA) as per Cruickshank *et al.* (1975). Defibrinated sheep blood was used for preparation of seven per cent sheep blood agar plates. Freshly grown broth cultures of each isolates (*Campylobacter* spp., *E.coli*, *Listeria monocytogenes* and *Salmonella* spp.) were streaked onto the blood agar plates and incubated at 37°C for 24-48 hrs except for *Campylobacter* spp.(42°C for 24-48 hrs in microaerophilic condition using Campy Gene packs). Zone of haemolysis around the colonies were identified as α-haemolysis and accordingly the isolates were designated as pathogenic.

3.8.2Congo red dye binding assay:

The ability of *E.coli* and *Salmonella* species isolates to bind Congo red (CR) dye was evaluated by streaking freshly grown culture onto the Congo Red BHI Agar plates (0.003 percent) and incubating at 37°C for 24-48 hrs. The positive results were indicated by formation of typical brick red colonies. The evaluation of the pathogenesity as +++, ++ and + was done based on intensity of brick red colour development in colonies. (Cruickshank *et al.,* 1975).

3.8.3 Christie, Atkins, Munch- Petersen (CAMP) Test

All the *Listeria* isolates were tested by CAMP test as per the method of ISO (1996). The standard strains of *Staphylococcus aureus* (MTCC 3160) *Rhodococcus equi* (MTCC1135) were streaked onto freshly prepared 5% SBA plates in such a manner that the streaks of *S. aureus* and *R. equi* were wide apart and parallel to each other. In between the two parallel streaks of *S. aureus* and *R. equi*, the *Listeria* isolates were streaked at 90degree angle leaving 2 to 3 mm apart. The plates were incubated at 37°C for 24 h and examined for enhancement of the haemolytic zone from partial hemolysis to a wider zone of complete haemolysis.The synergistic effect of the haemolysis; if any, between a *Listeria* strain and the *S. aureus* or *R. equi* strain were considered as CAMP-positive reaction.

The *Listeria* isolates with CAMP-positivity against *S. aureus* were characterized as *L. monocytogenes* giving a spade shaped haemolytic zone and those with CAMP positivity against *R. equi* would be characterized as *L. ivanovii*. The isolates showing typical character of *Listeria* spp. were differentiated using haemolysis and CAMP.

3.8.4Phosphatidylinositol–specific Phospholipase C (PI-PLC) assay

Phosphatidylinositol-specific phospholipase C (PI-PLC) based assay has been reported to be more reliable indicator to discriminate pathogenic and non-pathogenic *Listeria* species (Notermans *et al.* 1991).

All biochemically characterized *Listeria* isolates were screened for PI-PLC activity as per the method by Yadav *et al*. (2010). The *Listeria* isolates were grown overnight onto 7% SBA plates at 37^0C. All *Listeria* isolates were streaked on L. mono differential agar (Hi Media Ltd, Mumbai, India) in order to assess PI-PLC activity. The inoculated plates were incubated at 37^0C in a humidified chamber for 24 hrs. The isolate showing light blue colonies at the inoculation site was considered as a positive for PI-PLC production.

3.8.5 Detection of virulent gene *hcp*+ and *gltA* gene for *Campylobacter*

Amplification of *hcp*+ and *gltA* gene in *Campylobacter* species is considered as virulent gene. All the samples of Campylobacter species were processed for detection of *hcp*+ and *gltA* gene as per the method suggested by Corcionivoschi *et al.* (2015) with modifications.

3.8.5.1 Preparation of DNA template

The DNA template was prepared as per the section 3.7.1

3.8.5.2 Primers:

The details of the primers employed for the characterization of the virulent gene *hcp*+ and *gltA* gene are given in Table 25.

Table 25. Details of Primers for Conventional PCR of *Campylobacter* species

Target Gene	Primer	Sequence (5' to 3')	Base Pair
hcp+ (*Campylobacter spp*.virulent gene)	F	CAAGCGGTGCATCTACTGAA	463
	R	TAAGCTTTGCCCTCTCTCCA	
glt A (*Campylobacter spp*.virulent gene)	F	GCCCAAAGCCCATCAAGCGGA	142
	R	GCGCTTTGGGGTCATGCACA	

3.8.5.3 Contents for Optimization of *hcp*+ gene

The PCR was set for 25 µL reaction volume for the detection of *hcp*+gene using following contents and detailed cyclic conditions in Table 26 and 27.

Table 26.Details of contents for PCR confirmation of *hcp+* gene of *Campylobacter* species

Contents		Volume (25 µl)
Mili-Q water sterile		10.5
2X Go Taq master mix		12.5
hcp+ (*Campylobacter spp*.virulent gene	Forward Primer (10 pmol/ µl)	0.5
	Reverse Primer (10pmol/ µl)	0.5
DNA Template		1
	Total	**25**

Table 27. Cycling conditions for PCR confirmation of *hcp+* gene of *Campylobacter* species

Stage		Temperature (^{0}C)	Time (min)	
Stage 1	Initial denaturation	95	5:00	1 cycle
Stage 2	Denaturation	95	0:30	35 cycles
	Annealing	55	0:30	
	Extension	72	0:30	1 cycle
Stage 3	Final extension	72	10:00	
Stage 4	Holding	4	∞	

The details of the primers employed for the characterization of the virulent gene *glt A* gene are given in Table 25.

3.8.5.4 Ingredients for Optimization of *gltA* gene

The PCR was set for 25 µL reaction volume for the detection of *glt A* gene using following contents and cyclic condition as mentioned in table 28 and 29.

Table 28.Details of contents for PCR confirmation of *glt A* gene of *Campylobacter* species

Contents		**Volume (25 µl)**
Mili-Q water sterile		10.5
2X Go Taq master mix		12.5
glt A (*Campylobacter spp.*)virulent gene	Forward Primer (10 pmol/ µl)	0.5
	Reverse Primer (10pmol/ µl)	0.5
DNA Template		1
	Total	**25**

Table 29. Cycling conditions for PCR confirmation of *glt A* gene of *Campylobacter* species

Stage		**Temperature (^{0}C)**	**Time (min)**	
Stage 1	Initial denaturation	95	5:00	1 cycle
Stage 2	Denaturation	95	0:30	35 cycles
	Annealing	60	0:30	
	Extension	72	0:30	
Stage 3	Final extension	72	10:00	1 cycle
Stage 4	Holding	4	∞	

PCR tube (0.2 ml) containing the reaction mixture was flash spun in a spinner (Labnet, Korea).The reaction was performed in a thermal cycler (ABS, USA) with a pre-heated lid.

3.8.5.5Agarose Gel Electrophoresis:

The PCR product were visualized on 1% agarose gel as detailed in 3.7.7

3.8.6. Detection of virulent *stx1* and *stx2* gene for *E.coli*

All the isolates of *E.coli* were processed for detection of stx1 and stx2 gene as per the protocol suggested by Azeem *et al.* (2013) and Dhanashree *et al.* (2008).

3.8.6.1 Preparation of DNA template

The DNA template was prepared as per the section 3.7.1

3.8.6.2 Primers:

The details of the primers employed for the characterization of the virulent gene *stx 1 and stx 2* are given in Table 30.

Table 30. Details of Primers for Multiplex PCR of *stx1* and *stx2* genes of *E.coli*

Target Gene	Primer	Sequence (5' to 3')	Base Pair
stx 1	*F*	ACACTGGATGATCTCAGTGG	614
	R	CTGAATCCCCCTCCATTATG	
stx 2	*F*	CCATGACAACGGACAGCAGTT	779
	R	CCTGTCAACTGAGCAGCACTTTG	

3.8.6.3 Ingredients for Optimization of *stx1* and *stx2* genes of *E.coli*

The PCR was set for 25 µL reaction volume for the detection of *stx 1* gene and following ingredients and conditions as given in table 31 & 32.

Table 31. Details of ingredients for PCR confirmation of *stx1* genes of *E. coli* :

Ingredients		Volume (25 µl)
Mili-Q water sterile		8.5
2X Go Taq green master mix		12.5
Stx 1	Forward Primer (10 pmol/ µl)	1
	Reverse Primer (10pmol/ µl)	1
DNA Template		2
	Total	**25**

Table 32. Cycling conditions for virulent *stx 1* gene of *E. coli* :

Stage		Temperature (^{0}C)	Time (min)	
Stage 1	Initial denaturation	95	5:00	1 cycle
Stage 2	Denaturation	95	1:00	35 cycles
	Annealing	54	1:30	1 cycle
	Extension	72	1:30	
Stage 3	Final extension	72	5:00	
Stage 4	Holding	4	∞	

PCR tube (0.2 ml) containing the reaction mixture was flash spun in a spinner (Labnet, Korea).The reaction was performed in a thermal cycler (ABS, USA) with a pre-heated lid.

The PCR was set for 25 µL reaction volume for the detection of *stx 2* gene and following ingredients and conditions as given in table 33 & 34.

Table 33. Details of ingredients for PCR confirmation of *stx2* genes of *E. coli*:

Ingredients		**Volume (25 µl)**
Mili-Q water sterile		8.5
2X Go Taq green master mix		12.5
Stx 2	Forward Primer (10 pmol/ µl)	1
	Reverse Primer (10pmol/ µl)	1
DNA Template		2
	Total	**25**

Table 34. Cycling conditions for virulent *stx 2* gene of *E. coli* :

Stage		**Temperature (^{0}C)**	**Time (min)**	
Stage 1	Initial denaturation	95	5:00	1 cycle
Stage 2	Denaturation	95	1:00	35 cycles
	Annealing	58	1:30	
	Extension	72	1:30	1 cycle
Stage 3	Final extension	72	5:00	
Stage 4	Holding	4	∞	

3.8.6.4Agarose Gel Electrophoresis:

The PCR product were visualized on 1% agarose gel as detailed in 3.7.7

3.8.7 Detection of virulent *hlyA* gene for *Listeria monocytogenes*

All the isolates of *Listeria* were processed for detection of *hlyA* gene as suggested by Domanska *et al*. (1999).

3.8.7.1 Preparation of DNA template Primers

The DNA template was prepared as per the section 3.7.1

3.8.7.2 Primers

The details of the primers employed for the characterization of the virulent gene *hlyA* are given in Table 35.

Table 35 Details of primers used for assessment of *hly*A genes of *L. monocytogenes* isolates

Primer name	Primer Sequence (5′ to 3′)	Size	Reference
hly A	Forward: GCA GTT GCA AGC GCT TGG AGT GAA	456bp	(Domanska *et al.* 1999)
	Reverse: GCA ACG TAT CCT CCA GAG TGA TCG		

3.8.7.3 Contents for Optimization of *hlyA* genes of *Listeria monocytogenes*

The PCR was set for 25 µL reaction volume for the detection of *hlyA* gene with following ingredients and conditions as given in table 36 & 37.

Table 36 Details of contents for PCR confirmation of *hlyA* genes of *Listeria monocytogenes*

Contents	Volume (µl)
Mili-Q water sterile	8.5
2X PCR Buffer	12.5
Forward Primer (20 pmol/ µl)	1
Reverse Primer (20 pmol/ µl)	1
DNA Template	2
Total	**25**

Table 37. Details of programme for *hlyA* gene of *Listeria monocytogenes*

Stage		**Temperature (^{0}C)**	**Time (min)**	
Stage 1	Initial denaturation	95	5:00	1 cycle
Stage 2	Denaturation	95	0:40	35 cycles
	Annealing	53	1:00	
	Extension	72	2.00	1 cycle
Stage 3	Final extension	72	10:00	
Stage 4	Holding	4	∞	

PCR tube (0.2 ml) containing the reaction mixture was flash spun in a cooling centrifuge (REMI, C-24). The reaction was performed in a thermal cycler (ABS, USA) with a pre-heated lid.

3.8.7.4Agarose Gel Electrophoresis:

The PCR product were visualized on 1% agarose gel as detailed in 3.7.7

3.8.8Detection of virulent *invA* gene for *Salmonella* species

All isolates of *Salmonella* species were processed for detection of virulent *invA* gene. Amplification of *invA* gene is taken as international standard for detection of Salmonella genus (Malorny *et al.* 2003). Further it is also known to contribute virulence to Salmonellae (Jamshidi *et al.* 2009). All the isolates of Salmonella species were confirmed for virulence as mentioned above in 3.7.5.

3.9 Antibiogram study of foodborne isolates

Antibiotic sensitivity ofisolates to various antibiotics and chemotherapeutic agents was studied by agar disc diffusion method using single antibiotic disc (Bauer *et al.* 1966). The selection of antibiotic was based on the routinely used antibiotic in the field.

3.9.1 Test proper

The overnight grown cultures of respectiveisolates in BHI broth were spread evenly on MHA agar plates by using sterile swabs or L shape loop spreader. The antibiotic disc were placed with the help of sterile forceps and plates were incubated at 37^0C 24 hrs for *E. coli*, *Listeria monocytogenes* and *Salmonella* whereas for C*ampylobacter* spp at 42^0c for 48 hrs. The characterization of strains as sensitive, moderately sensitive and resistant was based on the size of zones of inhibition around each disc according to manufacturer's instruction (Hi-Media, Mumbai).Details of the antibiotics disc are given in following tables with their concentration (mcg/ml).

3.9.1.1 *Campylobacter* species*:*

Table 38. Details of Antibiotics used for *Campylobacter* species with concentration in µg (mcg) per disc

Sr. No.	Antibiotics	Abbreviation	Concentrations (mcg/ unit)
1	Ampicillin	A	10
2	Ciproflaxacin	Ce	30
3	Erythromycin	E	15
4	Gentamicin	G	30
5	Norfloxacin	Nx	10
6	Tetracycline	TE	30
7	Nalidixic acid	NA	30

3.9.1.2 *Escherichia coli:*

Table 39. Details of Antibiotics used for *E.coli* with concentration in µg (mcg) per disc

Sr. No.	Antibiotics	Abbreviation	Concentrations (mcg/ unit)
1	Ampicillin	A	10
2	Amikacin	Ak	30
3	Azithromycin	At	30
4	Ceftazidime	CE	10
5	Cephotaxime	CTX	10
6	Erythromycin	E	15
7	Doxycycline	Do	10
8	Gentamicin	G	30
9	Neomycin	N	10
10	Nalidixic acid	NA	30
11	Penicillin-G	P	10
12	Streptomycin	S	30
13	Tetracycline	TE	30
14	Trimethoprim	TR	10

3.9.1.3 *Listeria monocytogenes:*

Table 40. Details of Antibiotics used for *Listeria monocytogenes* with concentration in µg (mcg) per disc

Sr. No.	Antibiotics	Abbreviation	Concentrations (mcg/ disc)
1	Ampicillin	A	10
2	Cephotaxime	Ce	10
3	Cloxacillin	Cx	30
4	Erythromycin	E	15
5	Gentamicin	G	30
6	Kanamycin	K	30
7	Vancomycin	VA	30
8	Norfloxacin	Nx	10
9	Penicillin-G	P	10 units
10	Rifampicin	R	5
11	Oxytetracycline	O	30
12	Ceftriaxone	CTR	30

3.9.1.4 *Salmonella species:*

Table 41. Details of Antibiotics used for *Salmonella* species with concentration in µg (mcg) per disc

Sr. No.	Antibiotics	Abbreviation	Concentrations (mcg/ unit)
1	Amikacin	AK	10
2	Ampicillin	A	30
3	Gentamicin	G	30
4	Kanamycin	K	30
5	Trimethoprim	TR	10

3.10 Minimum Inhibitory Concentration (MIC)

The MIC, or minimum inhibitory concentration, is the lowest concentration (in µg/ml) of an antibiotic that inhibits the growth of a given strain of bacteria. A quantitative method of susceptibility testing, an MIC helps determine which class of antibiotic is most effective. This information can lead to an appropriate choice of an antibiotic that will increase chances of treatment success and help in the fight to slow antibiotic resistance.

3.10.1 Test Proper

All the confirmed isolates were inoculted in BHI broth and then respective isolates were spreaded on MHA plates with the help of sterile swabs. Then with the help of sterile forceps the MIC strips of each antibiotics were placed and plates were incubated at 37^{0}C 24 hrs for *E. coli*, *Listeria monocytogenes* and *Salmonella* whereas for c*ampylobacter* spp at 42^{0}c for 48 hrs. Reading was directly noted from the scale in terms of µg/mL at the point where the edge of the inhibition ellipse intersects the strip.

3.10.1.1 *Campylobacter species:*

Table 42. Details of MIC strips with concentration in µg (mcg/ml) per strips

Sr No	Antibiotic strips	Symbol	RANGE(CONC-mcg/ml)
1	Ampicillin	A	0.016 – 256
2	Ciproflaxacin	Ce	0.016 – 256
3	Erythromycin	E	0.016 – 256
4	Gentamicin	G	0.016 – 256
5	Norfloxacin	Nx	0.016 – 256
6	Tetracycline	TE	0.016 – 256
7	Nalidixic acid	NA	0.016 – 256

3.10.1.2 *Escherichia coli:*

Table 43. Details of MIC strips with concentration in µg (mcg/ml) per strips

Sr No	Antibiotic strips	Symbol	RANGE(CONC-mcg/ml)
1	Ampicillin	A	0.016 – 256
2	Amikacin	Ak	0.016 – 256
3	Azithromycin	At	0.016 – 256
4	Ceftazidime	CE	0.016 – 256
5	Cephotaxime	CTX	0.016 – 256
6	Erythromycin	E	: 0.016 – 256
7	Doxycycline	Do	0.016 – 256
8.	Gentamicin	G	0.016 – 256
9.	Neomycin	N	0.016 – 256
10.	Nalidixic acid	NA	0.016 – 256
11.	Penicillin-G	P	0.016 – 256
12.	Streptomycin	S	0.016 – 256
13	Tetracycline	TE	0.016 – 256
14	Trimethoprim	TR	0.016 – 256

3.10.1.3 *Listeria monocytogens:*

Table 44. Details of MIC strips with concentration in µg (mcg/ml) per strips

Sr No	Antibiotic strips	Symbol	RANGE(CONC-mcg/ml)
1	Ampicillin	AMP	0.016 – 256
2	Ceftriaxone	CTR	0.002 – 32
3	Cefotaxime	CTX	0.002 – 32
4	Cefotetan / Cefotetan + Cloxacillin	CTN	0.5 – 32
5	Erythromycin	ERY	0.016 – 256
6	Gentamicin	GEN	: 0.016 – 256
7	Kanamycin	KAN	0.016 – 256
8.	Norfloxacin	NOR	0.016 – 256
9.	Tetracycline	TET	0.016 – 256
10.	Penicillin	PEN	0.016 – 256
11.	Rifampicin	RIF	0.002 – 32
12.	Vancomycin	VAN	0.016 – 256

3.10.1.4 *Salmonella* species*:*

Table 45. Details of MIC strips with concentration in µg (mcg/ml) per strips

Sr No	Antibiotic strips	Symbol	RANGE(CONC-mcg/ml)
1	Ampicillin	AMP	0.016 – 256
2	Amikacin	AK	0.016 – 256
3	Gentamicin	GEN	0.016 – 256
4	Kanamycin	KAN	0.016 – 256
5	Trimethoprim	TR	0.016 – 256

RESULTS AND DISCUSSION

4.1 Isolation and Identification of Foodborne pathogens in pig and poultry:

In present investigation, a total of 300 samples from pigs and poultry comprising of meat, faecal, blood (50 each from both species) collected from different retail slaughter shops in and around Nagpur city were included. Meat and faecal samples were processed for cultural isolation whereas blood sample were directly processed for DNA extraction for molecular identification.

4.1.1 *Campylobacter* species

Isolation and identification of *Campylobacter* spp. was carried out as per standard protocol described by OIE (2008), Aradwad (2005), Pallavi and Kumar (2014). The fecal and meat samples from both the species were processed for isolation and identification of *Campylobacter* species employing Preston Enrichment Broth and mCCDA Selective Plating under microaerophilic environment attained by CampyPack gas generating packs (Oxoid). The plates showing typical grey, flat, moist type of colonies with metallic sheen (ceramic type) were selected (Plate 1) and the typical colonies were pickup and subjected to biochemical and sugar fermentation test for identification of *Campylobacter* spp. The characterization of the *Campylobacter* isolates up to species level was carried out by hippurate hydrolysis, indoxyl acetate hydrolysis, sensitivity to nalidixic acid and cephalothin discs (Table 46).

Of the total 100 meat and fecal samples of pigs processed for isolation and identification of *Campylobacter* spp., 6 and 5 samples showed typical grey, flat, moist type colonies with metallic sheen on mCCDA from pork and feces respectively. Gram's staining of these isolates showed 10 isolates (5 each from pork & faecal) with Gram negative rods. On subsequent motility test three from pork, five from faecal exhibited typical cork screw, darting type motility at 42^0C. Three isolates of pork and five of pig faecal showed catalase and oxidase positive. Three isolates of pork and two isolates of faecal showed IMViC pattern as (-,+,-,+). Three isolates of pork and two isolates of faecal showed nitrate negative. These isolates (2 each of pork and faecal) were TSI & urease positive. On sugar fermentation these isolates (2 each from pork and faecal) showed glucose, sucrose, lactose and sorbitol negative whereas xylose as positive.

Plate 1 Characteristics colonies of *Campylobacter spp.* on mCCDA plates

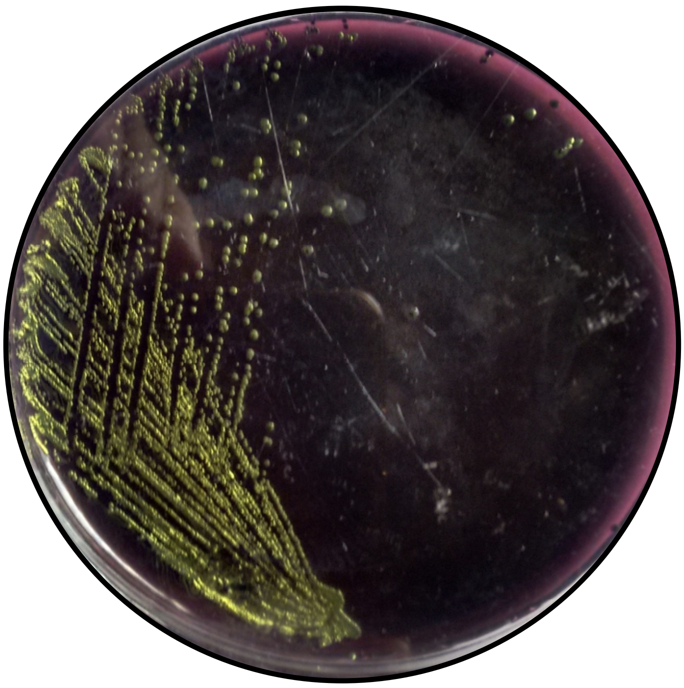

Plate 2 Characteristics colonies of Escherichia coli on EMB agar

Likewise a total of four isolates (2 each from pork & faecal) were confirmed as *Campylobacter* species from pig samples exhibiting the overall prevalence of four percent in pigs (Table 46 & Fig. 1).

On further processing of these four isolates from pig for species identification by growing at different temperature (25^0C, 30.5^0C & 42^0C), hippurate hydrolysis, indoxyl acetate hydrolysis, sensitivity to nalidixic acid and cephalothin discs tests two isolates (SP1C, SP40C) were confirmed as *C. jejuni*, one (SF31C) as *C. coli* and one isolate (SF46C) showed variation in hippurate hydrolysis (Table 47, Plate 5 and 6).

Overall prevalence of *Campylobacter jejuni* from pig samples was to the tune of four percent and for *C. coli* as two percent. Only Pork sample revealed presence of *C. jejuni* and no prevalence in pig fecal sample. Whereas for *C. coli* only one pig faecal sample was positive and no pork sample showed positivity (Table 47 & Fig 2).

Zhao *et al.* (2001) revealed the presence of *C. jejuni* in pork as 1.7% which is in partial agreement of present results showing four per cent prevalence in pork. Ghimire *et al.* (2014) reported 76% prevalence of *C. coli* which is towards higher side as compared to our results i.e. 2%. The deviation in the results may be because of the changes in sample collection places, its hygienic processing, animal health and management practices and antibiotics used (Ghimire *et al.* 2014).

