EPIDEMIOLOGY AND MOLECULAR CHARACTERIZATION OF MULTIDRUG-RESISTANT *ESCHERICHIA COLI* ISOLATED FROM FISH

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Roll No: 0121/16

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A thesis submitted in the partial fulfillment of the requirements for the degree of Master of Public Health

One Health Institute

Chattogram Veterinary and Animal Sciences University

Chattogram -4225, Bangladesh

MARCH 2023

Authorization

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This is to certify that we have examined the above Master's thesis and have found that is complete and satisfactory in all respects, and that all revisions required by the thesis examination committee have been made.

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Abbreviations	***Elaborations***
%	*Percentages*
>	*Greater than*
<	*Less than*
≥	*Greater than or equal to*
≤	*Less than or equal to*
=	*Equal to*
$	*US dollar*
°C	*Degree Celsius*
95% CI	*95% confidence interval*
AMR	*Antimicrobial resistance*
E. coli	*Escherichia coli*
MDR E. coli	*multidrug resistant E. coli*
ESBLs	*extended-spectrum β-lactamases*
ESBL-EC	*extended-spectrum beta-lactamase -producing E. coli*
PAmpC	*plasmid-mediated AmpC β-lactamase*
RNA	*Ribonucleic Acid*
DNA	*De-oxy Ribonucleic Acid*
et al.,	*and others*
Ltd.	*Limited*
Inc.	*Incorporated*
mg	*Milligram*
mL	*milliliter*
µg	*Microgram*
µm	*micrometer*
µL	*Microliter*
pmol	*picomole*

MARI	*Multiple Antibiotic Resistance Index*
AST	*Antimicrobial–Sensitivity Testing*
GPS	*Global Positioning System*
CVASU	*Chattogram Veterinary and Animal Sciences University*
DPBP	*Department of Physiology, Biochemistry and Pharmacology*
MT	*Metric Ton*
ha	*Hectare*
CLSI	*Clinical and Laboratory Standard Institute*
MGEs	*Mobile Genetic Elements*
WHO	*World Health Organization*
FAO	*Food and Agriculture Organization*
ARGs	*Antibiotic-Resistant Genes*
FY	*Fiscal Year*
GDP	*Gross Domestic Product*
MM	*Mater Mix*
sq. km.	*square kilometre*
GI	*Gastrointestinal*
ICU	*Intensive Care Unit*
df	*Degree of freedom*
UV	*Ultra Violet*
lbs	*Pounds*

Abstract

Antimicrobial Resistance (AMR) has become an alarming global concern due to the widespread dissemination of multi-drug resistant (MDR) bacteria. These bacteria pose a serious threat as they cause infections that are increasingly challenging to treat with existing antibiotics. The surge in the use of antimicrobial compounds across human, animal, and agricultural sectors, coupled with their subsequent release into the environment, has contributed to the emergence of MDR bacteria as a natural bacterial phenomenon. To investigate the prevalence of MDR *Escherichia coli (E. coli)* in commercial fish samples in Chattogram, a cross-sectional study was designed. Over a six-month period from March to August 2022, field sampling was conducted at ten randomly selected prominent fish markets in the Chattogram District. A total of 450 fish samples, including commercial fish, shrimp, and seafood, were purchased from randomly selected vendors. Various methods were employed to analyze the samples, including bacteriological culture-based techniques and the disc diffusion method following the Clinical and Laboratory Standard Institute (CLSI) guidelines. Additionally, uniplex, duplex, and multiplex Polymerase Chain Reaction (PCR) tests were used to determine the occurrence of resistance genes in *E. coli* isolates. The antibiotic sensitivity profiles of all identified isolates were established against a panel of 20 selected antibiotics belonging to 11 classes. Isolates that exhibited phenotypical resistance against at least 1 agent in 3 or more antimicrobial categories were classified as MDR. The study revealed an overall prevalence of *E. coli* in fish samples of 41.78% (188/450), with 38.67% prevalence in total study fish samples (174/450). Notably, 92.55% of total fish *E. coli* isolates (174 out of 188) were identified as multidrug resistant (MDR), exhibiting resistance against antibiotics from multiple categories. The highest resistance was observed against ampicillin, cefalexin, cephalothin, tetracycline, and trimethoprim-sulfamethoxazole. The Multiple Antibiotic Resistance Index (MARI) ranged from 0.15 to 1.00, with an average of 0.50. Of the MDR isolates, 89.36% (168 out of 174) exceeded the maximum MARI value benchmarked at 0.2 or above. The highest MARI value was observed in one MDR *E. coli* isolate from catfish. The MARI values indicated that the isolates originated from environments with a high-risk source of contamination and significant antimicrobial exposure. Molecular characterization via the Polymerase Chain Reaction uncovered various β-lactam encoding genes, ESBLs, p_{AmpC}, and other non-β-lactam encoding genes of the phenotypically resistant isolates. Among isolates antibiotic resistant genotype prevalence is 83.51% (157/188) and no gene was traced in 16.49% isolates (31/188).

Among antibiotic resistance gene prevalence of non-β-lactam encoding gene *tet-A* is highest 85.83%, following *sul-2* is 70.09%. Merely, $bla_{TEM}$is most prevailing among all β-lactam encoding gene with a prevalence of 63.53% following the prevalence of co-existence of the *bla* $OXA_{-1\ like}$ gene& $bla_{OXA-2\ like}$ gene is 52.38 % detected as ESBLs. Though, no amplification of *tet-C* and $bla_{ACC-1}$was detected. Genotypic resistance patterns revealed the co-existence of resistance genes of β-lactam encoding genes, $P_{ampC,}$ ESBLs, non-β-lactam encoding genes in same or altered fish *E. coli* isolates. Maximum antibiotic resistant patterns observed were unique. Highest seven resistance gene including bla_{TEM}, *PampC*, $bla_{CTX,}$ bla_{CMY-2} *like gene*, bla_{CMY-2} *like gene*, *tet-D, sul-2* was noticedin one isolate following six resistance genes was detected in six isolates. Genotypic resistance patterns were observed most frequently in shrimp and commercial fish rather than seafoods. The research's conclusions underscore the significance of fish as sources of MDR *E. coli* and resistance genes. The data generated could inform the development of mitigation strategies based on public health ethics to avert the emergence of MDR *E. coli*, addressing the global One-Health challenge posed by this alarming issue.

Keywords: Fish, MDR *E. coli* prevalence, MDR pattern, Multiple antibiotic resistance index (MDRI), Antibiotic Resistant Genotype pattern (ARG pattern).

Chapter-1: Introduction

Antimicrobial resistance (AMR) is recognized as an undoubtedly global health and developmental threat to treating common infections. Nowadays, AMR turns into incredibly alarming, especially for the rapid spread of multi- and pan-resistant bacteria (also known as “superbugs”) throughout the world, which cause infections that exert thriving challenges to cure with existing antibiotics and have been identified by the WHO as one of the top 10 urgent public health concerns from last few decades (WHO, 2021). AMR rates to clinically relevant antibiotics have been increasing terribly reported during the last 30 years (De Oliveira et al., 2020) is a global health problem affecting all humans, animals, and environments (Asaduzzaman et al., 2022). Currently, at least 700 thousand people die of drug-resistant diseases each year, among which more than 230 thousand people die of multidrug-resistant tuberculosis (Murray et al., 2022; Shao et al., 2021). By 2030, drug resistant diseases will throw 24 million people in extreme poverty. Even if remain unchecked, will cause 10 million deaths a year along with put the global economy into crisis by 2050 (Shao et al., 2021).

In the 1950s, as a means of addressing the rising demand for food, the first use of antibiotics in food animals was put into practice (Van et al., 2020). Currently, Antibiotics have been used in human and veterinary medicine for many years to minimize morbidity and mortality and the economic effect of bacterial infections (Ema et al., 2022). Apart from therapeutic and prophylactic purposes, antibiotics are also administered in agriculture and animal husbandry at subtherapeutic levels as growth promoters in bird, swine, beef and fish food even sprinkled on crops and fruit trees (Adenaike et al., 2016; Sivaraman et al., 2020; Zhou et al., 2019) with an estimated increase in utilization of 67% by the year 2030 (Van et al., 2020). Common agricultural (veterinary and aquaculture) antibiotics include beta-lactams, tetracyclines and sulfonamides, fluoroquinolones, aminoglycosides, trimethoprim, polymyxins, tylosin, and chloramphenicol leaving few antibiotics in reserve group (Sivakumar et al., 2021).

The role of the environment as an important reservoir for the transmission of AMR to both humans and animals is detected on a global scale with thriving concerns regarding the one health threat of multisectoral imprudent uses (Altayb et al., 2022; Fletcher & medicine, 2015). Augmented use of antimicrobial compounds in human, animal and agricultural

sectors (both terrestrial and aquatic food animal production) and their subsequent release into the environment has promoted the emergence of multidrug-resistance as a natural bacterial phenomenon (Asaduzzaman et al., 2022; Hassen et al., 2020; Jeon et al., 2019; Sivaraman et al., 2021). Most often horizontal antibiotic-resistant genes (ARGs) transfer (Adenaike et al., 2016; Marathe et al., 2016; Silva et al., 2019; Yin et al., 2013) occurs among environmental non-pathogenic bacteria exposed to virulent bacteria containing unmetabolized pharmaceuticals or their metabolites (urine and feces of treated patients and animals carries up to 90% of active metabolites, fish feces carry up to 75%) (Adenaike et al., 2016; Elhadi & Alsamman, 2015) or natural reservoir (predator birds, water effluents, lakes, river) (Delannoy et al., 2022; Hassen et al., 2020; Marathe et al., 2016; Sapugahawatte et al., 2020; Zhou et al., 2019) and anthropogenic reservoirs (human and animal sewage, waste water, urban and agricultural runoff) (Hassen et al., 2020; Zurfluh et al., 2015).

Aquaculture is the world's fastest-growing sector for food of animal origin (Ryu et al., 2012), About half of all fish and seafood products now originate from farming operations and is poise to further expand its production to meet the increasing global demand (Dewi et al., 2022). Food fish production has expanded almost 12 times in the last 30 years (Boss et al., 2016). Ninety percent of aquaculture production occurs in developing countries whereas, Southeast Asia c ontributes 22% of world production (Reza et al., 2020) State of the World Fisheries and Aquaculture report (FAO, 2022), biennial flagship report of the Food and Agriculture Organization (FAO) of the United Nations, recognized Bangladesh as the world's ranked third largest producer of captured fish from inland open waters (FAO, 2022), whether people also depend on fishing, fish farming, processing, and trading for their income (Akter et al., 2022). Bangladesh saw 1.25 million tons (11% of the total global yields) of captured fish production in 2020 (FAO, 2022). There are 260 freshwater, 475 marine fish species and about 12 exotic species are beingcultured in Bangladesh.

The GDP growth in the fisheries sector is 2.08 percent and the contribution of the fisheries sector in the overall agriculture sector is 21.83 percent in FY 2021-22. Fish provides 60% of national animal protein consumption (DoF, 2023) along with in FY 2021-2022, fisheries sector contributed 2.51% to national GDP and 21.82% to the agricultural GDP (BBS, 2022) and 1.5% to foreign exchange earnings by exporting fish and fish products in 2017-2018 (DoF, 2023). Due to the dense population and poor sanitation facilities, the people of Bangladesh are more susceptible to microbial attacks and face the challenges of

fecal contamination (Singh et al., 2018). A large group of the population involved in unhygienic sanitary practices mostly live near surface water source (haor, baor, beel, pond and river). Open defecation and inadequately treated or untreated domestic sewage disposal may contaminate these natural water bodies with several human pathogenic microbes (Akter et al., 2022). Fish industry in Bangladesh are being oppressed mostly withal occasionally experience of huge economical losses through production of low-quality fish due to microbial infections. These bacteria mostly inhabit in contaminated water, or found in the body of apparently normal fish (Ahmad et al., 2022).

Escherichia coli, A member of coliform group of the Enterobacteriaceae family, is found in the intestinal tract of birds, animals, humans, and fish microbiota (Ahmad et al., 2022; Akter et al., 2022; Sekhar et al., 2017). Inside the intestine of fish, *E. coli* commonly resident as non-pathogenic but when expanding outside the intestine, it can be responsible for causing disease, resulting in enterotoxigenic, whereas 18 toxigenic *E. coli* were isolated (Reza et al., 2020). It is widely accepted as indices of hazardous conditions (human or animal fecal contamination indicator organism) of fish and water (Dewi et al., 2022). The contamination of fish with pathogenic *E. coli* probably occurs through production process, handling, transportation and loading channels (Adenaike et al., 2016; Sekhar et al., 2017). It can be easily disseminated in various living ecosystems through the food chain and water and interchange genetic material with other bacterial communities that may lead to the emergence of pathogenic resistant bacteria that cause various human (Ryu et al., 2012) and animal diseases ends directly or indirectly in more than 2 million deaths each year (Adebowale et al., 2022; Akter et al., 2022).

Very recently, several workers have isolated multidrug-resistant *E. coli* (MDR-EC) and extended-spectrum beta-lactamase -producing *E. coli* (ESBL-EC) from community and public health care hospitals (Wielders et al., 2017), wild animals, horse, swine, pet animals, livestock, turkey (Jeon et al., 2019), broiler raw meat, vegetable salad, egg surfaces, unpasteurized milk, raw fish, and water, indicating serious public health threat (Elhadi & Alsamman, 2015; Laube et al., 2013; Moremi et al., 2016a; Sapugahawatte et al., 2020; Silva et al., 2019; Sivakumar et al., 2021). Potential routes of transmission of ESBL-EC to humans from and other cascades are via the food chain, by direct contact with animals or indirectly via the environment (Ahmad et al., 2022; Hassen et al., 2020; Nguyen et al., 2014; Wielders et al., 2017). Fish and Seafood grown in aquaculture farms than wild-caught fish and seafood are more likely to be exposed to antimicrobials, which

are fed to fish and shellfish to combat disease resulting from intensive husbandry practices (Tate et al., 2022). Use of antimicrobials as an irresponsible manner (inadequate use, overuse and improper use/misuse) in aquaculture activities may led to the development of large reservoir of multidrug-resistant *E. coli* in aquatic niche (Ahmad et al., 2022; Al-Bahry et al., 2012; Ryu et al., 2012; Silva et al., 2019). This consequences of antibiotics-resistance in aquaculture could limit the action of antibiotics resulting in major loss to this fish sector (Ahmad et al., 2022) and shifting of multi-drug resistant bacteria to the human gut commensal flora or associated human pathogens when the fish are eaten (Abgottspon et al., 2014; Sivaraman et al., 2020; Zurfluh et al., 2013).

Beta-lactam antibiotics are one of the main groups used to combat Gram-negative and Gram-positive bacteria and account for sixty percent of the antibiotics used globally (Almeida et al., 2017). The predominant mechanism for resistance to β lactam antibiotics in gramnegative bacteria is by synthesis of βlactamases. Among the β-lactamases, Strains of extended-spectrum *β*-lactamases (*ESBLs*) and plasmid-mediated AmpC *β-lactamase* (P_{AmpC}) are the most common (Karadiya et al., 2016). ESBLs and p_{AmpC} producing *E. coli* pose a threat to public health because of their ability to efficiently hydrolyze the β-lactam ring of (Jeon et al., 2019; Karadiya et al., 2016; Laube et al., 2013; Zurfluh et al., 2015) oxyimino group derivates (Laube et al., 2013; Tumbarello et al., 2007),i.e., penicillin, cephalosporins (Hassen et al., 2020; Nguyen et al., 2014), monobactam and aztreonam (Baothman et al., 2020; Karadiya et al., 2016; Silva et al., 2019; Sekhar et al., 2017) which are commonly used to treat serious infections caused by members of the Enterobacteriaceae family (Nguyen et al., 2014; Thongkao & Sudjaroen, 2019). Further, ESBL inhibited by the *β-lactamase* inhibitor clavulanic acid, sulbactam and tazobactam (Karadiya et al., 2016; Silva et al., 2019), mediate resistance to all β-lactams except fourth-generation cephalosporins and carbapenems (Benlabidi et al., 2021)-encoding genes are usually located on mobile genetic material transferable plasmids, transposons and integrons, these acts as a carrier of resistance genes for other classes of antimicrobials and genes in pathogenesis (Almeida et al., 2017; Jeon et al., 2019; Laube et al., 2013; Marathe et al., 2016). Such resistance is caused by an increasing number of different point mutation variants of ESBLs of family cephalosporin. (Hassen et al., 2020; Zurfluh et al., 2013). This multi-resistance phenotype of ESBL-producer *E. coli* dramatically limits the therapeutic options (Karadiya et al., 2016; Laube et al., 2013; Thongkao & Sudjaroen, 2019) in the field of modern antibiotics application worldwide (Ahmad et al., 2022;

Hassen et al., 2020; Jeon et al., 2019; Nguyen et al., 2014; Silva et al., 2019). ESBLs are categorized as TEM, and SHV, CTX-M, -lactamase families and encoded by beta-lactamase (*bla*) like b*la*$_{TEM}$ (Temoniera β-lactamase), *bla*$_{SHV}$(sulfhydryl variable), *bla*$_{CTX-M}$ (Cefotoximase Munich) genes (Khan et al., 2021; Sekhar et al., 2017) are detected most commonly (Hassen et al., 2020), whereas other groups, OXA encoded by *bla*$_{OXA}$(oxacillinase) and PER -lactamases have been described more recently (Karadiya et al., 2016; Khan et al., 2021; Yin et al., 2013; Zurfluh et al., 2013). Among the AmpC beta-lactamases, AmpC encoded by *bla*$_{AmpC}$, CMY encoded by *bla*$_{CMY}$ represents by far the most frequent beta-lactamase in livestock (Laube et al., 2013; Yin et al., 2013).

Tetracyclines and sulfonamides (SAs) synthetic antibiotics, deserve special attention due to their extensive use, high excretion rate, high solubility, and persistence in the environment (Harnisz et al., 2015; Hoa et al., 2020). However, continues and widespread use of antibiotics has led to the development of tetracycline and sulfonamides resistant *E.coli* from fish feed to water in all fish farming operations (Harnisz et al., 2015) Tetracyclines in particular, used to control furunculosis in salmonids, and oxytetracycline is a drug of first choice in the Polish fish farming industry (Liu et al., 2012).Thirty-eight tetracycline resistance genes have been found in aquatic environments. The efflux genes, *tet-A, tet-B, tet-C, tet-D,* and *tet-E* are frequently detected in various environmental compartments, including sewage treatment plant, surface water, fish farming ponds, dairy and pig farm (Agersø & Petersen, 2007; Ahmad et al., 2022; Su et al., 2011), marine sediments, irrigation ditches, wastewater, animal feedlot lagoons and manure (Liu et al., 2012; Luo et al., 2010). Regarding Sulfonamides, work by inhibiting folate biosynthesis by competing with the natural substrate p-amino-benzoic acid for binding to dihydropteroate synthase (DHPS), an enzyme in the folic acid synthesis pathway. Through this process, sulfonamides inhibit the formation of dihydrofolic acid. Bacterial resistance to sulfonamides, however, can occur through mutations in the chromosomal DHPS gene (folP) or through the acquisition of an alternative DHPS gene (*sul*), whose product has a low affinity for sulfonamides. Of the two pathways, the *sul* genes are the most prevalent mechanism of sulfonamide resistance (Hoa et al., 2008). Among sulfonamides resistance genes in four types including *sul-1, sul-2, sul3,* and s*ul-A* encoding dihydropteroate synthase (Liu et al., 2012; Luo et al., 2010), so far detected most from water and sediment in aquaculture settings (Agersø & Petersen, 2007; Hoa et al., 2008; Su et al., 2011).

