60% in broilers, 12% in foods of animal origins that are sold in the market (Rasrinual *et al.,* 1988).

Some species of *Campylobacter* cause reproductive diseases; some of its species causing it are *campylobacter fetus* species *fetus* and *campylobacter fetus* ssp.*venerealis*. There has been increased incidence of antimicrobial resistance appearing in various parts of the world. Antimicrobial resistance has been seen in both humans and animals. It has been seen that it has become resistant to some of the antimicrobial agents, including fluoroquinolones, ciproflaxacin and nalidixic acid (Pezzotti *et al.,* 2003). It is believed that antimicrobial use in food animal production may contribute to increase resistance of these bacteria in humans.

The given study has been done to determine the prevalence and serotypes of *Campylobacter* with antimicrobial resistance from different food samples.

Campylobacter causes food borne disease called Campylobacteriosis (Thomrongsuwannakij *et al.,* 2017). It has been a maniac all over the world. 96 million cases of gastroenteritis and 21 thousand deaths per year are reported worldwide.

Around 50 to 80% of camphylobacteriosis in humans is due to chicken reservoirs and their handling, preparation and consumption .Gastroenteritis problems may occur due to this species which may not require specific treatments. Severe and prolonged gastroenteritis problems in adults or in children may lead to the need of antimicrobial therapy (Allos *et al.,* 2017*).*

The organism is Gram negative rod, exhibiting corkscrew motility, cytochrome oxidase positive, and microaerophilic and is present in the intestine of domestic and wild animals mainly in some avian species like poultry. Healthy animals can also be carriers of this pathogen due to the intestinal colonization.

The first report about *Campylobacter* was by Theodore Escherich in 1886; he observed and described non-culturable spiral shaped bacteria (Vandamme, 2000; Vandamme *et al*., 2010). After this *Campylobacter* was identified for the first time in 1906 by two veterinarian who reported the presence of large number of peculiar organisms in the uterine mucous of a pregnant sheep(Skirrow, 2006; Zilbauer *et al*., 2008). These microorganisms were also reported from aborted bovine foetuses by McFadden and Stockman in 1913.

Campylobacter jejuni is a major cause of gastrointestinal problems worldwide. It may also cause some autoimmune disorders like Miller Fisher Syndrome and Guillain Barre Syndrome (GBS). In humans it may cause colorectal cancer, Barrett's oesophagus and inflammatory bowel disease. In some individual cases, meningitis, reactive arthritis, lung infection, brain absecces, bacteraemia have also been reported. Patients of this infection are known to suffer from fever, weight loss, bloody diarrhea and cramps that may last on an average for 6 days. The symptoms may occur after 24 to 48 hours of ingestion.

The incidences of Campylobacter infection have increased in countries like Australia, North America, and Europe. Although epidemiological data from Middle East, Asia and Africa are still incomplete, data shows that *Campylobacter* infections in these areas are endemic.

Materials and Methods :

Collection of food samples : In the present study different food samples (chicken, egg and vegetable) were collected from different stores of Patna. Each sample were collected aseptically and placed in separate sterilized plastic bags and was transferred to the lab within 4 hours of collection for the isolation of desired microorganism.

Isolation of bacteria from collected food sample : The samples was processed immediately upon arrival using aseptic technique, each sample

was placed in enrichment broth (Preston broth) (Vanderzant and Splittstoesser *et al*, 1992) and was incubated in anaerobic jar at 37°C at microaerophilic condition for 24 to 48 hours (Aurain *et al,* 2003).

After incubation in enrichment broth for 48 hours, 0.1ml of sample was spreaded on selective media (Karmali agar) (Simor *et al*, 1986) plates and *Campylobacter* CVA Agar. These plates were again incubated for 24 to 48 hours in anaerobic jar at microaerophilic condition at 42°C for isolation of desired micro organism.

Screening of isolated organism on charcoal based selective media : The isolated strain/organism which was obtained on Karmali agar CVA Agar plates were then streaked on selective charcoal based media and incubated at 42°C in microaerophilic condition for 24 hours.

Maintenance of culture : The isolated culture was streaked on Karmali and CVA Agar plates and incubated at 37°C in microaerophilic condition and was stored in refrigerator at 4°C for further use.

Identification : The suspected colonies were picked and identified on the basis of cultural, morphological and biochemical test according to the Bergy's Manual of Determinative Bacteriology.

Cultural characteristics : The isolates were identified on the basis of the colony characteristics such as color, margin, texture, elevation and opacity.