Table 46 Biochemical characterization of *Campylobacter* isolates from Pigs and Poultry

Source	Sample	Total no.	Characteristics on mCCDA	Cultural, Morphological, Biochemical Characterization											Sugar fermentation					Total Isolates and prevalence
				Grams staining (-)	Motility (+)	Catalase (+)	Oxidase (+)	Indole (-)	MR (+)	VP (-)	Citrate (+)	Nitrate (-)	TSI (+)	Urease (+)	Glucose (-)	Sucrose (-)	Lactose (-)	Sorbitol (-)	Xylose (+)	
Pig	**Pork**	**50**	6	5	3	3	3	3	3	3	3	3	2	2	2	2	2	2	2	2(4%)
	Faecal	**50**	5	5	5	5	5	4	4	2	2	2	2	2	2	2	2	2	2	2(4%)
Poultry	**Chicken**	**50**	7	7	6	6	6	6	6	3	3	3	3	3	3	3	3	3	3	3(6%)
	Faecal	**50**	11	10	7	7	7	7	7	7	6	5	4	4	4	4	4	4	4	4(8%)
	Total	**200**	29	27	21	21	21	20	20	15	14	13	11	11	11	11	11	11	11	11(5.5%)

MR- Methyl red test, VP- Voges Proskauer test, mCCDA- modified charcoal cefoperazone deoxycholate agar, TSI- Triple Sugar Iron test

Table 47 Differentiation of *Campylobacter* species

Sr. No.	Isolate No.	Species	Species Characteristics						*Campylobacter* species
			Growth at 25°C	Growth at 30.5 °C	Growth at 42 °C	Hippurate hydrolysis	Cephalothin 30 µg disc	Nalidixic acid	
1	**SP1C**	**Pig**	-	-	+	+	R	S	***C. jejuni***
2	**SP40C**		-	-	+	+	R	S	***C. jejuni***
3	**SF31C**		-	+	+	-	R	S	***C. coli***
4	**SF46C**		-	-	+	V	R	S	***V***
5	**PC1C**	**Poultry**	-	-	+	+	R	S	***C. jejuni***
6	**PC22C**		-	-	+	+	R	S	***C. jejuni***
7	**PC45C**		-	-	+	+	R	S	***C. jejuni***
8	**PF6C**		-	-	+	+	R	S	***C. jejuni***
9	**PF8C**		-	+	+	-	R	S	***C. coli***
10	**PF32C**		-	-	+	+	R	S	***C. jejuni***
11	**PF45C**		-	+	+	-	R	S	***C. coli***

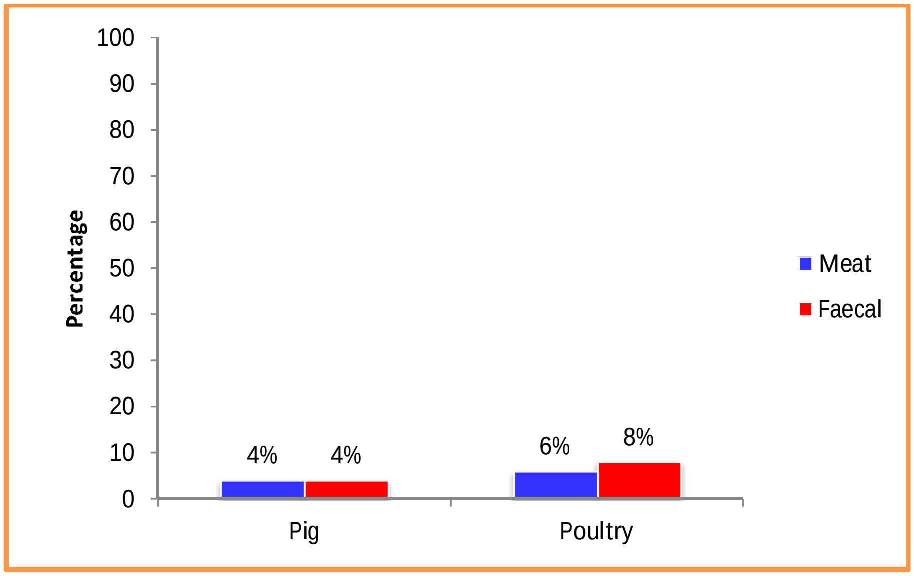

Fig.1 Prevalence of *Campylobacter* spp. by biochemical characterization

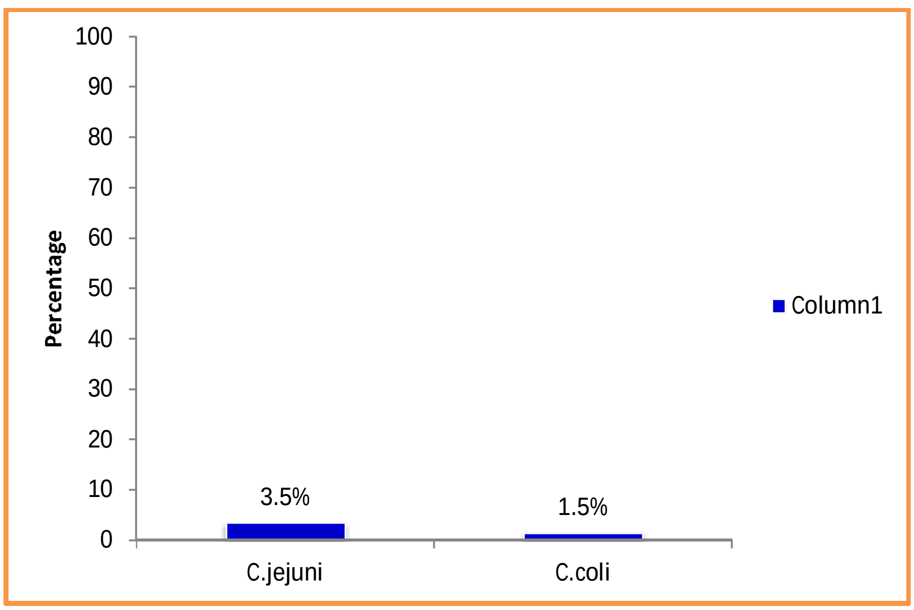

Fig. 2 Prevalence of *Campylobacter jejuni* and *campylobacter coli* by biochemical characterization

In present study, 100 samples of poultry (50 each of chicken & faecal) were processed for isolation and identification of *Campylobacter* spp. From 100 samples seven of Chicken and 11 samples of faecal showed typical grey, flat, moist type colonies with metal sheen on mCCDA. Processing these 18 isolates to Gram staining exhibited Gram negative rod in nature by 17 (7 chicken & 10 faecal) isolates. On subsequent test of motility; 6 from chicken and five from faecal exhibited typical corkscrew, darting type motility at 42^0C. For catalase and oxidase test six isolates of chicken and seven isolates of faecal showed positive results.Three isolates from chicken and six isolates of poultry faecal exhibited IMViC pattern as (-,+,-,+). On further processing for nitrate reduction test, TSI and urease test three isolates of chicken and four isolates of faecal showed specific results (-,+,+) as per standard.For sugar fermentation results three isolates of chicken and four isolates of poultry faecal showed positivity for xylose and negative for glucose, sucrose, lactose & sorbitol. As a result three isolates of chicken and four isolates of poultry faecal were confirmed as *Campylobacter* species giving the prevalence as six and eight percent respectively. The overall percentage in poultry sample was to the tune of seven percent including chicken and faecal sample.

On subsequent processing of seven campylobacter isolates of poultry up to species level five isolates (PC1C, PC22C, PC45C, PF6C & PF32C) were confirmed as *C. jejuni* giving overall prevalence of ten percent and two isolates (PF8C & PF 45C) as *C. coli* showing four percent prevalence. Chicken samples revealed only *C. jejuni*. Whereas poultry faecal samples revealed two each *C. jejuni* and *C. coli* (Table 47 & Fig. 2).

The results of present study i.e. five isolates (10%) of *C. jejuni* in poultry can be correlated with the findings of Singh *et al* (2011) who reported eight isolates of *C. jejuni* from poultry faecal swabs.

Ghimire *et al*. (2014), Sivasankari *et al.*(2015) and Dabiri *et. al.*(2014) reported 24%, 44.94% & 79%, prevalence of *Campylobacter jejuni* from poultry samples respectively which is very high as compared to the present study results.

Thus prevalence of *Campylobacter* species was four per cent each for pork and pig faecal samples. Whereas six and eight per cent prevalence in chicken and poultry faecal was reported.Therefore the overall prevalence of

Campylobacter species in present study (pig and poultry) was 5.5% (11 isolates).One poultry sample (No.45) was positive for co infection of *C. Jejunii* &*C. coli* from chicken and faecal source respectively.

Dabiri *et. al.* (2014), Zhao *et al.* (2001), Vaishnavi *et al.* (2015), Ghirmire *et al*. (2014) and Pallavi *et al.*(2014) reported variation in overall prevalence of *Campylobacter* species from 26 % to 70% from different meat, milk and faecal samples. The present study result of 11% can be correlated with the above researchers finding the variation in results from lower to the higher side may be because of the different sources of sample collection. These differences may be due to slaughtering practices, intrinsic carriage rates. Some of the differences in prevalence rates may also reflect differences in methods used to culture the *Campylobacter* (Ghimire *et al.*2014).

Campylobacters are a normal inhabitant of the intestinal tract of animals and birds and are excreted in large numbers in feces. The fecal contamination of foods of animal origin occurs during unhygienic slaughtering practices. These *Campylobacters* are further transferred to humans due to uncooked or partially cooked foods consumption (Pallavi and Kumar, 2014).

4.1.2 *Escherichia Coli.*

Isolation and identification of *E. coli* was carried out as per standard protocol described by OIE (2008). The sample viz; meat and faecal were processed for cultural isolation by employing MacConkey broth as enrichment and Eosin Methylene Blue (EMB) selective plating. After incubation, plates with typical round colonies with greenish metallic sheen were further characterized by standard biochemical test and sugar fermentation pattern suggested by OIE (2008) and Saba (2015).

Of 100 pig samples (50 each pork and faecal) processed for isolation of *E.coli*, 16 samples from pork & 21 from faecal showed typical colonies with metallic sheen on EMB (plate 2.). Processing these 37 isolates to Gram staining exhibited Gram negative coccobacillary nature by 37(16 pork, 21 faecal) isolates and variation in motility(±). On first preliminary identification test 16 pork and 20 pig faecal showed catalase positivity, whereas15 of pork and 18of faecal showed oxidase negativity. Of these 15 isolates of pork and 18 isolates offaecal showed IMViC pattern as (+,+,-,-). All these isolates showed nitrate positive and urease negative. Whereas TSI test revealed gas production and acidity in slant and butt. Ten pig samples showed presence of *E. coli* in meat and faecal samples both (Table 48 and plate 7)

On subsequent sugar fermentation test 15 isolates of pork and 17 isolates of faecal expressed standard sugar fermentation result as positive for glucose, sorbitol. and dulcitol whereas negative for lactose, sucrose, salicin, adonitol, inositol (Table 48 Fig.3).The pork samples showed 30% prevalence and pig faecal samples revealed 34% prevalence. Thus overall prevalence of *E. coli* in pig samples was 32% (Total=32 isolates; pork-15 & faecal-17).

The results of present study are in agreement with the results of Trotz-Williams *et al.* (2012) who reported 37.93% prevalence of *E. coli* from pork samples. Likewise Zhao *et al.* (2001), Magwedere *et al.* (2016) who reported 16.3%, 18.82% prevalence of *E. coli* from pork samples which is partially less as compared to present study results. Boonyasiri *et al.* (2014) reported 76.7% prevalence from pork samples.

In this study, 100 samples (50 each chicken, poultry faecal) were processed for isolation of *E. coli*, 21 isolates from chicken, 23 isolates from poultry faecal showed typical metalic sheen colonies on EMB (Table 48). Processing of these 44 isolates to Gram staining exhibited Gram negative coccobacillary nature by 42 isolates (21 each of chicken & poultry faecal). On subsequent test, 20 chicken and 21 faecalisolates showed catalase positive and oxidase negative results. When all these 41 isolates processed for IMViC tests showed (+, +,-,-) pattern. When further processed for nitrate and urease test the all isolates revealed nitrate positive and urease negative. Triple sugar iron test results exhibited gas production and acidity in slant and butt.

On further processing of these isolates 19 isolates of chicken and 21 isolates of poultry faecal turned acidic in nature after fermentation of glucose, sorbitol and dulcitol and negative for lactose, sucrose, salicin, inositol, adonitol. Individually chicken and faecal samples exhibited 38% & 42% prevalence respectively. Whereas, overall prevalence was to the tune of 40 per cent among poultry samples. Eight poultry samples showed presence of *E. coli* in both meat and faecal basis (Table 48, Fig.3).

Table 48 Biochemical characterization of*E. coli*isolates from Pigs and Poultry

Animal	Sample	Total no.		cultural, morphological, biochemical characterization											Sugar fermentation								Total Accepted Isolates
			EMB	GS (-ve)	Motility(+)	se	se	Indole (+)	MR (+)	(-)	e	e	Ureas	TSI (+)	Glucose	Sorbit	Dulcit	Adonitol	Salici	Sucro	lactos	ol (+)	
Pig	Pork	50	16	16	V	16	15	15	15	15	15	15	15	15	15	15	15	15	15	15	15	15	15 (30%)
	Faecal	50	21	21	V	20	18	18	18	18	18	18	18	18	17	17	17	17	17	17	17	15	17 (34%)
Poultry	Chicken	50	21	21	V	20	20	20	20	20	20	20	20	20	19	19	19	19	19	19	19	19	19 (38)
	Faecal	50	23	21	V	21	21	21	21	21	21	21	21	21	21	21	21	21	21	21	21	21	21 (42%)
	Total	200	81	79	V	77	74	74	74	74	74	74	74	74	72	72	72	72	72	72	72	72	72 (36%)

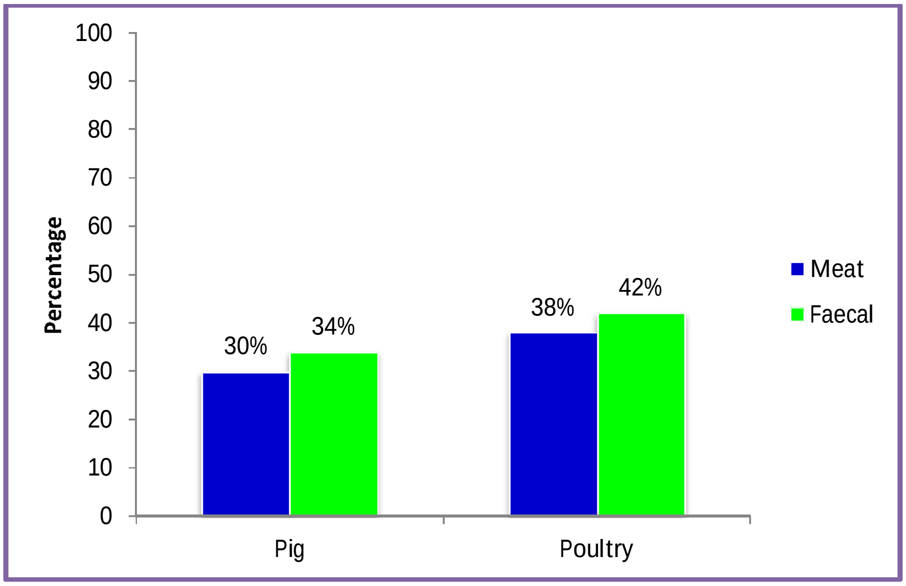

Fig. 3 Prevalence of *E. coli* by biochemical characterization

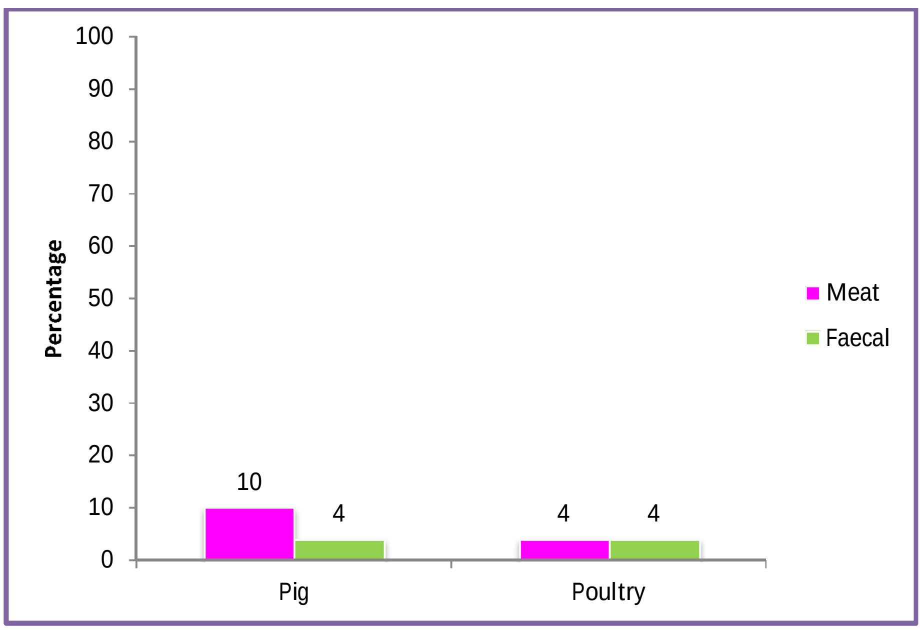

Fig. 4 Prevalence of *Listeria monocytogenes* by biochemical characterization

The results (38% prevalence in chicken) are completely in agreement with the findings of Zhao *et al.* (2001) who reported 38.7% prevalence in chicken samples from retail shops of greater Washington area. Magwedere *et al.* (2016) reported 18.82% prevalence from chicken which is partially less as compared to the present results. This may be due to hygienic slaughtering practices and healthy supply of birds.

An interesting finding of the present study was that the rates of enteric organism contamination of retail meats, particularly chicken carcasses, were significantly different. The possible explanations for this findings include differences in store handling practices, sampling times, and product batches (Zhao *et al.*2001).

4.1.3 *Listeria monocytogenes*

The isolation and identification was carried out as per standard protocol described by Curtis and Lee (1995), USDA (2011) and United States Food and Drug Administration (2012). The samples *viz*. meat and faeces of poultry and pigs were processed with two step enrichment using University of Vermont I (UVM-I) and University of Vermont II (UVM-II) following selective plating onto Polymixin Acriflavin Lithium Chloride Ceftazidime Aesculin Mannitol (PALCAM) agar as per the protocol described by USDA and McClain and Lee (1998). After incubation, the plates with typical colonies exhibiting black diffuse zone of Aesculin hydrolysis with greenish yellow colonies of about 0.5 mm diameter were identified as *Listeria.* The colonies were further characterized by testing it for Gram staining followed by biochemical tests and sugar fermentation pattern.

In present study, 100 samples (50 each pork, pig faecal)were processed for isolation and identification of *Listeria monocytogenes*. From these, eight samples of pork and five samples of faecal showed aesculin hydrolysis on PALCAM plates(plate 3). Six isolates of pork and four isolates of faecal showed Gram positive small cocobacilli. Of these 10 isolates seven isolates (five of pig & two of faecal) revealed tumbling motility at 25^0c.All these seven isolates were catalase positive and oxidase negative. On further processing, all the isolates (five of pig & two of faecal) revealed MR as positive, VP as negative and nitrate negative.Further all these isolates were processed for sugar fermentation test in which all the isolates showed variation in results. Thus ten and four percent

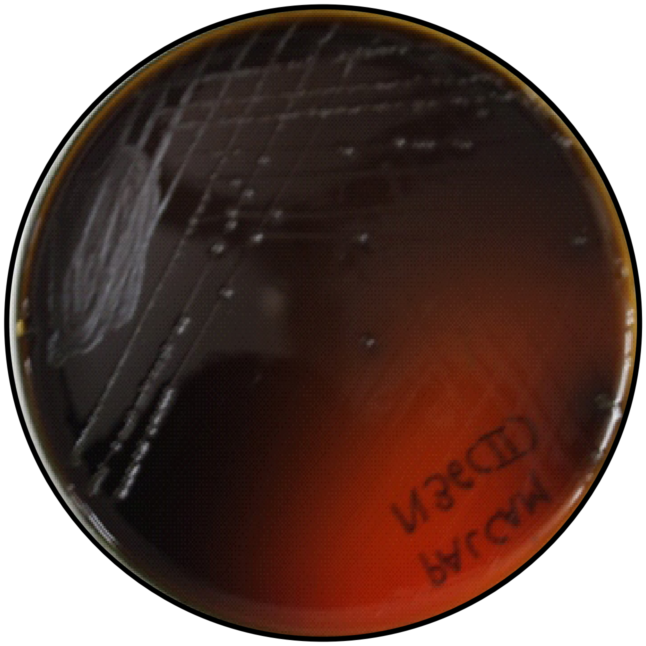

Plate 3 Characteristics colonies of Listeria monocytogenes on PALCAM agar

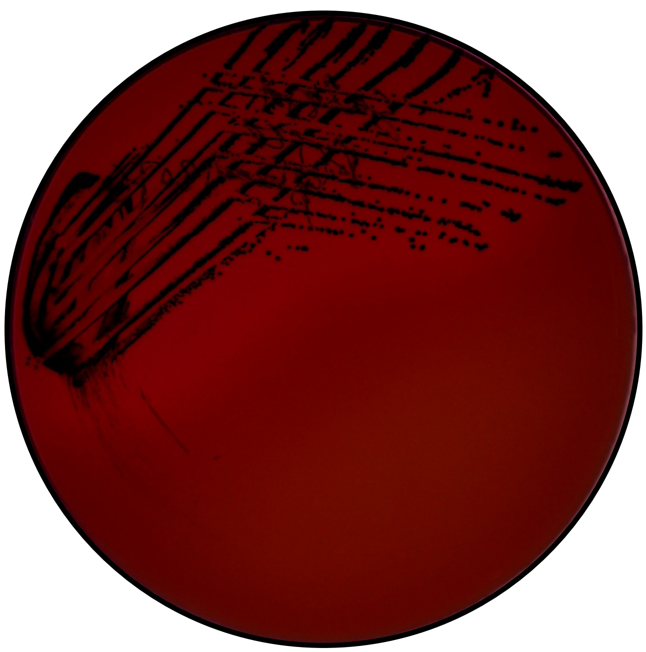

Plate 4 Characteristics colonies of Salmonella spp. on XLD agar

prevalence in pork and pig faecal were reported. Likewise a total of seven isolates (14%) were identified as listeriae (Table 49,Fig.4).

Krzysztof Kwiatek (2004), Dudhe *et al,* .(2012)& Gamboa Martin *et al.*(2012) reported 9.41%, 8% & 5% prevalence of *Listeria monocytogenes* from pork samples which can be correlated with the present study results. Uyttendaele *et al.* (1999) & Kanuganti *et al.*(2002) reported 38.2% &45%prevalence in pork samples respectively. The researchers reported higher presence of *Listeria monocytogenes* in pork samples. Whereas Zhou & Jiao (2006) reported 0.95% prevalence in pork which is very less as compared to the present study reports. The less percentage of prevalence may be due to the degree of *L. monocytogenes* contamination of pork and beef varies dependently on an examined area.

Probably, the presence of this pathogen resulted from a secondary post processing contamination. It has been proved in some studies that the most often this type of contamination takes place during unhygienic slicing and lack of good hygienic conditions (Krzysztof Kwiatek, 2004).

The presence of the pathogen in swine is associated to the fact that these animals are carriers of *L. monocytogenes*. For example it has been shown that pork lodge this bacterium in tonsils and intestine. For this reason if during evisceration there is organ rupture, the carcass can be contaminated with *L. monocytogenes*. Although it is frequent for *L. monocytogenes* to contaminate carcasses and processing plants, subsequent washing and disinfecting procedures should get rid of the contamination. Pathogen-free product depends on the effectiveness of these processes (Gamboa Martin *et al.* 2012).

A total of 100 samples (50 each chicken and poultry faecal) were processed for isolation of *Listeria*. Of these five isolates of chicken and four isolates of faecal showed aesculin hydrolysis. When these nine isolates stained with Grams stain five isolates (three of chicken and two of faecal) showed Gram positive reaction. Two isolates each of chicken and faecal origin showed tumbling motility at 25^0C. These four isolates were catalase positive and oxidase negative. on further processing these isolates revealed MR as positive, VP and nitrate as negative. All these isolates when screened for sugar fermentation, there was variation in sugar fermentation results.Thus all four isolates were identified as

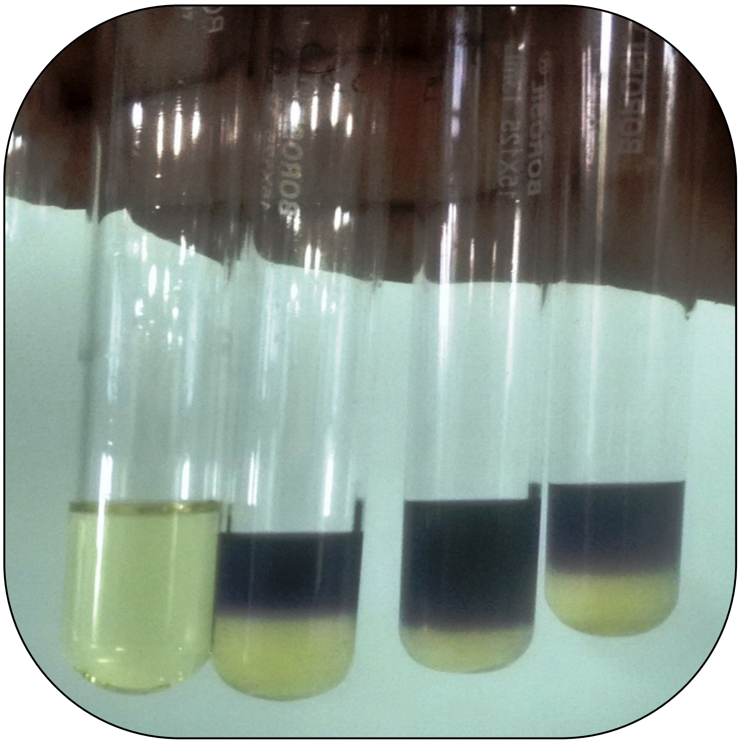

Plate 5 Formation of purple colour indicative of positive hippurate hydrolysis

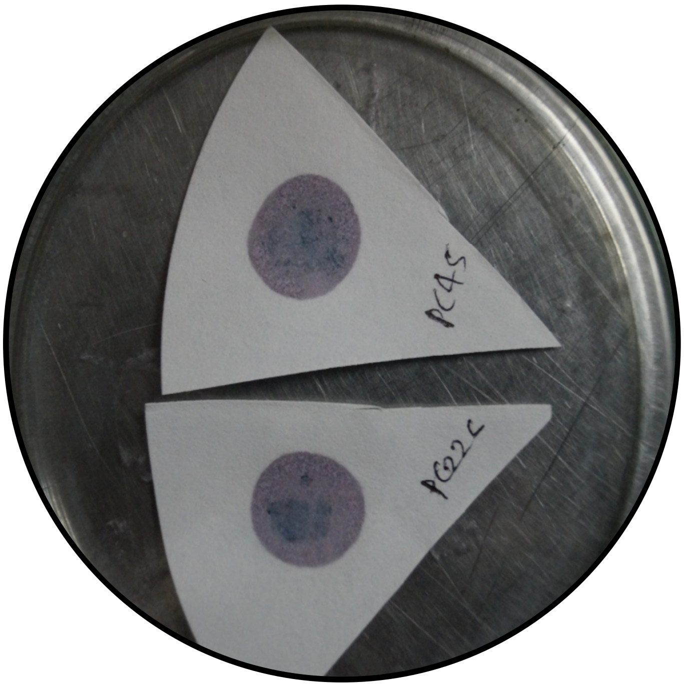

Plate 6 Indoxyl acetate hydrolysis by Campylobacter spp.