Contamination of fish with MDR bacteria in aquatic environment could demonstrate the risk of the persistence of these bacteria in the fish gut flora and explain possible human gut contamination (Hassen et al., 2020) through consumption of fish (Ellis-Iversen et al., 2020). Ultimately, it makes a significant influence on the products quality of fish due to its possibility of causing diseases and transmitting multidrugresistance *E. coli* in surrounding environment, predators and humans (Akter et al., 2022; Elhadi & Alsamman, 2015).

Therefore, hypothesis formulated to perform this research based on public health importance of fish consumption could be state as **"MAR index, multidrug resistance patterns and antimicrobial resistance genotype patterns varies significantly among MDR *E. coli* isolates of fish samples**".

1.1 Purpose (s) of the study:

Hence, this research work, was performed to visualize the current scenario of the prevalence of MDR *E. coli* along with their resistance profile in fish samples at wet market level in Chattogram, Bangladesh. The specific objectives, those are attained from this study are as follows-

- To estimate the prevalence of MDR *E. coli* in fish samples.
- To assess the MDR patterns against isolated *E. coli* from fish samples.
- To investigate the occurrence and evaluate the epidemiology of extended-spectrum β-lactamases (ESBLs) -resistance genes (bla_{TEM}, bla_{SHV}, bla_{CTX}, bla_{OXA-1}, bla_{OXA-2}, P_{AmpC}, bla_{CMY-1}, bla_{CMY-2}), tetracycline-resistance genes (*tet-A, tet-B, tet-C and tet-D*) and sulfonamide resistance genes (*sul-1, sul-2*) of isolated MDR *E. coli* from fish samples of this study, in accordance with one health perspectives.

Chapter-2: Review of Literature

This chapter reviews the relevant literature to describe the history of fish harvesting in Bangladesh, including definition, epidemiology and associated risk factors at different levels, molecular characterization, prevalence, transmission, economic and public health impacts, management, control and prevention strategies withal one health initiatives against MDR *E. coli* throughout the globe. The purpose of this chapter is to provide an update of previous research on this topic, identify gaps, and justify the importance of conducting current research.

2.1 Fish Farming Status in Bangladesh

Bangladesh, a densely populated developing country, is improving its economic status by making significant efforts in the agricultural sub-sector, mainly through increased production of crops, livestock and fish harvesting (Aditi et al., 2017). The continuous growth of the population and the pressure on agricultural land require designing strategies that can deal with the new challenges and opportunities to make agriculture more resilient, versatile and efficient (Palash et al., 2018). The fisheries sector is one of the most productive and dynamic industries which has tremendous potential for future development in the agrarian economy of Bangladesh. As an agri-food powerhouse, the contribution of fisheries to the national economy has always been significant, serving as an important source of animal protein, employment, food security, foreign earnings and socioeconomic development (Shamsuzzaman et al., 2020).

Bangladesh has a vast and diverse range of fisheries resources, which are broadly divided into inland water fisheries and sea fisheries. Inland fisheries include beers, rivers, estuaries, Kaptai lakes and floodplains covering an area of 39.27 lakh ha, while inland cultures include ponds, ditches baor, pen culture/ corral farming, cage farming, shrimp/prawn farms, seasonal cultured water body covering an area of 8.33 lakh ha (Shamsuzzaman et al., 2020). In contrast, marine capture fisheries cover an area of approximately 166,000 sq. km. water area including Exclusive Economic Zone (EEZ) of 200 nautical miles from the baseline (DoF, 2023).70.1% land area and 48.4% population of Bangladesh are devoted to agriculture that availed 17.5% share of agriculture including fishing in GDP (FAO, 2023). The entire fisheries sector directly or indirectly supports the livelihoods of more than 18 million people in the country. About 1.4 million women make a living from the fisheries sector through fishing, farming, handling and

processing of fish (Shamsuzzaman et al., 2020). In FY2020-21, fishing GDP of Bangladesh at Current Prices is 918,215 (In Million TK.) also fishing implicit on GDP and Sectorial Deflators is 127.59% (BBS, 2022).

Fish production in FY 2020-21 stood at 46.21 lakh metric tons, which is 50.91 % more than the total production (30.62 lakh MT) in FY 2010-11 and production increment rate is 2.73% than previous year (DoF, 2023). It may be mentioned that, the total fish production in the country was 7.54 lakh MT in FY 1983-84. Fish production has increased more than 6 times in 38 years (BEW, 2022) According to FAO report 'The State of World Fisheries and Aquaculture 2020', Bangladesh ranked 3rd in inland open water capture production and 5th in world aquaculture production (FAO, 2023). In the last 10 years, Bangladesh has risen to the second position in terms of growth rate of fish production in inland water bodies. Moreover, Bangladesh ranked 1st among 11 Hilsa producing countries in the world. Currently Bangladesh ranks 4th in Tilapia production in the world and 3rd in Asia (BEW, 2022). At present there are 143 government fish farm and 1,055 private farms throughout the country. Fish and fish products of Bangladesh are exported to 52 countries of the world including European Union countries, United States, Japan, Russia and China. In FY 2020-21, Bangladesh earned Tk. 4,088.96 crore by exporting 76,591.69 MT fish and fishery products (BEW, 2022; DoF, 2023).

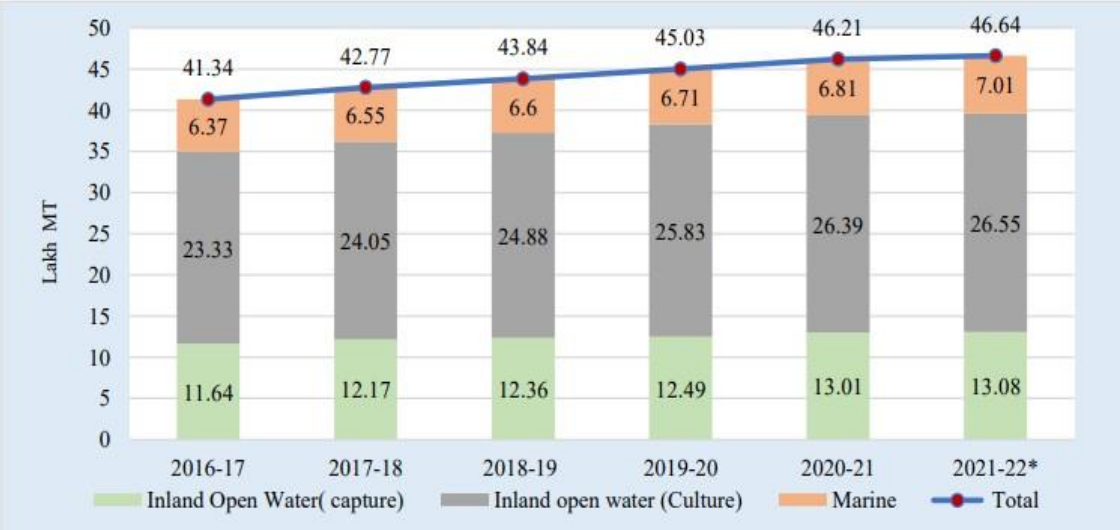


Figure-2.1. Fish Production Trends in Different Resources from FY 2016-17 to FY 2021-22 (Source: Department of Fisheries, Ministry of Fisheries and Livestock. *projected.)

In addition, a rapid increase in aquaculture production in Bangladesh has lowered fish prices, increased protein consumption and reduced poverty. This high production of fish brought the accreditation of 'Blue Revolution' in Bangladesh. However, the use of lime and other chemicals to clean the pond water is increasing and the quality of commercially produced fish feed in factories is also questionable. Moreover, there is no assessment and monitoring of to what extent chemicals are used in the ponds and the fish feed. Ensuring quality fish will be a major challenge, especially when it comes to exports (Rashid & Zhang, 2019).

2.2 Fish Farming and aquaculture status in Chattogram

Chittagong, a major coastal city in Bangladesh, has a long history of fish farming and aquaculture. The region is blessed with a vast coastline, a variety of rivers, and ponds, which makes it an ideal location for aquaculture activities. Today, Chattogram is a leading center for aquaculture production in Bangladesh, with both freshwater and marine aquaculture activities taking place in the region. Prawn, tilapia, and carp are some of the most commonly farmed species, with a range of farming systems used including pond-based, cage-based, and pen-based systems (DoF, 2023).

However, there're prevailing many constraints in fish farming, without eliminating those barriers, it is difficult to attain satisfactory growth in fish and aquaculture farming in Chattogram. Among those, major barriers include poor quality and supply of fish fry and fingerlings, lacking of extension service and updated information, unavailability of balanced feed material lack of proper technical education of fish farming as well as disease management and antibiotic uses, poor market facilities (Barua & Sarker, 2010), low market value of fish and lack of balance measurement, lack of modern infrastructure facility in fish landing and market, poor transportation and preservation system, unhygienic market environment (Rahman et al.,2013), influences of middlemen and terrorists (Barua & Sarker, 2010; Rahman et al., 2013) prevalence of fish diseases (Barua & Sarker, 2010; Faruk et al., 2008). Also, age and family size have significant positive relationships with farming. In the case of farmers having more of age, supported by the experiences gained over the years, impact of the barriers in respect of the successful pursuit of pond fish culture would get neutralized considerably in contrast to the level of problems faced by younger farmers. However, as younger farmers are more dynamic and cosmopolitan, they can easily collect essential technical information and they can also consult with Upazila Fisheries Officer (UFO) and other extension personnel in difficult

situations. So, the intensity of the barriers they face also become less, bringing them closer to the older set of fish farmers and for family size, the farmers having a large family have a higher impact of the barriers in the pursuit of pond fish culture (Barua & Sarker, 2010).

The government of Bangladesh has also implemented various policies and initiatives to support the development of the aquaculture industry in the region, including providing financial incentives to farmers and promoting the adoption of best management practices (BEW, 2022). Despite these efforts, there are still challenges facing the aquaculture industry in Chittagong, including water pollution, sanitation and hygiene status, disease outbreaks, and inadequate infrastructure (Sunny et al., 2021). However, with ongoing support and investment, the industry has the potential to continue to grow and contribute to the local economy and food security in the region. An area of 3,188 sq. km., adjacent to Nijhum Island under Hatia Upazila, has been declared as Marine Protected Area (MPA). The government of Bangladesh has undertaken a pilot project of deep-sea fishing to extract tuna and similar pelagic fish. A Crab hatchery has been set up at Kalatali in Cox's Bazar. Besides, sea weed and oyster culture is being piloted in 0.8 hectares coastal area of Sadar upazila, Teknaf, Maheshkhali and Ukhia upazila of Cox's Bazar district. This opens up new horizons for the blue economy (BEW, 2022).

2.3 Description of *E. coli*

E. coli is a Gram-negative, rod-shaped with a measurement of 1.1-1.5 x 2.0-6.0 μm, capsulated, oxidase- negative, non-spore forming facultative anaerobic bacterium (Lim et al., 2010; Scheutz, 2005). This microorganism was first discovered in the human colony in 1885 by German bacteriologist Theodor Escherich. Dr. Escherich also showed that certain strains of the bacterium were responsible for infant diarrhea and gastroenteritis, an important public health discovery. *E. coli* bacteria were initially called Bacterium coli, the name was later changed to Escherichia coli to honor its discoverer (Kaper et al., 2004). Most *E. coli* strains harmlessly colonize the gastrointestinal tract of humans and animals as normal flora can cause extra-intestinal infections (Brenner et al., 2005; Lupindu, 2017). Prior to the identification of specific virulence factors for pathogenic strains, *E. coli* was primarily classified based on serological identification of O (lipopolysaccharide, LPS) and H (flagellar) antigens. Based on the type of virulence factor present and host clinical symptoms, *E. coli* strains are classified into pathogenic types (pathotypes are defined as a group of strains of the same species causing a common

disease) (Allocati et al., 2013). All the strains are not harmless but some strains such as enterotoxigenic, enteropathogenic, enter invasive, or enterohaemorrhagic and can cause diarrhea, food poisoning in humans along with detrimental effect on body as well as fatal diseases in animals and birds particularly under conditions of poor hygiene (Moriel et al., 2012). Pathogenic strains of *E. coli* are depicted in short in **Figure 2.2.**

Pathotype (acronym)	Diseases	Symptoms	Virulence factors
Enteric E.coli			
EnteroPathogenic *E. coli* (EPEC)	Diarrhoea in children	Watery diarrhoea and vomiting	Bfp, Intimin, LEE
EnteroHaemorrhagic *E. coli* (EHEC)	Haemorrhagic colitis, HUS	Bloody diarrhoea	Shiga toxins, Intimin, Bfp
EnteroToxigenic *E. coli* (ETEC)	Traveler's diarrhoea	Watery diarrhoea and vomiting	Heat-labile and sheat-stable toxins, CFAs
EnteroAggregative *E. coli* (EAEC)	Diarrhoea in children	Diarrhoea with mucus and vomiting	AAFs, cytotoxins
Diffusely Adherent *E. coli* (DAEC)	Acute diarrhoea in children	Watery diarrhoea, recurring UTI	Daa, AIDA
EnteroInvasive *E. coli* (EIEC)	Shigellosis-like	Watery diarrhoea; dysentery	Shiga toxin, hemolysin, Cellular invasion, Ipa
Adherent Invasive *E. coli* (AIEC)	Associated with Crohn disease	Persistent intestinal inflammation	Type 1 fimbriae, Cellular invasion
Extraintestinal E. coli (ExPEC)			
UroPathogenic *E. coli* (UPEC)	Lower UTI and systemic infections	Cystitis, pyelonephritis	Type 1 and P fimbriae; AAFs, hemolysin
Neonatal Meningitis *E. coli* (NMEC)	Neonatal meningitis	Acute meningitis, sepsi	S fimbrie; K1 capsule
Avian Pathogenic *E. coli* (APEC)	Probable source of food-borne disease	-	Type 1 and P fimbriae; K1 capsule

Bfp: Bundle-forming pili; LEE: Locus for enterocyte effacement; HUS: haemolytic-uraemic syndrome; CFA: colonization factor antigen; AAF: aggregative adherence fimbria; Daa: diffuse adhesin; AIDA: adhesin involved in diffuse adherence; Ipa: Invasion plasmid antigen.

Figure-2.2. A compilation *E.coli* pathogenic type. Along with diseases, symptoms and responsible virulence factors (Source: (Allocati et al., 2013))

2.4 Fish diseases and antimicrobials usage aspects

Disease is considered one of the important factors to decrease in fish production, both in farming system and in wild condition. Also, regarded as a major constraint on the development and sustainability of aquaculture like other farming sectors. Fish production costs are increased by disease outbreaks because of the investment lost in dead fish, cost of treatment, and decreased growth during convalescence. World Bank in 2006 reported global loss of about US $ 3billion per year to aquaculture production and trade due to disease (Alfred et al., 2020). Large-scale mortality of fish usually occurs in ponds due to

environmental stress followed by parasitic invasion and bacterial, fungal, protozoan and monogenean infections (Aftabuddin et al., 2016).

2.4.1 Forms of fish diseases

There are two broad forms of diseases affecting fish which is given below:

2.4.1.1 Infectious Diseases

It is also called a biotic disease. Infectious diseases are caused by living pathogenic microbes found in the aquatic environment or carried by other fish (viruses, bacteria, fungi or parasites). Fish become susceptible to pathogenic infections when there are stress factors (environmental disturbances, deterioration of water quality, unbalanced nutrition or physical injuries), that weaken the natural resistance of the fish (immune system). The emergence of infectious diseases is usually caused by ecological changes, often related to human activities, such as translocation of organisms, environmental degradation, agricultural practices or technology. Infections can occur internally and externally and affect fish tissues, organs and other body parts. These are mostly infectious diseases and some type of treatment may be necessary to control outbreaks. Bacterial diseases are responsible for high mortality in both wild and farmed fish. They can infect a single fish and multiply rapidly, causing significant fish mortality within days or weeks. Bacterial diseases are often internal infections and usually require treatment (by adding antibiotics to food or water). Also, bacterial diseases can be external, which can be caused by rough handling or the effects of parasitic infection (Alfred et al., 2020).

2.4.1.2 Non-infectious diseases

Non-infectious diseases can be broadly categorized as environmental, nutritional and genetical.Some frequently occurring environmental diseases in aquaculture includes gas bubble disease, swim bladder stress syndrom, asphyxiation/hypoxia, sunburn disease, brown blood disease , acidosis and alkalosis disease etc.Some frequently occurring nutritional diseases in aquacultureincludes lipodosis, fish scurvy ,broken back syndrome, steatitis and white fat disease, avitaminosis.Genetical diseases are mainly happens due to inbreeding (Alfred et al., 2020).

2.5. An Epigram on Antibiotics

To begin, the definition of “antibiotic,” was first proposed by Selman Waksman (the discoverer of streptomycin and a pioneer in screening of soils for the presence of microbes), it is simply a description of a use, a laboratory effect, or an activity of a chemical compound.

The generic term “antibiotic” is used to denote a class of organic molecules that inhibit or kill microorganisms through specific interactions with bacterial targets regardless of the source of the particular compound or class. Purely synthetic therapeutics are therefore considered antibiotics. Finally, they interact with receptors to trigger specific cellular responses and biochemical mechanisms of pathogen cross-resistance. Fluoroquinolones (FQs), sulfonamides, and trimethoprim are good examples. Since the introduction of the first effective antimicrobial agent, the sulfonamides, in 1937, the development of specific resistance mechanisms of resistance has plagued their therapeutic use. Sulfonamide resistance was first reported in the late 1930s, and the same mechanism is at work about 70 years later. Alexander Fleming discovered penicillin in 1928, and several years before the introduction of penicillin as a therapeutic in 1940, two members of the penicillin discovery team identified a bacterial penicillinase. Once the antibiotic was used widely, resistant strains capable of inactivating the drug became prevalent, and synthetic studies were carried out to chemically modify penicillin to prevent it from being cleaved by penicillinase (β-lactamase). Interestingly, the identification of a bacterial penicillinase before the use of the antibiotic can now be appreciated in light of recent findings that numerous antibiotic resistant genes are components of natural microbial populations (Davies & Davies, 2010).

2.5.1 Antimicrobial resistance

Antimicrobial resistance is the resistance of a microbe to an antimicrobial agent that was used effectively in treating or preventing an infection caused by that microbe. When the infectious agent is bacteria then the more specific term is antibiotic resistance or antibiogram. The WHO report released on April 2014 stated that, “this serious threat is no longer a prediction for the future, it’s happening right now in every region of the world and has the potential to affect anyone, of any age, in any country. This antimicrobial resistance is considered as one of the three greatest threats to public health (Marnoor, 2017; WHO,2014).” A group of international experts of the European Centre for Disease Prevention and Control (ECDC) and the Centers for Disease Control and Prevention (CDC) was defined MDR (multidrug resistance) as acquired non-susceptibility to at least one agent in three or more antimicrobial categories, XDR (extensively drug resistance) was defined as nonsusceptibility to at least one agent in all but two or fewer antimicrobial categories (i.e. bacterial isolates remain susceptible to only one or two categories) and

PDR (pan drug resistance) was defined as non-susceptibility to all agents in all antimicrobial categories (Magiorakos et al., 2011).