Morphological characteristics : Inoculum from the plates was taken to perform gram staining. A thin smear of the culture was prepared and heat fixed. Crystal violet which is a primary stain was applied for 1 minute and then washed with distilled water. Gram's iodine which is a mordant was applied for 2 minutes and then washed with ethanol (decolourizer), then rinsed with distilled water. Safranin (counter stain) was then applied for 1 minute and then rinsed with distilled water and air dried the slide. The slide was observed under microscope at 40X objective to characterize the isolates as Gram negative or Gram positive and the shape and arrangement of cell was observed.

Biochemical tests : The isolates were subjected to different biochemical tests for identification.

Motility test : The test culture was inoculated on SIM agar media tubes and incubated at 37°C for 48 hours; diffused growth along with the line of stab indicates positive result whereas a negative result is indicated by restricted growth along the stab line.

Triple sugar iron (TSI) Agar test : TSIA slant were prepared having thick lower tip. Thereafter, the isolated organism was inoculated with the help of inoculating needle to TSIA by first streaking the surface of the slant and then stabbing the medium again to the thick tip or the butt region. Slant was incubated at 37°C for 24 to 48 hours in microaerophilic condition.

This test checks the ability of microorganisms to ferment sugars. The slants were also observed for the production of acid and gas. Yellow color both in butt and in the slant means lactose is fermented. Yellow butt and red slant demonstrate that lactose is not fermented only glucose is fermented. If hydrogen sulphide gas is produced, the black colour of ferrous sulphide is seen and absence of colour change means negative result.

Catalase test : One drop of 3% H2O2 was taken on a clear glass slide and with the help of a sterilized glass rod a loop full of culture was inoculated to it. Immediate evolution of gas bubble indicates a positive result whereas no gas bubble indicates negative result.

Oxidase test : Filter paper soaked with the substrate tetramethyl p- phenylenediamine dihydrochloride and was moisten with distilled water, the colony to be tested was picked up by platinum wire and smear was made on filter paper. Inoculated area of paper was observed for a colour change within 10 to 30 second, positive result show

colour change to deep blue or purple whereas negative result shows no colour change.

Hippurate Hydrolysis test : A loop full of bacteria was suspended in a 0.5ml of sodium Hippurate solution in test tube and was incubated at 37°C for 2 hours, after incubation 0.2ml of ninhydrin solution was added and tube was again incubated at 37°C for 10 minutes and colour change was observed. Positive result shows deep blue colour and negative result shows no colour change or slightly pale blue colour.

Nitrate reduction test : Nitrate broth was inoculated and then incubated with test organism at 37°C for 24 hours. After incubation 6 to 8 drops of nitrite reagent A and 6 to 8 drops of nitrite reagent B was added and colour change was observed within a minute, If colour change is not seen zinc powder is added and observed for at least 3 minutes for red colour to develop. Development of red colour on addition of Zinc powder shows positive result and absence of red colour after addition of zinc powder shows negative result.

Results and Discussion :

Isolation from different food samples : In this study, we found that all the 3 samples were positive for *Campylobacter.* Our sample for isolation was chickens, leafy vegetables and eggs. Campylobacter All samples were placed on Karmali Agar and *Campylobacter* CVA Agar incubated at 37°C in microaerophilic conditions for 24-48hours (Avrain *et al,* 2003). From the analysis of 3 different samples, large numbers of colonies were obtained. *Campylobacter* prevalence at the farm level is significantly higher. On Karmali Agar media four colonies were selected and named as K1, K2, K3 and K4 and from *Campylobacter* CVA Agar 4 colonies were selected and named as C1, C2, C3, C4.

Identification : The isolated organisms were further characterized by cultural, morphological and biochemical analysis. (Table1) (Table2)

Cultural and Morphological characteristics : All the isolates were gram negative bacteria. Then these isolates were identified as *Campylobacter* species by performing gram staining and different biochemical tests. (Table3) (Table4)

Antibiotic Susceptibility pattern : Antibiotics susceptibility pattern of the isolated c*ampylobacter* was done by different antibiotics by disk diffusion method, and evaluation of susceptibility and resistant pattern was according to the Clinical and Laboratory Standard Institute (CLSI 2014). The assayed Antibiotics: Nalidixic Acid (30µg), Erythromycin (10 µg), Gentamicin (10 µg) and Ampicillin (30 µg). The isolated bacteria (*Campylobacter*) showed significant susceptibility towards all the antibiotics. *Campylobacter* showed more resistance against ampicillin, gentamicin and erythromycin and showed more susceptible to nalidixic acid. Similar results were also showed by (Manel Gharbi *et al,* 201*8*) (Table5) (Figure2).