Listeria monocytogenes giving the overall prevalence of eight percentie. four percent each in chicken and poultry faecal samples(Table 49,Fig4).

Kuan *et al.* (2013) reported higher prevalence of *L. monocytogenes* in chicken which is very high as compared to the presence of present study reports (4%).

On the other side Kanarat *et al.* (2011) reported 2.5% prevalence in frozen chicken meat and 0.2% in ready to eat chicken products. *L. monocytogenes* is ubiquitous in nature and because of that may originate from the birds itself entering slaughterhouses or due to contamination arising from workers, equipment and/or the processing plants environments (Kanarat *et al.* 2011).

Among all the samples from all sources, the higher incidence of *L. monocytogenes* was noticed with meat samples. In developing countries like India, the possible reason behind this may be the use of chopping boards, mincing machines, cleaning clothes and carcasses, which are frequently used in butcheries and kept for an indefinite period of time (Khan *et al.* 2013).

Table 49 Biochemical characterization of *Listeria* spp. isolates from Pigs and Poultry

Animal	Sample	Total no.	cultural, morphological, biochemical characterisation								Sugars			Total isolates (Prevalence percent)
			AH (+)	Grams Staining (+)	Motility (+)	Catalase (+)	Oxidase (-)	MR (+)	VP (-)	Nitrate (-)	L-Rhamnose (+)	D-Xylose (–)	α-methyl D-mannoside (+)	
Pig	Pork	50	8	6	5	5	5	5	5	5	V	V	V	5 (10%)
	Faecal	50	5	4	2	2	2	2	2	2	V	V	V	2 (4%)
Poultry	Chicken	50	5	3	2	2	2	2	2	2	V	V	V	2(4%)
	Faecal	50	4	2	2	2	2	2	2	2	V	V	V	2(4%)
	Total	200	22	15	11	11	11	11	11	11	V	V	V	11 (5.5%)

MR- Metyl Red test, VP-Voges Proskauer.

Kuan *et al.* (2013) reported higher prevalence of *L. monocytogenes* in chicken which is very high as compared to the presence of present study reports (4%).

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Among all the samples from all sources, the higher incidence of *L. monocytogenes* was noticed with meat samples. In developing countries like India, the possible reason behind this may be the use of chopping boards, mincing machines, cleaning clothes and carcasses, which are frequently used in butcheries and kept for an indefinite period of time (Khan *et al.*2013).

4.1.4 *Salmonella* species

The isolation and identification was carried out as per standard protocol described by Agarwal *et al.* (2005), OIE (2008), Kalambe (2012), Sable (2015), andBergey's manual .The samples *viz*. meat and faeces in pig & poultrywere processed with two step enrichment using BPW and RV following selective plating onto Xylose lysine deoxylate(XLD) agar as per the protocol described by Sable (2015). After incubation, the plates with typical colonies exhibiting black centered colonieswere identified as *Salmonella* spp*.* The colonies were further characterized by testing it for Gram staining followed by biochemical tests and sugar fermentation pattern.

From 100 samples of pig (50 each of pork and faecal) eight and six isolates of pork and pig faecal showed typical black centered colonies on XLD agar (Plate 4). Of which only six isolates of pork and five isolates of faecal were Gram negative and expressed variation in motility using hanging drop method. From these Gram negative eleven isolates, nine isolates (pork=5, faecal=4) were catalase positive and oxidase negative. All nine isolates revealed IMViC pattern as (-,+,-,+) which is considered as a standard pattern for *Salmonella*. Five isolates of pork and three isolates of faecal were nitrate negative and TSI positive. On further processing for sugar fermentation five isolates of pork and

Plate 7 MR test shown by E coli isolates

Plate 8 Typical pattern of Salmonella growth on TSI agar

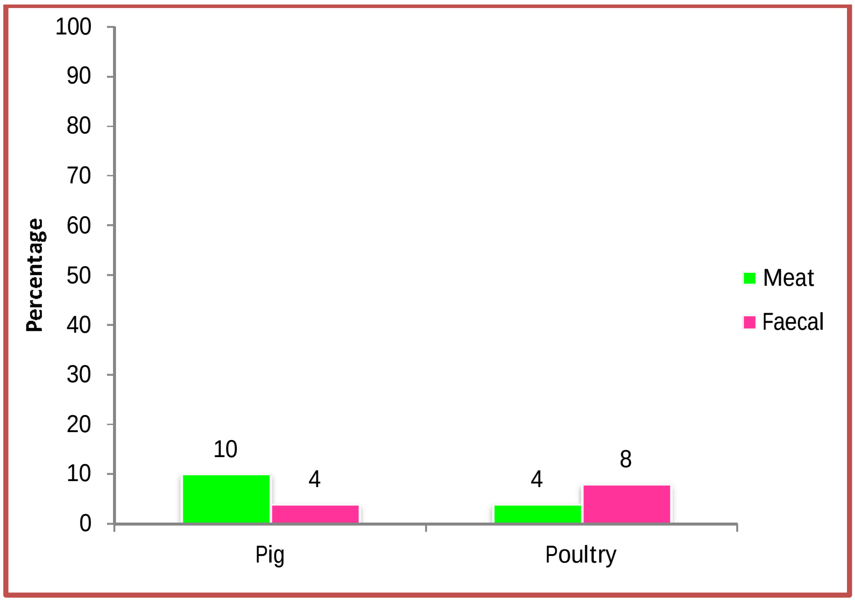


Fig. 5 Prevalence of *Salmonella spp.* by biochemical characterization

two isolates of faecal exhibited glucose positive and lactose, sucrose, adonitol & salicin negative (Table 50, Plate 8, Fig.5).

All these results of different preliminary and secondary biochemical test confirms the *Salmonella* to the tune of ten percent (5 isolates) in pork and four percent (2 isolates) in faecal. Thus the overall prevalence of *Salmonella* species in pig was seven percent. Zhao *et al.* (2001) reported 7% prevalence of *Salmonella* from pork which is correlating to the present study reports. Ejeta*et al.* (2004) reported 16.4% prevalence from pork of retail supermarkets in Addis Ababa, Ethiopia. Davis *et al.* (2004) reported 23% prevalence from faecal swabs of pigs slaughtered in Great Britian. The difference in prevalence rates may have occurred because the current study was conducted at local shops where cross-contamination was likely to occur during slaughter, distribution, processing and packaging.

The prevalence reported by these researchers are high as compared to our findings. It is difficult to compare these results with findings in other countries as there have been few truly representative national surveys and there are considerable variations in sampling frames and methods as well as in Salmonella culture techniques (Davies *et al.* 2004).

On processing of 100 poultry samples (50 each chicken, poultry faecal) for isolation and identification of *Salmonella* species nine samples of chicken and 11 samples of faecal showed black centered colonies on XLD agar. Five from chicken and seven from faecal isolates showed Gram negative result and variation (±) in motility. Three isolates of chicken and seven isolates of faecal showed catalase positive and oxidase negative result. Two isolates of chicken and seven isolates of faecal showed (-,+,-,+) pattern for IMViC. On further processing of these isolates for nitrate reduction and TSI showed negative and positive result respectively. Of these only six isolates (two of chicken and four of faecal) exhibited proper pattern for sugar fermentation as negative for lactose, sucrose, salicin & adonitol whereas positive for glucose (Table 50, Fig.5). One poultry sample (No.48) was positive for salmonella from both the sources of meat and faecal.

Thus in poultry samples; the overall prevalence of *Salmonella* species based on biochemical characterization was reported to be 12 per cent (4% in chicken & two percent in faecal). Zhau *et al.* (2011) reported nine percent prevalence from poultry in Washigton which is in agreement with the present findings. The difference could be due in part to the types of samples analyzed (whole birds versus steaks; fresh versus frozen).

On the basis of biochemical identification methods the pig samples showed the co-infection of more than one organism mostly in pork samples. Two samples of pork were positive for *Campylobacter* and *Salmonella* contamination (Sample no. 1 & 40), two samples for *E. coli* and *Listeria* (Sample no 28 & 43) and one sample (Sample No11). Whereas two samples (Sample no. 31 & 46) of faecal showed mixed infection of campylobacter and *E. coli* (Table 50).

Table 50 Biochemical characterization of *Salmonella* isolates from Pigs and Poultry

Animal	Sample	Total no.	cultural, morphological, biochemical characterisation											Sugar					Total isolate (Prevalence percent)
			XLD	GS (+)	Motility (+/-)	Catalase (+)	Oxidase (-)	Indole (-)	MR (+)	VP (-)	Citrate (+)	Nitrate (-)	TSI (+)	Lactose (-)	Sucrose (-)	Salicin (-)	Adonitol (-)	Glucose (+)	
Pig	Pork	50	8	6	V	5	5	5	5	5	5	5	5	5	5	5	5	5	5 (10 %)
	Faecal	50	6	5	V	4	4	4	4	4	4	3	3	2	2	2	2	2	2 (4%)
Poultry	Chicken	50	9	5	V	3	3	3	3	3	2	2	2	2	2	2	2	2	2 (4%)
	Faecal	50	11	7	V	7	7	7	7	7	7	7	7	4	4	4	4	4	4 (8%)

GS-Grams staining, MR- Methyl Red., VP- Voges Proskaeur.

Similarly in case of poultry; two chicken samples (sample no. 1,9 & 48) showed infection more than one organism viz. *Campylobacter* , *E. coli, Listeria monocytogenes&*salmonella species. Whereas chicken sample from one bird (sample no 22) showed coinfection of all four organisms. On the contrary six poultry faecal sample (Sample no 6, 8,32, 42,43,45) showed positivity for mixed pathogens (Table 51).

The present findings are in agreement with the Zhao *et al* (2001) reported mixed infection in retail meat samples of Washington area detailing five pork samples contained *E. Coli* and *Salmonella,* whereas only one turkey sample contained *E. coli* and *Salmonella*. Two turkey samples were contaminated with Campylobacter and *E. coli*, and one turkey sample was contaminated with *Campylobacter* and *Salmonella*.

Campylobacter, *Salmonella*, and pathogenic *E. coli* all colonize the gastrointestinal tracts of a wide range of wild and domestic animals, especially animals raised for human consumption. Food contamination with these pathogens can occur at multiple steps along the food chain, including production, processing, distribution, retail marketing, and handling or preparation. Numerous epidemiological reports have implicated foods of animal origin as the major vehicles associated with illnesses caused by food-borne pathogens. Contaminated raw or undercooked poultry and red meats are particularly important in transmitting these food-borne pathogens. Other sources of human infections with *Campylobacter, Salmonella*, and STEC include contaminated produce and contact with farm animals and pets. Person-to-person transmission has also been described. (Zhao *et al* 2001).

4.2 Molecular characterization of foodborne pathogens from pig and poultry

4.2.1 *Campylobacter* species

In present study, four Isolates from pig were biochemically characterized as *Campylobacter*. These biochemically identified isolates were processed for molecular characterization by employing conventional PCR by targeting genus specific *16S rRNA* gene of 816 bp.

In present study, two isolates each from pork & pig faeces showed positivity for genus specific *Campylobacter 16S rRNA* gene of 816 bp. While two

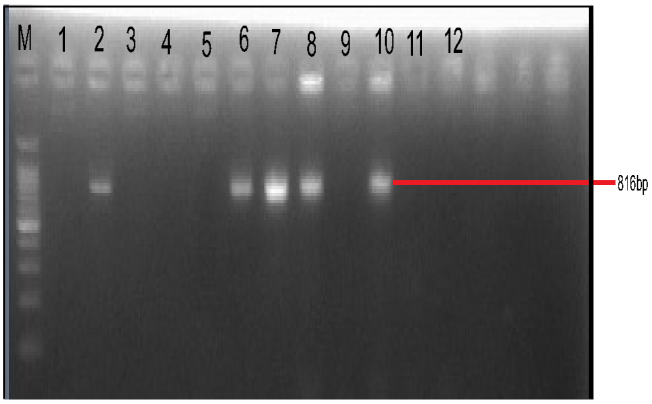


Plate 9 PCR for 16S rRNA gene of Campylobacter spp.

Lane . 1 Negative Control
Lane . 2 ATCC -1153 *(Campylobacter Jejuni)*
Lane . 6 PC1C
Lane . 7 PC45C
Lane . 8 PF6C
Lane . 10 PF8C
Lane . M - Ladder

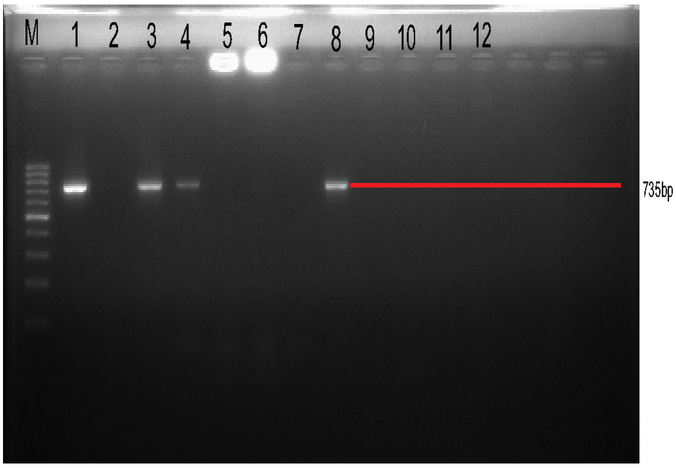


Plate 10 PCR for hip O gene of Campylobacter jejuni

Lane . 1 ATCC -1153 *(Campylobacter Jejuni)*
Lane . 2 Negative Control
Lane . 3 PC1C
Lane . 4 PC45C
Lane . 8 PF6C
Lane . 10 PF8C
Lane . M - Ladder

isolates (SP1C, SP40C) from pork turned positive for *Campylobacter jejuni* by targeting *hip O* gene (735 bp). One isolate (SF31C) of pig faecal found positive for *Campylobacter coli* by targeting *asp K* gene (500 bp) and another isolate (SF46C) could not be identified till species level using *hip O* &*asp K* gene. By molecular identification methods i.e. using PCR techniques overall four percent prevalence of *Campylobacter* species was confirmed in which two per cent *Campylobacter jejuni* and one per cent *Campylobacter coli* were identified in pigs (Table 51, Plate 9.).

Table 51 Molecular characterization of Campylobacter isolates from Pigs and Poultry

Sr. No.	Animals	Sources	Isolate No.	PCR			Total prevalence (Campylobacter spp)
				***16S rRNA* (Campylobacter spp.)**	***hipO* (C. jejuni)**	***asp K* (C. coli)**	
1	**Pig**	**Pork**	**SP1C**	+	+	-	**4(4%)**
2			**SP40C**	+	+	-	
3		**Faecal**	**SF31C**	+	-	+	
4			**SF46C**	+	-	-	
5	**Poultry**	**Chicken**	**PC1C**	+	+	-	**7(7%)**
6			**PC22C**	+	+	-	
7			**PC45C**	+	+	-	
8		**Faecal**	**PF6C**	+	+	-	
9			**PF8C**	+	-	+	
10			**PF32C**	+	+	-	
11			**PF45C**	+	-	+	
		Total		**11 (5.5%)**	**7 (3.5%)**	**3 (1.5%)**	**11 (5.5%)**

Seven isolates of poultry showed positivity on biochemical characterization and then processed for molecular identification by employing PCR techniques targeting *16S rRNA*, *hipO* &*asp K* gene for genus and species. In present study, three isolates (PC1C, PC22C, PC45C) from chicken and four (PF6C, PF8C, PF32C, PF45C) from faeces showed positivity for genus

Campylobacter. On further processing for species level all three isolates (PC1C, PC22C, PC45C)of chicken and two (PF6C, PF8C) isolates from faeces turned positive for *Campylobacter jejuni* by targeting *hip O* gene (735 bp) whereas two isolates (PF32C, PF45C) isolates from faecal found positive for *Campylobacter coli, asp K* gene showing 500bp band. Overall seven percent prevalence of *Campylobacter* was observed in poultry samples; classifying six percent among chicken and eight percent among faecal samples whereas five percent *Campylobacter jejuni* and two percent *Campylobacter coli* found in Poultry (Table 52, Plate 10,11).

Seven isolates (7%)of poultry showed positivity on biochemical characterization and then processed for molecular identification by employing PCR techniques targeting *16S rRNA*, *hip O* &*asp K* gene for genus and species. In present study, three isolates (PC1C, PC22C, PC45C) from chicken and four (PF6C, PF8C, PF32C, PF45C) from faeces showed positivity for genus *Campylobacter*. On further processing for species level all three isolates (PC1C, PC22C, PC45C)of chicken and two (PF6C, PF8C) isolates from faeces turned positive for *Campylobacter jejuni* by targeting *hip O* gene (735 bp) whereas two isolates (PF32C, PF45C)isolates from faecal found positive for *Campylobacter coli, asp K* gene showing 500bp band. Overall prevalence of *Campylobacter* confirming six percent among chicken and eight percent among faecal samples whereas five percent *Campylobacter jejuni* and two percent *Campylobacter coli* found in Poultry. One poultry bird (sample No. 45) showed positivity in faecal (PF45C) and chicken(PC45C) both with different campylobacter species. Chicken sample was contaminated with *C. jejuni* and faecal with *C. coli* (Table 51, Fig. 6).

In present study for pig and poultry samples, overall four & seven per cent prevalence of *Campylobacter* spp. was reported which can be correlated to Zhao *et al.*(2001) who studied the prevalence of *Campylobacter* spp., *Escherichia coli* and *Salmonella* serovars in retail Chicken, Turkey, Pork, and Beef from the Greater Washington, D.C., Area. The worker reported 1.7% prevalence among pork by targeting ORF common gene for *Campylobacter*spp (256 bp) and hippurasegene (735 bp) for *C. jejuni* and ORF for *C. coli* of 500 bp.

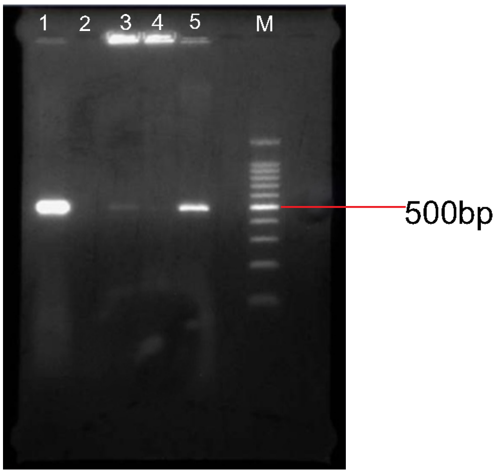


Plate 11 PCR for PCR for asp k gene of Campylobacter coli

Lane 1: SF31C
Lane 3: PF8C
Lane 5: PF45C.
Lane M: Marker

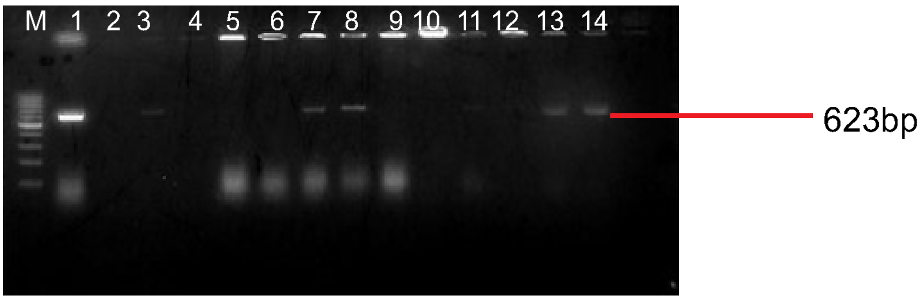


Plate 12 PCR for *uid A* gene of *Escherichia coli*

Lane 1: *E. coli* (ATCC 25922)

Lane 2: Negative Control

Lane 3: SP15E

Lane 7: SP42E

Lane 8: SF28E

Lane 11: SF44E

Lane M: Marker

Vaishanvi *et al.*(2015) studied the isolation of *Campylobacters* from intestinal tract of poultry in northern region of India. *Campylobacter* was isolated from 57/127 (44.9%) of the samples All culture isolates (100%) were reconfirmed as *Campylobacter* by 16S rRNA polymerase chain reaction. Molecular identification of isolates revealed the presence of *C. jejuni* in 45 (79.0%), *C. coli* in 1 (1.8%) and co-infection of *C. coli* and *C. jejuni* in 11 (19.3%). The researchers observed higher prevalence as compared to the present study findings (seven percent). In present study, cent percent biochemically identified isolates showed positivity as *Campylobacter*spp. by employing 16S rRNA polymerase chain reaction out of which 63.63 % were *C. jejuni* and 37.27 % as *C. coli*. Correspondingly the co-infection of both species of *C. jejuni* &*C. coli* is also observed in the present study.

Similarly Stoyanchev (2004) detected 40.8% *Campylobacter* spp. prevalence in fresh chilled poultry and poultry products using PCR of 16S rRNA gene which is again very high as compared to present study reports. Linton *et al.* (1997) also reported higher prevalence (20 isolate) of *Campylobacter* in human faecal sample from which 17 were confirmed as *C. jejuni*, two as *C. coli* and one isolate showed both co-infection of both the species. Similarly in present study report the *C. jejuni* were isolates maximum followed by *C. coli*. The researchers also observed the concurrence with culture and phenotypic identification to species level by employing genus (*16S rRNA*) and species specific (*hip O* and *asp K*) PCR which was also noted in present study reports.

Ushijima (2014) studied sensitive and rapid detection of campylobacter species from stools of children with diarrhea in Japan by the loop-mediated isothermal amplification method . Fourteen of the 20 samples were identified as *C. jejuni* by the cultural method. All 20 samples were also positive for *C. jejuni* by the PCR method by employing *hip O* gene i.e. 100 % positivity by PCR from cultural isolates. The confirmation of Campylobacter species is towards higher side as compared to present reports.

Campylobacter jejuni and *C. coli* are generally considered commensals of livestock, domestic pet animals and birds (OIE, 2008).The increased percentage may be because of the different reasons such as season of collection of samples, farm management practices, dechlorinated or contaminated water

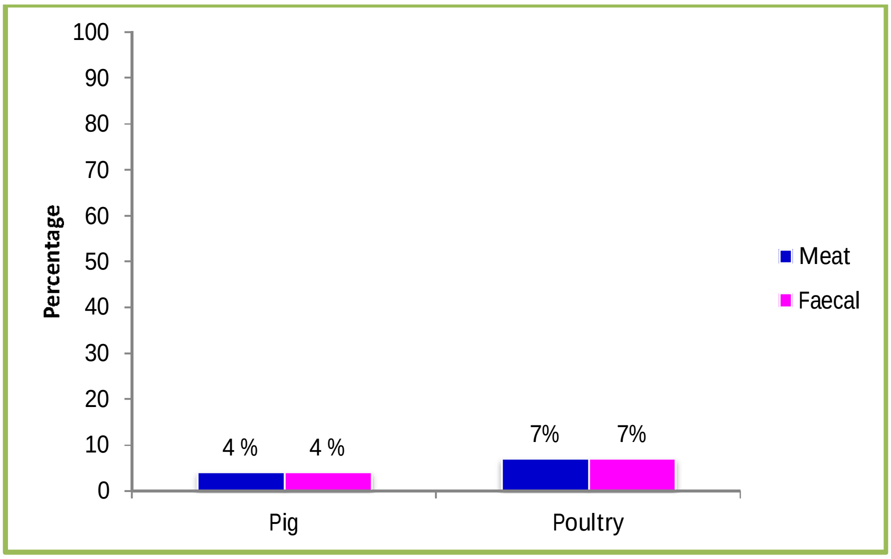


Fig.6 Molecular Characterisation of *Campylobacter* spp.

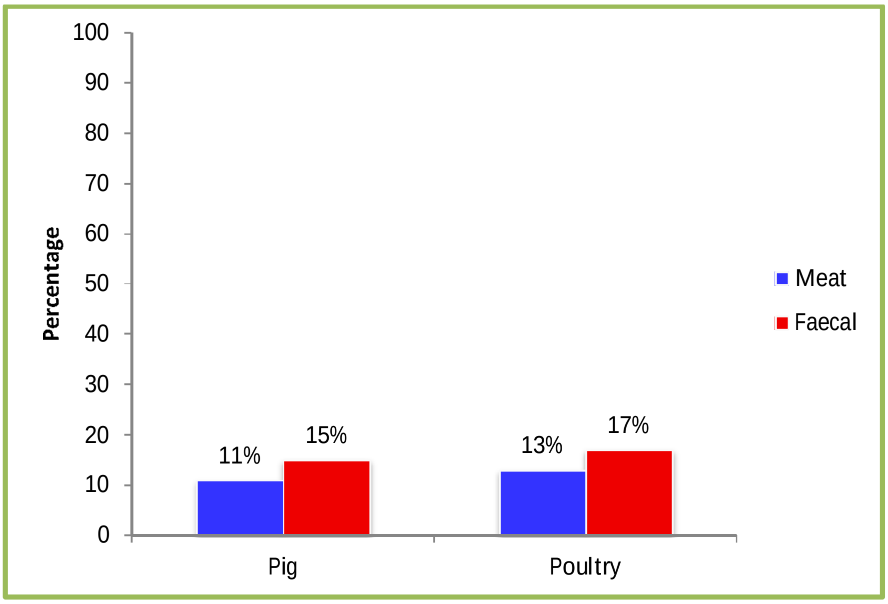


Fig.7 Molecular Characterisation of *E.coli* isolates

supply to birds. In chicken samples most important source of campylobacter contamination is because of unhygienic slaughter techniques & specifically during intestinal content cleaning. (Shan 2000).