2.5.2 Mechanisms of Antimicrobial resistances

Resistance mechanisms can be intrinsic, acquired or adaptative. Intrinsic resistance is when some strains of bacteria have a lower permeability of the membrane, or the presence of efflux pumps. Acquired resistance is developed when mobile genetic elements, such as plasmids, transposons, and naked DNA, are integrated into the bacteria's genetic material. Adaptative resistance is usually caused by metabolic changes in the bacteria in response to environmental pressures and exposure to sub-inhibitory concentrations of antibiotics. Adaptative changes can be plasmid or chromosomal-mediated, and the location of resistance genes on mobile genetic elements (MGEs) facilitates their spread to other bacteria. These genes enable bacteria to survive exposure to antibiotics. These changes can be plasmid or chromosomal-mediated, and the presence of MGEs makes it easier for the genes that confer resistance to spread to other bacteria (Cerceo et al., 2016; Nikaido, 2009; San Millan, 2018) (Figure2.3).

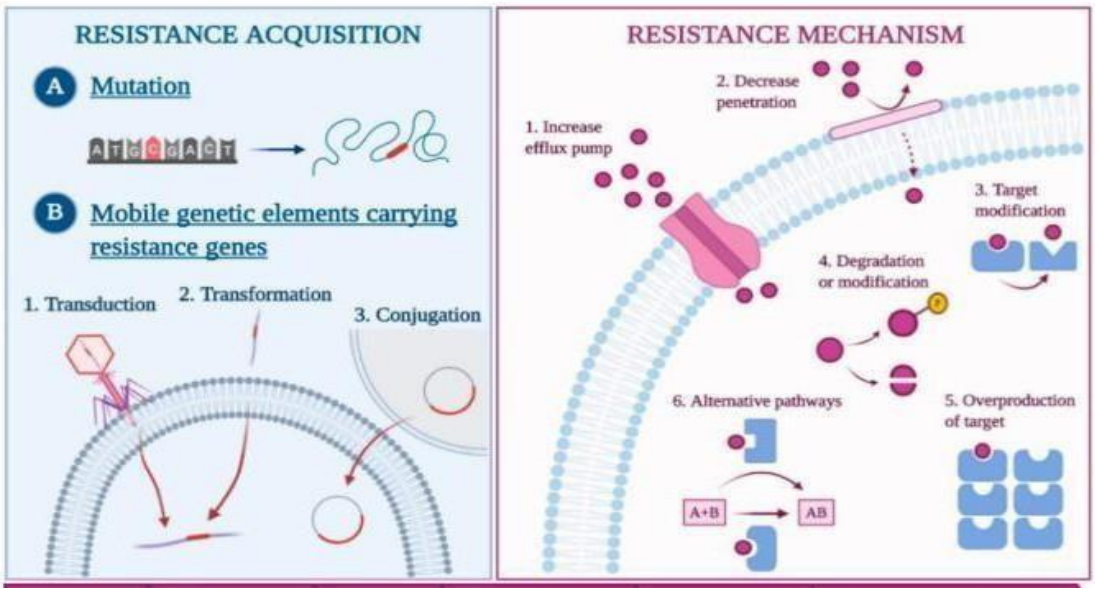


Figure-2.3. Resistance acquisition and consequent mechanisms expressed by resistant organisms (Source: (Silva et al., 2019))

2.5.2.1 Mutational alteration of target protein

This is typically a mutation process that involves alteration (usually acetylation or methylation of a specific nucleic base) of a nucleic acid, ineffective an antibiotic compound with a proteic target. One example of resistance attributable to protein modification is that conferred by the *erm* gene, which is usually plasmid coded and produces methylation of adenine at position 2058 of the 50S rRNA, causing resistance

to macrolides, lincosamide and streptogramin, also known as the macrolide lincosamide-streptogramin (MLS) phenotype (Nikaido, 2009).

2.5.2.2 Enzymatic inactivation of drug

Common resistance mechanism to naturally occurring antibiotics such as aminoglycosides (kanamycin, tobramycin and amikacin), which are inactivated by enzymatic phosphorylation and enzymatic hydrolysis of beta-lactams through beta-lactamases (penicillin, cephalosporins and carbapenems such as imipenem) also prevent their bioactive action (Nikaido, 2009).

2.5.2.3 Acquisition of exogenous resistance genes

Sequencing for the genes coding for the targets of penicillin, DD-transpeptidase or penicillin binding proteins (PBP), revealed that penicillin resistance among *Streptococcus pneumoniae* was due to the production of *mosaic* proteins, parts of which came from other organisms. Note that *S. pneumoniae* is capable of natural transformation and may import foreign DNA. A case of this scenario is the generation of MRSA. MRSA contains a new methicillin-resistant PBP, called PBP-2A, whose expression is induced by methicillin or other beta-lactams. The gene for this new PBP apparently came from an organism other than *S. aureus* and contains other antibiotic resistance genes. *S. aureus* is not naturally transformable and it is unclear how this horizontal transfer of a large DNA segment occurred (Cerceo et al., 2016; Nikaido, 2009).

2.5.2.4 Bypassing the target

Vancomycin, a fermentation product from *Streptomycetes*, has an unusual mode of action. Instead of inhibiting an enzyme, it binds to a substrate, the lipid linked disaccharide pentapeptide, a precursor of the cell wall peptidoglycan. Because of this mechanism, many would assume it would be impossible to generate resistance against vancomycin. However, vancomycin resistance is now prevalent in. When vancomycin resistance was studied, it was found that the substrate to which vancomycin binds was replaced in the resistant strain by an ester structure, which is not bound by vancomycin (Cerceo et al., 2016; Nikaido, 2009).

2.5.2.5 Preventing drug access to targets

Drug access to target can be reduced locally but also by active efflux by multidrug efflux pumps. In Gram-negative bacteria, access can be reduced generally by decreasing influx across outer membrane (Cerceo et al., 2016; Nikaido, 2009).

2.5.2.6 Local inhibition of drug access

TetM or *TetS* proteins, produced by plasmid-coded *tet* genes in Gram-positive bacteria, bind to ribosomes with high affinity and change ribosomal conformation, preventing association of tetracyclines to ribosomes. Plasmid-coded Qnr proteins protect DNA topoisomerases from (fluoro)quinones (Cerceo et al., 2016; Nikaido, 2009).

2.5.2.7 Non-specific inhibition of drug access

Mutation within coding sequences of porins, reducing permeation rates of bulky beta-lactams without affecting those of smaller nutrient molecules (Cerceo et al., 2016; Nikaido, 2009).

2.5.3 Problems of antibacterial resistance

Antibacterial resistance (ABR) has become one of the most important public health problems of the 21st century, affecting all health sectors and thus society as a whole (Dadgostar, 2019; Ventola, 2015). After the discovery of antibiotics, Sir Alexander Fleming recommended to use antibiotics properly because he had already observed bacterial resistance after prolonged exposure. Since then, antibiotic resistance has been detected against almost all antibiotics developed and used in healthcare settings (Dadgostar, 2019; Klein et al., 2018; Nikaido, 2009; Ventola, 2015).

The exacerbated use of antibiotics in various sectors such as agriculture, animal husbandry, and healthcare, coupled with poor management and misinformation about appropriate use, has led to a continuous selective pressure on bacterial communities promoting the development of resistance ADR is a major cause of death and morbidity worldwide (Klein et al., 2018), as it threatens the prevention and treatment of various infections, causes severe illness and longer hospital stays, and increases health care costs and recurrent treatment failures (Dadgostar, 2019). Resistant pathogenic strains such as methicillin-resistant Staphylococcus aureus and multidrug-resistant Pseudomonas aeruginosa, members of the Enterobacteriaceae family such as *Escherichia coli*, *Klebsiella pneumoniae* and *Acinetobacter baumannii* are some of the diverse bacteria that pose serious health threats (Cerceo et al., 2016).These problems has long been recognized by the WHO, which in 2001 provided a global framework for interventions to slow the emergence and reduce the spread of resistant organisms(Adenaike et al., 2016). In 2014, the WHO warned that the antibiotic resistance crisis is becoming dire (Ventola, 2015) and declared humanity is beyond the antibiotic era along with proposed prevention and control strategies encompassing individuals, policymakers and health care professionals those are needed to control the development and spread of AMRs (Cerceo et al., 2016).

Global antibiotic use has increased dramatically over the past decade by 65%, if the same trend continues an overall consumption increase of 200% compared to 2015 by 2030 compared to 2015. Additionally, a general increase in consumption of newer and last-resort of antibiotics such as glycylcyclines, oxazolidinones, carbapenems and polymyxins has been observed. A concern is low- and middle-income countries catching up to high-income countries' consumption levels. Further globally, the consumption of last-resort antibiotics, particularly carbapenems and colistin, is increasing, consistent with the well-documented increase in pathogens resistant to antibiotics (Klein et al., 2018). Overuse of antibiotics promotes the development of resistance and epidemiological studies show a direct link between antibiotic use and the emergence of resistant strains (Dadgostar, 2019; Klein et al., 2018). Antibiotics eliminate sensitive competitors, allowing surviving resistant strains to spread as a result of natural selection (Ventola, 2015).Sub-inhibitory and sub-therapeutic concentrations can promote the development of resistance through genetic alterations, resulting in changes in gene expression, horizontal gene transfer (HGT), and mutagenesis (San Millan, 2018; Ventola, 2015).

2.5.4 How widespread antibiotic resistance is?

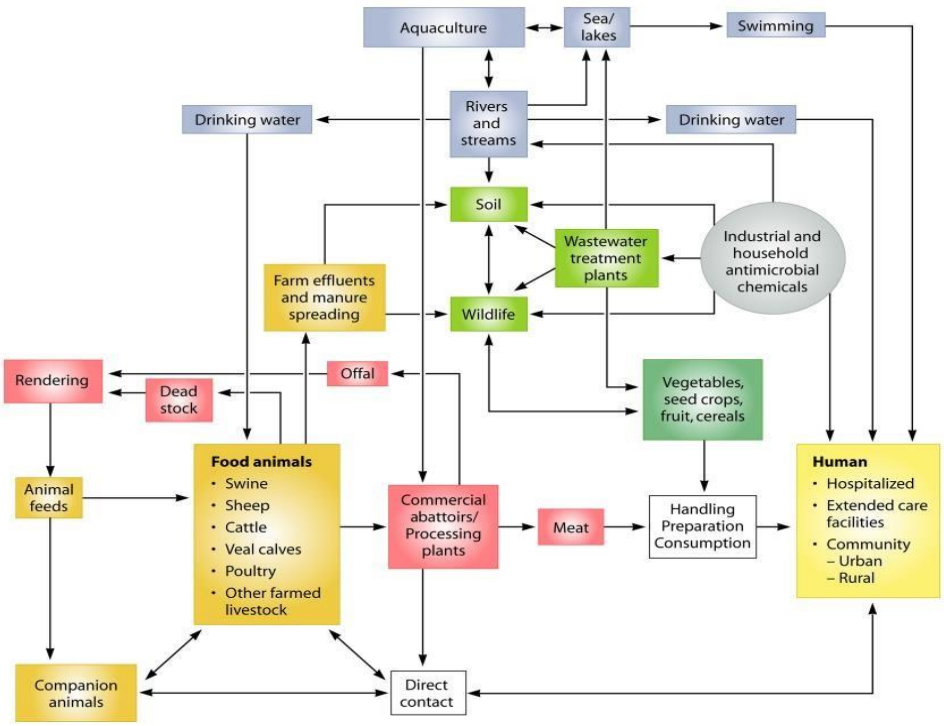


Figure-2.4. Dissemination of antibiotics and antibiotic resistance within agriculture, community, hospital, wastewater treatment, and associated environments (Source: (Davies & Davies, 2010)).

In agriculture, antibiotics are widely used as growth supplements to improve overall animal health and produce higher yields and quality products, as well as to prevent infections. Antibiotics used for non-therapeutic purposes such as growth promotion have been shown to select for resistance to high concentrations of antibiotics in both pathogenic and commensal bacteria (Ventola, 2015). Applying antibiotic-contaminated manure to soil is an agricultural practice in Europe, the United States, China and other countries around the world, which transfers antibiotics to agricultural land. This has been documented for example for sulfamethazine, tetracycline and chlortetracycline, as well as tylosin. Manure introduces bacteria that spread various combinations of multiple resistance genes, that can efficiently transfer on broad-host- range plasmids or other conjugative elements to many species in soil and then to other habitats, thereby promoting horizontal transfer of antibiotic resistance genes in soil (Heuer et al., 2011). In addition, the air surrounding these farms is also a source of antibiotic-resistant bacteria, with inhalation of air serves as another exposure route to transfer of antibiotic resistant bacteria (Antunes et al., 2020; Chapin III et al., 2005; Heuer et al., 2011) represented in Figure-2.5.

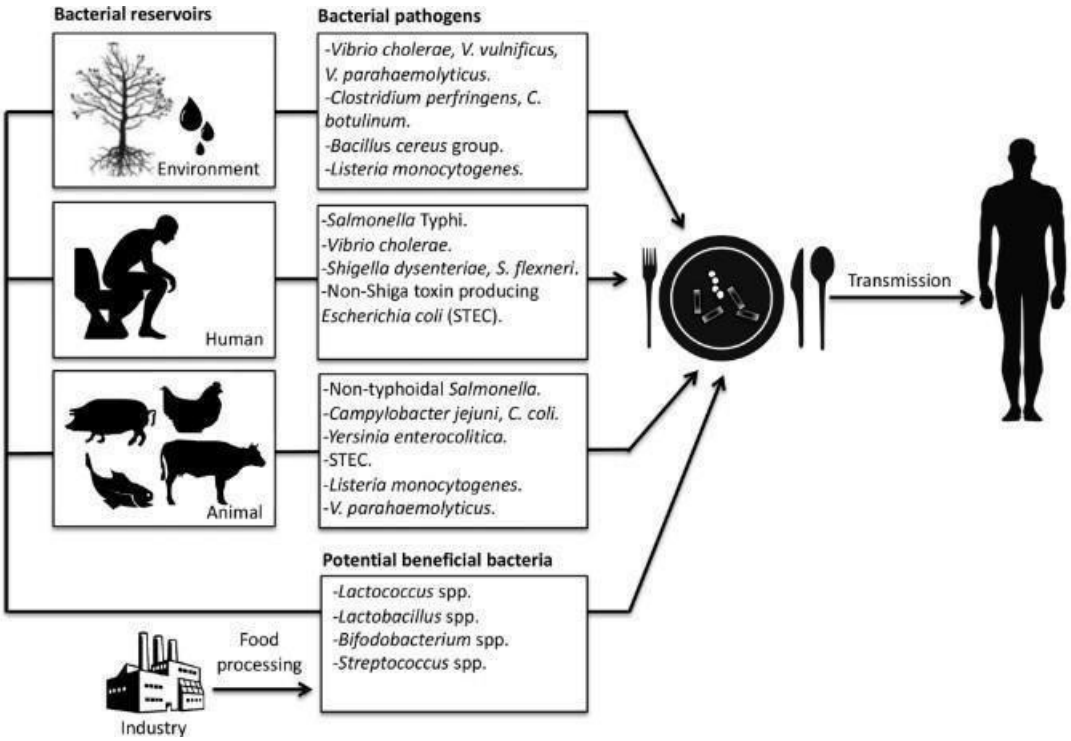


Figure-2.5. Transmission of bacterial organisms (pathogenic or commensal) to humans (Source: (Antunes et al., 2020)).

Aquaculture, the newest area of food production, may promote the emergence of resistance via similar methods to agriculture. According to research, the majority of

antibiotics used in agriculture and aquaculture are same as those utilized to treat people. Moreover, antibiotic classes that the WHO considers crucial are frequently used in aquaculture and agriculture. As a result, certain zoonotic bacteria found in seafood were resistant to a number of antibiotics on the WHO list (Done et al., 2015). Antibiotics have improved animal health, increasing economic gain for farmers, as pathogens are greatly reduced with antibiotic usage (Chapin III et al., 2005; Done et al., 2015). Though there has been increasing awareness regarding antibiotic usage in farmed species, becoming further under scrutiny because of increasing concern regarding antimicrobial resistance. These imprudent patterns of antibiotic prescribing and use represent a potential risk to human and animal health (Manyi-Loh et al., 2018). In both agriculture and aquaculture, development resistance can occur when these bacteria are exposed to sub-therapeutic concentrations of antibiotics.

2.6. Extended-spectrum *β-lactamases (ESBLs)*

ESBLs-producing *E. coli* have increasingly been detected in humans since the early 1990s and in animals since 2000. Resistance in bacteria of animals and its impact on human health have drawn much attention worldwide. Increasing use of antimicrobials has been associated with the emergence of resistant bacterial strains with mutated β-lactamases to hydrolyze the extended spectrum of β-lactams. The first ESBL was detected at the teaching hospital of Clermont-Ferrand, France, in July 1984, was the cefotoximase *TEM/CTX-1* (Shah et al., 2004).

2.6.1 Categories of *ESBLs*

Most ESBLs are derivatives of TEM or SHV enzymes. There are now > 90 TEM-type *βlactamases* and >25 SHV-type enzymes. TEM- and SHV-type ESBLs are mostly found in *E. coli* and *K. pneumonia* (Shah et al., 2004; Smet et al., 2010).

2.6.1.1 TEM-type *β-lactamases*

More than 150 TEM-type *β-lactamases* have been found, and all of them are derivatives of TEM-1 or TEM-2 by point mutations. TEM-1 was first demonstrated in 1965 in an Escherichia coli isolate from a patient in Athens, Greece, named Temoneira (designation TEM). In contrast to the majority of TEM β-lactamases, TEM-1, TEM-2 and TEM-13 are not ESBLs and are only able to hydrolyze penicillins. Some TEM derivatives have been found to have a reduced affinity for β-lactamase inhibitors and are known as

inhibitor resistant TEM. These enzymes have negligible activity against ESBLs (Shah et al., 2004; Smet et al., 2010).

2.6.1.2 SHV-type *β-lactamases*

The SHV-1 β-lactamase is most commonly found in *K. pneumoniae* and is responsible for up to 20% of the plasmid-mediated ampicillin resistance in this species. In 1983, a *Klebsiella ozaenae* strain was isolated in Germany possessing an SHV-2 enzyme that efficiently hydrolyzed cefotaxime and, to a lesser extent, ceftazidime. Unlike the TEM-type βlactamases, there are relatively few derivatives of SHV-1. Yet, the majority of SHV-type derivatives possess the ESBL phenotype. However, one variant, SHV-10, is reported to have an inhibitor-resistant phenotype; (derived from SHV-5). The majority of SHV-type ESBLs are found in *K. pneumoniae, Citrobacter diversus, E. coli*, and *P. aeruginosa* (Shah et al., 2004; Smet et al., 2010).