Conclusion :

This work was mainly based on the isolation and antimicrobial resistant pattern of *Campylobacter species* isolated from different food samples like chicken, vegetables and eggs. Bacterial contamination cannot be detected by flavour, odour or vision. Hence, proper biochemical tests are done which includes Catalase test, Sugar Utilization Test (TSI), Sodium Hippurate Test and Nitrate Reduction Test. Promising results were obtained when biochemical tests were performed. Antibiotic Susceptibility Tests is usually carried out to determine and it is most successful in treating the bacterial infection in vivo. Small discs containing antibiotics are placed on the plates upon which bacteria are growing in selective media. If the bacteria are sensitive to the antibiotic, a clear zone of inhibition is visualized around the disc indicating poor growth; otherwise it shows resistance towards bacteria.

Table 1. Characterization of isolates on *Campylobacter* CVA media plates

S.No	Food Samples	Colony characterisation on Campylobacter CVA Agar			
		colour	texture	Gram staining	Shape under microscope
1	Chicken	White	smooth	Gram negative	comma
2	Vegetable	White	smooth	Gram negative	comma
3	Egg	White	smooth	Gram negative	comma

Table 2. Characterization of isolates on Karmali Agar media plates

S.No	Food Samples	Colony characterisation on Karmali Agar			
		colour	texture	Gram staining	Shape under microscope
1	Chicken	Grey	smooth	Gram negative	comma
2	Vegetable	Grey	smooth	Gram negative	comma
3	Egg	Grey	smooth	Gram negative	comma

Table 3. Characterization of isolates under microscope

S.No	Source	Characteristics		Gram Staining
		shape	colour	
1	Chicken	Comma or curved shape	Pink	Gram negative
2	Vegetable	Comma or curved shape	Pink	Gram negative
3	Egg	Comma or curved shape	Pink	Gram negative

Table 4. Biochemical tests for *Campylobacter* Species

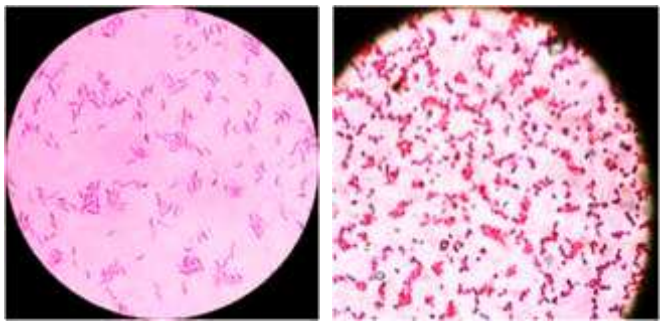
Sample No.	Motility test	TSIA test H_2S production	Catalase test	Oxidase test	Hippurate hydrolysis test	Nitrate reduction test	Organism
1	+ve	-ve	+ve	+ve	+ve	+ve	*C. jejuni*
2	+ve	+ve	+ve	+ve	-ve	+ve	*C.coli*
3	+ve	+ve	-ve	+ve	+ve	+ve	*C. mucosalis*

Table 5. Antibiotic sensitivity test for *Campylobacter* Species

Isolates	Antibiotics	Disk diameter for susceptible organism (mm)	Sensitivity Test
Campylobacter (chicken sample)	Nalidixic Acid(NA)	30-39	Resistant
	Erythromycin(ER)	17-27	Intermediate
	Gentamicin(GE)	11-14	Susceptible
	Ampicillin(A)	8-11	Susceptible
Campylobacter (vegetable sample)	Nalidixic Acid(NA)	24-37	Resistant
	Erythromycin(ER)	17-22	Intermediate
	Gentamicin (GE)	11-14	Susceptible
	Ampicillin(A)	7-12	Susceptible
Campylobacter (Egg sample)	Nalidixic Acid(NA)	20-34	Resistant
	Erythromycin(ER)	18-20	Intermediate
	Gentamicin(GE)	12-16	Susceptible
	Ampicillin(A)	8-9	Susceptible

(A) **(B)**

Fig. 1. Streak Plates on Karmali and CVA media



(A) **(B)**

Fig. 2. Campylobacter species under microscope

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