Prevalence of *Campylobacter* varies between countries depending on the level of hygienic measures followed. This variation in getting a low number of isolates positive for targeted gene may be due to the primers used, the prevalence of inhibitors, laboratory condition, seasonal variations as well as geographic diversity in the distribution of *C. jejuni* and *C. coli* isolates (Begum *et al.* 2015)

4.2.2 *Escherichia coli:*

In present study, 32 pig isolates which showed positivity on biochemical characterization were processed for molecular characterization by employing PCR and targeting genus specific *uidA* gene primers of 623 bp. For confirmation of shiga toxin producing *E.colistx1* and *stx2* genes were targeted for 614bp & 779bp.Present study revealed 11 isolates from pork and 15 from pig faeces for *Escherichia coli by employing uidA gene.* Overall 26% prevalence of *E.coli* was noted in the study using PCR technique of which 10 % showed positivity from both the sources i.e. meat &faecal (Table 53).Similarly when screened for detection of shiga toxin producing *stx1* and *stx2* genes, all isolates of pig were negative (Table 52, Fig. 7).

Table 52 Molecular identification *E. coli* isolates from pigs by employing Conventional PCR (*uid A* gene)

Sr.No	Animals	Sources	Isolate No.	PCR (*uid A* 623 bp)	Prevalence percent
1	Pig	Pork	SP4E	+ve	11(22 percent)
2			SP10E	+ve	
3			SP11E	+ve	
4			SP13E	+ve	
5			SP15E	+ve	
6			SP17E	+ve	
7			SP28E	-ve	
8			SP31E	+ve	
9			SP36E	-ve	
10			SP37E	-ve	
11			SP42E	+ve	
12			SP43E	+ve	
13			SP44E	+ve	
14			SP47E	+ve	
15			SP48E	-ve	
16		Faecal	SF2E	-ve	15 (30 percent)
17			SF4E	+ve	
18			SF10E	+ve	
19			SF11E	+ve	
20			SF13E	+ve	
21			SF15E	+ve	
22			SF21E	+ve	
23			SF25E	+ve	
24			SF28E	+ve	
25			SF30E	+ve	
26			SF31E	+ve	
27			SF42E	+ve	
28			SF43E	+ve	
29			SF44E	+ve	
30			SF46E	+ve	
31			SF47E	+ve	
32			SF49E	-ve	

On processing of 40 biochemically confirmed poultry isolates for molecular characterization targeting *uid A*gene of 623 bp; 13 isolates from chicken and 17 from faeces showed positivity for *E. coli.* On the whole 30 % prevalence of *Escherichia coli* could be registered among poultry samples. Of that 10 % birds showed positivity in chicken and faecal both. Independently; 26% (13 isolates) in chicken and 32%(16 isolates) among faecal samples(Table 55)When these positive isolates screened for virulent shiga toxin producing *stx1* and *stx2* genes, all isolates showed negativity for the targeted product (Table 53, Plate 12).

Table 53 Molecular identification *E. Coli* isolates from poultry by Conventional PCR (*uid A* gene)

Sr No	Animals	Sources	Isolate No	PCR (µid A)	Prevalence percent
1	Poultry	Chicken	PC1E	+ve	13 (26 percent)
2			PC4E	-ve	
3			PC6E	+ve	
4			PC8E	+ve	
5			PC9E	+ve	
6			PC11E	-ve	
7			PC15E	+ve	
8			PC17E	-ve	
9			PC22E	+ve	
10			PC24E	-ve	
11			PC27E	+ve	
12			PC31E	-ve	
13			PC32E	+ve	
14			PC38E	+ve	
15			PC39E	+ve	
16			PC40E	-ve	
17			PC41E	+ve	
18			PC46E	+ve	
19			PC48E	+ve	

20			PF1E	+ve	
21			PF3E	-ve	
22			PF6E	+ve	
23			PF8E	+ve	
24			PF15E	+ve	
25			PF17E	+ve	
26			PF18E	+ve	
27			PF23E	+ve	
28			PF26E	+ve	
29			PF27E	+ve	
30		**Faecal**	PF29E	+ve	**17 (34 percent)**
21			PF32E	+ve	
32			PF34E	- ve	
33			PF37E	+ve	
34			PF39E	+ve	
35			PF41E	+ve	
36			PF42E	-ve	
37			PF43E	+ve	
38			PF45E	-ve	
39			PF46E	+ve	
40			PF50E	+ve	

Azeem *et al.*(2013) confirmed *E. coli* species by targeting PCR for *uidA species specific* gene from different raw milk, rectal swabs and stool samples of children with a percentage of 2%, 1.42% and 6.7% respectively. Similarly; Dhanashree *et al.*(2008) detected only one shiga-toxigenic *Escherichia coli* from stool and meat samples from Manglore region of India for all STEC genes like *stx*1 gene (614 bp), *stx*2 gene (779 bp), *eae* gene (384 bp), *rfbO157* gene (497 bp), *hlyA* gene (166 bp). Zhao *et al.*(2001) reported 38.7%, 19.0%, 16.3% and 11.9% prevalence of *E. coli* using *stx*1 and *stx*2 genes in chicken, beef, pork and turkey samples respectively from the greater Washington, D.C., area.

The present study report is totally negative for *stx1* and *stx2* genes of *E. coli* from pig and poultry samples which cannot be correlated with the findings of the above researchers findings where they reported varying percentage of virulent *E. coli* from different sources. However, the absence of stx genes in these isolates could be due to the fact that stx gene is bacteriophage coded and the isolate would have lost the same during preservation (Dhanashree *et al.* 2008).

4.2.3 *Listeria monocytogenes*

In present research, 11 isolates from pig showed positivity towards biochemical characterization and then processed further for molecular characterization by employing PCR targeting genus specific *prs* gene of 370 bp and species specific *isp* gene of 711 bp. On processing of these; three isolates from pork and one from faecal sample showed presence of *prs* gene targeting genus *Listeria* while two isolates from pork and one isolate from faeces turned positive for *Listeria monocytogenes*. Prevalence of *Listeria* spp could be 6 percent among pork and two percent among faecal samples in which four percent and two percent *Listeria* monocytogenes found in pork and faecal samples respectively. Separately prevalence of *Listeria spp* and *Listeria monocytogenes* among pig were reported 4% and 3% respectively (Table 54, Plate 13,14)

In present study, 4% and 2% *Listeria monocytogenes* were reported from pork and faecal samples respectively which is on lower side than Vaidya (2013) who reported 8%,12% *L. monocytogenes* from pig sample 50 each of pork, and faecal sample collected from pig slaughter in Nagpur region. The worker reported isolation of *L. monocytogenes* from 8% pork and 12% faecal samples on employing conventional PCR by targeting *prs*(370 bp) and *isp* (711 bp).Dudhe (2012) confirmed 12 *List*eria isolates (2 from goats, 4 from cattle, and 6 from pigs) targeting *prs* gene and *isp* gene to confirm *Listeria monocytogens*(Fig. 8).

Khawase (2015) studied the prevalence of *Listeria monocytogenes* in clinical cases of small ruminants by targeting *prs* genus and *isp* species specific primers. Worker reported overall prevalence of *L. monocytogenes* in goats was 8.7 per cent whereas in sheep it was 14.28 per cent , environmental samples no isolations were recorded.

Four isolates from poultry showed positivity on biochemical characteristization and on further processing for molecular characterization by employing conventional PCR by targeting genus specific *prs* gene (370 bp) and isp (711 bp). In present study, single isolates from chicken and two from faecal showed positivity for genus *Listeria* while single isolate from each chicken and faeces turned positive for *Listeria monocytogenes*. Prevalence of *Listeria* spp could be 2 percent among chicken and four percent among faecal samples

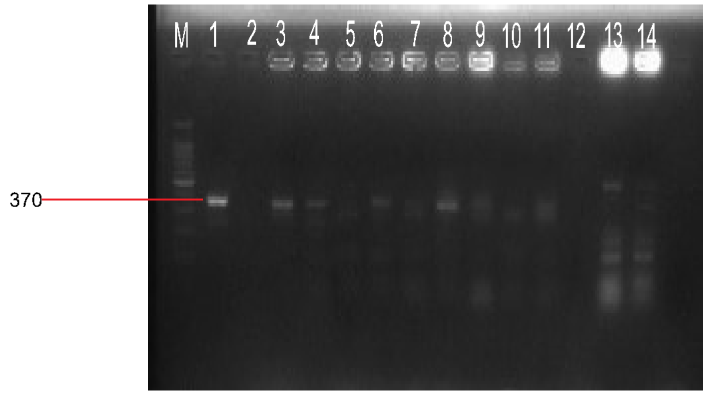


Plate 13 PCR for prs gene of Listeria spp.

Lane . 1 ATCC -19118 (*Listeria monocytogenes)*
Lane . 2 Negative control
Lane . 3 SP6L
Lane . 4 SP8L
Lane . 6 SF37
Lane . 8 PC9L
Lane . M - Ladder

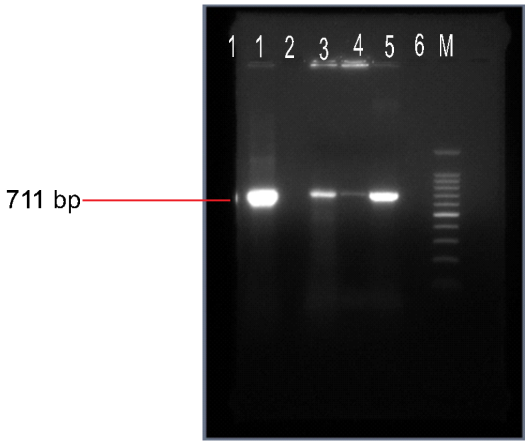


Plate 14 PCR for isp gene of L. monocytogenes

Lane 1: L. monocytogenes (ATCC 19118)
Lane 2: Negative Control
Lane 3: SP6L
Lane 4:SP8L
Lane 5: SF37L
Lane M: Marker

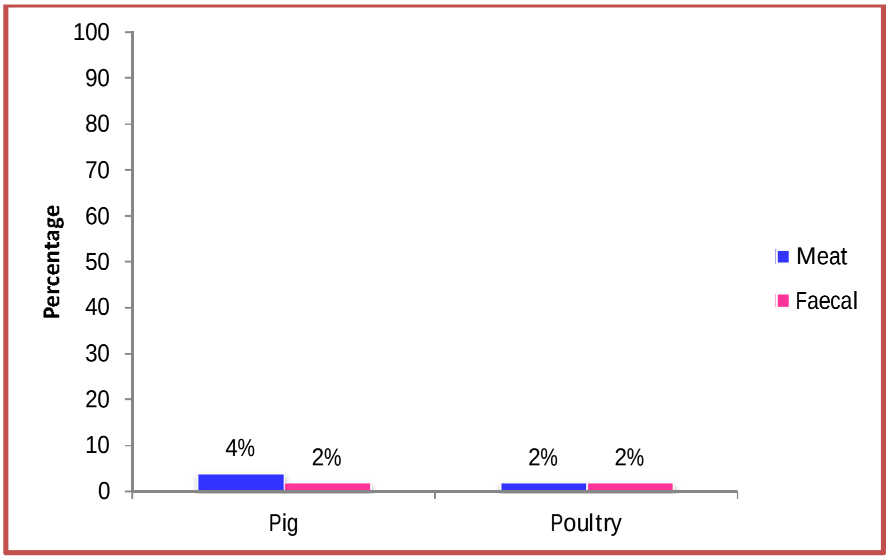


Fig.8 Molecular Characterisation of *Listeria monocytogenes* isolates

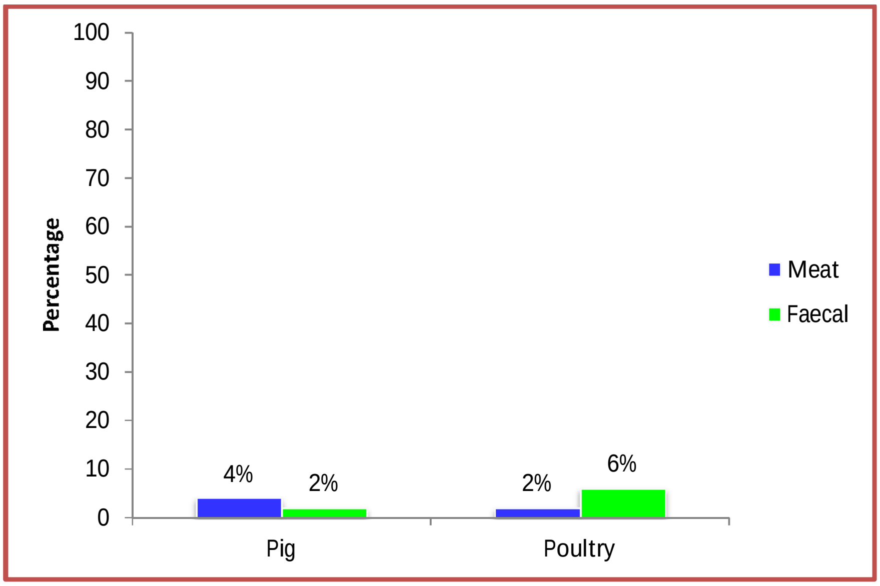


Fig.9 Molecular Characterisation of *Salmonella* spp. isolates

whereas two percent *Listeria* monocytogenes found in chicken and faecal samples respectively.Overall prevalence of *Listeria* spp and *Listeria monocytogenes* among poultry were reported 3% and 2% respectively (Table 54).

Table 54 Molecular profile of *Listeria monocytogene* isolates from Pigs and Poultry

Sr. No	Animals	Sources	Isolate no.	PCR	
				***Prs* 370 bp (Genus specific)**	***Isp* 711 bp (species specific)**
1	**Pig**	**Pork**	SP6L	+	+
2			SP8L	+	+
3			SP26L	–	–
4			SP28L	+	–
5			SP43L	–	–
6		**Faecal**	SF26L	–	–
7			SF37L	+	+
8	**Poultry**	**Chicken**	PC9L	+	+
9			PC22L	–	–
10		**Faecal**	PF32L	+	–
11			PF43L	+	+
	Total			**7 (3.5%)**	**5(2.5%)**

The researchers Lotfollahi *et al.*(2014) attempted isolation of *Listeria* spp. from dairy and meat product in Iran and reported 10 % and 0% *Listeria monocytogenes* from chicken concentrates and sausages. Kalorey *et al.* (2006) screened 50 faecal samples from different animals in Nagpur region and confirmed eight (16%) as *L. monocytogenes* by *prs* and *Isp g*ene. Nikas *et al.*(2013) reported 13% *L. monocytogenes* from chicken samples which is on higher side than present investigation and showed incomplete agreement with our study.

4.2.4 ***Salmonella* species:**

On processing of biochemically confirmed seven Salmonella isolates employing PCR technique targeting genus specific *inv A*(284); two isolates (SP11S, SP40S) from pork and one (SF23S) from pig faeces showed positivity for *salmonella* species. Prevalence of *salmonella* could be four percent among pork and two percent among faecal samples.

Similarly when six chicken isolates showed positivity on biochemical characterization and then processed for molecular confirmation employing conventional PCR & targeting genus specific *inv A* of 284bp; one isolates (PC48S) from chicken and three (PF11S, PF36S & PF48S) from faeces showed positivity for *Salmonella.* Overall prevalence among poultry would be considered as 4 % by confirming PCR by targeting genus specific *invA* (284 bp) gene. One poultry sample (no. 48) showed positivity in both the samples ie. in chicken and faecal sample (Table 55, Plate 15).

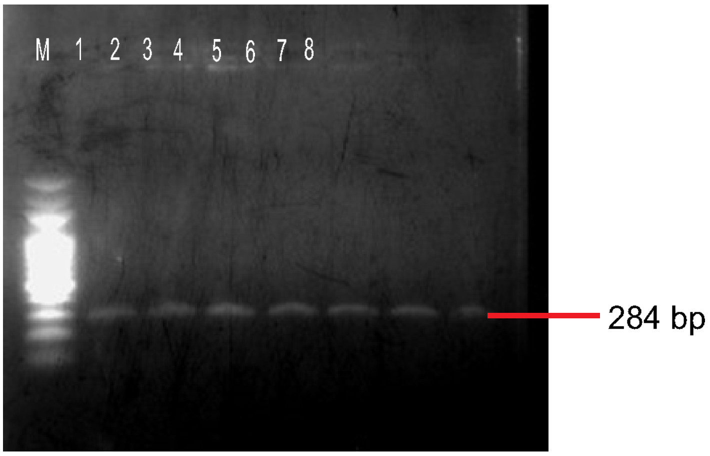


Plate 15 PCR for inv A gene of Salmonella spp.

Lane 1: S. enteridis (ATCC 13076)
Lane 2: SP11S
Lane 3: SF23S
Lane 4:PC48S
Lane 5: PF36S
Lane 6 : SP40S
Lane M: Marker

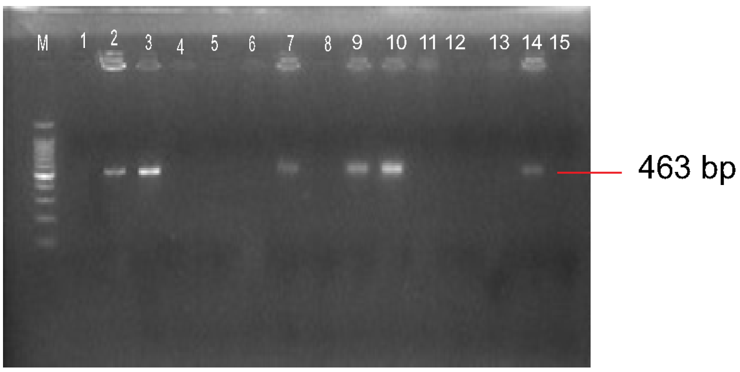


Plate 16 PCR for hcp+ gene of Campylobacter spp
.
Lane M: Marker
Lane 2: PF6C
Lane 3: SP40C
Lane 7: PF8C
Lane 9: PC45C
Lane 14: Negative Control
Lane 15: Campylobacter jejuni (ATCC BAA 1153)
Lane M: Marker

Table 55. Molecular profile of *Salmonella spp.* isolates from Pigs and Poultry

Sr. No	Animals	Sources	Isolate no.	PCR for *Inv A* (284 bp)	Total isolates and prevalence
1	Pig	Pork	SP1 S	-	2 (4%)
2			SP11 S	+	
3			SP32 S	-	
4			SP23 S	-	
5			SP40 S	+	
6		Faecal	SF3 S	-	1 (2%)
7			SF23 S	+	
8	Poultry	Chicken	PC22 S	-	1(2%)
9			PC48 S	+	
10		Faecal	PF11 S	+	3(6%)
11			PF36 S	+	
12			PF42 S	-	
13			PF48 S	+	
Total				**7(3.5%)**	**7(3.5%)**

In present study, four percent overall prevalence reported among poultry which is towards lower side when compared to Kaushik *et al.* (2014) who studied the isolation and prevalence of Salmonella from chicken meat and cattle milk collected from local markets of Patna, India and detailed 18.42% prevalence employing PCR for *invA* gene of 284bp .Zhau *et al.* (2001) studied total of 184 Chicken samples and 209 of Pork samples and revealed the prevalence of Salmonella as 2% and 3.3% in chicken and pork samples respectively in Washington D.C. In contrast, Salmonella was isolated from only 3.0% of the 825 meat samples, and chicken had the highest rate of Salmonella contamination (4.2%) and goes parallel with present finding of 2 % salmonella in chicken samples and confirmed by PCR (Fig. 9).

Kalambe *et al.*(2016) studied the prevalence, antibiogram, pathogenicity of Salmonella spp. from foods of animal origin. A total of 10 isolates of Salmonella spp. from meat (3 from cattle, 1 from buffaloes and 6 from pigs) with an overall prevalence of 5% among food animals was recorded by employing genus specific virulent gene inv A (284) for *Salmonella* spp. which are in complete agreement with our present study.

Shanmugasamy *et al.*(2011) processed 60 samples of poultry carcasses from the commercial broiler slaughtering facility in Namakkal, for cultural isolation, followed by PCR study using *invA* gene for *Salmonella* spp. and found 8.3% of poultry carcasses contaminated with Salmonella organism which goes on higher side than present work.

4.2.5 Correlation between cultural and molecular techniques

All biochemically identified isolates (04) of campylobacter species were confirmed using molecular techniques (PCR) except one (SF46C). Of 32 isolates of E. coli from pork and pig faecal samples only 26 were confirmed using uidA primers. From seven biochemically identified listeria species three isolates were confirmed as Listeria monocytogenes using prs and isp genes. Seven isolates of salmonella were identified using biochemical tests from pig samples and only three were confirmed employing invA gene.(Table 56.)

Table 56. Correlation of biochemical and molecular characterization of food borne isolates obtained from meat, faecal and blood of pigs

Sample No.	*Campylobacter* spp.				*E. coli*				*Listeria monocytogenes*				*Salmonella* spp.			
	Pork		Faecal		Pork		Faecal		Pork		Faecal		Pork		Faecal	
	Biochemical	PCR	Biochemical	PCR	Biochemical	PCR	Biochemical	PCR	Biochemical	PCR	Biochemical	PCR	Biochemical	PCR	Biochemical	PCR
1	+	+											+			
2							+									
3															+	
4					+	+	+	+								
6									+	+						
8									+	+						
10					+	+	+	+								
11					+	+	+	+					+	+		
13					+	+	+	+								
15					+	+	+	+								
17					+	+										
21							+	+								
23													+		+	+
25							+	+								
26									+		+					
28					+		+	+	+							
30							+	+								
31			+		+	+	+	+								
32													+			
36					+											
37					+						+	+				
40	+	+											+	+		
42					+	+	+	+								
43					+	+	+	+	+							
44					+	+	+	+								
46			+	+			+	+								
47					+	+	+	+								
48					+											
49							+									
Total	2	2	2	2	15	11	17	15	5	2	2	1	5	2	2	1

All blood samples were turned negative for PCR.

Similarly all biochemically identified foodborne isolates of poultry origin were screened for molecular confirmation. All isolates of campylobacter were recognized as Campylobacter jejuni and C. coli using hipO and aspK genes primers. From 40 isolates of E.coli 30 isolates were confirmed using conventional PCR. When listeria isolates of poultry origin were used for isp and prs genes only two isolates were confirmed as Listeria monocytogenes. When six isolates of salmonella were processed for PCR method only four isolates were documented as salmonella species. (Table 57).

All blood samples from pigs were negative for molecular identification of all foodborne pathogenes.

Vaishanvi et al. (2015) reconfirmed the isolates of campylobacter using 16S rRNA polymerase chain reaction. In present study, cent percent biochemically identified isolates showed positivity as Campylobacter spp. by employing 16S rRNA polymerase chain reaction out of which 63.63 % were C. jejuni and 37.27 % as C. coli. Correspondingly the co-infection of both species of C. Jejuni & C. coli is also observed in the present study.

In present study, 11 isolates of *E. coli*, one isolates of each *Salmonella* and *Listeria* spp showed positivity among pork and faecal samples of pigs which is could be contributed due to faecal contamination during post slaughtering processes whereas none of blood sample turned positive for any of the targeted pathogen (Table 59).

Table 57. Correlation of biochemical and molecular characterization of food borne isolates obtained from meat, faecal and blood of poultry

Sample No.	*Campylobacter* spp.				*E. coli*				*Listeria monocytogenes*				*Salmonella* spp.			
	Chicken		Faecal		Chicken		Faecal		Chicken		Faecal		Chicken		Faecal	
	Biochemical	PCR	Biochemical	PCR	Biochemical	PCR	Biochemical	PCR	Biochemical	PCR	Biochemical	PCR	Biochemical	PCR	Biochemical	PCR
1	+	+			+	+	+	+								
3							+									
4					+											
6			+	+	+	+	+	+								
8			+	+	+	+	+	+								
9					+	+			+	+						
11					+										+	+
15					+	+	+	+								
17					+		+	+								
18							+	+								
22	+	+			+	+			+				+			
23							+	+								
24					+											
26							+	+								
27					+	+	+	+								
29							+	+								

31					**+**											
32			**+**	**+**	**+**	**+**	**+**	**+**			**+**					
34							**+**									
36															**+**	**+**
37							**+**	**+**								
38					**+**	**+**										
39					**+**	**+**	**+**	**+**								
40					**+**											
41					**+**	**+**	**+**	**+**								
42							**+**								**+**	
43							**+**	**+**			**+**	**+**				
45	**+**	**+**	**+**	**+**			**+**									
46					**+**	**+**	**+**	**+**								
48					**+**	**+**							**+**	**+**	**+**	**+**
50							**+**	**+**								
Total	**3**	**3**	**4**	**4**	**19**	**13**	**21**	**17**	**2**	**1**	**2**	**1**	**2**	**1**	**4**	**3**

All blood samples were turned negative for PCR.

4.3 *In-vitro* pathogenicity test:

In-vitro pathogenic characters were studied by using haemolysis, congo red dye assay, PI-PLC and targeting different virulent genes for respective pathogens.

4.3.1 *Campylobacter* species:

4.3.1.1Haemolysin Production:

In the study, three isolates (SP40C, SF31C, and SF46C) from pig and six isolates (PC1C, PC22C, PC45C, PF6C, PF8C, PF45C) from poultry showed characteristics haemolysis around the colonies of *Campylobacter* spp. on sheep blood agar. Overall 4.5% of campylobacter isolates showed haemolysis on SBA with a clear zone of haemolysis around the colonies and accordingly were designated as pathogenic (Table 58).

Misawa *et.al*.(1994) detected the alpha and beta hemolytic like activity from *Campylobacter jejuni* in 40 strains of *C. jejuni* (29 human and 11 nonhuman strains) affected by pH, temperature and incubation of the pathogenes. Similarly in present study the campylobacter isolates showed alpha and beta haemolytic activity.