2.6.1.3 Inhibitor-resistant *β-lactamases*

B-Lactamase inhibitors were primarily developed to overcome resistance caused by the TEM and SHV-type due to their acquisition. Initially, most strains of *E. coli* and *K. pneumoniae* producing TEM-1, TEM-2 or SHV-1 type, were sensitive to *β-lactamase* inhibitor/β-lactam drug combinations. Over the time period, resistance has now been developed against inhibitors. Most of the resistance is due to the high production of TEM1, TEM-2 or SHV-1 *β-lactamases*. A second less commonly encountered mechanism of resistance to inhibitor/drug combinations involves the appearance of derivatives of TEM1 type. Inhibitor-resistant TEM *β-lactamases* were found mainly in clinical isolates of *E. coli*, along with some strains of *K. pneumoniae, K. oxytoca, P. mirabilis*. The inhibitorresistant TEM variants also resistant to inhibition by clavulanic acid and sulbactam, thereby showing clinical resistance to the β-lactam and β-lactamase inhibitor combinations of amoxicillin-clavulanate, ticarcillin–clavulanate and ampicillin– sulbactam although they remain susceptible to inhibition by tazobactam and subsequently the combination of piperacillin and tazobactam (Shah et al., 2004; Smet et al., 2010).

2.6.1.4 CTX-M-type *β-lactamases*

CTX-M-type, is a novel family of plasmid-mediated ESBLs, with the ability of hydrolyzing cefotaxime, were first isolated in Munich. CTX-M enzymes have 40% or less identity with TEM and SHV-type ESBLs. So far,>70 CTX-M enzymes have been

isolated. Based on the amino acid sequences they are divided into 5 clusters, these are: CTX-M-1, CTX-M-2, CTX-M-8, CTX-M-9 and CTX-M-25. Till the date, CTX-M-type β-lactamases are the highest among the ESBLs have been isolated from many parts of the world, including Europe, South America, and Asia (Shah et al., 2004; Smet et al., 2010).

2.6.1.5 OXA-type *β-lactamases*

The OXA-type enzymes are another growing family of ESBLs. These enzymes are quite different from the TEM and SHV enzymes. The OXA-type β-lactamases confer resistance to ampicillin and cephalothin, also characterized by their super hydrolyzing capability against oxacillin and cloxacillin and the fact that they are poorly inhibited by clavulanic acid. While most ESBLs have been found in *E. coli, K. pneumoniae*, and other Enterobacteriaceae, the OXA-type ESBLs also found in *P. aeruginosa* at a greater extent (Shah et al., 2004; Smet et al., 2010).

2.6.1.6 AmpC *β-lactamases*

Another large group of broad-spectrum *β-lactamases* are the AmpC enzymes, which are typically encoded on the chromosome of many Gram-negative bacteria. AmpC type *βlactamases* may be carried on plasmids of bacterial species lacking the chromosomal *ampC* gene, such as in *E. coli*, *Klebsiella spp*. These enzymes have been named with inconsistency as of typical *β- lactamase* nomenclature – according to the resistance produced to cephamycins (CMY,43 varieties), cefoxitin (FOX, 7 varieties), moxalactam (MOX, 3 varieties) or latamoxef (LAT, 4 varieties), according to the type of enzyme ACC (Ambler class C), 4 varieties of ACT (AmpC type), 3 varieties are according to the site of discovery, such as the Miriam Hospital in Providence (MIR-1) or the Dhahran Hospital in Saudi Arabia (DHA, 2 varieties). BIL-1 was named after the patient (Bilal) who provided the original sample (Shah et al., 2004; Smet et al., 2010).

2.6.1.7 Other ESBLs

While the majority of ESBLs are derived from TEM or SHV β-lactamases, a few other ESBLs have also been reported that are not closely related to any of the established families of β-lactamases. The PER-1 β-lactamase was first discovered in strains of *P. aeruginosa* isolated from patients in Turkey. Later, it was also found among isolates of *S. enterica* serovar *Typhimurium* and *Acinetobacter baumanii*. The (PER-Pseudomonas extendedresistant), PER-1 strain of β-lactamase is widespread across Turkey and up to 60% of ceftazidime-resistant strainsof *A. baumanii* was found, which represent 46% of the total isolates. A related enzyme, PER-2, which has 86% amino acid homology with

PER-1, was first found among *S. enterica* serovar *Typhimurium* strains in Argentina.Another enzyme, VEB (Vietnam extended-spectrum β-lactamase), VEB-1, was first isolated from *E. coli* in a patient from Vietnam, but was subsequently also found in a *P. aeruginosa* isolate in Thailand. A third related enzyme is CME-1, which was isolated from *Chryseobacterium meningosepticum*. TLA (Tlahuicas Indians), TLA-1, which was identified in an *E. coli* isolate from a patient in Mexico, SFO-1, was first detected in *Serratia fonticola*, which is strongly related to β-lactamase but cannot hydrolyze cephamycins and properly inhibited by clavulanic acid. GES (Guiana extended-spectrum), GES-1 is another uncommon ESBL enzyme that is not closely related to any other plasmid-mediated β-lactamase but shows 36% homology to a carbenicillinase from *Proteus mirabilis* (Shah et al., 2004; Smet et al., 2010).

2.6.2 Risk factors associated with ESBLs spread to human

ESBLsproducing strains are usually found in those areas of hospitals where antibiotic use is frequent and the patient's condition is critical. Various risk factors have been associated with the selection and spread of ESBL-producing strains are stated below as mentioned prior (Oteo et al., 2010; Shah et al., 2004) :

- Emergency intra-abdominal surgery.
- Through central venous or arterial catheter, gastrostomy or jejunostomy tube, urinary catheter.
- GI colonization.
- Prolonged ICU or hospital stay.
- Prior antibiotics including 3rd generation cephalosporins (Number and duration of antibiotic therapy probably most important factors)
- Prior nursing home stay.
- Consumption of contaminated animal originated food products and inhalation of contaminated air.
- Mechanical activities.

2.7 Epidemiology of MDR *E. coli* in public health aspects

Multi-resistant *Escherichia coli* have been evolved by the use of broad-spectrum antimicrobials in both livestock and humans (Singh et al., 2014). The development of MDR *E. coli* create problems due to their propensity to disseminate antimicrobial resistance

genes. The emergence and diffusion of MDR strains of *E. coli* is complicating the treatment of several serious infections. Enterobacteriaceae, particularly *E. coli*, are the most frequent cause of hospital- and community-acquired infections. Besides, MDR *E. coli* strains are also commonly isolated from animals and food products. The use of antibiotics in animals contributed to the emergence and spread of the number of antibiotic-resistant strains, including *E. coli*, which can also infect humans through either direct contact with animals or through consumption of contaminated food. *E. coli* is able to survive and adapt in various extraintestinal habitats and to spread resistances between humans, animals, their products and the environment through several transmission pathways. Environment plays a key role in the spread of antimicrobial resistance serving as an unlimited reservoir of antimicrobial resistance genes. Therefore, *E. coli* may acquire other drug resistance traits from environmental bacteria and conversely it can spread its resistance genes to potential pathogens in different habitats. The epidemiology of MDR *E. coli* associated infections varies widely depending on the type of strain involved. In the last years in Europe, *E. coli* outbreaks were mainly caused by various EHEC strains. STEC *E. coli* O104:H4 has been responsible for a large number of outbreaks in the recent years. *E. coli* O25b:H4/ST131 (sequence type 131) is an emerging disseminated multidrug-resistant EPEC strain, causing a broad spectrum of diseases, mainly urinary tract infections. *E. coli* O25b:H4/ST131 is widely distributed in Europe, with Spain and Italy most prominently affected (Allocati et al., 2013).

Moreover, the prevalence of ESBLs producing *E. coli* clearly increasing, and in many parts of the world 10–40% of strains of *E. coli* express as ESBLs. The incidence of ESBLs varies according to spatial and temporal pattern. In the USA the incidence in Enterobacteriaceae ranges from 0 to 25%. In Europe, the prevalence of ESBL production among isolates of Enterobacteriaceae varies greatly from country to country. In the Netherlands, a survey of 11 hospital laboratories showed that <1% of *E. coli* possessed an ESBL. In Europe, 5.4% for E coli. In Japan, the percentage of *β-lactam* resistance due to ESBL production in *E. coli* remained very low (<0.1 and 0.3%, respectively). In other parts of Asia, the percentage of ESBL production in *E. coli* from 4.8% in Korea to 8.5% in Taiwan, 12% in Hong Kong and up to 50% in India. It is interesting that specific ESBLs appear to be unique to a certain country or region. For example, TEM-10 ESBL- producing organisms have recently been reported in the United States and Europe. While TEM-3 is common in France, TEM-47producing organisms in Poland, TEM-102 in Ireland and a prevalence of TEM-52, SHV12, and SHV-2a *β-lactamases* in Korea. In contrast, the SHV-5 *β-lactamase* is

commonly encountered worldwide. In 6.3% of all Enterobacteriaceae tested produced ESBLs, 42.3% of which were TEM-derived enzymes. CTX-M- type ESBLs, bla (CTX-M-2) type and bla (CTX-M-3) type were found in four countries where they had not been described previously are Australia, Belgium, Turkey, and South Africa (Oteo et al., 2010; Smet et al., 2010).

An overall literature review on Prevalence of *E. coli* in fish samples worldwide, antimicrobial resistance (%) in fish samples worldwide and Prevalence of MDR *E. coli* resistance gene isolated from fish samples worldwide are enlisted in Table-2.1, Table-2.2 and Table-2.3 respectively.

Table-2.1. Prevalence of *E. coli* in fish samples worldwide

Country	**Fish Species**	**Sample**	***E. coli* Prevalence**	**Study area**	**Reference**
Bangladesh	Koi, Poa, Loitta, Sorputi, Bata, Taki	Fish Muscle	> 90%	Dhaka	(Akter et al., 2022)
Korea	Fish,Shellfish, Mollusk, Crustaceans	Mashed/Filet Fish	6.7%	Seoul	(Ryu et al., 2012)
India	Food Fish	gut portions with muscle tissue	48%	Assam	(Sivaraman et al., 2020)
	Carp, Catfish & shellfish (Shrimp)	Fish Muscle, exoskeleton, finfish flesh	80.70%	Kolkata, West Bengal	(Dutta, 2016)
	Fish	Fish Muscle	38%	Chhattisgarh State	(Khan et al., 2021)
	Catla fish	Fish Muscle	69.3%	Andhra Pradesh	(Sekhar et al., 2017)
Switzerland	shrimps		64%	Berne	(Boss et al., 2016)

	pangasius	Fresh, frozen, or thawed raw filets	17%		
Denmark	Pangasius fillets and prawns	Fish flesh and prawn	22.3%	retail shops around Denmark	(Ellis-Iversen et al., 2020)
France	Freshwater fishes	Fish flesh	0 to 92%	Ouche river, Burgundy	(Bollache et al.,2019)
Saudi Arabia	raw frozen mackerel fish	fish gills, intestines parts and skin	51.1%	Eastern Province of Saudi Arabia	(Elhadi & Alsamman, 2015)
Nigeria	catfish, tilapia and crab	Fish Muscle	44.1%	Lagos state	(Odumosu et al., 2021)
	African Cat Fish	GIT samples	17.5 %	Jos Main Fish Market	(Akande et al., 2019)
	smoked fish	smoked fish	16%	Samaru-Zaria	(Adenaike et al., 2016)

Table-2.2. Antimicrobial Resistance (%) in fish samples worldwide

Country	Fish species	Antibiotics	Resistance %	Study area	Reference
Korea	Fish, shellfish, mollusk, crustaceans	Tetracycline	30.7%	wholesale and retail markets in Seoul	(Ryu et al., 2012)
		Streptomycin	12.8%		
		Cephalothin	11.7%		
		Ampicillin	6.7%		
		Ticarcillin	6.1%		
Saudi Arabia	Raw frozen mackerel fish	Ampicillin	100%	Eastern Province of Saudi Arabia	(Elhadi & Alsamman, 2015)
		Piperacillin	96.7%		
		Cefotaxime	93.3%		
		Ceftriaxone	93.3%		
		Tetracycline	53.3%		
		Nalidixic acid	40%		
		Trimethoprim	30%		
		Piperacillin	96.7%		
Bangladesh	Koi, Poa, Loitta, Sorputi, Bata, Taki	Penicillin	100%	Dhaka	(Akter et al., 2022)
		Erythromycin	39%		
		Rifampicin	34%		
	Tilapia and Mrigal	Erythromycin	81.25 %	Sylhet city	(Reza et al., 2020)
		Novobiocin	87.5 %		
		Imipenem	38%		
		Meropenem	30%		

		Chloramphenicol	40%		
		Azithromycin	49%		
		Tetracycline	55%		
		Cotrimoxazole	62%		
		Piperacillin-tazobactam	60%		
India	Fish	Aztreonam	68%	Chhattisgarh State	(Khan et al., 2021)
		Ceftazidime	57%		
		Cefotaxime	54%		
		Ampicillin	43%		
	Catla Fish	Cefotaxime	12.5%	Andhra Pradesh	(Sekhar et al., 2017)
		Ceftriaxone	11.5%		
		Ceftazidime	6.7%		
		Aztreonam	5.7%		
Nigeria	African Cat Fish	Cefoxitin	77.1%	Jos Main Fish Market	(Akande et al., 2019)
		Amoxicillin-clavulanic acid	74.3%		
		Amoxycillin	51.4%		
		Sulfamethoxazole/trimethoprim	42.9%		
	Crab	Tetracycline	100%	Lagos state	(Odumosu et al., 2021)
		Trimethoprim	76.5%		
		Cefotaxime	44.1%		
		Colistin	44.1%		
	Catfish	Tetracycline	100%		
		Trimethoprim	88.6%		

		Cefotaxime	40%		
		Colistin	40%		
	Tilapia	Tetracycline	100%		
		Trimethoprim	75%		
		Colistin	44.4%		
		Gentamicin	30.6%		
		Ciprofloxacin	30.6%		

Table-2.3. Prevalence of MDR *E. coli* resistance gene isolated from fish samples worldwide

Country	**Species and sample**	**Resistant gene**	**Prevalance**	**Study area**	**Reference**
Korea	Fish, Shellfish mollusk, crustaceans	*Tet-B*	41.4%	wholesale and retail markets in Seoul	(Ryu et al., 2012)
		Tet-D	20%		
		blaTEM	21.4%		
Pakistan	Commercial Fish	*blaCTX-M*	40%	Peshawar	(Ahmad et al., 2022)
		blaSHV	60%		
		blaOXA-10	0%		
		BlaNDM-1	6.0 %		
India	Food Fish	*tetA*	18%	Assam	(Sivaraman et al., 2020)
		tetB	0%		
		tetG	0%		
		blaCTX-M-15	98%		
	Tilapia	*blaCTX-M*	38%	Pune	(Marathe et al., 2016)
		blaSHV	31%		

		blaOXA	7%		
	Fish	*blaTEM*	13.2%	Chhattisgarh State	(Khan et al., 2021)
		blaSHV	1.3%		
		blaCTX-M	0%		
	Catla Fish	*blaAmpC*	12.5%	Andhra Pradesh	(Sekhar et al., 2017)
		blaTEM	12.5%		
		blaCTX-M	8.64%		
		blaSHV	5.77%		
		blaOXA	3.85%		
Saudi Arabia	Raw frozen mackerel fish	*blaCTX-M*	82%	Eastern Province of Saudi Arabia	(Elhadi & Alsamman, 2015)
		blaTEM	0%		
		blaSHV	0%		
France	Freshwater fishes	*blaCTX-M*	0 to 85%	Ouche river, Burgundy	(Bollache et al.,2019)

2.8 Impact of MDR *E. coli* in the ground one health

β-lactamases were first detected in the early 1980s in humans, and their presence and diversity have been increasing ever since. The first-time cephalosporin resistance was noted in animals was in early 2000. As presence of ESBLs producing microbes in animals is increasing, and it is not unrealistic to expect that this will have an impact on human health. Resistance may be transferred in two ways. Due to close contact or consumption of animal meat, a *β-lactam* resistant zoonotic strain *E. coli* 0157:H7, may betransferred directly from animal to human, thus possibly causing zoonotic infections, has been revealed earlier(Vijayan et al., 2023). As for direct transfer of resistance, the use of antimicrobial agents already ineffective to resistant bacteria may be the most important factor. What's more , resistance may possibly be acquired indirectly, through the transfer of resistance genes from bacteria of animal origin to bacteria infecting humans. TEM-52-isolated from Salmonella sp. is not only spreading between poultry and humans through direct transfer, but the stable plasmid may carry this gene or may spread among

various Salmonella serotypes, thus indicating a chance for indirect resistance transfer. Dispersion of CMY-2 producing *E. coli* from cattle and pigs to humans, or vice versa, due to the association of this gene with ISEcp1, represents the opportunity of indirect transfer of resistant genes throughout the environment (Smet et al., 2010). In case of, food-producing animals, some ESBLs strains were isolated from specific individual countries, such as TEM-106 in Belgium, CTX-M-8, SHV-5 in Tunisia and many CTX-M enzymes in China. Other resistant strains have been found to be more widely distributed. So far, for TEM-52- and SHV-12 producing isolates, isolated especially from poultry, have only been described in European (Mahmood & Bal, 2014; Smet et al., 2010). ESBLs such as CTX-M-1, CTX-M-2 and CTX-M-14 have been found in many European countries, being associated with E. coli mainly from poultry (Smet et al., 2010).The CTX-M-15 enzyme, the most widely diffused enzyme among human Enterobacteriaceae, was only recently detected among *E. coli* from poultry and pigs (Diab et al., 2017). MDR and ESBL producing *E. coli* isolated from the fecal microflora of healthy pets. CTX-M-1 have been isolated from commensal of healthy dog and cat pets in Europe and Latin America along with CMY-2 producing *E. coli* found in faces of healthy dogs in Italy (Rocha-Gracia et al., 2015). Also, AmpC β-lactamase, CTXM-1, CMY-2, CMY-7 strains of resistant *E. coli* isolated from UTIs of pet and healthy wild animals, homologous to strains of livestock (Costa, 2013). CTX-M-1 strain is most predominant among both healthy and sick human, food animals as well as pets(Ben Sallem et al., 2012), indicating commensal *E. coli* of humans may constitute a reservoir of $bla_{CTX\text{-}M}$ (Messai et al., 2008). It seems that ESBLs producing *E. coli* have been evolving and spreading at a rapid rate among humans, animals and the environment worldwide limiting the treatment options.

2.9 Preventive and control strategies to combat MDR *E. coli* emergence in fish

Management of infections by MDR *E. coli* has been arduous erratically. A single approach to the prevention and control of MDR in aqua-cultural settings is not successful alone. Rather a combination of different strategies is effective. These include-

2.9.1 Setting up a national or regional information exchange between farmers and responsible parties is compulsory.

- The FAO, the World organization for Animal Health (OIE), and WHO are cooperating to control antimicrobial use in animals. Global monitoring systems such

as the National Antimicrobial Resistance Monitoring System (NARMS) for Enteric Bacteria and the European Antimicrobial Resistance Surveillance Network are making efforts to collect information on antimicrobial consumption and antimicrobial resistance. The theme for World Health Day 2011 was "combating drug resistance: no action today, no cure tomorrow," which was meant to urge all countries around the world to take more proactive steps against bacterial resistance. Further, in the World Health Assembly 2015, a global action plan was adopted to tackle antimicrobial resistance. Moreover, the 2016 UN High-Level Meeting on antimicrobial resistance and the G20 Summit in Hangzhou have made strong commitments to control antibiotic resistance.

- Governments all over the world should work together to regulate and reduce antibiotics misuse effectively. Controls over antibiotic use in agriculture, hospitals and the community need to be dramatically tightened an to restrict or ban the use of antimicrobials (especially antibiotics which are licensed in human medicine) in food animals as growth promoters according to WHO recommendation so as to prevent upcoming MDR *E. coli* catastrophe universally.
- In order to effectively reduce the abuse of antibiotics and to reverse MDR *E. coli* emergence in livestock farming and aquaculture, national stewardship that need to mandate are -

  - The government should strictly implement the relevant national animal husbandry and aquaculture farming, WHO and OIE laws and regulation policies regarding animal health, antibiotics usage, and formulate specific supporting implementation measures, guidelines on rewards and punishments, supervision and management rules, so as to restrict the abuse of antibiotics.
  - Attention to the regulation on antibiotic misuse in livestock husbandry and aquaculture. Government can set a restrictive floating price range for antibiotics, and a government-sponsored labeling program can reduce the use of antibiotics. Moreover, the government should promulgate circulars, regulations on veterinary prescription as well as advocate for the importance of prescriptions to veterinary medicine suppliers and fisheries farmers.
  - Additionally, government can establish systematic policies on antibiotic regulation and market regulation. Especially for antibiotic enterprises that do not comply with the regulations, the government regulatory departments should

severely punish them and order them to stop related business activities if necessary.

➢Also, for the farmers who illegally purchase should bring under punishment. At the same time, it is very necessary to organize training programs for farmers on -

a. Scientific culturing guidelines and how to establish efficient and healthy farming patterns rather than relying heavily on antibiotics?
b. How to adjust the nutritional structure of feed?
c. How to choose functional additives and more easily digested feed?
d. How to prove the farms' management level and sanitary conditions?
e. How to introduce advanced animal husbandry and aquaculture? technology, and to reduce the pathogens spread by mosquitoes and flies?

➢Besides, government departments should strictly supervise the treatment of wastewater discharged from livestock farming and aquaculture, monitoring the antibiotic content of the rivers, lakes, and other aquatic environments near farms in real-time, to ensure the quality and safety of agricultural products, and to protect the ecological environment from MDR emergency.

➢The government should report on the use and residues of antibiotics every year, so as to comprehensively grasp the first-hand data of antibiotic use and residues in the animal husbandry and aquaculture. In the whole process of livestock farming and aquaculture, the government should use advanced technology for supervision and regulation

➢Finally, the government should strengthen the publicity of science popularization education to make people fully aware of the serious harm to human health and ecological environment from the abuse of antibiotics, and to raise the public's attention to the rational use of antibiotics.

2.9.2 Biosecurity Measures in Aquaculture

Biosecurity is a management action to prevent the introduction of disease-causing agents to aquaculture farming. Farm-level biosecurity measures involve the application of a combination of activities more or less which includes strict quarantine measures, sanitation of equipment, disinfection of egg, traffic control, water treatments, use of clean feed, disposal of dead appropriately. These protocols should be implemented during the introduction of new stock as well as implementing them for reducing pathogens and to avoid transferring pathogens from one stock to another. Most diseases of aquaculture can be overcome by the meticulous application of biosecurity measures. Stocking density reduction is one of the most important approaches to control diseases of fish in aquaculture. Low stocking densities are a very useful first step measure when ectoparasite infections break out, along with increasing water flow, to achieve a greater fishing effect. Therefore, improved sanitary farm conditions as well as the maintenance of farm biosecurity are important alternatives that could be adopted by farmers instead of depending on antibiotic drugs for disease control and prevention. These would essentially serve as a means of preventing the entry and dissemination of pathogens in farms.

2.9.2.1 Quarantine and Restriction of Movement in Aquaculture

Quarantine is confining aquatic animals that are introduced from outside and they are with unknown health status before introducing to the stock. During this time strict observation of animals and using the appropriate diagnostic test is required. The duration of quarantine may range from five days to 3 months. After a correct diagnosis of the disease in question, treatment should be given with efficacious agents for the appropriate period of time. Prophylactic treatments can inhibit developing clinical signs and appropriate use of antibiotics can prevent development bacterial resistance genes and drug resistance among fishes.

2.9.2.2 Disinfectants and Pesticides in Aquaculture

Disinfection involves the use of physical or chemical agents to remove microorganisms usually on inanimate objects. In aquaculture, disinfectants can also include compounds used to destroy microorganisms living on the surface of fish eggs. These agents are used in aquatic animal rearing facilities as part of biosecurity protocols to control the spread of aquatic animal pathogens. The cleaning and drying of ponds properly can be phenomenal in controlling of many diseases of fish in aquaculture. A pond that has clean well- aerated water is important in producing healthy fish. Quaternary ammonium

compounds, formaldehyde, hydrogen peroxide, isopropyl alcohol, gluco-protamine, chlorine iodine, and iodophors, are mostly used as a disinfectant in aquaculture. Apart from being toxic to fish quaternary ammonium compounds are effective in killing organisms in inanimate objects. Chlorine can also be used but it must be neutralized adequately to avoid killing of fish. Equipment disinfected with iodine-containing compounds must also be rinsed of prior to use because they can be toxic.

2.9.3 Surveillance for Diseases of Fish in Aquaculture

Any aquatic health plan or any policy development for aquatic animal health is not possible without quality health data. This data can be used for disease control, quarantine, and health certification which can be achieved by conducting aquatic animal surveillance. Surveillance to avoid the introduction of disease is an important element of any biosecurity strategy to identify the possible route of disease introduction to aquatic farms and to detect the emergence of a new disease which will ensure that control strategies can be implemented before the pathogen becomes widespread. It is important to conduct surveillance regularly in order to reduce the risk of the spread of pathogens. Disease surveillance should be an integral and key part of all government aquatic animal health services.

2.9.3.1 Passive Surveillance

Data collected for other propose can be utilized to know aquatic animal health status and to plan appropriate measures to reduce the incidence of disease. Data can be obtained from laboratories, field visits, research projects, farmers, and aqua culturists. Passive surveillance is useful for the early detection of emerging diseases. Its limitation is that it does not allow estimation of disease incidence and prevalence and it cannot be used to demonstrate freedom from disease (df of surveillance).

2.9.3.2 Active Surveillance

Active surveillance involves surveys to know the status of a particular disease in question. Evidence of disease in a specified population, and in some instances, provides the data to prove that the specified population is free of a specific disease. Results of active surveillance may be biased unless properly designed and analyzed. Appropriate analyses can provide valid measures of incidence and prevalence of disease in particular area. Its advantages include information better in quality, it is faster and cheaper and more reliable to collect information than passive surveillance.

2.9.4 Importance Diagnostic Tests in Prevention and Control of Infectious Disease in Aquaculture

Sensitive and specific diagnostic tests are invaluable to assure healthy fish though diagnosing aquatic animals by the clinical sign is almost impossible because fishes live in water and move fast that which them impossible to visualize closely and inspect them for any clinical deviations. This makes rapid and accurate diagnostic methods to be important for the prevention and control of infectious diseases. Diagnostic tests for identification of fish diseases include conventional microbiological, immune-serological, and molecular methods. Rapid and accurate molecular-based methods have become important diagnostic tools. Lateral flow immunoassays, DNA microarrays, proteins, or glycans can also be immobilized on a solid surface of the microarray to probe different target molecules labeled with fluorescence. In diagnosing disease of fish, the detection of the pathogen in a tissue sample is conducted by lethal sampling rather than the detection of antibodies that are an indicator of a particular disease, but in case of, high valued fishes like ornamental fish, nonlethal sampling is recommended. Diagnostic tests are not expected to be 100% sensitive and specific. To avoid misinterpretation, diagnostic test protocols should be selected and interpreted based on their performance under the conditions of use. In the context of biosecurity programs, diagnostic tests are used to detect the emergence and follow the progression of infectious agents in fish stocks. There are four main biosecurity-related objectives for which diagnostic tests are commonly used: to demonstrate freedom from infection in aquaculture for obtaining or maintaining infection-free certification, to screen fish before introduction to the receiving facility, to detect infected fish as early as possible during a quarantine period, and to confirm suspicious or clinical case.

2.9.5 Application of preventive and protective therapies

The worldwide emergence of multidrug-resistant bacteria has dramatically limited the number of antibiotics that retain activity against these pathogens. This problem has been further amplified by the dearth of novel classes of antibiotics. Therefore, development of novel therapeutic strategies for infectious diseases is high demand. Regarding these following alternatives may be practiced:

2.9.5.1 The Use of Probiotics and prebiotics in fish farming

Boosting the natural defense of a fish is one of the researchable areas with many beneficial advantages. The main substances which can be incorporated in feed and

delivered orally to fish or may be injected biological disease prevention in aquaculture is among the best approaches in infectious disease control.

Probiotics are live microorganisms, administered to hosts to develop a protective immune status. After being administered to fish they multiply themselves to occupy the gut of the fish, help normal microflora and maintain microbial balance in the hosts. Many microorganisms have been evaluated as probiotics in aquaculture. *Bacillus subtilis*, *Lactobacillus acidophilus*, *Lactobacillus sakei*, and *Shewanella putrefaciens* are few of them. These can be used in fish and other cultured animals to prevent disease and promote weight gain. Probiotics can be applied to the feed, or they can be added to the water directly. The other administration strategy is encapsulation or Microencapsulation such as cells of *Shewanella putrefaciens* in calcium alginate. Encapsulation helps by improving nutritional values and proper delivery of the microbe to the host without waste of live organisms. Further, Probiotics can be added directly to the feed in the water tank. Prebiotics are resistant to attack by endogenous enzymes and hence can reach the site of action to promote the proliferation of gut microflora. Some of the prebiotics, that are currently used in animal feed, are mannan-oligosaccharides (MOS), fructo-oligosaccharides (FOS), and mixed oligo-dextran.

2.9.5.2 The use of medicinal plant product in fish farming

The use of medicinal plant product application in fish farming for disease control is another promising alternative to antibiotics. Certain medicinal plants are well are known to contain antimicrobial, and Medicinal plant-based extracts stimulate the immune system of fish, avoid stress, and act as antibacterial and antiparasitic agents due to their active chemical ingredients. And so far, their antioxidant properties could help us to replace the use of antibiotics in food animal production. Red clover extract, a plant-derived product with an abundance of active biological compounds, has recently been discovered as an alternative to antibiotic growth promoters in food animals. Furthermore, the ability of organic acids to exhibit bactericidal and bacteriostatic characteristics based upon the physiological conditions of the organism as well as the physicochemical features of the environment argue for their use as alternatives to antimicrobial elements in food animals. They can be administered by extracting their active component or the whole plant material can be added to the aquarium directly. Depending on the type of plant part used and the season of harvest of the plant material, their active ingredient may be varied. Medicinal plants can be administered to fish by injection or oral administration and through immersion or baths. Injecting the extracted material is an effective method for large fish.

Chapter-3: Materials and Methods

Fundamentally, this chapter comprises a narrative description of the study area, species of the sample, sampling, transportation, preservation, standard laboratory test procedure and statistical analysis of the research findings of this study, etc.

3.1 Elucidation of the study area

Chattogram, the largest port city of Bangladesh, is pinpointed on the banks of the river Karnaphuli (Mia et al., 2015), in the south-eastern part of Bangladesh, accompanied by the GPS coordinates of 22.20°N and 91.50° E (Anonymous, 2023). Chattogram is blessed with huge culture and capture fisheries, especially for the marine fishery. Besides, the famous Kaptai lake fishery is situated in the Chattogram division. Due to its geographical location on the coastline, fish were landed here and there in convenient locations along the coast and riverbanks. Several shrimp and prawn hatcheries have also been built in this area. As a result, all sea-caught and farmed fish in this region are landed and distributed to markets in major cities and other nearby areas, in the capital city as well as the northern and northwestern regions of the country (Ahmed & Rahman, 2005; Rahman et al., 2013).

3.1.1 Fish Harvesting of Chattogram

Chattogram Division yields a leading number of 7,79,703 metric tons (nearly 20% of national production) of fish per annum from its inland areas consisting of aquaculture (pen, cage, season), farm (shrimp/prawn and crab), Kaptai lake, river, beels, flood plains and ponds comparing with other divisions in the whole country. Whereas the foremost amount is harvested from the pond 3,95,761 metric tons and from the flood plain 1,69,774 metric tons respectively (BBS, 2022). Owing to apical fish production and landing Chattogram has the utmost no of landing centers. A former study reported that 76 landing centers, 821 fish dealers, 5786 wholesalers, 17134 retails and 809 fish markets (71% year-round coupled with 29% seasonal) are existing here. Pivotal fish production territories of this area are Kaptai lake (culture fishery), Chattogram and Cox's Bazar (Marine Capture Fishery) as well as Chandpur (Riverine Capture Fishery) (Rahman et al., 2013). A significant number of fish dealers, fish markets and harvesting zone denote the massive trade flow including economic movement of Chattogram, in addition to made this district worthy to conduct the present study on *MDR E. coli* in selected commercial fish market.

3.2 Study design, period and sampling site

A cross-sectional study was designed from February 2022 to December 2022 in order to conduct this study. Ten prominent fish markets in Chattogram city (Bahaddarhat Bazar, Fishery Ghat, Agrabad Bazar, Modhunaghat Bazar, Reazuddin Bazar, Pahartali Bazar, Jhautala Bazar, Oxygen Bazar, Chawkbazar, 2no gate Bazar) were selected randomly for the study (Figure 3.2.). It may be mentioned here that the term market is Bazar in Bengali which is used for the name of wet market in this study. These markets receive fishes from different fishing sites and fish production zone within Chattogram.

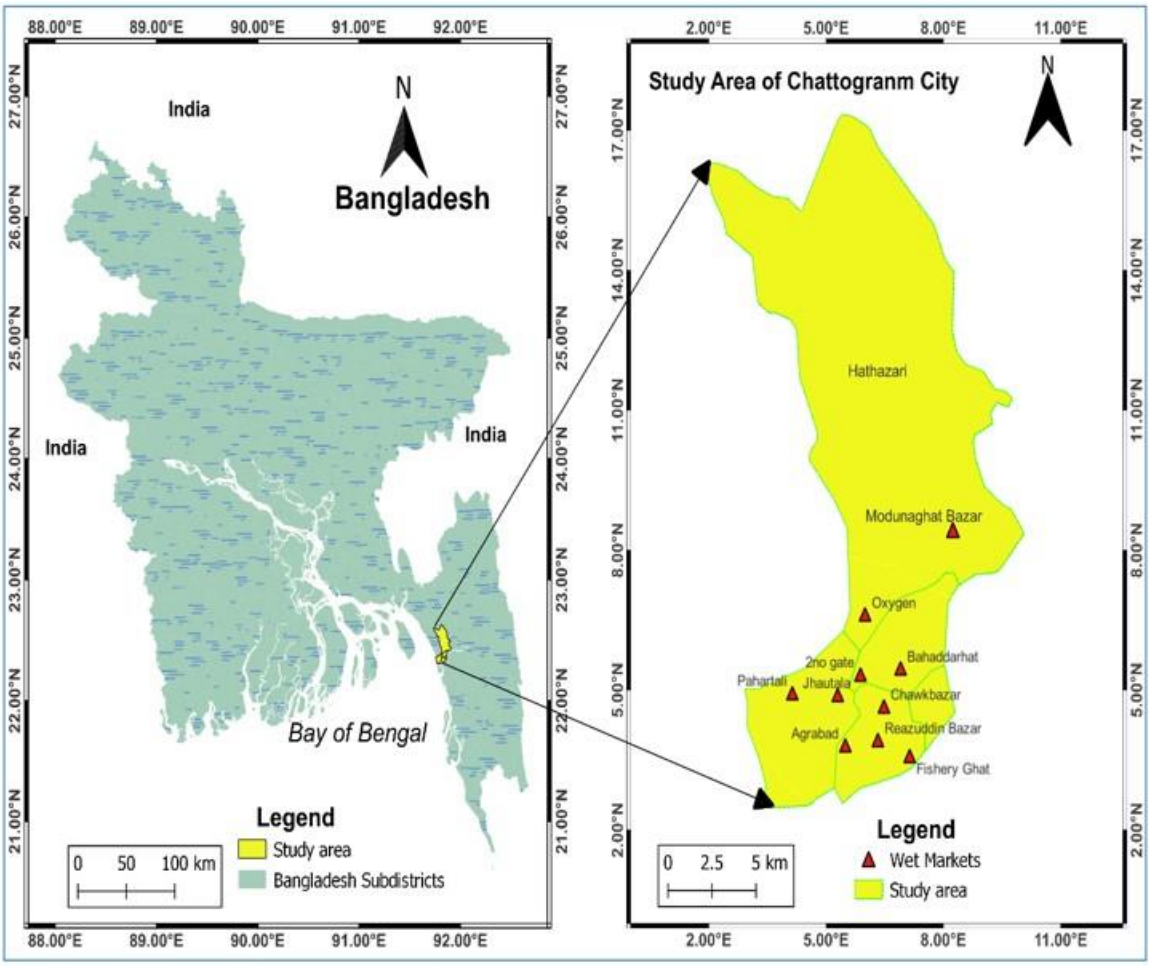


Figure-3.1. Map of the sampling sites. A map of Bangladesh highlighting the study area with yellow color (in left side) and the red triangles of study area (in right side) indicate the GPS coordinates of the fish markets that have been designated as sampling points in Chattogram City.

Fishery Ghat is one of the largest fish landing stations and wholesale fish markets in Bangladesh (Siddiqua et al., 2022). Fishery Ghat, Modhunaghat Bazar and Pahartali Bazar served both fish supply and wholesale purposes in Chattogram district and elsewhere. Along with the wholesale fish trade, Reazuddin Bazar is one of the largest retail markets in

the Chattogram area. These are also common features of other markets in this study namely Bahaddarhat Bazar. The retail markets assessed in this study were Agrabad Bazar, Jhautala Bazar, Oxygen Bazar, Chawkbazar, 2no gate Bazar.

3.3 Description of this research work

3.3.1 Sample collection and transportation

Field sampling was done over a 6-month period from March to August 2022. The study was conducted by analyzing a total of 450 fish samples (45 from each market) purchased from randomly selected vendors. The samples included in this study were the following eight different categories: 1. Torrent Catfish (*Amblyceps mangois*) (n=60,6 from each market & different vendors), 2. Koi (*Anabas testudineus*) (n=60,6 from each market & different vendors), 3. Loitta (*Harpadon nehereus*) (n=60,6 from each market & different vendors), 4. Pangus (*Pangasius pangasius*) (n=70,7 from each market & different vendors), 5.Poa (*Otolithoides pama*) (n=50,5 from each market & different vendors), 6. Pabda (*callichrus pabda*) (n=50,5 from each market & different vendors), 7.Prawn *(Penaeus monodon & Penaeus indicus)*(n=50,5 from each market& same or different vendors), 8. Tilapia (*Oreochromismossambicus &Oreochromis niloticus*) (n=50,5 from each market & different vendors). Fish samples were identified based on morphometric Features (Shafi & Quddus, 1982). A sample was defined as the unit that was purchased and may have varied by purchase. Such as, a quarter kilogram of shrimp was purchased. All samples were fresh, frozen or thawed. Each specimen was collected aseptically into a separate Ziplock bag containing peptone water to wash the surface of their bodies and labeled with information (sample ID, sample name, location of fish market (ICMSF, 1978). Afterward, placed in a cool box, the samples are immediately transported to the Research Lab. at the Department of Physiology, Biochemistry and Pharmacology (DPBP), CVASU where samples were analyzed immediately within 2 hours maintaining microbial integrity.