4.3.1.2 Determination of Virulent *hcp*+ gene and *gltA* gene :

Present study revealed total 11 isolates (pig=4, poultry=7) of *Campylobacter*, of which eight isolates of *Campylobacter* turned positive for *hcp*+as a virulent gene by employing conventional PCR. *hcp*+ gene is a specific gene for T6 secretion system of *Campyloacter* spp. which code for haemolysin co-regulated protein. Whereas *gltA* gene is an housekeeping gene for all *Campylobacter* spp and turned positive in all *Campylobacter* species (Table 58, Plate 16,17).

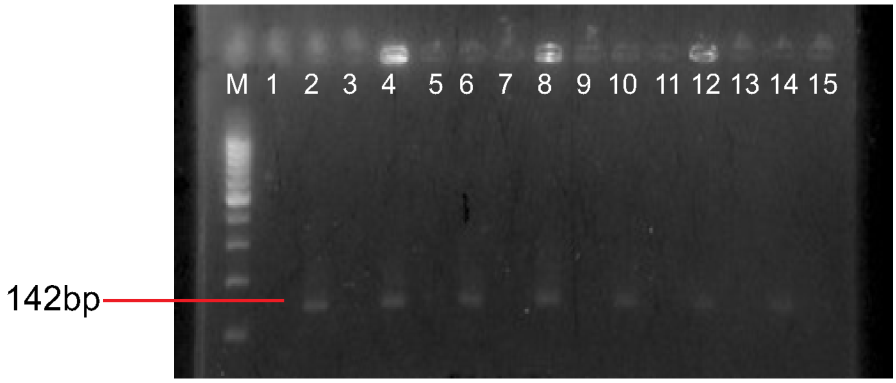


Plate 17 PCR for glt A gene of Campylobacter spp.
Lane M: Marker
Lane 1: Negative Control
Lane 2: Campylobacter jejuni (ATCC BAA 1153)
Lane 4: PF8C
Lane 6: PC45C
Lane 8: SP40C
Lane 10: PF6C

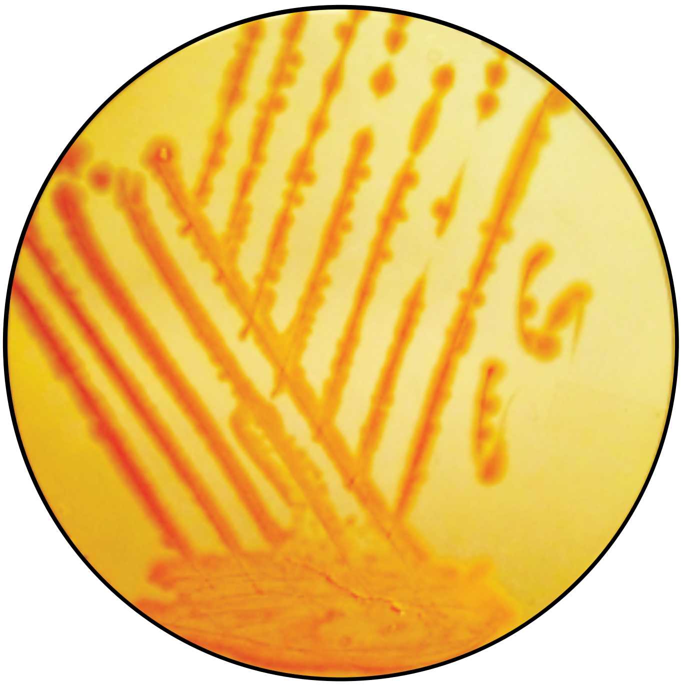


Plate 18 Typical (Brick red) colonies on Congo red agar by E. coli

Table 58. ***In-Vitro*** **pathogenecity of Campylobacter isolates from Pigs and Poultry**

Sr. No.	Animals	Sources	Isolate No.	Haemolysin on SBA	PCR	
					hcp +	***glt A***
1	**Pig**	**Pork**	**SP1C**	-	-	+
2			**SP40C**	+	+	+
3		**Faecal**	**SF31C**	+	+	+
4			**SF46C**	+	+	+
5	**Poultry**	**Chicken**	**PC1C**	+	+	+
6			**PC22C**	+	-	+
7			**PC45C**	+	+	+
8		**Faecal**	**PF6C**	+	+	+
9			**PF8C**	+	+	+
10			**PF32C**	-	-	+
11			**PF45C**	+	+	+
		Total		**9 (4.5%)**	**8 (4%)**	**11 (5.5%)**

According to the results of Corcionivoschi *et al.*(2015), in present study also all the isolates of *Campylobacter* were positive for housekeeping gene i.e. *gltA* studied the virulence of *Campylobacter* isolates by PCR targeting *hcp*+ gene and *gltA* gene. The workers observed 56.1% *C. coli* and 28.8% *C. jejuni* positive isolates which goes on parallel side of the present study where 62.5% *Campylobacter jejuni* and 37.5% *Campylobacter coli* showed positivity for *hcp*+ gene and considered as pathogenic.

Harrison *et.al*.(2014) studied identification of possible virulence marker from *Campylobacter jejuni* isolates and reported 33.33% of *Campylobacter jejuni* strains from chicken were positive for *hcp*+ gene (463 bp) which is to lower side than our present study results.

This variation in virulent characteristics may be because of pathogenic characteristics of the isolates which is mainly depend upon flagellar motility of bacteria, is essential for cell adhesion and invasion but its absence indicates severe reduction in motility and intestinal mucosa colonization of humans and chicken (Melo *et al.* 2013).

All the isolates of campylobacter of present study were positive for *gltA* housekeeping gene. Of these two isolates (SP1C & PF32C) of *Campylobacter jejuni* were negative for haemolysin production and *hcp*+ virulent gene indicating nonpathogenic nature. One isolate (PC22C) showed haemolysin production but negativity towards *hcp*+ virulent gene. Of total 11 isolates of campylobacter five & three isolates of *C. jejuni* &*C. coli* respectively were pathogenic in nature (Table 60) All the pathogenic isolates showed motility followed by positivity for haemolysin & hcp+ gene which confirms the virulent characteristics of the pathogenes (Corcionivoschi *et al.*2015).

4.3.2 *Escherichia coli*

4.3.2.1 Haemolysis on sheep blood agar (SBA):

In present study, 19 per cent isolates from pigs while nine per cent from poultry showed haemolysis on SBA with clear zone around colony and accordingly designated as pathogenic (Table 59& 60).

Table 59. *In-vitro* Pathogenicity test for *Escherichia coli of* pigs isolates

Sr. No.	Animals	Sources	Isolate No	Haemolysin (SBA)	Congo red dye Test	PCR	
						***Stx* 1**	***Stx* 2**
1	Pig	Pork	SP4E	+	+ve	-	-
2			SP10E	+	-ve	-	-
3			SP11E	-ve	-ve	-	-
4			SP13E	-ve	-ve	-	-
5			SP15E	+	+ve	-	-
6			SP28E	+	+ve	-	-
7			SP31E	-ve	-ve	-	-
8			SP42E	+	+ve	-	-
9			SP43E	+	+ve	-	-
10			SP44E	+	-ve	-	-
11			SP47E	++	-ve	-	-
12		Faecal	SF4E	+++	+ve	-	-
13			SF10E	+	-ve	-	-
14			SF11E	++	-ve	-	-
15			SF13E	+	-ve	-	-
16			SF15E	+	+ve	-	-
17			SF21E	-ve	-ve	-	-
18			SF25E	-ve	-ve	-	-
19			SF28E	+	+ve	-	-
20			SF30E	-ve	-ve	-	-
21			SF31E	–	-ve	-	-
22			SF42E	+	-ve	-	-
23			SF43E	+	-ve	-	-
24			SF44E	+	+ve	-	-
25			SF46E	+	+ve	-	-
26			SF47E	++	+ve	-	-
Total				**19 (19%)**	**11 (11%)**	**0**	**0**

Table 60 *Invitro* pathogenicity test for *Escherichia coli* of Poultry isolates

Sr. No.	Animals	Sources	Isolate no.	Haemolysin (SBA)	Congo red dye	PCR Stx1	PCR Stx2
1	Poultry	Chicken	PC1E	-ve	-ve	-	-
2			PC6E	-ve	-ve	-	-
3			PC8E	+ve	-ve	-	-
4			PC9E	+ve	+ve	-	-
5			PC15E	-ve	-ve	-	-
6			PC22E	-ve	-ve	-	-
7			PC27E	-ve	-ve	-	-
8			PC32E	-ve	-ve	-	-
9			PC38E	++	+ve	-	-
10			PC39E	-ve	-ve	-	-
11			PC41E	-ve	-ve	-	-
12			PC46E	-ve	-ve	-	-
13			PC48E	-ve	-ve	-	-
14		Faecal	PF1E	-ve	-ve	-	-
15			PF6E	+	-ve	-	-
16			PF8E	-ve	-ve	-	-
17			PF15E	-ve	+ve	-	-
18			PF17E	-ve	-ve	-	-
19			PF18E	+ve	+ve	-	-
20			PF23E	-ve	-ve	-	-
21			PF26E	-ve	-ve	-	-
22			PF27E	+ve	+ve	-	-
23			PF29E	-ve	-ve	-	-
24			PF32E	+ve	+ve	-	-
25			PF37E	-ve	-ve	-	-
26			PF39E	+ve	-ve	-	-
27			PF41E	-ve	-ve	-	-
28			PF43E	++	+	-	-
29			PF46E	-ve	-ve	-	-
30			PF50E	-ve	-ve	-	-
	Total			**9 (9%)**	**7(7%)**	**0**	**0**

Deshmukh *et al.*(2005)observed that 29 out of 53 *E. coli* isolates from diarrheic cases were haemolytic on 5% washed sheep blood agar. Similarly Jaulkar *et al.*(2009) revealed 13(86.66%) isolates haemolytic among which 10(66.66%) isolates were α -haemolytic and, three (20%) were β-haemolytic whereas two (13.33) isolates were negative for haemolysin production.

4.3.2.2 Congo red dye binding test:

In present investigation, 11 percent isolates from pig and seven percent from poultry showed cono red dye binding capacity (Table 61 & 62).

Table 61 *In Vitro* pathogenecity profile of *Listeria monocytogenes* isolates from Pigs and Poultry

Sr. No	Animals	Sources	Isolate no.	Haemolysis on SBA	CAMP		PI-PLC	*hly A*
					S. aureus	***R. equi***		
1	**Pig**	**Pork**	SP6L	+	+	–	+	–
2			SP8L	+	+	–	+	+
3			SP28L	–	–	–	-	–
4		**Faecal**	SF37L	+	+	–	+	+
5	**Poultry**	**Chicken**	PC9L	+	+	–	+	+
6		**Faecal**	PF32L	+	–	–	–	+
7			PF43L	+	+	–	+	+
	Total			**6 (3%)**	**5 (2.5%)**	**0 %**	**5 (2.5%)**	**5 (2.5%)**

Table 62 *In Vitro* Pathogenicity profile of *salmonella spp.* isolates from Pigs and Poultry

Sr. No	Animals	Sources	Isolate no.	Haemolysin on SBA	Congo red dye test
1	Pig	Pork	SP1 S	-	-
2			SP11 S	+	+ +
3			SP32 S	-	+
4			SP23 S	-	-
5			SP40 S	+ +	+ + +
6		Faecal	SF3 S	-	-
7			SF23 S	+ +	+
8	Poultry	Chicken	PC22 S	-	-
9			PC48 S	+	+ +
10		Faecal	PF11 S	+	+
11			PF36 S	+	+ + +
12			PF42 S	-	+
13			PF48 S	+	+
Total				**7(3.5%)**	**9 (4.5%)**

Chausalkar *et al. (*2004) reported positivity of an 22 *E. coli* isolates from healthy pigeon but only one isolate was congo red positive. Chaudhari *et al.* (2007)studied the pathogenic nature of *E. coli* from smoked meat by congo red adsorption (CRDA) test. The study reported positivity of all isolates for congo red binding**.** On the contrary Jaulkar *et al.*(2009) screened 15 isolates of *E.coli* from foods of animal origin and five isolates showed positivity to Congo red binding assay(Plate 18).

The findings of present study are in accordance with the above researchers reports. Congo red dye binding (CR) assay could be used as phenotypic marker to distinguish invasive and noninvasive strains of *E. coli* (Parul *et al.*2014).

4.3.2.3 Determination of virulent gene:

In the present study all *E.coli* isolates from poultry and pig samples were negative for STEC genes like *stx*1 and *stx*2 (Table 59& 60).

The results of Dhanashree *et. al*. (2008) detected only one *E. coli* isolate positive for all STEC genes like *stx*1 gene (614 bp), *stx*2 gene (779 bp), *eae* gene (384 bp), *rfbO157* gene (497 bp), *hlyA* gene (166 bp) from stool and meat samples from Manglore (India). Similarly Zhao *et al* (2001) reported (38.7%) *E. coli* from chicken samples, 19 percent from beef, 16.3 per cent from pork and 11.9 percent from turkey employing virulent gene *stx1* and *stx2* for *E coli.* The results of present study are totally dissimilar with the above findings of researchers.

From all the isolates of pig, 11 isolates (five from chicken & six from faecal) of *E. coli* showed positivity for congo red dye assay and haemolysin but negative for STEC virulent *stx1* and *stx2* genes. Likewise six isolates (two from chicken & four from faecal) of *E. coli* from poultry showed same results (Table 59& 60).

4.3.3 *Listeria monocytogenes*

Pathogenicity of *Listeria monocytogenes* is studied by haemolysin production on Sheep Blood Agar (SBA); Christie-Atkins-Munch-Peterson test (CAMP), phosphatidyl inositol-phospholipase-C (PI-PLC) assay, PCR targeting virulent gene *hly A*.

4.3.3.1 Haemolysis on sheep blood agar (SBA)

In present study, each three isolates of *Listeria monocytogenes* among pig and poultry showed haemolysis on 7% sheep blood agar (Table 61).

Chaudhari *et al*. (2004) reported 2.4% *L. monocytogenes* from buffalo beef samples collected at Bareilly, India and all isolates were pathogenic with characteristic haemolysis on SBA. Cetinkaya *et al.*(2014) confirmed 3.9% *L. monocytogenes* positive for haemolysin assay and were designated as pathogenic out of 512 food samples including raw milk, dairy products, meat and meat products, chicken meat, sea food and raw vegetables collected at Turkey. The results are in concurrence with the present findings.

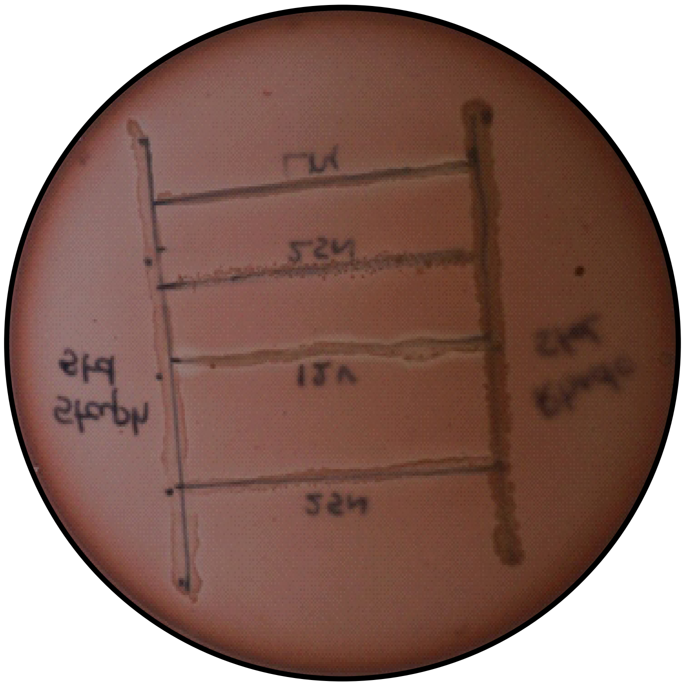


Plate 19 CAMP test shown by L. monocytogenes

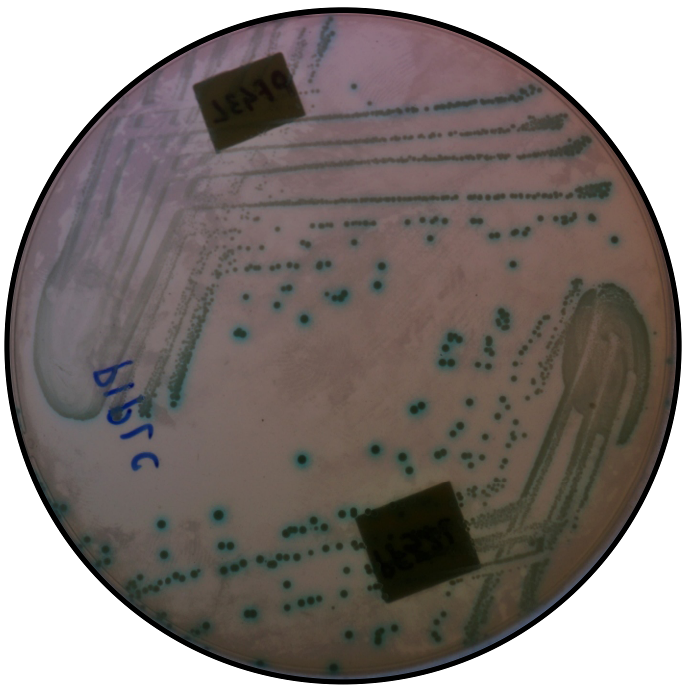


Plate 20 PI-PLC assay for Listeria monocytogenes

Waskar *et al.*(2013) processed 736 meat samples collected from Mumbai. The workers employed the haemolysis test for determination of pathogenicity of isolates (51) obtained from 736 meat samples and reported 6.92 % pathogenic *Listeria monocytogenes* which is higher than present study reports. The haemolytic characteristics depends on the pathogenicity of the isolates.

4.3.3.2 Christie, Atkins, Munch-Petersen (CAMP) test

In present study, three isolates from pig and two isolates from poultry showed CAMP test positive and revealed enhanced zone of haemolysis against *S. aureus* and confirmed as *L. monocytogenes* (Table 61,Plate 19).

In present work, five positive *L. monocytogenes* from seven *Listeria* spp. showed positivity on CAMP test which is in agreement with Chaudhari *et al.*(2004) who reported 5 positive pathogenic. *L. monocytogenes* from 11 isolates of *Listeria* spp. obtained from buffaloes at Bareilly by conducting CAMP test. Similarly Molla *et al.*(2004) screened 316 food samples and reported 5.1% prevalence of *L. monocytogenes* confirmed by CAMP test.

4.3.3.3 Phosphatidylinositol-specific phospholipase C (PI-PLC) Assay:

In present work, five isolates of *L. monocytogenes* showed characteristics light blue colored colonies on L. mono Differential agar indicating PI-PLC activity (Table 61, Plate 20).

The present study results are in concurrence with the results of Dudhe (2012) who isolated 12 *Listeria* strains from different food animals and confirmed the pathogenicity on the basis of PI-PLC production. The worker reported all isolates positive for PI-PLC test. Vaidya (2013) screened 17 isolates of *L. monocytogenes* for pathogenicity by PI-PLC test employing L. mono differential agar and reported all isolates positive for PI-PLC test. Waskar *et al.*(2013) examined pathogenicity of 53 isolates of *L. monocytogenes* obtained from 736 processed meat samples .Of those 46 isolates were positive while 5 isolates as negative for PI-PLC test.

4.3.3.4 Determination of *hly A* gene by PCR

Of seven listeria species, five isolates showed positivity for *hlyA* gene and designated as pathogenic *Listeria* spp. (Table 61,Plate 21).

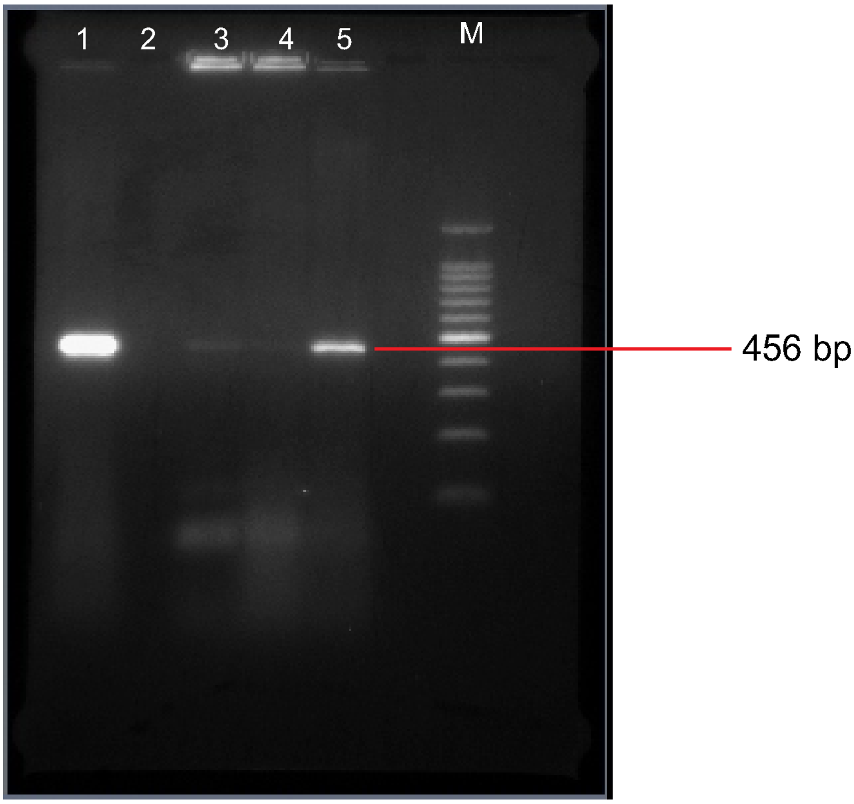


Plate 21 PCR for hly A of L. monocytogenes
Lane 1: L. monocytogenes (ATCC 19118)
Lane 2: Negative Control
Lane 3: SP6L
Lane 4:SP8L
Lane 5: SF37L
Lane M: Marker

The results are in concurrence with Vaidya (2013) who studied the pathogenicity of 17 isolates of *L. monocytogenes*(7 from goats and 10 from pigs) in Nagpur region. The researcher reported all 17 isolates positivity for the *hly*A gene at 456 bp. In the same way Thomas (2013) reported 18 isolates comprising 9 each from meat and faecal samples obtained from the cattle slaughtered in Nagpur region bearing *hly*A gene at 456 bp and accordingly positive isolates were considered as pathogenic. Also Paziak-Domaneska *et al.*(1999) studied pathogenicity of 46 isolates of *L. monocytogenes* obtained from meat and sausage and confirmed all as pathogenic by PCR using primer against *hlyA*.

Swetha *et al.*(2013) processed 150 samples (25 each of chicken, swab of chicken, pork, swab of pork, fish, swab of fish) and revealed 17 samples positive for *hly*A gene. The results are on higher side than present study were 2% reported.

Overall two isolates each from pig and poultry showed positivity for all virulent tests viz. haemolysin, PIPLC, CAMP &*hlyA* gene (Table 61) confirming the pathogenic nature.

The positive CAMP and haemolysis assay and the presence of *hlyA* gene by PCR in the isolates suggest that these virulent strains may have the potential to invade host cells and cause listeriosis.

4.3.4 *Salmonella* spp.

In order to evaluate the pathogenicity of Salmonella isolates, all were subjected to *in-vitro* pathogenicity tests including haemolysis on sheep blood agar (5 per cent) and Congo red dye binding assay (0.003 per cent) and assessment of *invA* gene. Thirteen isolates of pig (fecal=2 & pork=5) and six from poultry (fecal=4 & chicken=2) were subjected for *in-vitro* pathogenicity tests.

4.3.4.1. Haemolysin production on Sheep Blood Agar (SBA)

Of total 19 isolates (13 from pig & 6 from poultry); seven isolates (three from pig and four from poultry) turned positive for haemolysin production on SBA and accordingly were designated as pathogenic. All isolates which were positive for *inv A* showed alpha (α) haemolysis around colonies and clear zone ofhaemolysis (Table 62).

Jaulkar *et.al*.(2009) verified haemolysis of salmonella isolates revealing overall 36.36% positivity among foodborne pathogenes from animal originated foods from Vidharbh region of Maharashtra . Among the total isolates, 36.36% produced α- haemolysis whereas none of the isolates showed the β- haemolysis which is at lower side than present study where 53.84% isolates showed positivity for haemolysis.

Agrawal *et.al*.(2005) determined haemolytic potential of 94 strains of Salmonella gallinarum through demonstration of haemolysin on blood agar & revealed production of two types of haemolysis viz., beneath the colony haemolysis (BCH) or contact haemolysis and clear zone haemolysis (CZH). The researchers further reported clear zone of haemolysis on blood agar prepared with washed erythrocytes of goat and a total of 12 per cent (11 of 94) different haemolytic patterns of strains revealing multiplicity of haemolysins in *S. gallinarum.* The present study found only one pattern of haemolysis i.e. beta haemolysis with variation in zone of haemolysis.

4.3.4.2 Congo red dye binding test

In present assay, nine isolates from total thirteen showed strong ability to bind with congo red dye and produce characteristics brick red colonies and considered as pathogenic. Two each isolates showed strong positive (+++) and moderate (++) while five showed weak (+) positivity (Table 62 ,plate).

In this study, Salmonella isolates showed high degree of binding ability to Congo red dye agar (0.003%) which was judged by degree of colour intensity produced by colony. All eight isolates showed characteristics brick red colonies on Congo red dye BHI agar which goes parallel with Kalambhe (2012) who evaluated total 10 isolates positive for Congo red binding assay suggesting their high virulent nature from animal and human origin to detect presence of virulent Salmonella Spp.

Sable (2015) determined In vitro pathogenicity of the Salmonella isolated from childhood diarrhea cases and 5 isolates showed varied degree of congo red binding ability which is near to present investigation.