3.3.2 Isolation, Molecular identification and preservation of isolates

3.3.2.1 Sample Processing, Enrichment and Isolation

All the samples were washed with normal saline **(0.90% w/v of NaCl),** to reduce microbial cross-contamination of the internal organs, following which a sterile surgical blade was used to open the carcass and to make a longitudinal incision along the gut (Moremi et al., 2016b; Sivaraman et al., 2021). The gut portions with muscle tissue of the samples were processed into small pieces by using sterile scissors. In case of the shrimp sample, the whole

mass was taken and cut into small pieces including exoskeleton, and finfish flesh (Dutta, 2016). After processing, Samples were transferred to each labeled sterile test tube containing buffer peptone water (BPW) (HIMEDIA, p^H:7.2±0.2, Mumbai, India) and incubated at 37°C overnight for primary enrichment.

Following enrichment, the samples were streaked on MacConkey agar medium (HIMEDIA, p^H: 7.1±0.2, Mumbai, India) and incubated at 37°C for 24 hours (Barbosa et al., 2014). Typical Bright pink-colored large dry colonies yielded on a MacConkey agar plate indicative of lactose-fermenting characteristics of *E. coli* were picked. These colonies were further streaked on a selective media, EMB (Eosin-Methylene Blue) agar (OXOID, p^H: 7.1±0.2) or Violet Red Bile (VRB) agar (HIMEDIA, p^H: 7.4±0.2) and incubated at 37°C for 24-48 hours. Colonies raised with moist green metallic sheen morphology yielded on EMB agar medium (Odumosu et al., 2021; Sivaraman et al., 2020) or characteristic pinkish-red or deep-red color colony with lactose fermentation and surrounding red precipitations of bile acids (colony appears as bluish fluorescence under UV-ray) on VRB agar medium(Mackie et al., 1996), suggestive of *E. coli*. Thereafter, the isolates were sub-cultured on blood agar (HIMEDIA, p^H: 7.3±0.2) plates and incubated at 37°C for 24 hours to check and virtually pure culture growth was obtained (Alshammari et al., 2019). After completion of the incubation period, colonies from blood agar were used for antibiogram assay and DNA extraction for polymerase chain reaction (PCR).

3.3.2.2 Molecular identification of *E. coli*:

All suspected phenotypically positive isolates on blood agar were subjected to molecular identification for the final confirmation through multiplex PCR assay with gene-specific primers for the *uid*A gene (which codes for the β-D-glucuronidase enzyme) and flanking region of the *usp*A gene. The detailed procedure are represented in the **Table-3.1, Table-3.2** & **Table-3.3.**

Table-3.1. Oligonucleotide primer sequences used for *E. coli* detection and confirmation

Primer	Primer Sequence (5′-3′)	Annealing	Fragment size (bp)	Reference
uspA Up	CCGATACGCTGCCAATCAGT	55.2°C	884	(Godambe et al., 2017)
uspA Down	ACGCAGACCGTAGGCCAGAT			
uidA Up	TATGGAATTTCGCCGATTTT		164	
uidA Down	TGTTTGCCTCCCTGCTGCGG			

*PCR reactions were performed in a final volume of 15 µl, and all primers were used at a concentration of 10 pmol/µl.

Table-3.2. Reagents used in PCR assay for molecular detection

Serial No	Name of the contents	Amount
1	OneTaq Quick-load 2X MM w/Std Buffer (Biolabs Inc., New England)	7.5µl
2	*usp*A Up	0.5µl
3	*usp*A Down	0.5µl
4	*uid*A Up	0.5µl
5	*uid*A Down	0.5µl
6	DNA template	1µl
7	Nuclease free water	4.5 µl
Total Volume		**15 µl**

Table-3.3. Thermocycling conditions of PCR for molecular detection

Serial No	Steps	Temperature and time	Reference
1	Initial denaturation	94°C for 5 minutes	(Godambe et al., 2017)
2	Final denaturation (35 cycles)	94°C for 10 seconds	
3	Annealing	55.2°C for 10 seconds	
4	Initial extension	72°C for 1 minute	
5	Final extension	72°C for 10 minutes	
6	Final holding	4°C	

*Thermocycler used for PCR: 2720 Thermal Cycler (**Brand:** Applied Biosystems™ 4359659) under DPBP, CVASU.

3.3.3 Preservation of the *E. coli* isolates

When *E. coli* pure culture isolates have fully developed on the blood agar, scrape it off with a loop and re-culture in brain heart infusion (BHI) broth. This was followed by overnight incubation at 37°C. The next day, 15% (v/v) glycerin stock was prepared as cryoprotectants (autoclaved and cooled). Then, 700 µl BHI broth culture (log-phase of bacteria) of each isolate was added to 300 µl of glycerin stock solution in a sterilized and properly leveled cryovial, followed by vortexing to mix the isolates evenly with the glycerol. Finally, Stored at -20 °C (Acharya, 2022).

3.3.4 Antibiogram assay

Antimicrobial sensitivity, multi-drug resistance (MDR) *E. coli* index and their interpretation for all resistant isolates were determined with the standard Kirby-Bauer disk diffusion method (Bauer, 1966), on Mueller-Hinton agar plates (HIMEDIA, p^H: 7.4±0.1), according to the guidelines of Clinical and Laboratory Standards Institute (Humphries et al., 2021), using twenty anti-microbial drugs of public health significance belonging to eleven different antimicrobial classes **(Table-3.4)**. These antibiotics are often recommended through the Center for Disease Control and Prevention (CDC) to treat infections mediated by Enterobacteriaceae. Details about used antimicrobials (anti-microbials name, respective class, abbreviation, disc/wafer potencies, zone of inhibition& manufacturers) are enlisted in table.

Table-3.4. List of antimicrobials used against resistant isolates along with their disc concentration and diffusion ZOI (Zone of Inhibition) (Humphries et al., 2018)

Group of antimicrobial agents	Antimicrobials	Abbr.	Disc Content	Zone of Inhibition (diameter in mm)			Manufacturers
				Escherichia coli			
				S	I	R	
Penicillin derivatives/ *β-lactams*	Amoxicillin	AML	10µg	≥18	14-17	≤13	Oxoid Limited, UK
	Ampicillin	AMP	10µg	≥17	14-16	≤13	Oxoid Limited, UK
1st gen cephalosporins	Cefalexin	CL	30µg	≥15	-	≤14	Oxoid Limited, UK
	Cephalothin	KF	30µg	≥18	15-17	≤14	Mast Group Ltd., UK
2nd gen cephalosporins	Cefoxitin	FOX	30µg	≥15	13-14	≤12	Mast Group Ltd., UK
3rd gen cephalosporins (*ESBLs*)	Cefotaxime	CTX	30µg	≥26	23-25	≤22	Mast Group Ltd., UK
	Ceftazidime	CAZ	30µg	≥21	18-20	≤17	Mast Group Ltd., UK
Third-generation cephalosporins +inhibitor (*(ESBLs*+ inhibitor)	Cefotaxime+ Clavulanic acid	CTX/CV	30/10µg	-	-	-	Mast Group Ltd., UK
	Ceftazidime+ Clavulanic acid	CAZ/CV	30/10µg	-	-	-	Mast Group Ltd., UK
Tetracyclines	Doxycycline	DO	30µg	≥15	11-13	≤10	Oxoid Limited, UK
	Tetracycline	TE	30µg	≥15	12-14	≤11	Oxoid Limited, UK
Sulfonamides	Trimethoprim/ Sulfamethoxazole	SXT	25µg	≥16	11-15	≤10	Oxoid Limited, UK
Aminoglycosides	Gentamycin	GM	1µg	≥15	13-14	≤12	Mast Group

								Ltd., UK
		Neomycin	NE	10µg	17	13-16	≤12	Mast Group Ltd., UK
Quinolones and fluoroquinolones	2nd Gen	Ciprofloxacin	CIP	5µg	≥21	16-20	≤15	Oxoid Limited, UK
		Enrofloxacin	ENF	5µg	≥21	17-20	≤16	Mast Group Ltd., UK
		Norfloxacin	NOR	10µg	17	13-16	≤12	Mast Group Ltd., UK
	3rd gen	Levofloxacin	LEV	5µg	≥18	15-17	≤13	Mast Group Ltd., UK
Amphenicols		Florfenicol	FFC	30µg	≥18	13-17	≤12	Mast Group Ltd., UK
Polymyxins		Colistin Sulfate	CT	10µg	≥11	-	≤10	Oxoid Limited, UK

*gen: generation

3.3.4.1 Antibiotic susceptibility test (AST) method involves the following steps:

i. Sub-culture of test organisms was done on blood agar and incubated at 37° for overnight to obtain a pure growth

ii. Sterile inoculating loop was used taking 4 to 5 pure colonies to dissolve into 2-3 ml sterile saline water and mix evenly with vortex, all strains were diluted in 0.85% saline to obtain turbidity equivalent to McFarland scale 0.5 standard (equivalent to growth of $1\text{-}2\times10^{8}$CFU/ml)

iii. After standardization of the inoculum, within 15 minutes sterile cotton swab was dipped into the inoculum and rotated against the side of the tube with firm pressure to remove excess fluid. The swab was streaked evenly over the entire dry surface of Mueller Hinton agar to seed lawn culture of isolates three times rotating the plate approximately at 60 degrees.

iv. After 15 minutes of streaking discs/wafers containing antibiotics were placed on the agar surface using sterile forceps. When all discs were dispersed, the agar plates were inverted and incubated at 37 ˚C for 18 hours.

v. Finally, after the ending of the incubation period, the size of the zone of inhibition (in mm) around a disc including the diameter of the disc was

measured using a standard meter rule and results were interpreted as "Resistant (R), Intermediate (I), or Susceptible (S)" according to CLSI,2018 (Humphries et al., 2018)

3.3.4.2 Screening for ESBLs and AmpC β-lactamases

The screening was done by disc diffusion technique using 3rd generation cephalosporins (ceftazidime, cefotaxime and ceftriaxone). As per CLSI recommendation, isolates showing resistance (zone ≤ 22 mm for ceftazidime and ≤ 25 mm for ceftriaxone) by disc diffusion method to more than one of these agents were considered potential ESBL producers and further preceded for confirmation (Karadiya et al., 2016). Isolates showing resistance to cefoxitin (inhibition zone < 18 mm) by disc diffusion method were considered potential AmpC producers (Karadiya et al., 2016; Nguyen et al., 2014).

3.3.4.3 Confirmation of ESBLs

For confirmation, all isolate of potential ESBL producers were subjected to the Phenotypic Confirmatory Disc Diffusion Test (PCDDT) as recommended by CLSI combination disc method (CDM), using Ceftazidime (30µg) alone and ceftazidime combined with clavulanic acid (30µg/10µg) as well as cefotaxime (30µg) alone and cefotaxime combined with clavulanic acid (30µg/10µg). Discs were placed independently 30 mm apart center to center on a lawn culture of 0.5 McFarland turbidity of the test isolate on Mueller-Hinton Agar plate and incubated for 18-24 hours at 37^{o}C. A difference in zone of inhibition by ≥5mm of either ceftazidime/clavulanic acid with ceftazidime alone and cefotaxime/clavulanic acid with cefotaxime alone was interpreted as confirmed ESBL producer (Akande et al., 2019; Karadiya et al., 2016; Khan et al., 2021).

3.3.5 Multidrug Resistance (MDR) & Multidrug-Resistance Index (MDRI) determination

Multi-drug resistance (MDR) was defined as acquired nonsusceptibility to at least one agent in three or more antimicrobial categories (Alkofide et al., 2020; Magiorakos et al., 2011). Multiple antibiotic resistance phenotype (MARP)s for all the isolates were assessed For MARI calculation because they exhibited resistance against three or more than antimicrobial classes out of the eleven classes as earlier mentioned. In addition, extensive drug resistance (XDR) was defined as resistance to all but two of the tested antimicrobial

categories; and finally, pan-drug resistance (PDR) was defined as resistance to all of the categories tested (Krumperman, 1983).

3.3.6 Molecular detection of resistant genes

3.3.6.1 Blood agar sub-culture

Isolates preserved in glycerol stocks were picked off from the freezer and thawed at room temperature. The isolates were then plated on blood agar and incubated at 37°C for 24 hours to resuscitate. When the incubation period was over, the blood agar colonies were used for DNA extraction.

3.3.6.2 Extraction of genomic DNA

Several different protocols are available for extracting DNA, starting with the first and bestknown simplified boiling procedure narrated by Marmur in the early 1960s (Marmur, 1961). The genomic DNA extraction was carried out via the boiling method, stated below-

A volume of 100 µl of nuclease-free water was dispensed into sterile screw-capped Eppendorf tubes, into which pure overnight colonies were dispersed. The resulting suspension was vortexed to generate uniform cell suspensions and boiled at 100 ˚C for 10 min. Immediately after boiling, the tubes were kept on ice for 5 minutes, and the cell lysate was extracted after centrifugation at 13,000 g for 4 min via the Labnet Prism R Refrigerated Microcentrifuge with Rotor (Labnet International, USA). Finally, 50 µL supernatant from each tube transferred into other sterile screw-capped Eppendorf tubes and stored at -20°C until use. This supernatant contained the DNA utilized in polymerase chain reaction (PCR) assays (Woźniakowski et al., 2021).

3.3.6.3 PCR Detection of resistant genes

On the basis of AST-SIR phenotypical bla_{TEM}, bla_{SHV}, $P_{AmpC.}$All ESBLs resistant isolates were tested for detecting $bla_{\text{CTX.}}$ Resistant isolates against ESBLs with inhibitor are tested for $bla_{OXA\text{-}1\ like}$Group genes &*bla*OXA-2 like Group genes, *blaCMY-1 like* Group genes &*blaCMY-2 like* Group genes, *blaACC-1* genes. In case of, tetracyclines-resistant isolates *tetA, tetB, tetC, tetD* genes were tested and sulfonamides or folate synthesis pathway inhibitors resistant isolates were tested for *Sul-I* and *Sul-II* genes. A combination of uniplex and multiplex PCRs were employed for determining MDR genes considering their amplification conditions (Akande et al., 2019; Karadiya et al., 2016; Nguyen do et al., 2016; Khan et al., 2021).

- Uniplex PCR was performed for: a) Sulfonamides resistant genes determinants (*Sul-I, Sul-II*) &ESBLs resistant gene determinants (bla_{ACC-1}, bla_{CTX}).
- Duplex PCRs for b-lactamase and ESBL resistant gene determinants (bla_{OXA-1} like group genes & bla_{OXA-2} like group genes, bla_{CMY-1} like group genes & bla_{CMY-2} like group genes)
- Three multiplex PCRs for b-lactamase and ESBLsresistant determinants: bla_{TEM}, bla_{SHV}, $P_{AmpC.}$
- Four multiplex PCRs for Tetracycline resistant gene determinants: *tet-A, tet-B, tet-C, tet-D.*

The primers used were adapted from previously published articles. The details of the genes tested, primer sequences, expected amplicon size and amplification conditions and reagent used for PCR assays are listed in the Table-3.5, Table-3.6&Table-3.7.

Table-3.5. Primer sequences & amplicon size of resistant genes of interest

Antibiotic Resistance	Gene	Primer Name	Primer sequence (5´- 3´)	Amplicon Size(bp)	Reference
β-lactamases	$bla_{TE\ M}$	bla_{TEM}-F	GCGGAACCCCTATTTG	964	(Hasman et al., 2005)
		bla_{TEM}- R	TCTAAAGTATATATGAGTAAACT TGGTCTGAC		
	bla_{SHV}	bla_{SHV} -F	TTCGCCTGTGTATTATCTCCCTG	854	(Hasman et al., 2005)
		bla_{SHV} - R	TTAGCGTTGCCAGTGYTCG		
	P_{ampC}	P_{ampC} -F	GTGAATACAGAGCCAGACGC	343	(Hasman et al., 2005)
		P_{ampC} - R	GTTGTTTCCGGGTGATGC		
	bla_{OXA-1} like group	bla_{OXA-1} like group -F	ATGAAAAACACAATACATATCAACTT CGC	820	(Hasman et al., 2005)
		bla_{OXA-1} like group -R	GTGTGTTTAGAATGGTGATCGCATT		
	bla_{OXA-2} like group	bla_{OXA-2} like group -F	ACGATAGTTGTGGCAGACGAAC	602	(Hasman et al., 2005)
		bla_{OXA-2} like group-R	ATYCTGTTTGGCGTATCRATATTC		
ESBLs	bla_{CTX}	bla_{CTX} -F	ATGTGCAGYACCAGTAARGTKATGGC	593	(Hasman et al., 2005)
		bla_{CTX} -R	TGGGTRAARTARGTSACCAGAAYCAG CGG		
	bla_{CMY-1} like group	bla_{CMY-1} like group -F	GTGGTGGATGCCAGCATCC	915	(Hasman et al., 2005)
		bla_{CMY-1}	GGTCGAGCCGGTCTTGTTGAA		

		like group -R			
	bla$_{CMY-2}$ *like group*	*bla*$_{CMY-2}$ *like group* -F	GCACTTAGCCACCTATACGGCAG	758	(Hasman et al., 2005)
		bla$_{CMY-2}$ *like group* -R	GCTTTTCAAGAATGCGCCAGG		
	bla$_{ACC-1}$	*bla* $_{ACC-1}$-F	ATYCTGTTTGGCGTATCRATATTC	818	(Hasman et al., 2005)
		bla$_{ACC-1}$-R	AGCCTCAGCAGCCGGTTAC		
Tetracyclines	*tet-A*	*tet-A* -F	CGCCTTTCCTTTGGGTTCTCTATATC	182	(Koo & Woo, 2011)
		tet-A -R	CAGCCCACCGAGCACAGG		
	tet-B	*tet-B*-F	GCCAGTCTTGCCAACGTTAT	975	(Koo & Woo, 2011)
		tet-B -R	ATAACACCGGTTGCATTGGT		
	tet-C	*tet-C* -F	TTCAACCCAGTCAGCTCCTT	560	(Koo & Woo, 2011)
		tet-C -R	GGGAGGCAGACAAGGTATAGG		
	tet-D	*tet-D*-F	GAGCGTACCGCCTGGTTC	780	(Koo & Woo, 2011)
		tet-D -R	TCTGATCAGCAGACAGATTGC		
Sulfonamides	*sul-1*	*sul-1*-F	CGG CGT GGG CTA CCT GAA CG	779	(Lanz et al., 2003)
		sul-1 -R	GCC GAT CGC GTG AAG TTC CG		
	sul-2	*sul-2*-F	CCTGTTTCGTCCGACACAGA	721	(Lanz et al., 2003)
		sul-2-R	GAAGCGCAGCCGCAATTCAT		

*All primers were procured from New England Biolabs, UK

Table-3.6 Amplification conditions of PCR assays for resistant genes of interest

Gene name	***bla*$_{TEM}$, *bla*$_{SHV}$, *P*$_{ampC}$**	***bla*$_{OXA-1}$ like group, *bla*$_{OXA-2}$ like group**	***bla*$_{CTX}$**	***bla*$_{CMY-1}$ like group, *bla*$_{CMY-2}$ like group**	***bla*$_{ACC-1}$**	***tet-A, tet-B, tet-C, tet-D***	***Sul-1***	***Sul-2***
Initial denature-tion	94°C for 3mins	94°C for 3mins	94°C for 3mins	94°C for 3mins	94°C for 3mins	94°C for 5mins	95°C for5 mins	94°C for 4 mins
Cyclic denature-tion	94°C for 1min	94°C for 1min	94°C for 1min	94°C for 1min	94°C for 1min	94°C for 30seconds	95°C for 1min	94°C for 1min
Cyclic annealing	50°C for 1min	62°C for 1min	60°C For 1 min	58°C for 1 min	53°C for 1 min	55°C for 30seconds	68°C for 1 min	66°C for 1min
Cyclic extension	72°C for 1min	72°C for 1min	72°C for 1min	72°C for 1min	72°C for 1min	72°C for 30seconds	72°C for 1min	72°C for 1min
Final extension	72°C for 10 mins	72°C for 10 mins	72°C for 7 mins	72°C for 10 mins	72°C for 10 mins	72°C for 5mins	72°C for 10 mins	72°C for 7 mins
Holding	4°C	4°C	4°C	4°C	4°C	4°C	4°C	4°C
Cycle number	25	25	25	25	25	35	35	35
References	(Hasman et al., 2005)	(Hasman et al., 2005)	(Hasman et al., 2005)	(Hasman et al., 2005)	(Hasman et al., 2005)	(Koo & Woo, 2011)	(Lanz et al., 2003)	(Lanz et al., 2003)

Table-3.7. Reagents used for the amplification of resistance genes

Serial No.	Name	Manufacturer
1	OneTaq® 2X Master Mix with Standard Buffer	New England Biolabs, Inc. (https://international.neb.com)
2	Molecular marker (Thermo Scientific Gene Ruler 1 kb DNA Ladder)	Thermo Fisher Scientific (https://www.thermofisher.com)
3	Ethidium bromide solution (0.625 mg/mL) (1%)	Thermo Fisher Scientific (https://www.thermofisher.com)
4	Electrophoresis buffer 50x TAE	Thermo Fisher Scientific (https://www.thermofisher.com)
5	Agarose powder	Thermo Fisher Scientific (https://www.thermofisher.com)
6	Nuclease free water	Thermo Fisher Scientific (https://www.thermofisher.com)

PCR tests were conducted with a final volume of 15 µl **(Table-3.8.)**

Table-3.8. Volume of PCR assay (uniplex or multiplex) for each test

Serial no	Name of the contents	Volume
1	Thermo Scientific Dream Taq PCR Master Mix (2x) ready to use	7.5µl
2	Forward primer (each gene 0.5µl*n)	(0.5n) µl
3	Reverse primer (each gene 0.5µl*n)	(0.5n) µl
4	DNA template	1µl
5	Nuclease free water	[15-(8.5+n)] µl
	Total volume	15µl

*Here, n (Number of resistant genes) = 1- 4 (according to the selected assay in this study).