Tiwari *et al.*(2000) studied differences in haemolysin expression in a strain of Salmonella enteric serovar Typhimurium definitive phage type (DT) 98 cultured under various conditions and observed colonies as smaller, with dark centers and wider zones of haemolysis on Congo red blood agar. The workers directly correlated Haemolysin production with Congo red binding in nutrient broth. However, they also reported Culture-cell-free haemolysin activity as higher, but cell-bound haemolysin activity was very low in growth medium supplemented with Congo red which can be correlated with present study were Salmonella isolates which showed haemolysis were turned positive for Congo red dye binding test.

4.3.4.3. Detection of *invA* gene among Salmonella isolates

In present investigation, two isolates from pork and one from pig faeces showed positivity for *salmonella.* Similarly, in poultry samples one isolates from chicken and three from faeces showed positivity for *salmonella* species using invA gene (284bp).Accordingly six percent pathogenicity collectively among pork and faecal samples of pig. Consequently four percent pathogenicity among pork & two percent among pig faecal samples. Pathogenicity among poultry was considered as two percent in chicken and six percent in faecal samples confirming four percent prevalence among poultry samples. Overall positivity for *invA* gene (284 bp) is three percent. Overall three percent pathogenicity was reported among pigs and poultry samples each (Table 55, Plate 15).

Jamshidi *et al.*(2009) analyzed 60 samples randomly collected from chilled broiler carcasses at Iran. The workers showed that five (8.3%) and one (1.6%) samples were contaminated with Salmonella spp. and Salmonella Typhimurium, respectively by using S139 and S141 primers that amplifies a 284 bp sequence of the *invA* gene and *Fli15* and Tym primers that amplifies a 559 bp sequence of the *fliC* gene which showed incomplete agreement with present work revealing four percent prevalence among poultry samples.

However the results are higher when compared to Karmi (2013) observed 16% of positivity for Salmonella isolation from meat and poultry products collected from shops and supermarkets of Egypt. Specifically; 26% (13/50) in meat samples and 6% (3/50) in poultry samples and all Salmonella isolates were positive for the *invA* gene showing 284 bp DNA fragment. Ezzat *et al.*(2014) detected *invA* gene from six isolated strains (*S. enteritidis, S. macclesfield, S. rissen, S. derby, S. magherafelt and S. enteric sub spp. salamae*) isolated from 1000 broiler chicken samples (40 apparently healthy, 80 diseased chickens and 80 freshly dead broiler chickens) of Dakahlia and confirmed as pathogenic.

Sable (2015) studied the pathogenicity of salmonella isolated from childhood diarrhea cases by employing *inv A* (284 bp) gene and confirmed the pathogenicity of Salmonella isolates which is in complete agreement with present study.

Collectively, two isolates (SP11S, SP40S) from pork and one isolate (SF23S) from pig faecal were positive for haemolysin, congo red dye binding assay and presence of virulent *invA* gene. Similarly two samples of poultry faeces (PF11S, PF36S) showed positivity for haemolysin, Congo red assay and *invA* gene. One sample (no. 48) of poultry was positive for all three different virulent tests from both the sources (meat & faecal).

4.4 Antibiogram study:

In present study all the positive isolates were screened for antibiogram study using different antibiotics suggested by Baur *et al* (1996).

4.4.1 *Campylobacter* spp.

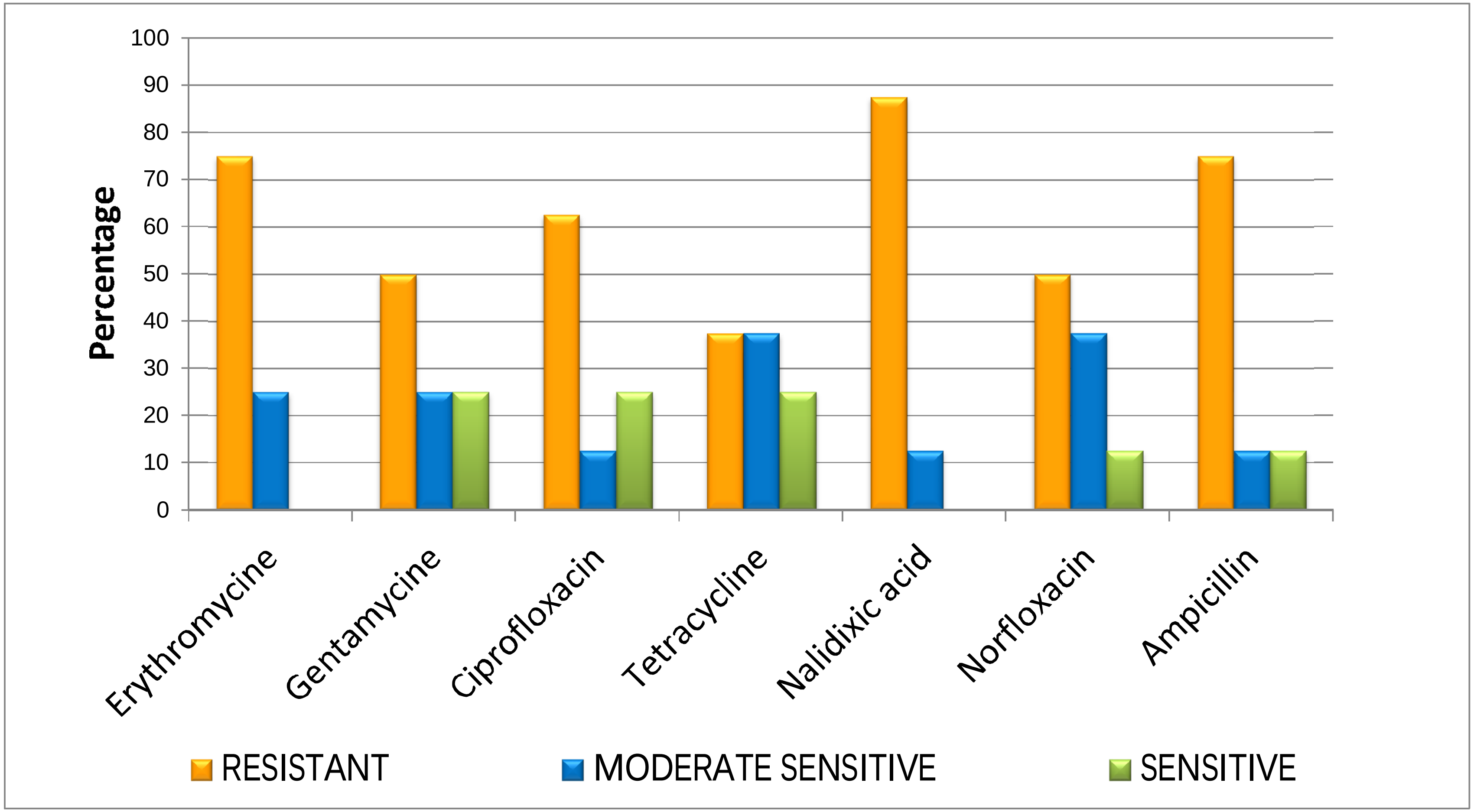
In the present investigation, all eight *hcp*+ virulent *Campylobacter* isolates were studied by agar disc diffusion method using seven antibiotics *viz*., ampicillin, ciprofloxacin, nalidixic acid, erythromycin, gentamicin, norfloxacin, and tetracycline. All eight *Campylobacter* isolates were characterized as sensitive, moderately sensitive and resistant based on the size of the inhibition zones according to manufacturer's instructions (HI Media). The overall antibiotic sensitivity pattern of *Campylobacter* isolates towards seven antimicrobial agents is detailed in table given below.

Table 63 Details of antibiogram study of *Campylobacter* isolates

Sr. No.	Antibiotics	No. of isolates(08)			Percentage (%)		
		Resistant	Moderately sensitive	Sensitive	Resistant	Moderately sensitive	Sensitive
1	Erythromycin	6	2	-	75	25	0
2	Gentamycin	4	2	2	50	25	25
3	Ciprofloxacin	5	1	2	62.5	12.5	25
4	Tetracycline	3	3	2	37.5	37.5	25
5	Nalidixic Acid	7	1	-	87.5	12.5	-
6	Norfloxacilin	4	3	1	50	37.5	12.5
7	Ampicillin	6	1	1	75	12.5	12.5

A total of eight isolates (five *C. jejunii* & three *C. coli*)were screened for antibiogram study against seven antibiotics of that seven isolates (87.5%) showed resistance towards nalidixic acid followed by six isolates each for erythromycin and ampicillin (75%) whereas five isolates to ciprofloxacin (62.5%) &four isolates each against gentamycin and norfloxacin (50%) and three isolates to tetracycline (37.5%) Moderate sensitivity was observed towards tetracycline and norfloxacin (37.5% each) by three isolates each, followed by erythromycin, gentamycin (25% each)by two isolates each and towards ciprofloxacin, nalidixic acid and ampicillin (12.5% each). Sensitive towards two isolates each for gentamicin, ciprofloxacillin and tetracycline, one isolate for norfloxacin and ampicillin with overall sensitivity of 25% each, 12.5% each respectively. (Table 65, Fig. 10).

Fig.10 Overall antibiotic pattern of *Campylobacter* Spp. isolates



The present finding of antibiogram study from pig and poultry isolates is in cent percent agreement with the report by Dalal *et al.*(2010), Baserisalehi *et al.* (2007) and Shivshankari *et al.*(2015) who reported maximum resistance towards nalidixic acid, ampicillin and erythromycin with different percentages. Likely for sensitivity of the campylobacter species Rahimi *et al.*(2011) & Dabiri *et al.* (2013),reported most sensitivity towards gentamycin and ciprofloxacin which is also observed in our findings.

4.5.4 *Escherichia coli*

In the present investigation, all twenty eight *E. coli* isolates were studied by agar disc diffusion method using fourteen antibiotics *viz.*, Ampicillin, amikacin, azithromycin, Ceftazidione, Cefotaxim, doxycycline, Erythromycin, Gentamicin, neomycin, nalidixic acid, penicillin G, streptomycin, tetracycline and trimethoprim. All twenty seven *E. coli* isolates were characterized as sensitive, moderately sensitive and resistant based on the size of the inhibition zones according to manufacturer's instructions (HI Media). The overall antibiotic sensitivity pattern of *E. coli* isolates towards twelve antimicrobial agents is detailed in table 66 given below.

Out of total twenty eight isolates screened for antimicrobial sensitivity test, 19 isolates were found to be resistant for ampicillin, erythromycin and neomycin (67.85%) whereas 16 isolates were resistant against ceftazidime, streptomycin (57.54% each) 14 isolates to cefotaxime (50%) thirteen isolates each to doxycycline and trimethoprim (46.42% each), 11 isolates to amikacin, gentamicin and nalidixic acid (39.28% each); eight isolates showing resistance towards azithromycin (28.57%) and seven to tetracycline (25%). However maximum resistance was observed towards penicillin-G i.e. 75 per cent. Moderate degree of sensitivity was noted towards amikacin (50%) followed by nalidixic acid (42.85%), Ceftazidime (35.11%), azithromycin and doxycycline (28.57 % each), ampicillin, gentamycin and streptomycin (17.85% each), erythromycin (10.71%) and trimethoprim (7.14%)(Fig. 11).

Maximum sensitivity was observed for tetracycline and trimethoprim (46.42 %each) and then decreasingly for azithromycin and gentamycin (42.85% each), cefotaxim, doxycycline and streptomycin (25% each), erythromycin (21.42%), nalidixic acid (17.85%), ampicillin (14.28%), amikacin& penicillin-G(10.71% each), Ceftazidime& neomycin (7.14% each).

Fig. 11 Overall antibiotic patterns of *E.coli* isolates

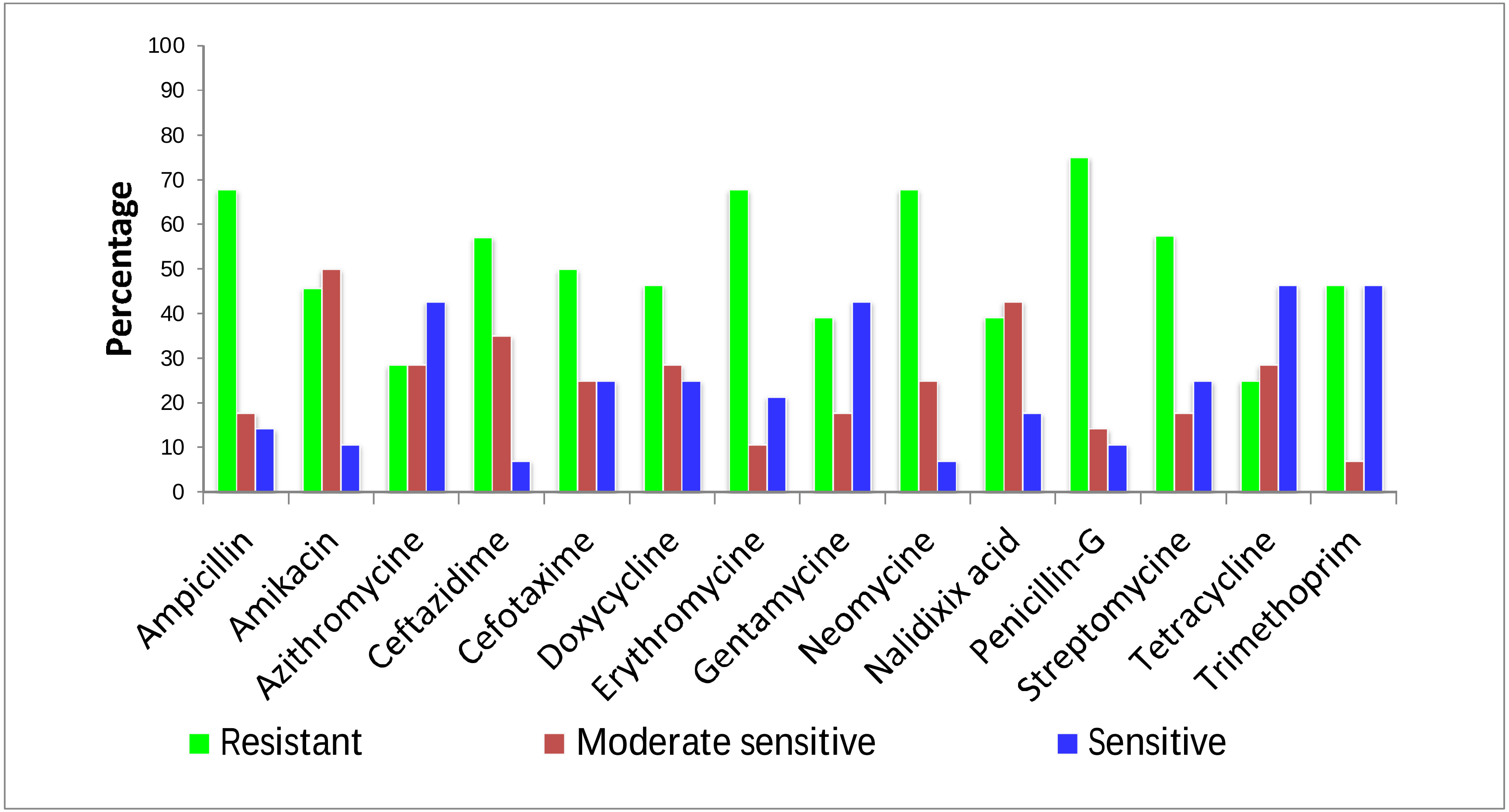


Table 64 Details of antibiogram study of *E. coli* isolates

Sr.No.	Antibiotics	No. Of isolates (28)			Percentage (%)		
		Resistant	Moderately sensitive	sensitive	Resistant	Moderately sensitive	Sensitive
1	Ampicillin	19	5	4	67.85	17.85	14.28
2	Amikacin	11	14	3	45.83	50	10.71
3	Azithromycin	8	8	12	28.57	28.57	42.85
4	Ceftazidime	16	10	2	57.14	35.11	7.14
5	Cefotaxime	14	7	7	50	25	25
6	Doxycycline	13	8	7	46.42	28.57	25
7	Erythromycin	19	3	6	67.85	10.71	21.42
8	Gentamycin	11	5	12	39.28	17.85	42.85
9	Neomycin	19	7	2	67.85	25	7.14
10	Nalidixic acid	11	12	5	39.28	42.85	17.85
11	Penicillin-G	21	4	3	75	14.28	10.71
12	Streptomycin	16	5	7	57.54	17.85	25
13	Tetracycline	7	8	13	25	28.57	46.42
14	Trimethoprim	13	2	13	46.42	7.14	46.42

Goswami *et al.*(2004) studied antibiogram of 51 strains of *E.coli* isolated from poultry and noticed highest degree of resistance to penicillin (100%) followed by tetracycline (98.3%), ampicillin (62.74%), cloxacillin (15.68%), erythromycin (7.84%) and nalidixic acid (1.96%). Likewise Jaulkar *et al.* (2009) observed cent percent resistant against penicillin and tetracycline followed by doxycycline hydrochloride (73.33%), ceftazidim (53.33%), ampicillin (46.66%), erythromycin and streptomycin (40% each). Similarly the present study reports are in agreement with the above researchers reports.

Maximum sensitivity in present study was observed towards tetracycline and trimethoprim which is conflicting with the findings of Bhong & Brahmabhat (2006), Jaulkar (2009) and Sheikh *et al* (2013) who reported sensitivity against different antibiotics viz. ciprofloxacin, nalidixic acid, norfloxacin, chloramphenicol and gentamycin.

4.4.3 *Listeria monocytogenes*

In the present investigation, all five *Listeria* isolates were studied by agar disc diffusion method using five antibiotics *viz.*, ampicillin, ceftriaxone, cefotaxim, cloxacilin, erythromycin, gentamicin, kanamycin, norfloxacin, oxytetracycline, penicillin G, rifampicin and vancomycin.. The overall antibiotic sensitivity pattern of *Listeria* isolates towards twelve antimicrobial agents is detailed in table given below.

Out of total five isolates screened for antimicrobial sensitivity test against twelve antibiotics, in which maximum resistance was observed towards gentamycin, kanamycin, norfloxacin (100% each) followed by penicillin-G and vancomycin (80% each); ceftiaxone, cloxacillin, erythromycin and rifampicin (60% each). Moderate degree of sensitivity was observed towards cephotaxim (60%), ampicillin, erythromycin and rifampicin (40% each) followed by ceftriaxone, cloxacillin, oxytetracycline, penicillin-G and vancomycin (20% each). Whereas maximum sensitivity was exhibited towards ampicillin and oxytetracycline (40% each) followed by ceftriaxone, cefotaxim and cloxacilin (20% each) (Table 67, Fig 12).

Fig.12. Overall antibiotic pattern of *Listeria monocytogenes* isolates

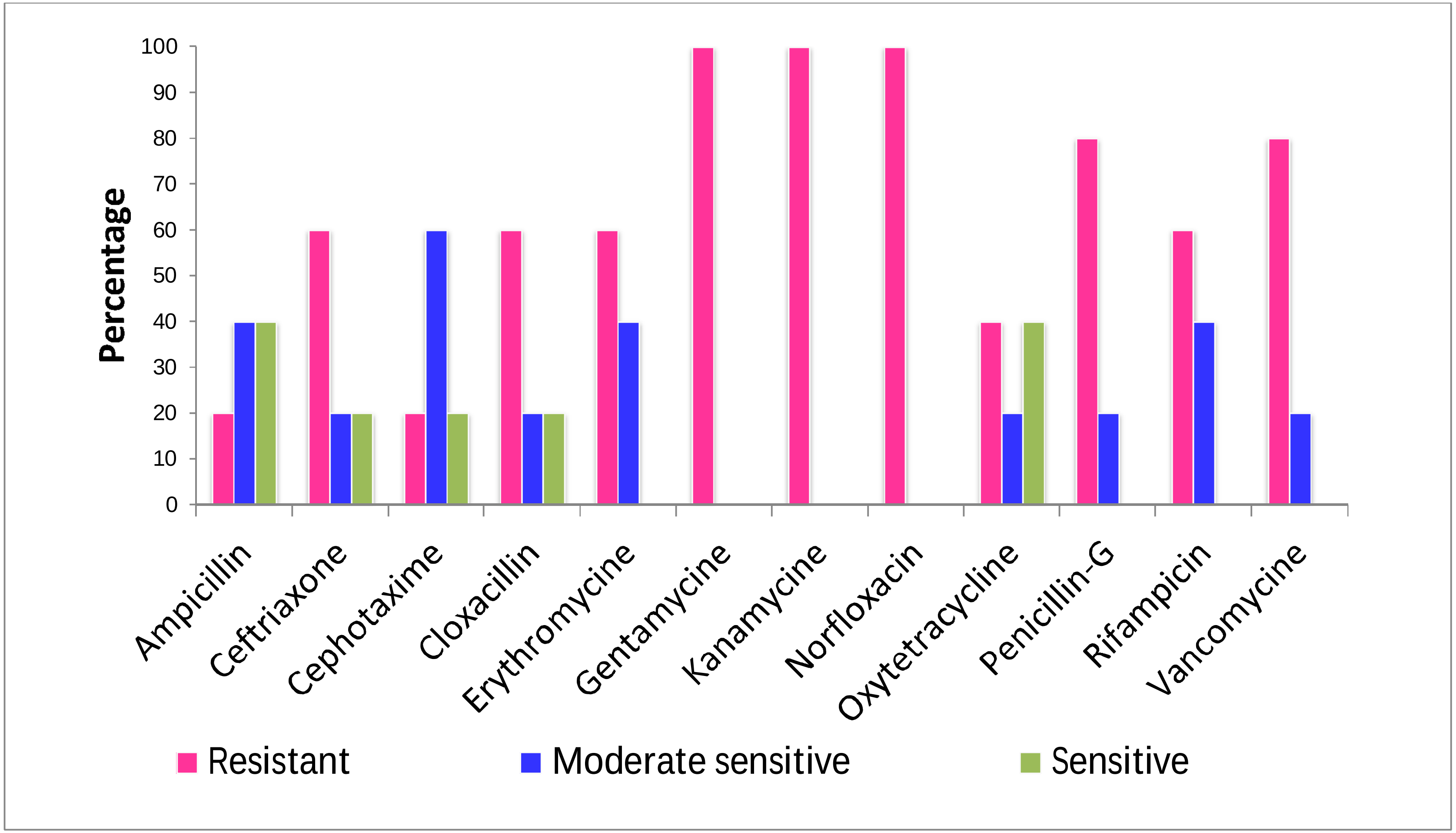


Table 65 Details of antibiogram study of *Listeria monocytogenes* isolates

Sr.No.	Antibiotics	No. Of isolates(five)			Percentage		
		Resistant	Moderately sensitive	Sensitive	Resistant	Moderately	Sensitive
1	Ampicillin	1	2	2	20	40	40
2	Ceftriaxone	3	1	1	60	20	20
3	Cephotaxime	1	3	1	20	60	20
4	Cloxacillin	3	1	1	60	20	20
5	Erythromycin	3	2	0	60	40	0
6	Gentamycin	5	0	0	100	0	0
7	Kanamycin	5	0	0	100	0	0
8	Norfloxacilin	5	0	0	100	0	0
9	Oxytetracycline	2	1	2	40	20	40
10	Penicillin-G	4	1	0	80	20	0
11	Rifampicin	3	2	0	60	40	0
12	Vancomycin	4	1	0	80	20	0

Plate 22 Antibiogram assay for Salmonella isolates

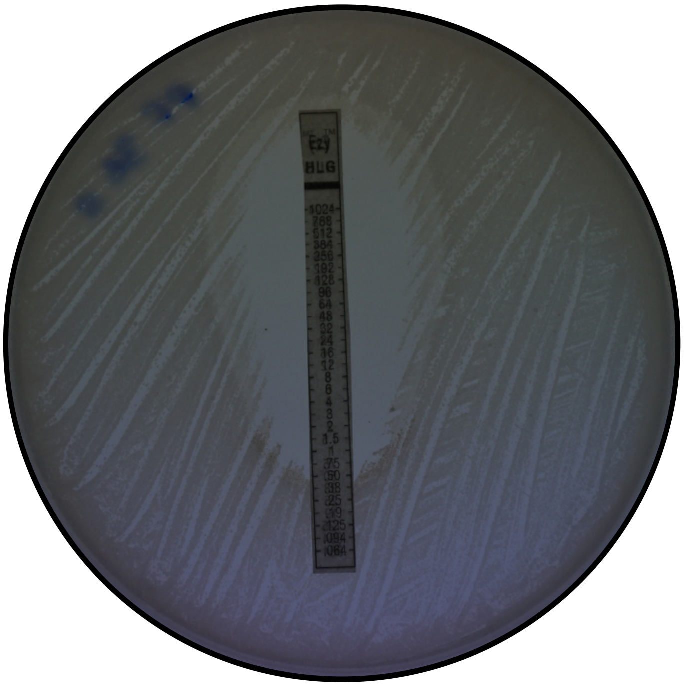


Plate 23 MIC assay for E. coli isolates

The results of antibiogram study of *L. monocytogenes* among pig and poultry were complete agreement with the findings of the researchers who conducted work in India and worldwide. Moreno *et al.*(2014) tested susceptibility of 46 *Listeria* species. isolates derived from slaughter house environment, pork and human infection using several antibiotics and found all *Listeria* isolates susceptible to penicillin, ampicillin, tetracycline, and erythromycin and resistant to clindamycin, daptomycin and oxacillin. Vasu *et al.*(2014) determined antibiogram of three isolates of *Listeria* spp. obtained from meat processing environment in Kerala. All isolates were sensitive to cefotaxime, chloramphenicol, doxycycline, erythromycin, streptomycin, vancomycinand gentamicin, intermediately sensitive to enrofloxacin and rifampicin, resistant to ampicillin and cloxacillin

4.4.4 *Salmonella* spp.