3.3.7 Visualization of PCR products by Agar Gel Electrophoresis:

Agarose gel electrophoresis is commonly used to assess the success of PCR reactions. The DNA fragments are separated lengthwise as they pass through the agarose matrix.1.5% (W/V) Agarose gel was used to visualize the PCR product. Briefly, the procedure is given below (Mullis, 1990):

- 0.75 g of agarose powder and 50 ml of 1X TAE buffer were mixed thoroughly in a conical flask and heated in a microwave oven until the agarose was dissolved.
- The agarose mixture was then cooled to 50°C in a water bath and a drop of ethidium bromide was added to the mixture after staining.
- Assemble the gel casting tray by sealing the ends of the gel chamber with tape and inserting an appropriate number of combs into the gel tray.
- The agarose-TAE buffer mixture was poured onto the gel plate and held for 20 minutes at room temperature to solidify, then the combs were removed and the gel was transferred to an electrophoresis vessel (horizontal gel electrophoresis buffer tank, Cleaver Scientific, UK). filled with 1X TAE buffer and held until the gel was completely submerged.
- An amount of 5 µl PCR product of a sample was loaded into an agarose gel hole.
- 3-5 µl of 1 kb DNA marker (O'Gene Ruler 1 kb plus) was loaded to compare the size of the amplicons then electrophoresis was run at 100-110 volts and 80-100 mA for 30-40 minutes via electrophoresis power supply (Cleaver Scientific, UK).
- Finally, the gel was visualized under a UV trans-illuminator for image acquisition and analysis.

3.4 Statistical analysis

All data were inserted as well as parameterizing, coding and integrity were checked into a spreadsheet program (Microsoft Office Excel Professional 2021) for validation and consistency according to fish and market variables. The dependent variables of the study were coded as dichotomous (positive or negative) and the sample size of each species was considered as a categorical variable. Descriptive statistics were performed to determine the prevalence and antimicrobial resistance profiles of the samples (expressed as percentage).

The multi-drug resistance index (MDRI) of *E. coli* isolates was calculated according to the formula described by Krumperman (Krumperman, 1983) The MDRI of a single *E. coli* isolate is defines as 'a/b', where; 'a' represents the number of antimicrobial agents to which the isolate was resistant to, and 'b' represents the number of antimicrobial agents to which the isolate was exposed to.

The MAR index of the sampling point (from which several isolates were taken) is defined as a/(bxc), where 'a' is an aggregate antibiotic resistance score of all isolates from the sampling point, 'b' is the number of antibiotics tested, and 'c' is the number of all isolates from the sampling point. When the MARI is higher in value than 0.2, it indicates that antibiotics are being used intensively in that area and implies an environment with a highrisk of AMR's proliferation (Krumperman, 1983; Nyandjou et al., 2019).

All the statistical analysis was performed in using STATA-IC 15.2 program (a "p-value" level of ≤ 0.05 was considered statistically significant during factorial association analysis and hypotheses checking) and the map was created using QGIS 3.28.3 (Firenze).

Chapter-4: Results

4.1 Demographic information of the present study

A snap of the demographic information of this research work, which recounts variable, sample category, sample size of each category, identified total *E. coli* in samples are stated in **Table 4.1**.

Table-4.1. Descriptive demography of the present study

Variable	Category	Number	% (95% CI)
Sample type	Poa	50	11.11% (8.36%-14.39%)
	Pabda	50	11.11% (8.36%-14.39%)
	Tilapia	50	11.11% (8.36%-14.39%)
	Shrimp	50	11.11% (8.36%-14.39%)
	Catfish	60	13.33% (8.36%-14.39%)
	Koi	60	13.33% (8.36%-14.39%)
	Loitta	60	13.33% (8.36%-14.39%)
	Pangus	70	15.56% (8.36%-14.39%)
Market Location	WM -1: Bahaddarhat	45	10% (7.39%-13.15%)
	WM -2: Fishery Ghat	45	10% (7.39%-13.15%)
	WM -3: Agrabad	45	10% (7.39%-13.15%)
	WM-4:Modhunaghat Bazar	45	10% (7.39%-13.15%)
	WM -5: Reazuddin Bazar	45	10% (7.39%-13.15%)
	WM -6: Pahartali	45	10% (7.39%-13.15%)
	WM -7: Jhautala	45	10% (7.39%-13.15%)
	WM -8: Oxygen	45	10% (7.39%-13.15%)
	WM -9: Chawkbazar	45	10% (7.39%-13.15%)
	WM -10: 2 no gate	45	10% (7.39%-13.15%)
E. coli	Positive	188	41.78% (37.18%-46.49%)
	Negative	262	58.22% (53.51%-62.82%)

*WM: Wet Market.

Table -4.1 narrates, Equal (n=45,10%, **95% CI** 7.39%-13.15%) number of fish samples were collected from each wet market and highest sample type was pangus fish (n=70,15.56%, **95% CI** 8.36%-14.39%) . Proportionate prevalence of positive *E. coli* is 41.78% (n=188, **95% CI** 37.18%-46.49%) and negative *E. coli* is 58.22% (n=262,**95%CI** 53.51%-62.82%) respectively.

4.2 *E. coli* isolates in fish

4.2.1 Detection of *E. coli* isolates from fish samples

A total of 450 fish samples from ten prominent wet markets of the Chattogram city area were investigated in this study. Amid all study samples 188 *E. coli* isolates (41.78%; **95% CI** 37.18%-46.49%) were identified, baes on phenotypical confirmation on the characteristic growth on a Blocker petri plate with MacConkey agar medium & EMB (Eosin-Methylene Blue) agar medium in Figure **4.1**. Characteristic growth on Violet Red Bile agar medium (pinkish- red colony with agar lactose fermentation along with bluish fluorescence appearance under UV-ray) in Figures **4.2** and blood agar medium are shown in Figures **4.3** respectively. **Figure 4.4,** represents, the molecular confirmation of the culture positive isolates via PCR assay.

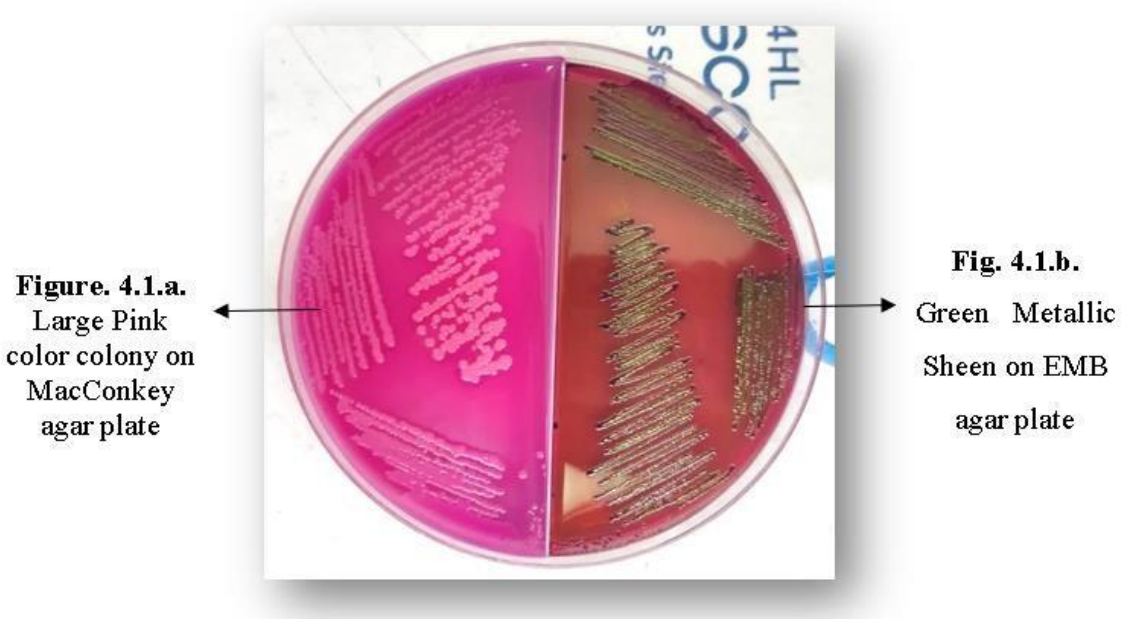


Figure 4.1. Growth of *E. coli* on MacConkey & EMB blocker agar plate

Figure- 4.2. Growth of *E. coli* on a VRB (Violet Red Bile) agar plate with characteristic deep red colony and red precipitation of bile surrounding the colonies

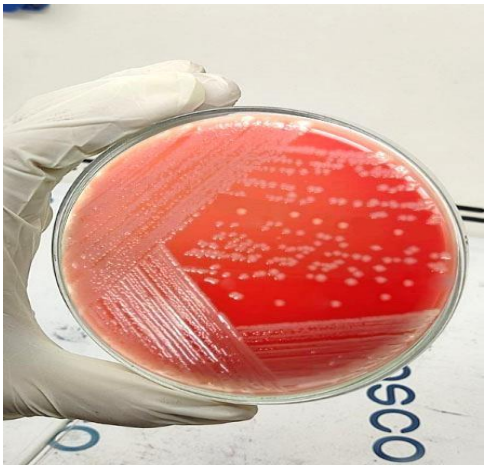


Figure- 4.3. Isolating pure colony of *E. coli* from Blood agar culture

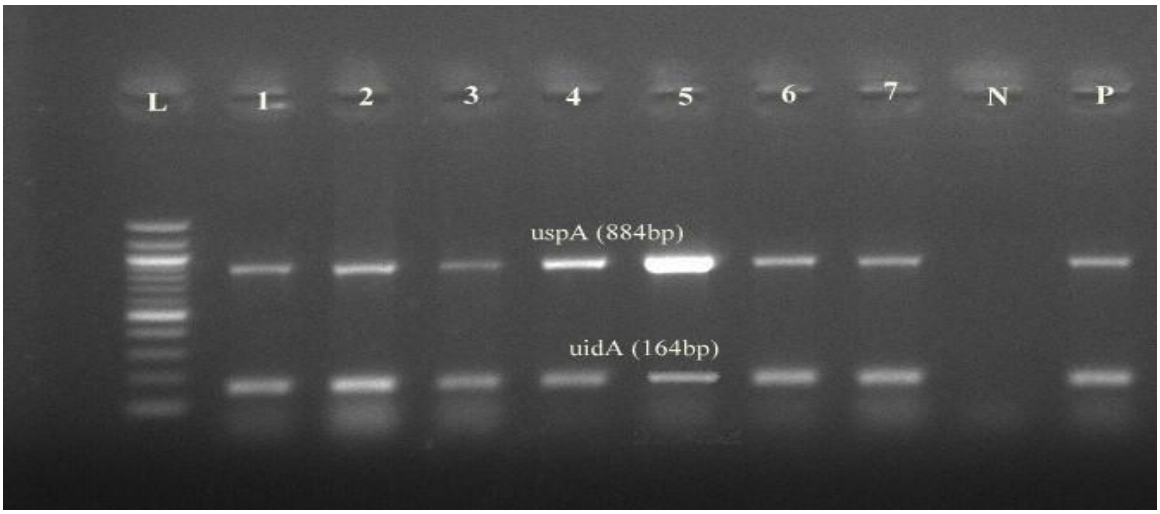


Figure- 4.4. Molecular confirmation isolates using *E. coli* specific molecular markers (uidA and flanking region of uspA). Lane L: DNA ladder; Lane P: Positive control; Lane N: Negative control.

Prevalence (%) of *E. coli* isolates in study sample with associated factors (sample type and wet market) are represented in table 4.2 (model was build based on considering a p-value of ≤0.05 as significant).

Table-4. 2. Univariable association of the presence of *E. coli* in the study samples with the associated factors (Sample type & Wet market).

Variable	Category (N)	Positive, n (%)	95% CI	*p-value* (χ^2 *test)*
Sample Type	Poa (50)	13, (26.00%)	14.63%– 40.34%	0.000
	Pabda (50)	16, (32.00%)	19.52%– 46.70%	
	Tilapia (50)	28, (56.00%)	41.25%– 70.01%	
	Shrimp (50)	33, (66.00%)	51.23%– 78.79%	
	Catfish (60)	31, (51.67%)	47.17%– 75.35%	
	Koi (60)	18, (30.00%)	22.92%– 50.81%	
	Loitta (60)	14, (23.33%)	16.23%– 42.49%	
	Pangus (70)	35, (50.00%)	55.39%– 82.14%	
Wet Market	WM -1: Bahaddarhat (45)	24, (53.33%)	3.79%– 68.34%	0.000
	WM -2: Fishery Ghat (45)	5, (11.11%)	3.71%– 24.05%	
	WM -3: Agrabad (45)	19, (42.22%)	27.66%– 57.85%	
	WM -4: Modhunaghat Bazar (45)	6,(13.33%)	5.05%– 26.79%	
	WM -5: Reazuddin Bazar (45)	18, (40.00%)	25.70%– 55.67%	
	WM -6: Pahartali (45)	27, (60.00%)	44.33%– 74.30%	
	WM -7: Jhautala (45)	25, (55.56%)	40.00%– 70.36%	
	WM -8: Oxygen (45)	17, (37.78%)	23.77%– 53.46%	
	WM -9: Chawkbazar (45)	25, (55.56%)	40.00%– 70.36%	
	WM -10: 2 no gate (45)	22, (48.89%)	33.70%– 64.23%	

*WM: Wet Market.

Table -4.2 represents, highly significant association (*P*= 0.000) among *E. coli* isolates with associated factors (sample type and wet market).

4.2.2 Wet market level

Maximum positive isolates were identified form wet market Pahartali (n=27) with a highest prevalence value of 60.00% (**95% CI** 44.33%– 74.30%),**(Table -4.2).** Elsewhere, least study prevalence of 11.11% (**95% CI** 3.71%– 24.05%) was observed from positive

isolates (n= 5) of Fishery Ghat Bazar, **(Table -4.2).** Besides, equivalent and second highest results of positive isolates (n=25) and prevalence value of 55.56% (**95% CI** 40.00%– 70.36%) is noticed both in wet market Chawkbazar and Jhautala, **(Table -4.2).**

4.2.3 Sample type

Most number of *E. coli* were isolated from pangus (n=35) sample, though foremost prevalence were revealed from shrimp 66.00% (**95% CI** 51.23%– 78.79%), **(Table -4.2).** On the contrary, fewest isolates were from Poa fish samples (n= 13) but the lowest prevalence was raised from loitta fish 23.33% (**95% CI** 16.23%– 42.49%), **(Table -4.2).**

4.3 Antimicrobial sensitivity profiling of *E. coli* isolates

AST of all the possitiveE. coli (188) isolates were performed to revealed there phenotypical antibiogram (SIR) profiling. **Figure-4.5**, depicts turbidity comparison with McFarland Standards, **Figure 4.6**, depicts AmpC *β-lactamases* screening for PCR, **Figure 4.7**, depicts AST (Antimicrobial–Sensitivity Testing) towards different antimicrobials and Figure 4.10 depicts, *ESBLs* screening for PCR test.