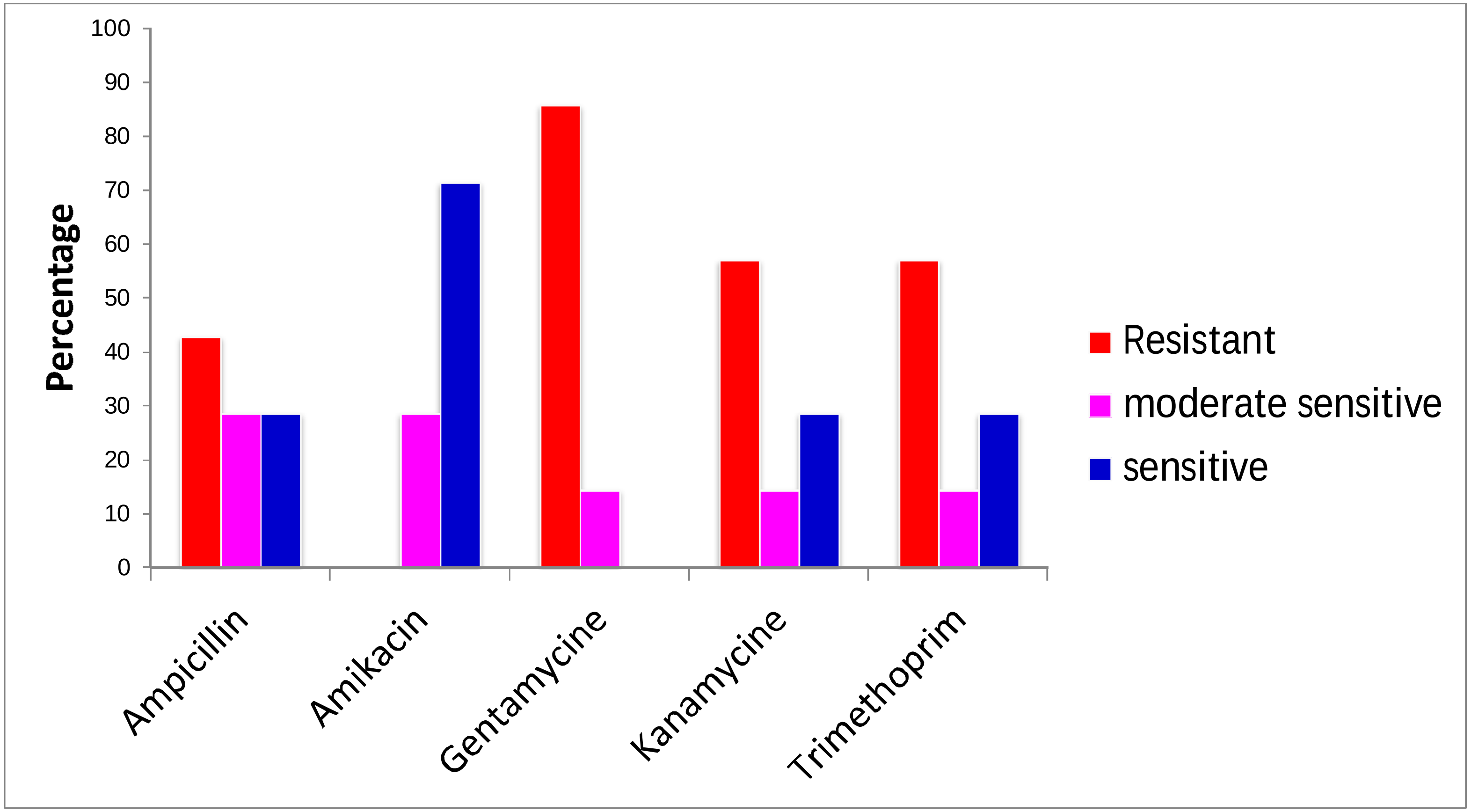
In the present investigation, all *invA* gene consisting virulent seven *Salmonella* isolates were studied by agar disc diffusion method using five antibiotics *viz*., ampicillin, gentamicin, kanamycin, amikacin and trimethoprim. The overall antibiotic sensitivity pattern of *Salmonella* isolates towards five antimicrobial agents is detailed in table given below. Of these six *Salmonella* isolates were resistant to gentamycin (85.7%) followed by kanamycin & trimethoprim (57.14% each) and ampicillin (42.85%) (Plate 22).

Table 66 Details of in-vitro antibiogram study of Salmonella isolates

Sr.No.	Antibiotics	No. Of isolates(7)			Percentage		
		Resistant	Moderately sensitive	Sensitive	Resistant	Moderately Sensitive	Sensitive
1	**Ampicillin**	3	2	2	42.85	28.57	28.57
2	**Amikacin**	0	2	5	0	28.57	71.42
3	**Gentamicin**	6	1	0	85.71	14.28	0
4	**Kanamycin**	4	1	2	57.14	14.28	28.57
5	**Trimethoprim**	4	1	2	57.14	14.28	28.57

Moderate degree of sensitivity was observed towards ampicillin &amikacin (28.57% each) followed by gentamycin, kanamycin & trimethoprim

Fig. 13 Overall antibiotic pattern of *Salmonella*spp. isolates



(14.28% each). Maximum Sensitivity was noted towards amikacin (71.42%) followed by ampicillin, kanamycin, trimethoprim (28.57% each). (Table 68, Fig).

Kalambe *et al.* (2016) reported overall resistance of 50% of salmonella isolates for trimethoprim followed by ampicillin (20%) which is in concurrence with the present findings. Kim *et al* (2011) revealed resistance of salmonella isolates for kanamycin (33.33%) which can be coroborated with the findings of present study where the 57.14% resistance was observed against kanamycin. Likewise Singh *et al* (2009) also exhibited the maximum resistance towards ampicillin (86.49%) which also partially in agreement with present results for ampicillin resistant (42.85%)(Fig. 13).

In present finding; the antibiogram study from pig and poultry isolates is in cent percent agreement with the report by Selvaraj *et.al.(*2010) who reported maximum sensitivity of salmonella isolates from poultry samples towards amikacin. Similarly Kalambe *et al.* (2016) reported maximum sensitivity for gentamycin followed by ampicillin and trimethoprim (40% each), amikacin (30%) and kanamycin (10%) which is also in agreement with the present study reports. Whereas Moon *et al.*(2011) reported maximum sensitivity towards ampicillin, colistin, piperacillin, netillin and norfloxacin & Highest resistance against ciprofloxacin and ofloxacin which is contradictory for present results.

4.6. Minimum Inhibitory Concentration (MIC)

Minimum inhibitory concentration (MICs) is defined as the lowest concentration of particular antimicrobial agent that will inhibit the visible growth of a micro-organism after overnight incubation when sub-cultured on to antibiotic free media. MICs are used by diagnostic laboratories, mainly to confirm resistance, but most often as a research tool to determine the *in-vitro* activity of new antimicrobials and data from such studies have been used to determine MIC breakpoints.

4.6.1 Minimum Inhibitory Concentration of *Campylobacter species isolates*

In the present study, isolates showed variable elliptical zone of inhibition around antibiotic strips. The highest MIC concentration was recorded for norfloxacin i.e. 32 mcg/ml, followed by 12 mcg/ml and 8 mcg/ml for one isolate each towards oxytetracycline. The highest MIC values in the present study can be correlated with Baserisalehi *et al.*(2007) who conducted a comparative study on antimicrobial susceptibility of *Campylobacter* spp. isolated from faecal sample of domestic animals and poultry from India and Iran observed lowest MIC values for ciprofloxacin (2 µg /ml) and highest MIC values for ampicillin and chloramphenicol with 256 µg/ml in case of all isolates in India and 64 µg/ ml in case of isolates in Iran.

Out of all the eight isolates under study cent percent resistance was reported by 5, 4, 2 and 1 isolate each towards Ceftriaxone, Erythromycine, Norfloxacin and Tetracycline, respectively. Out of eight isolates three isolate showed 6, 2 and 1 mcg/ml MIC concentration whereas four isolates showed 0.125, 0.19, 0.25 and 6 mcg/ml MIC concentration towards Ceftriaxone and erythromycin respectively which are in concurrence with finding of Guevremonte *et al.*(2006) who has reported Breakpoint values of 4 µg/mL and 16 ug/mL forenrofloxacin and gentamicin and tetracycline, respectively.

Two isolates each showed MIC concentration of 2, 3, 4 and 6 mcg/ml towards gentamicin. The highest MIC was reported towards norfloxacin i.e. 12, 0.75, 0.25 mcg/ml. was shown by one isolate each whereas lowest concentration of 0.19 mcg/ml was revealed by two isolates. Of the eight isolates 50% isolates were resistant towards tetracycline whereas remaining showed 4 and 8 mcg/ml MIC(by two isolates each) the variability in result is comparable with antibiotic resistance pattern of *Campylobacter* species isolated from foods of animal origin i. e MICs of erythromycin sensitive isolates ranged between 0.01 and 0.1 µg/ml, MIC level of 0.01 µg/ml in 10 isolates and 0.1 µg/ml in 19 isolates. MICs ranging between 0.1 and 1 µg/ml fornorfloxacin sensitive isolates, MIC level of 0.1 µg/ml, 0.5 µg/ml and 1 µg/ml for 10, 6 and 13 isolates, respectively as per reports of Pallavi and Kumar (2014).

In the present investigation MIC_{50} value for gentamycin and norfloxacin was recorded to the tune of 3 mcg/ml and 0.19 mcg/ml, respectively.

Table 67 Minimum Inhibitory Concentration of *Campylobacter* species isolates

Sr no.	Antibiotic	MIC Concentration (mcg/ml)							
		PF 6C	PC 1C	SP40 C	SF46 C	PF8 C	PC45 C	PF45 C	SF31 C
1	**Ceftriaxone**	-	-	-	2	-	6	1	-
2	**Erythromycin**	-	0.125	-	6	0.19	-	0.25	-
3	**Gentamicin**	2	3	3	2	6	4	4	6
4	**Norfloxacin**	12	32	-	0.19	0.19	0.75	0.25	-
5	**Tetracycline**	-	4	-	8	4	-	4	-

4.6.2 *Escherichia coli*

In the present study, 28 isolates of *E coli* were analyzed for MIC concentration studies which showed variable elliptical zone of inhibition around antibiotic strips.

The highest MIC concentration i.e. 64 mcg/ml was recorded for penicillin followed by lowest concentration of 0.25 mcg/ml towards erythromycin whereas resistance was reported for all the four antibiotics except gentamicin (Table 70). The MIC concentration of ampicillin was in the range of 0.75,1.5,2,4, 32 mcg/ml and MIC concentration which were exhibited by two, ten, three, two and seven isolates, respectively. The less than 100 mcg/ml in the present study towards ampicillin is in agreement with OIE *et al.*(1997) who has studied in vitro susceptibility of *Escherichia coli* O157 to several antimicrobial agents and reported that MIC value of ampicillin and tetracycline was less than 100 µg/ml in some strains.

The MIC concentration of erythromycin was recorded as 0.25, 6 and 8 mcg/ml for twelve, six and eight isolates whereas two isolates were resistant to erythromycin. The MIC concentration for gentamycin ranges between 0.50-8 mcg/ml for all the isolates except one which was resistant for gentamicin.

In the present investigation highest MIC value was reported towards penicillin i.e. 16, 24, 32, 48 and 64 mcg/ml by one, four, seven, three and three isolates, respectively however; 10 isolates revealed complete resistance.

In the present investigation $MIC5_0$ value for ampicillin was recorded to the tune of 1.5 mcg/ml whereas for erythromycin it was 0.25 mcg/ml. The MIC_{90} and MIC_{50} value of gentamicin was 0.75 and 1.5 mcg/ml respectively. Whereas MIC_{50} value for penicillin was recorded as 24 mcg/ml; and MIC_{50} for tetracycline was 3 mcg/ml. The results are in concurrence with the MIC_{50}, MIC_{90} values for ceftriaxone i.e. 8 and 64 mg/l, respectively and MIC50 and MIC90 value of ciprofloxacin as 8, 32 mg/l, respectively against *E. coli* isolates (Boonyasiri *et al.*2014)(Plate 23).

Table 68. Minimum Inhibitory Concentration of *E. coli* isolates

Sr no.	Animal	Source	Isolate no.(28)	Antibiotic				
				Ampicillin	Erythromycin	Gentamicin	Penicillin	Tetracycline
1	Pig	Pork	SP4E	32	8	1	24	2
2			SP10E	1.5	8	1.5	32	0
3			SP15E	0	6	1	0	0
4			SP28E	32	6	1.5	32	3
5			SP42E	1.5	0.25	0.75	24	0
6			SP43E	0	8	1	0	3
7			SP44E	32	6	0.75	32	2
8			SP47E	1.5	0.25	1.5	64	8
9		Faecal	SF4E	32	6	1.5	24	2
10			SF10E	1.5	0.25	0.75	0	3
11			SF11E	0	8	0.75	0	0
12			SF13E	32	0.25	1.5	24	2
13			SF15E	1.5	0.25	0.75	0	3
14			SF28E	1.5	0.25	1.5	32	8

15			SF42E	32	0.25	0.75	32	2
16			SF43E	1.5	6	1.5	0	3
17			SF44E	0	8	1.5	0	0
18			SF46E	1.5	0.25	0.75	0	4
19			SF47E	32	8	1.5	0	6
20	Poultry	Chicken	PC8E	4	0.25	1.5	32	2
21			PC9E	1.5	8	8	32	3
22			PC38E	2	6	1.5	48	6
23		Faecal	PF6E	0.75	0.25	8	0	6
24			PF18E	4	1.5	2	16	8
25			PF23E	2	0.25	1.5	64	4
26			PF27E	0.75	0	0.50	64	6
27			PF43E	2	0	0.50	48	4
28			PF39E	1.5	0	1.5	48	6

4.6.3 *Listeria monocytogenes*

In the present study five isolates of *Listeria monocytogenes* were studied for MIC value determination which revealed variable elliptical zone of inhibition around antibiotic strips. The highest MIC concentration was recorded for tetracycline i.e. 48 mcg/ml whereas cent percent resistance was reported for four isolates towards different antibiotics.

The MIC concentration of ampicillin was in the range of 0.25-0.75 mcg/ml for four isolates whereas; one isolate showed resistance. The MIC concentration of cephotaxime ranges between 0.4-3 mcg/ml for four isolates whereas one isolate was resistant. Of the five isolates all isolates showed 0.50 mcg/ml minimum inhibitory concentration towards cloxacillin (Table 71, Plate 24).

The MIC concentration of 4 and6 mcg/ml was exhibited by two and one isolates towards erythromycin respectively however; remaining two isolates were resistant. All the isolate showed variable MIC concentration towards gentamicin i.e. 0.50, 1.2, 1.5, 2 and 4 mcg/ml.

The highest MIC was recorded kanamycin (48 mcg/ml) followed by 32 mcg/ml and 6 mcg/ml by one isolate each, whereas remaining two isolates exhibited cent percent resistance. The MIC concentration of 0.25, 2 and 24 mcg/ml was recorded for two isolates each and one isolate respectively towards norfloxacin.

In the present study MIC concentration for penicillin-G ranges between 0.75-3 mcg/ml. however none of the isolate showed resistance. The lowest MIC concentration was recorded for Rifampicin i.e 0.016 mcg/ml. The MIC concentration of vancomycin was in range of 1-1.5 mcg/ml for four isolates while one isolate revealed complete resistance. Two isolates revealed resistance towards ceftriaxone followed by 0.5, 0.75, 2 mcg/ml of MIC concentration by one isolate each.

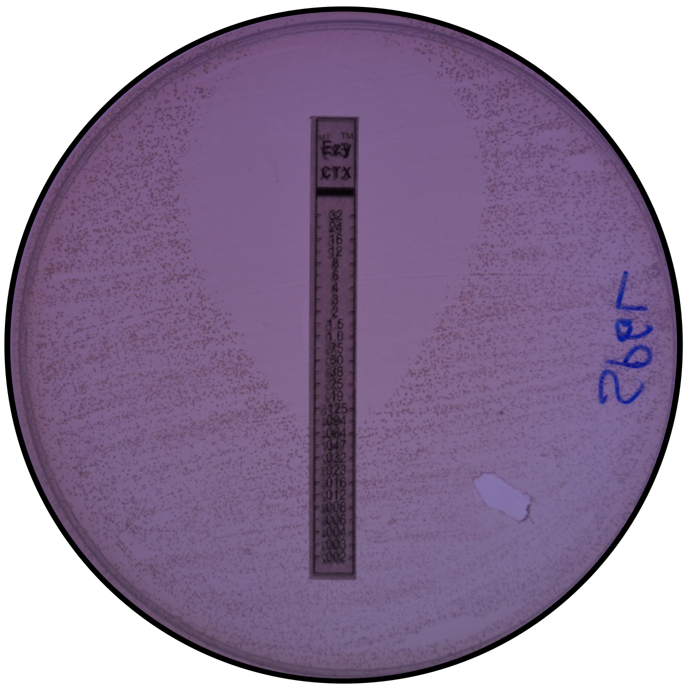


Plate 24 MIC assay for L. monocytogenes isolates

Table 69 Minimum Inhibitory Concentration of *L. monocytogenes* isolates

Sr. No.	Antibiotic	MIC Concentration (mcg/ml)				
		SP6L	**SP8L**	**SF37L**	**PC9L**	**PF43L**
1	Ampicillin	-	0.5	0.75	0.25	0.5
2	Cephotaxim	0.94	0.4	0.75	3	0
3	Coxacillin	0.5	0.5	0.5	0.5	0.5
4	Erythromycin	0	4	0	4	6
5	Gentamicin	1.2	0.5	2	4	1.5
6	Kanamycin	32	48	-	6	-
7	Norfloxacin	2	2	0.25	0.25	24
8	Penicillin-G	3	1.3	1.5	0.75	1.5
9	Rifampicin	0.016	1	0	0.50	2
10	Vancomycin	1	1.5	-	1	1.5
11	Ceftriaxone	0.5	0.75	2	0	0
12	Tetracycline	1	-	48	24	1

4.6.4 *Salmonella* spp.

Minimum Inhibitory Concentration of *Salmonella species isolates*

In the present study, three isolates of *Salmonella* showed variable elliptical zone of inhibition around antibiotic strips. The highest MIC concentration was recorded for kanamycin i.e. 48 followed by 32, 16 mcg/ml for one, two, and one isolate each whereas; lowest concentration ranging from 4 -1 mcg/ml was reported in remaining three isolates (Table 72). The MIC concentration of gentamicin was ranging between 0.50-8 mcg/ml for all the isolates whereas; MIC concentration of ampicillin was 0.38, 0.50, 0.75, 1.5,3 and 8 mcg/ml for two and one isolate each respectively. The MIC values reported in the present study are at lower level when compared with results MIC value of >256 µg/ml for ampicillin as reported by Saha *et al.* (2006).The results are also comparable with Akhtar *et al.*(2010) who has processed 14 *S. enteritidis* isolates obtained from poultry for estimation of minimum inhibitory concentration against different antimicrobial drugs and observed resistance to ampicillin and chloramphenicol at higher concentration i.e. (100 µg/10 µl).

Table 70 Minimum Inhibitory Concentration of *Salmonella species isolates*

Sr no.	Antibiotic	MIC Concentration (mcg/ml)						
		SP11S	**PF48S**	**SF23S**	**SP40S**	**PC48S**	**PF11S**	**PF36S**
1	Ampicillin	1.5	3	0.38	0.38	0.50	0.75	8
2	Gentamicin	0.75	0.75	6	3	6	8	0.50
3	kanamycin	1	2	32	48	16	32	4

None of the isolate showed resistance to any of the antibiotics. The MIC_{50} values were recorded to the tune of 0.75, 3 and 16 mcg/ml for Ampicillin, Gentamicin and kanamycin respectively. The results are also comparable with Capoor *et al.*(2009) who carried out estimation of Minimum Inhibitory Concentration of variable antibiotics by E-test strip against 210 *Salmonella* spp. recovered from enteric fever and septicemia cases and reported MIC_{90} of the carbapenems (imipenem and meropenem) for *Salmonella* Typhi and *Salmonella Paratyphi* A was 0.064 mg $ml^{-}1$. MIC_{90} of faropenem was 0.25 mg $ml^{-}1$ for *S.* Typhi*, S.* Paratyphi *A* and *Salmonella* Typhimurium and the MIC_{90} of azithromycin for all *Salmonella* spp. ranged from 8 to 16 mg $ml^{-}1$. Due to less number of isolates in the present study MIC_{90} value could not be calculated.

SUMMARY AND CONCLUSIONS

Food borne diseases are important and major growing public health concern and problem with economic implications worldwide. Millions of people suffer from foodborne illnesses yearly in developed and developing countries. Though many studies have been targeted to evaluate bacteria responsible for foodborne zoonosis by subsequent isolations from chicken and pork, it is very difficult to obtain accurate estimates of incidences of microbiological food borne diseases. The major public health concern pathogens of food borne are *Salmonella* species, *Campylobacter* species, *Escherichia coli* and *L. monocytogenes.*

Present study was designed with an aim to study prevalence of *Campylobacter spp*, *E. coli*, *Listeria monocytogenes* and *Salmonella* pathogens in pork and chicken, study their *in-vitro* pathogenicity, antibiogram and evaluate Minimum Inhibitory Concentration (MIC) determination of the isolates. In order to accomplish this work, a total of 300 samples 50 each of meat, faecal, blood samples from pigs and poultry (50 each) slaughtered in and around Nagpur were included in the study.

Meat and faecal samples were processed for isolation and characterization of *Campylobacter* spp., *Escherichia coli*, *L. monocytogenes, Salmonella spp.* with selective media and incubation conditions whereas blood samples were processed for DNA extraction and subsequently processed for PCR. Isolations of *Campylobacter* spp. was attempted by employing combination of Preston enrichment broth and subsequent streaking onto modified charcoal cefoperazone deoxycholate agar (mCCDA) as a selective agar. Combination of MacConkey broth and Eosin Methylene Blue (EMB) agar was employed for isolations of *Escherichia coli*. Isolations of *L. monocytogenes* were attempted by employing enrichment with University of Vermont (UVM-I and II) and subsequent streaking onto Polymixin-Acriflavin- Lithium chloride Ceftazidime Aesculin-Mannitol (PALCAM) while for isolations of *Salmonella spp*. combination of Buffered Peptone Water (BPW) and Rappaport vassiliadis (RV) and subsequent streaking onto Xylose Lysine Deoxycholate (XLD) agar medium as a selective agar was employed. The tentative isolates were characterized biochemically and by molecular characterization technique.

In order to estimate the prevalence among meat samples, processing of 100 samples (50 each of pork and chicken) revealed cultural positivity for *Campylobacter* spp by two (4%), for *E.coli* by 15 (30%), for *Listeria monocytogenes* by 2 (4%) and for *Salmonella* spp by 2(4%) pork samples. Screening of chicken samples (50) confirmed isolations of *Campylobacter* spp from 3 (6%), *E.coli* from 13(26%), *L. monocytogenes* from one (2%) and *Salmonella* spp. from one (2%) samples.

A total of 100 faecal samples (50 each of pigs and poultry) were screened for isolations. The screening of 50 faecal samples from pigs revealed isolations of *Campylobacter* spp. from 2 (4%), *E.coli* from 15 (30%), *L. monocytogenes* and *Salmonella* spp. from each one (2% each) samples. The faecal swabs from poultry (50) revealed cultural positivity by 4(8%) for *Campylobacter* spp. by 17(34%) for *E.coli*, by one (2%) for *L. monocytogenes* and by 3(6%) for Salmonella spp.

The isolates of *Campylobacter* spp. (11) originated from pigs (4) and poultry (7) were characterized by PCR as *C. jejuni* (7, including 2 from pigs and 5 from poultry) and *C. coli* (3, including one from pig and 2 from poultry) while one isolate remained uncharacterized. Isolates of E.coli (56) originated from pigs 26 and 30 from poultry were further confirmed by PCR targeting *uid A* gene (623 bp). The total isolates of *L. monocytogenes* (05) obtained from 3 pigs and 2 poultry were confirmed by *isp* 711 bp. Whereas 7 isolates of *Salmonella* spp. (3 obtained from pigs and 4 from poultry) were confirmed by *inv A* 284bp.

The isolates of *Campylobacter* spp. (11) were subjected to in vitro pathogenicity assay like haemolysis on SBA and detection of *hcp* + and *glt A* gene which revealed, 9 Haemolytic isolates, 8 with *hcp*+ and 11 bearing *glt A.* Among these 11 isolates, 8 were found positive to all these assays, thus were designated as highly pathogenic.

Of the 56 *E.coli* isolates, 28 turned haemolytic, 18 were congo red binders while none turned positive for *stx*1 and *stx*2 genes. The isolates with simultaneous positivity among haemolysis and congo red were 17 and accordingly were designated as highly positive. None of the isolates were found bearing shiga toxin 1 and 2 genes.

Subjecting 5 isolates of L. monocytogenes resulted all as haemolysin producers, with CAMP positivity, PI-PLC producers and bearing *hly A* gene, thus were designated as highly pathogenic.

Processing of all isolates of *Salmonella* spp (7) in in vitro pathogenicity assay like Congo red binding, haemolysis on SBA and inv A detection revealed all with haemolytic nature , congo red binders and bearing *invA* gene. Accordingly they are designated as pathogenic.

The processing of 100 blood samples (50 each of pigs and poultry) for blood PCR revealed negativity in all.

The pathogenic isolates of *Campylobacter* (08) were processed for antibiogram using 7 antibiotics *viz*., erythromycin, gentamycin, ciprofloxacin, tetracycline, nalidixic acid, ampicillin. The highest degree of resistance towards nalidixic acid (87.5%), erythromycin and ampicillin (75% each) were noted, whereas highest degree of sensitivity towards gentamycin, ciprofloxacin and tetracycline (25%) followed by cloxacillin, ceftriaxone , cephotaxim (20% each).

Antibiogram of all 28 isolates of *Escherichia coli* against 7 antibiotics *viz*., ampicillinamikacin, azithromycin, ceftazidime, cefotaxime, doxycycline, erythromycin, gentamycin, neomycin, nalidixic acid, penicillin-G, streptomycin.Screening of 28 *Escherichia coli* isolates exhibited highest degree of resistance towards neomycin, erythromycin and amphicillin (67.85% each) whereas highest degree of sensitivity towards azithromycin (42.85%) followed by erythromycin (21.5 % each).

Antibiogram of all 5 isolates of *L. monocytogenes* against 12 antibiotics *viz*., ampicillin, ceftriaxone, cephotaxime, cloxacillin, erythromycin, gentamycin, kanamycin, norfloxacilin, oxytetracyclin, penicillin-g, rifampicin, and vancomycinexhibited highest degree of resistance towards kanamycin, gentamycin and norfloxacillin (100% each), whereas highest degree of sensitivity towards amphicillin and oxytetracycline (40%) followed by cloxacillin, ceftriaxone, cephotaxim (20% each).

Antibiogram of all 7 isolates of *Salmonella* spp. against amikacin, gentamycin, kanamycin, trimethoprim, amphicillin exhibited highest degree of resistance towards gentamycin (85.7.% each) and highest degree of sensitivity towards amikacin (71.42%), followed by amphicillin, kanamycin, trimethoprim (20% each).

Minimum Inhibitory Concentrationwas alsocalculated by using 5 antibiotic strips loaded with variable concentrations commonly used in the region *viz;* ceftriaxone, erythromycin, gentamicin, tetracyclin, norfloxacillin for *Campylobacter* spp.which showed highest MIC concentration for norfloxacillin as 32 mcg/ml. The isolates of *E.coli* showed highest MIC concentration for norfloxacillin as 64 mcg/ml while isolates of *L. monocytogenes* recorded highest MIC concentration for kanamycin i.e. 32 mcg/ml and isolates of *Salmonella* exhibited highest MIC concentration for kanamycin i.e. 32 mcg/ml.

Conclusions:

1) Preston enrichment broth and mCCDA agar is satisfactory media for isolation of *Campylobacter* spp. under microaerophilic condition. Likewise EMB, PALCAM, XLD are satisfactory media for isolation of *E.coli*, *Listeria monocytogenes* and *Salmonella* spp. respectively for routine laboratory protocols.

2) In the present investigation food borne pathogen with biochemical variations are reported thus recommends use of set of tests for characterization and confirmation of isolates.

3) Highly pathogenic nature of the *Campylobacter*, *E.coli*, *L. monocytogenes* and *Salmonella* isolates obtained from food animals in the region is a matter of concern from public health point of view.

4) The highly resistant nature of the isolates to the antibiotics signifies entry of residues in food chain which is alarming situation and needs special attention.

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APPENDIX-I

(A) *Campylobacter species*.

1) Prestone Enrichment broth

Nutrient broth No.2	: 12.5 g
Distilled water	:1000ml
	: 7.4 ± 0.2 (at 25ºC)

Preparation:-

Preston enrichment broth was prepared by dissolving 12.5 g of Nutrient broth No.2 (CM0067B) media in 470ml of distilled water and autoclaved at 15lbs pressure at 121ºC for 15 min, cooled to 50 ºC and 25ml of lysed sheep blood was added aseptically along with one vial of reconstituted Prestone Campylobacter Selective Supplement (SR00117E).

Prestone Campylobacter Selective Supplement (SR00117E).
Oxoid, UK laboratories. Its composition is:

Polymyxin B	:2,500IU
Rifampicin	:5.0 mg
Trimethoprim lactate	:5.0 mg
Cyclohexymide	:50.0mg
The content of 1 vial rehydrated aseptically with 2ml of sterile distilled water. Mixed properly and aseptically mixed with above mentioned broth	

2) mCCDA (modified Charcoal Cefoperazone Deoxycholate Agar)

Nutrient broth No.2	:25.0 g
Charcoal bacteriological	: 4.0 g
Casein enzymic hydrolysate	:3.0 g
Sodium deoxycholate	:1.0 g
Ferrous sulphate	:0.25 g
Sodium pyruvate	:0.25 g
Agar	:12.0 g
Distilled water	:1000 ml
pH	: 7.4 ± 0.2 (at 25ºC)

Preparation:-

modified Charcoal Cefoperazone Deoxycholate Agar (CM0739B) was prepared by dissolving 22.75 gms of media in 500 ml distilled water and autoclaved15lbs pressure at 121ºC for 15 min, cooled to 50 ºC and rehydrated content of 1 vial of CCDA supplement (FD135) was added aseptically .Mix well and pour into sterile Petri plates.

CCDA selective supplement (SR0155E): Oxoid, UK laboratories. Its composition is

Cefoperazone	:16.0 mg
Amphotericin B	:5.0 mg
The content of 1 vial rehydrated aseptically with 2ml of sterile distilled water. Mixed properly and aseptically mixed with above mentioned broth.	

3) CampyGen 2.5 Lit (CN0025A) : A product of BD, Oxoid, UK laboratories.

(B) E.coli species

3) MacConkey broth

MacConkey Broth	: 40.07gms
Distilled Water	: 1000 mL
pH	: 7.4 ± 0.2
Autoclaved at 121°C for 15 min.	

4) EMB (Eosine Methylene Blue) Agar

EMB Agar powder	: 35.96 g
Distilled Water	: 1000mL
pH	: 7.4 ± 0.2

(C) Salmonella species

5) Buffer peptone Water

Peptone	: 10.0 g
Sodium chloride	: 5.0 g
Disodium hydrogen phosphate dodecahydrate (Na2HPO4.12H2O)	: 9.0 g
Potassium dihydrogen phosphate (KH2PO4)	: 1.5 g
Distilled Water	: 1000 mL
pH	: 7.0
Sterilize dispense into suitable flasks and autoclaved at 121°C for 20 min.	

6) Rappaport-Vassiliadis *Salmonella* Enrichment (RV) Broth

RV Broth	: 26.62 gms
Distilled Water	: 1000 mL
pH	: 7.4 ± 0.2

Autoclaved at 115°C for 15 min.

7) Xylose Lysine Desoxycholate (XLD) Agar

XLD agar powder	: 54.8gms
Distilled Water	: 1000 ml
pH	: 7.4 ± 0.2

Dissolve the components in the water. Heat under constant stirring until the medium starts to boil. Avoid over-heating. Immediately transfer the solution to a water bath at about 500 C, continue stirring until the medium has reached about 50ºC.

(D) Listeria species

8) University of Vermont (UVM) Medium

A. UVM Base

Broth base	: 27.17 gm
Distilled water	: 500ml
pH	: 7.4 ± 0.2

Dissolved ingredients completely and sterilize by autoclaving at 15 lbs pressure (121˚C) for 15 min. Cool to around 45-50˚C and aseptically add rehydrated contents of one vial (5 ml) of *Listeria* selective supplements.

B. Complete medium

a. For UVM-1

Allow the base to cool and then aseptically add rehydrated contents of one vial (5ml) of *Listeria* selective supplements (UVM-FD136).

b. For UVM-2

To the base, add rehydrated contents of one vial (5ml) of *Listeria* selective supplements (UVM- FD 137).

9) PALCAM

***Listeria* identification agar base**

Agar base	: 34.5 gm
Distilled water	: 500 ml
pH	: 7.0 ± 0.2

Dissolved ingredients completely and sterilize by autoclaving at 15lbs pressure (121˚C) for 15 min. Cool to around 45-50˚C and aseptically add rehydrated contents of one vial (5 ml) of *Listeria* selective supplements (PALCAM-FD061). Mix well and pour into sterile petri plates.

10) Brain Heart Infusion (BHI) Broth

BHI broth powder	: 37.0 gm
Distilled water	: 1000 ml
pH	: 7.2 ±0.2
Autoclaved at 121^{0}C for 20 min	

11) Brain Heart Infusion (BHI) Agar

BHI agar powder	: 47.0 gm
Distilled water	: 1000 ml
pH	: 7.2
Autoclaved at 121^{0}C for 20 min	

12) Mueller Hinton agar (MHA)

MHA powder	: 38.0 gm
Distilled water	: 1000 ml
pH	: 7.2
Autoclaved at 121^{0}C for 20 min	

13) L. mono Differential agar base

L. mono differential agar powder	: 36.02 gm
Distilled water	: 460 ml
pH	: 7.2
Autoclaved at 121^{0}C for 20 min	

Dissolved ingredients completely and sterilize by autoclaving at 15lbs pressure (121˚C) for 15 min. Cool to around 45-50˚C and aseptically add sterile rehydrate content of 1 vial each of L.mono selective supplement I (FD 212), L.mono selective supplement II (FD 213),L.mono enrichment supplement I (FD 214). Mix well and pour into sterile petri plates.

14) Sheep blood agar (SBA)

A. Base

Sheep blood agar base	: 40.5 gm
Distilled water	: 1000 ml
pH	: 7.3±0.2

Dissolved ingredients completely and sterilize by autoclaving at 15lbs pressure (121˚C) for 15 min.

B. Complete Medium

Cooled the autoclaved base to 46-48^{0}C and aseptically added 50 ml of defibrinated sheep blood mixed properly and then poured into Petri dishes.

15) Congo red binding agar (0.003%)

BHI agar	: 37.0 gms
Congo red dye	: 0.03 gms
Distilled water upto	: 1000 ml
Autoclaved at 1210C for 20 min.	

16) Triple Sugar/Iron Agar (TSI agar)

TSI Agar powder	: 64.46 gms
Distilled water	: 1000 ml
pH	: 7.4
Autoclaved at 1210C for 10 min.	

17) Sugar fermentation Test

A. Peptone water base

Peptone bacteriological	: 10 gm
NaCl	: 5 gm
Distilled water	: 1000 ml

B. Indicator Solution

Bromocresol purple (0.2%)	: 25 ml
Test compound	: 50ml

C. Sugar Solution (10%)

Sugar	: 1 gm
Distilled water	: 10 ml
Filtered by passing solution through cellulose Acetate 0.20 µm	

D. Complete media

Peptone water base (A)	: 950ml
Indicator solution (B)	: 25ml
Adjusted pH to	: 7.2 to7.3
Sterilized by autoclaving at 121^{0}C for 20 min. cooled and poured into test tube @ 5ml/ tube.	
added 256µl of sugar (10%)(C) per 5ml of peptone media	

10% sugar solution was added in peptone water with 0.2% Bromocresol purple as an indicator.The sugars used were L-Rhamnose,D-Xylose, and α-methyl-D- mannoside.

18) Hippurate Hydrolysis

I) Hippuric acid solution (1%)

Hippuric acid	:1 gms
Distilled water	:100 ml
pH	:8.0

II) Ninhydrin solution

Ninhydrin	:3.5g
Acetone	:50ml
Butanol	:50ml
Ninhydrin was dissolved in 100 ml mixture of 1:1 (v/v) acetone and butanol. It was dispensed in 10 ml amount in to screw capped test tubes, wrapped in aluminimum foil, stored at 4ºC and used within 2 weeks.	

19) Indoxy Acetate Hydroxyl (10%, W/V)

Indoxy Acetate	:10gms
Distilled water	:100 ml

20) Oxidase Reagent

Tetra methyl-p-phenylenediamine dihydro-chloride	: 0.1 g
Distilled water	: 10 mL
Dissolve the chemical in the water. Prepare freshly before use.	

21) Catalase Reagent (3% H2O2)

Hydrogen per oxide	: 3 ml
Distilled water	100 mL
Store at 4ºC	

22) Citrate test:

Simmons Citrate Agar	: 2.42 gms
Distilled water	: 100 mL
Autoclaved at 10 lbs pressure, 115°C for 20 minutes	

23) Indole Test:

A. Tryptone Water:

Tryptone	: 10.0 gms
Sodium chloride	: 5.0 gms
Distilled water	: 1000 ml
pH	: 7.2 ±0.2
Autoclaved at 1210C for 20 min.	

B. Kovac's Reagent for Indole:

p – dimethylaminobenzaldehyde	: 5.0 gms
Amyl Alcohol	: 75 ml
Conc. HCl	: 25 ml
Dissolve the aldehyde in the alcohol by gentle warming in water bath at about 50-55ºC. Cool and add the acid. Protect from the light and store at 4ºC.	

24) Methyl Red test

A. Medium (Glucose phosphate peptone water)

Peptone	: 5.0gm
Dipotassium hydrogen phosphate	: 5.0gm
Distilled water	: 1000ml
Glucose(10% solution)	: 50 ml
pH	: 7.6 ± 0.2
Autoclaved at 121^{0}C with 15 lbs for 15 minutes	

B. Methyl Red Indicator Solution

Methyl red	: 0.1 gm
Ethanol (95%)	: 300 ml
Distilled water	: 200 ml

25) Voges- Proskauer Test

Same as per Methyl Red Test

A. Voges- Proskauer indicator solution

a. 40% potassium hydroxide solution

Potassium hydroxide	: 40.0 gm
Distilled water	: 100 ml

b. 5% α-Napthol solution

α-Napthol	: 5.0gm
Absolute ethanol	: 100 ml

26) Nitrate Reduction Test

A. Nitrate broth

Potassium nitrate	: 1.0gm
Beef extract	: 10.0 gm
Peptone	: 10.0 gm
Sodium chloride	: 5.0 gm
Distilled water	: 1000ml
pH	: 7.2 ± 0.2
Autoclaved at 121 ^{0}C at 15 lbs for 15 minutes	

B. Test Reagent

a. Solution A

0.8% Sulphanilic acid in 5 N acetic acid

(Dissolved by gentle heating)

b. Solution B

0.6% dimethyl- α- napthylamine in 5N acetic acid

(Dissolved by gentle heating)

Sterile nitrate broth 5 ml was inoculated with a heavy growth of the test organism and incubated at 37^{0}C for 24hrs. subsequently add 0.1ml of test reagent to the culture in broth. The development of red colour with in 1 minute was taken as positive reaction.

27) Phosphate Buffer Solution (PBS)

Disodium hydrogen phosphate (anhydrous) (Na_2HPO_4)	: 1.16 gm
Potassium dihydrogen phosphate (KH_2PO_4)	: 0.2 gm
Potassium chloride (KCl)	: 0.2 gm
Sodium chloride (NaCl)	: 8.0 gm
Distilled water	: 1000 ml
pH	: 7.2 ± 0.2
Autoclaved	

APPENDIX- II

Reagents for Polymerase Chain Reaction (PCR)

1) DNA extraction

Organisms which showed tumbling motility were processed for DNA extraction.

2) PCR buffer

KTT-20	100 ml
Proteinase –K	10 mg
Store at 4°C.	

APPENDIX- III

.**Reagents for agarose gel electrophoresis:**

1) **Tris-glacial acetic acid EDTA (TAE) buffer (50X stock solution):**

Tris base/Tris buffer	292 gm
Glacial acetic acid	57.1 ml
0.5M EDTA (pH 8.0)	100 ml
Distilled water	upto 1000 ml
Stored at room temperature. For working solution, dilute 1:50 (1X TAE) with distilled water for agarose gel electrophoresis. Store at room temprature	

2) **Gel loading dye:**

Bromophemol blue	0.25%
Sucrose in water	40.0%
Store at 4°C.	

3) **Ethidium bromide solution (10mg/ml):**

Ethidium bromide	0.1 gm
Distilled water	10 ml
Store at 4°C	

VITA

The author, **Dr. BIDGAR GANESH JIBHAU** born on 11th May, 1991 at Nashik of Maharashtra State, India.

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THESIS ABSTRACT

a) Title of thesis	:	**BIOCHEMICAL AND MOLECULAR CHARACTERIZATION OF COMMON FOOD BORNE ORGANISMS IN PORK AND CHICKEN**
b) Full name of student	:	**BIDGAR GANESH JIBHAU**
c) Name and address of major Advisor	:	**Dr. W. A. Khan** Assistant Professor Department Of Veterinary Public Health, Nagpur Veterinary College, Nagpur**.**
d) Degree to be awarded	:	**Master of veterinary sciences**
e) Year of award of degree	:	**2016**
f) Major subject	:	**Veterinary public health**
g) Total number of page in the thesis	:	**137**
h) Number of words in the abstract	:	**466**
i) Signature of the student	:	
j) Signature, Name and address of forwarding authority	:	

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Nagpur.

ABSTRACT

In present investigation, a total of 300 samples from food animals comprising 50 each of meat, faecal, blood samples from slaughtered pigs and poultry (50 each) were screened for presence of *Campylobacter* spp., *Escherichia coli, Listeriamonocytogenes*, *Salmonella* spp. Preston enrichment

broth and modified cefoperozone deoxycholate agar (mCCDA) were used for cultural isolation of *Campylobacter* spp. while MacConkey broth and Eosin Methylene Blue (EMB) were for *Eschrichia coli* and UVM-I and UVM-II followed by PALCAM for *L.monocytogenes* while Buffered Peptone Water and Rappaport vassiliadis (RV) and XLD agar as a selective agar for *Salmonella* spp.

Processing of 50 each of pork and chicken revealed cultural positivity for *Campylobacter* spp by two (4%), for *E. coli* by 15 (30%), for *Listeria monocytogenes* by 2 (4%) and for *Salmonella spp.* by 2 (4%) pork samples. Screening of chicken samples (50) confirmed isolations of *Campylobacter* spp from 3 (6%), *E.coli* from 13 (26%), *L.monocytogenes* from one (2%) and *Salmonella* spp. from one (2%). Similarly processing of 100 faecal samples (50 each of pigs and poultry) revealed isolations of *Campylobacter* from 2 (4%), *E.coli* from 15 (30%), *L. monocytogenes* and *Salmonella* spp. from each one (2% each) samples. The faecal swabs from poultry (50) revealed cultural positivity by 4(8%) for *Campylobacter* spp, by 17(34%) for *E. coli*, by one (2%) for *L. monocytogenes* and by 3(6%) for *Salmonella spp*. These isolates of food borne pathogens were further confirmed by molecular test ie., PCR.

Of the 11 *Campylobacter* spp. 9 turned haemolytic, 8 with *hcp*+ and 11 bearing *glt A*. Out of 56 *E.coli* isolates, 28 turned hemolytic, 18 as congo red binders while none turned positive for *stx*1 and *stx2* genes. Of the 5 isolates of *L.monocytogenes* all were haemolytic, with CAMP positivity, PI-PLC producers and bearing *hly A* gene. Processing of all isolates of *Salmonella* spp (7) revealed all with haemolytic nature, congo red binders and bearing *invA* gene.

The processing of 100 blood samples (50 each of pigs and poultry) for blood PCR revealed negativity in all.

The antibiogram of Campylobacter isolates revealed highest degree of resistance towards nalidixic acid (87.5%), whereas highest degree of sensitivity towards gentamycin, ciprofloxacin and tetracycline (25%). The isolates of *Escherichia Coli .* exhibited highest degree of resistance towards neomycin, erythromycin and amphicillin (67.85% each) whereas highest degree of sensitivity towards azithromycin (42.85%). The isolates of *L. monocytogenes* turned resistance towards kanamycin, gentamycin and norfloxacillin (100% each), whereas sensitive towards amphicillin and oxytetracycline (40%). *Salmonella* *i*solates showed highest degree of resistance towards gentamycin (85.7.% each) and highest degree of sensitivity towards amikacin (71.42%).

The Minimum Inhibitory Concentrationfor *Campylobacter spp.* was recorded to be for norfloxacillin as 32 mcg/ml while for *E.coli* as 64 mcg/ml. The MIC for *L. monocytogenes* was recorded for kanamycin as 32 mcg/ml and for *Salmonella* as kanamycin at 32 mcg/ml.

ic/k lkjk×k

v- ic/kkp f'k"kd % **Mdjkp o dkcMhp eklke/; lkekU;r% [kk|kU;kr vk<G.kkj ftok.kp tojlk;fud o vf.od x.ko.ku**

c- fo|kF;kp i.k uko % **fcMxj x.k'k ftHkkÅ**

d- ekxn'kdkp uko o iRrk % **Mk- MCy- ,- [kku lgk¸;d ik/;kid i'ko|d lkefgd LokLF;] foHkkx] ukxij i'ko|d egkfo|ky;] ukxij**

M- inku dj.;kr ;.kkjh inoh % Lukrdkrj inoh ¼,e-Ogh-,l-lh-½

b- inoh inku dj.;kp o"k % 2016

Q- e[; fo"k; % **i'ko|d lkefgd LokLF;**

x- ic/kkrhy ,d.k i"B % 137

g- lkjk'kkrhy ,d.k 'kCn % 386

b- fo|kF;kph lgh %

t- vxf"kr dj.kkÚ;k vf/kdkÚ;kph lgh] uko vkf.k iRrk %

¼Mk- ,u- ih- nf{k.kdj ½
lg;kxh vf/k"Bkrk
ukxij i'ko|d egkfo|ky;
ukxij

---

ic/k lkjk'k

iLrr l'kk/ku vH;klkr [kk|kU Eg.ku mi;kxkr ;.kkÚ;k ojkg] dDdV ;klkj[;k ik.;kp dRry dj.;kr vkY;kurj ekl] fo"Bk rlp jDrueu;kp iR;dh 50 ueu ldyhr dj.;kr vky- o R;kpk dEik;ykcDVj] b-dkyk;] fyLVfj;k ekuklk;Vktul] lkYekuuyk b- itkrhP;k ftok.kp iFkDdj.k dj.;klkBh iLVu bufjpeV ckFk o l/kkfjr flQkijk>ku fMvkDlhdkyV vxkj ¼,eflflfM,½ ;kpk okij d:u dEik;ykcDVj itkrhp ftok.k] edkdh ckFk vkf.k bvkflu feFkhyhu CY; ¼b-ch-,e½ pk b-dkyk;] ;-f0g-,e-&1 o ;-f0g-,e-&2 o ikYdepk mi;kx fyLVfj;k ekuklk;Vktul rj cQM ilVku okVj o jiikV oLlhfyfMl vkj-f0g- vkf.k ,Dl-,y-fM-pk mi;kx flyfDV0g vxkj Eg.kqu dj.;kr vkyk-

dDdV o ojkgkiklu ldyhr iR;dh 50 ueu g ifd;k dj.;kr vkY;kurj R;kidh ojkgkP;k ueU;ke/ku dEik;ykcDVj p 2 ¼4VDd½] b-dkyk; p 30 ¼15 VDd½]fyLVfj;k ekuklk;Vktulp 2¼4VDd½] ldkjkRed vk<Gu vky- R;kpiek.k dDdVikl u ldyhr ueU;kidh dEik;ykcDVj p 3 ¼6VDd½]b-dkyk; p 13 ¼26 VDd½]fyLVfj;k ekuklk;Vktulp 1 ¼2VDd½] lkYekuYyk p 1 ¼2VDd½ ldkjkRed vk<Gu vky- dDdVe/khy fo"Bp 50 ueU;kidh dEik;ykcDVj p 4 ¼8VDd½]b-dkyk; p 17 ¼34 VDd½]fyLVfj;k ekuklk;Vktulp 1 ¼2VDd½]lkYekuYyk p 3 ¼6 VDd½ dYpj ldkjkRed vk<Gu vky- lnj iFkdj.k dyy ueu g][kk|kUu fo"kkDr dj.;kl dkj.khHkr vlY;kp i<hy ikyhej> 'k[kyk vfHkfd;k Eg.ktp ih-lh-vkj- ;k rikl.khr fl/n >ky-

mijkDr ueU;kidh dEik;ykcDVj p 11 idh 9 ueu g jDrHktd] 3 g ,p-lh-ih-$]o lo vdjk ueU;ke/; th-,y-Vh-,- pk leko'k vlY;kp vk<Gu vky-b-dkyk;P;k 56 iFkdr ueU;kidh 28 jDrHktd] dkxk jM c/k fuek.k dj.kkj rj ,l-vk;-,Dl- 1 o 2 dfjrk dk.krp ueu ldkjkRed vk<Gu vky ukgh-fyLVfj;k ekuklk;VktulPk lo ikp ueUk g jDrHktd] dEi pkp.khl ldkjkRed] ih- ih-&ih-,y- lh- ldkjkRed rlp ,p-,y-ok;- tudkpk vrHkko vlY;kp fun'kukl vky- lkYekuYyk itkrhp 7 ueu g jDrHktd] dkxk jM c/k fuek.k dj.kkj o vk;-,u-f0g-,-tudkpk vrHkko vlY;kp fun'kukl vky-

ojkg]dDdV ;kp iR;dh 50 vl 100 ueu g ih-lh-vkj- rikl.khr udkjkRed vk<Gu vky-

dEik;ykcDVj p ueU;kuh ufyfMfD>d vflM ;k ifrtfodkfo:/n lokPk irhpk ifrjk/k n'kfoyk 87-5 VDd] rj tVkek;flu]flikQkfYdLu o VVkIk;fDyu ;k ifrtfodkirh 25 VDd brdh ldkjkRedrk n'kfoyh-b-dkyk;P;k ueU;kuh fuvkek;flu] bfjFkkek;flu o vEihflyhu irh 67-85VDd brdk ifrjk/k n'kfoyk rj vf>Fkkek;flu ifr 42-85 VDd ldkjkRedrk n'kfoyh- fyLVfj;k ekuklk;Vktul P;kueU;kuh dukek;flu] tVkek;flu o ukj¶YkDlflu fo:/n 'krifr'kr ifrjk/k n'kfoyk rj vEihflyhu o VVklk;fDyu ;k ifrtfodkirh 40VDd brdh ldkjkRedrk n'kfoyh- lkYekuYyk itkrhP;k ueU;kuh tVkek;fluirh 87-5VDd brdk ifrjk/k rj vfedkflu ifr 71-42 VDd ldkjkRedrk n'kfoY;kp vk<Gu vky-

dEik;ykcDVj itkrhP;k ueU;kuh ukj¶YkDlflu irh y?kRre ifrjk/k iek.k 32 ek;dkxe @fe-yh] b-dkyk;P;k ueU;kuh 64 ek;dkxe@ fe-yh n'kfoyk- fyLVfj;k ekuklk;VktulP;k vkf.k lkYekuYyk itkrhP;k ueU;kuh dukek;flu irh iR;dh 32 ek;dkxe @fe-yh y?kRre ifrjk/k iek.k n'kfoyk-