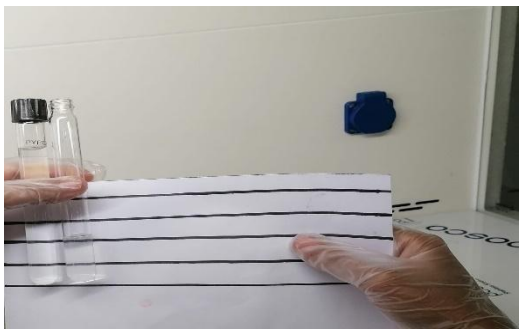


Figure-4.5. Composition of turbidity suspension of *E. coli*inoculum

McFarland Standard

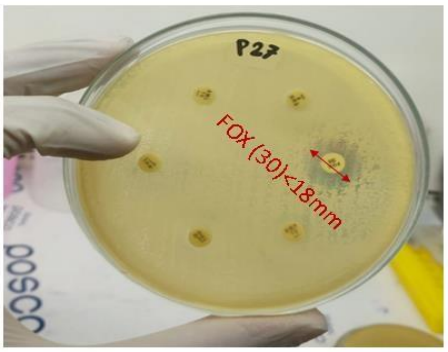


Figure-4.6. AmpC *β-lactamases* screening

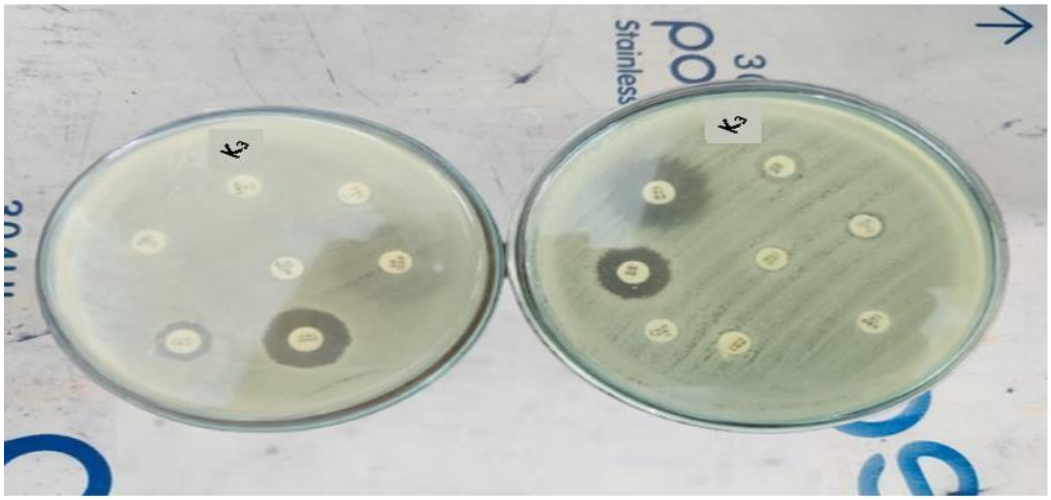
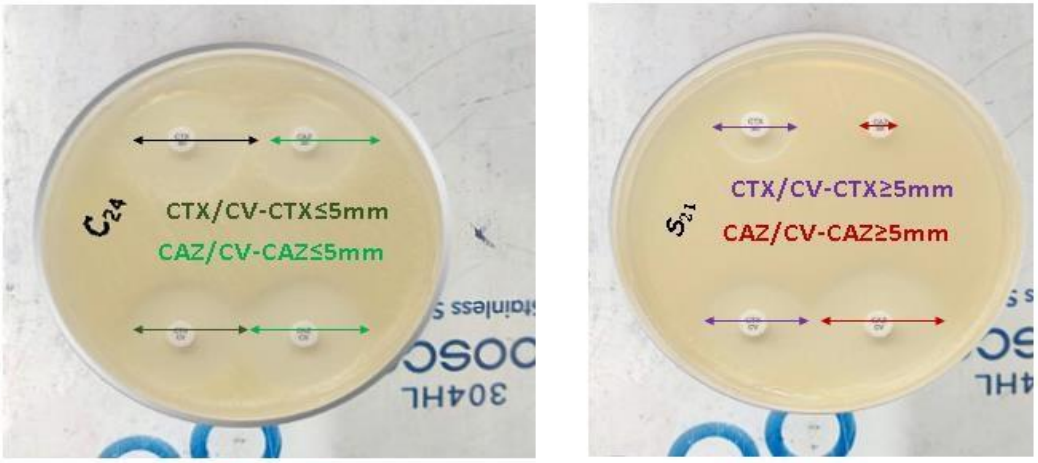


Figure- 4.7. AST (Antimicrobial –Sensitivity Testing)



CTX(30) = Cefotaxime 30mg; CTX/CV= Cefotaxime 30mg+Clavulinic Acid 10 mg
CAZ(30)= Ceftazidime 30mg; CTX/CV= Ceftazidime 30mg+Clavulinic Acid 10 mg

Figure -4.8. *ESBLs* screening

Antimicrobial sensitivityresults of all the isolates are demonstrated in **Table-4.3** (results were interpreted following the guidelines of Clinical and Laboratory Standard Institute, CLSI).

Table-4.3. Antibiotic susceptibility testing result of *E. coli* isolates

Antibiotics (code)	**Sensitive% (n), (95% CI)**	**Intermediate%(n), (95% CI)**	**Resistant%(n), (95% CI)**
Amoxicillin (AML10)	4.26% (8), (1.85% - 8.21%)	15.96% (30), (11.03% - 21.99%)	79.79% (150), (73.33% - 85.28%)
Ampicillin (AMP10)	11.17% (21), (7.05% - 16.57%)	5.85% (11), (2.96% - 10.23%)	82.98% (156), (76.83% - 88.06%)
Cefalexin (CL10)	11.70% (22), (7.48% - 17.18%)	0.00% (0), (0.00% - 1.94%)	88.30% (166), (82.82% - 92.52%)
Cephalothin (KF10)	8.51% (16), (4.94% - 13.45%)	11.17% (21), (7.05% - 16.57%)	80.32% (151), (73.91% - 85.75%)
Cefoxitin (FOX30)	48.94% (92), (41.59% - 56.31%)	14.36% (27), (9.68% - 20.20%)	36.70% (69), (29.81- 44.02%)
Cefotaxime (CTX30)	63.83% (120), (56.52% - 70.69%)	9.57% (18), (5.77% - 14.71%)	26.60% (50), (20.43% - 33.52%)
Ceftazidime (CAZ30)	64.89% (122), (57.61% - 71.70%)	10.11% (19), (6.20% - 15.33%)	25.00% (47), (18.98% - 31.82%)
Cefotaxime+ Clavulanic acid (CTX-CV)	88.83% (167), (83.43% - 92.95%)	0.00% (0), (0.00% - 1.94%)	11.17% (21), (7.05%-16.57%)
Ceftazidime+ Clavulanic acid (CAZ-CV)	89.89% (169), (84.67% - 93.80%)	0.00% (0), (0.00% - 1.94%)	10.11% (19), (6.20% - 15.33%)
Doxycycline (DO30)	29.79% (56), (23.35% - 36.87%)	27.66% (52), (21.40% - 34.64%)	42.55% (80), (35.39% - 49.96%)
Tetracycline (TE30)	26.60% (50), (20.43% - 33.52%)	4.79% (9), (2.21% - 8.89%)	68.62% (129), (61.46% - 75.17%)
Gentamycin (GM 10)	33.51% (63), (26.81% - 40.75%)	8.51% (16), (4.94% - 13.45%)	57.98% (109), (50.58% - 65.13%)
Neomycin (NE30)	19.15% (36), (13.79% - 25.51%)	30.85% (58), (24.33% - 37.98%)	50.00% (94), (42.64% - 57.36%)
Ciprofloxacin (CIP 5)	43.62% (82), (36.41% - 51.02%)	17.55% (33), (12.40% - 23.76%)	38.83% (73), (31.82% - 46.19%)
Norfloxacin (NOR 10)	60.64% (114), (53.27% - 67.67%)	13.83% (26), (9.24% - 19.60%)	25.53% (48), (19.46% - 32.39%)
Enrofloxacin (ENF 5)	21.28% (40), (15.66% - 27.83%)	31.38% (59), (24.83% - 38.54%)	47.34% (89), (40.03% - 54.74%)
Levofloxacin (LEV 5)	74.47% (140), (67.61% - 80.54%)	10.64% (20), (6.62% - 15.95%)	14.89% (24), (8.35% - 18.40%)

Trimethoprim/Sulfamethoxazole (SXT 25)	26.60% (50), (20.43% - 33.52%)	5.85% (11), (2.96% - 10.23%)	67.55% (127), (60.36% - 74.19%)
Colistin Sulfate (CT10)	40.96% (77), (33.86% - 48.35%)	0.00% (0), (0.00% - 1.94%)	59.04% (111), (51.65% - 66.14%)
Florfenicol (FFC 30)	31.91% (60), (25.32% - 39.09%)	16.49% (31), (11.49% - 22.58%)	51.60% (97), (44.21% - 58.93%)

Table -4.3 represents, highest number of *E. coli* isolates were resistant to Cefalexin (n=166, 88.30%, **95% CI** 82.82% - 92.52%) following Ampicillin (n=156, 82.98%, **95% CI** 76.83% - 88.06%) and most sensitive to Ceftazidime+ Clavulanic acid (n=169, 89.89%, **95% CI** 84.67% - 93.80%) and Cefotaxime+ Clavulanic acid (n=167, 88.83%, **95% CI** 83.43% - 92.95%) followed by Levofloxacin (n=140, 74.47%, **95% CI** 67.61% - 80.54%).

Over against, Lowest number of *E. coli* isolates were resistant to Ceftazidime+ Clavulanic acid (n=19, 10.11%, **95% CI** 6.20% - 15.33%) and Cefotaxime+ Clavulanic acid (n=21, 11.17%, **95% CI** 7.05% - 16.57%) following Levofloxacin (n=24, 14.89% ,**95% CI** 67.61% - 80.54%). Whereas, minimum sensitivity was displayed towards Amoxicillin (n=8, 4.26%, **95% CI** 1.85% - 8.21%) subsequently, Cephalothin (n=16, .51%, **95% CI** 4.94% - 13.45%).

4.3.1 Antimicrobial sensitivity profiling of *E. coli* isolates of fish sample type

Figure- 4.9. Heat map representing Antibiotic sensitivity Profile of *E. coli* isolates of fish sample type. The color code of the antimicrobial sensitivity profiles indicates the phenotypes of the isolates to specific antibiotics. Red- Resistance, Yellow- Intermediate resistance, Green- Sensitive

*AML: Amoxicillin, AMP: Ampicillin, CL: Cefalexin, KF: Cephalothin, FOX: Cefoxitin, CTX: Cefotaxime, CTX-CV: Cefotaxime+ Clavulanic acid, CAZ: Ceftazidime, CAZ-CV: Ceftazidime+ Clavulanic acid, DO: Doxycycline, TE: Tetracycline, GM: Gentamycin, NE: Neomycin, CIP: Ciprofloxacin, NOR: Norfloxacin: Enrofloxacin, LEV: Levofloxacin, SXT: Trimethoprim/Sulfamethoxazole, CT: Colistin Sulfate, FFC: Florfenicol.

4.4 Multidrug-resistance of *E. coli* isolates

Table-4.4 and **Table-4.5** (**Table-4.5** has been attached in **Appendix-B**) narrates, multidrug resistant (MDR) pattern of antimicrobial classes (≥ 3 groups of antimicrobials). In **Table-4.5**, an aggregated 131 MDR patters was displayed with highly significant association (P=0.000) among MDR patters of *E. coli* isolates and number of antimicrobial classes showing resistance. Resistance percentage towards 6 classes of antimicrobials was utmost, therein 27 (20.61% **95% CI** 16.04%-28.55%) number of patterns was revealed. Additionally, 9 isolates (9.68% **95% CI** 3.19%-12.64%) was showing resistance towards all 11 antimicrobial classes.

Table-4.4. Multidrug resistance of antimicrobial classes

No. of multidrug resistance antimicrobial classes	Number of observed patterns n, (%)	95% CI
3	1 (0.76%)	0.02%-4.18%
4	12 (9.16%)	4.82%-15.45%
5	23 (17.56%)	11.47%-25.17%
6	27(20.61%)	16.04%-28.55%
7	16(12.21%)	7.15%-19.08%
8	19 (14.50%)	8.96%-21.72%
9	19 (14.50%)	8.96%-21.72%

10	9 (6.87%)	3.19%-12.64%
11	9 (6.87%)	3.19%-12.64%
Total number of MDR Patterns observed	**131 (100%)**	**97.22%-100%**

Just the opposite, nominal number of MDR patters was displayed only towards 3 antimicrobial classes, wherein only 1 (0.76% **95% CI** 0.02%-4.18%) pattern was revealed.

Table-4.6 states, the overall multidrug resistance of *E. coli* isolates. Among all, a total of 174(92.55%, **95% CI** 87.82% – 99.87%) isolates wereexhibited multidrug resistance. However, 14 (7.45%, **95% CI** 4.13% – 12.19%) isolates didn't exhibit any MDR pattern.

Table-4.6. Percentage of multidrug resistance to *E. coli* isolates

No of multidrug-resistance antimicrobial	**n, (%)**	**95% CI**
MDR_Yes	174, (92.55%)	87.82% – 99.87%
MDR_No	14, (7.45%)	4.13% – 12.19%
3	7, (4.02%)	1.63% – 8.11%
4	5, (2.87%)	0.94% – 6.58%
5	8, (4.60%)	2.01% – 8.86%
6	13, (7.47%)	4.04% – 12.44%
7	23, (13.22%)	8.57% – 19.17%
8	22, (12.64%)	8.10% – 18.51%
9	13, (7.47%)	4.04% – 12.44%

10	15, (8.62%)	4.91% – 13.82%
11	15, (8.62%)	4.91% – 13.82%
12	5, (2.87%)	0.94% – 6.58%
13	8, (4.60%)	2.01% – 8.86%
14	11, (6.32%)	3.20% – 11.03%
15	8, (4.60%)	2.01% – 8.86%
16	6, (3.45%)	1.28% – 7.35%
17	4, (2.30%)	0.63% – 5.78%
18	9, (5.17%)	2.39% – 9.59%
19	2, (1.15%)	0.14% – 4.09%
20	1, (0.57%)	0.01% – 3.16%

4.4.1 Multiple Antibiotic Resistance Phenotypes & Multiple Antibiotic Resistance Index

The discrete Multiple Antibiotic Resistance Phenotypes (MARPs) and the Multiple Antibiotic Resistance Index (MARIs) Displayed by *E. coli* isolates are arrayed in **table 4.7.** The generated multiple antibiotic resistance indices ranged from 0.15 to 1.00, amongst all the highest value noticed in one MDR *E. coli* isolates from catfish which displayed resistance against all the antibiotics assayed **(MAR index is calculated as the ratio between the number of antibiotics that an isolate is resistant to and the total number of antibiotics the organism is exposed to)**. Isolates having a MAR index ≥ 0.2 originated from a high-risk source of contamination where several antibiotics are used desperately. In this study, 89.36%, (n=168, **95 % CI** 80.05%93.38%) of *E. coli* isolates having MAR index of 0.2 and above, is worrisome.

Table-4.7. The multiple antibiotic resistance phenotypes and multiple antibiotic resistance index of *E. coli* isolates with associated factors**.**

No of antibiotics showing resistance	multiple antibiotic resistance phenotypes (MARPs)	No of phenotype observed n,(%),95%CI	Sample Type								MAR index	*P- Value*
			Catfish	KOI	Loitta	Pangus	POA	PABDA	PRAWN	Tilapia		
3	DO, CT, FFC	1(0.57%), (0.01%-3.16%)	-	-	-	-	1	-	-	-	0.15	0.260
	DO, TE, NE	1(0.57%), (0.01%-3.16%)	-	-	-	1	-	-	-	-		
	AML, CL, KF	2(1.15%), (0.14%-4.09%)	-	-	-	-	-	-	2	-		
	AML, KF, TE	1(0.57%), (0.01%-3.16%)	-	-	-	-	-	-	1	-		
	AML, CL,KF	1(0.57%), (0.01%-3.16%)	-	-	-	-	-	-	-	1		
	AML, CL,DO	1(0.57%), (0.01%-3.16%)	-	1	-	-	-	-	-	-		
4	AML,CL,TE,CT	1(0.57%), (0.01%-3.16%)	-	-	-	-	-	-	-	1	0.20	

	AML,AMP,CL,KF	1(0.57%), (0.01%-3.16%)	-	-	-	-	-	-	-	1		
	AML,KF,TE,GM	1(0.57%), (0.01%-3.16%)	-	-	-	-	-	-	-	1		
	AML,AMP,SXT,CT	1(0.57%), (0.01%-3.16%)	-	-	-	-	-	-	-	1		
	AML,AMP,TE,SXT	1(0.57%), (0.01%-3.16%)	-	-	-	-	-	-	-	1		
5	CL, KF, TE,NE,CT	2(1.15%), (0.14%-4.09%)	-	2	-	-	-	-	-	-	0.25	
	CL, KF,TE,NE,SXT	1(0.57%), (0.01%-3.16%)	-	-	-	1	-	-	-	-		
	CL, KF,FOX,CIP,CT	1(0.57%), (0.01%-3.16%)	-	-	-	-	-	-	-	1		
	AML,KF,TE,GM,NE,	1(0.57%), (0.01%-3.16%)	-	-	-	-	-	-	-	1		
	AML,AMP,CL,KF,ENF	2(1.15%), (0.14%-4.09%)	-	2	-	-	-	-	-	-		
	AML,FOX,TE,CIP,SXT	1(0.57%), (0.01%-3.16%)	-	-	1	-	-	-	-	-		
6	CL,KF,TE,NE,SXT,FFC	1(0.57%), (0.01%-3.16%)	-	-	-	1	-	-	-	-	0.3	
	AMP,CL,KF,TE,NE,CT	1(0.57%), (0.01%-3.16%)	-	-	1	-	-	-	-	-		
	AML, AMP,CL,KF,NE,ENF	1(0.57%), (0.01%-3.16%)	-	-	-	1	-	-	-	-		

	AML, AMP,CL,KF,DO, SXT	1(0.57%), (0.01%-3.16%)	-	-	-	1	-	-	-	-		
	AML, AMP,CL,KF,TE,SXT	2(1.15%), (0.14%-4.09%)	-	-	2	-	-	-	-	-		
	AMP,KF,FOX,GM,NE,CIP	1(0.57%), (0.01%-3.16%)	-	-	-	-	-	1	-	-		
	AML,AMP,DO,TE,GM,CT	1(0.57%), (0.01%-3.16%)	-	-	-	-	-	1	-	-		
	AML,AMP,KF,GM,SXT,CT	1(0.57%), (0.01%-3.16%)	-	-	-	-	-	-	-	1		
	AML,AMP,CL,KF,FOX,CIP	1(0.57%), (0.01%-3.16%)	-	-	-	-	-	-	-	1		
	AML,AMP,TE,GM,SXT,CT	2(1.15%), (0.14%-4.09%)	-	-	-	-	-	-	-	2		
	AML, AMP,DO,TE,SXT,FFC	1(0.57%), (0.01%-3.16%)	-	-	-	-	1	-	-	-		
7	CL,KF,DO,TE,ENF,SXT,CT	1(0.57%), (0.01%-3.16%)	-	-	-	-	1	-	-	-	0.35	
	FOX,TE,GM,NE,CIP,SXT,CT	1(0.57%), (0.01%-3.16%)	-	-	-	-	-	-	-	1		
	KF,TE,GM,NE,SXT,CT,FFC	1(0.57%), (0.01%-3.16%)	-	-	-	-	-	-	-	1		
	KF,FOX,TE,CIP,ENF,SXT,CT	1(0.57%), (0.01%-3.16%)	-	-	-	-	-	-	-	1		
	AML,CL,KF,FOX,NE,CIP,CT	1(0.57%), (0.01%-3.16%)	-	-	-	-	-	-	-	1		

	AMP,CL,KF,DO,TE,NE,SXT	1(0.57%), (0.01%-3.16%)	-	1	-	-	-	-	-	-		
	AML,AMP,CL,KF,TE,ENF,SXT	2(1.15%), (0.14%-4.09%)	-	-	-	-	-	-	2	-		
	AML,AMP,CL,KF,ENF,SXT,FFC	1(0.57%), (0.01%-3.16%)	-	-	-	-	-	-	1	-		
	AML, AMP,CL,KF,SXT, CT, FFC	1(0.57%), (0.01%-3.16%)	-	-	-	-	-	-	1	-		
	AML, AMP,CL,KF,TE,NE,ENF	1(0.57%), (0.01%-3.16%)	-	-	1	-	-	-	-	-		
	AML,CL,CTX, CAZ,TE,NOR,CT	1(0.57%), (0.01%-3.16%)	-	-	-	-	1	-	-	-		
	AML, AMP,KF,DO,TE,SXT,CT	1(0.57%), (0.01%-3.16%)	-	-	-	1	-	-	-	-		
	AML, AMP,CL,KF,DO,TE,SXT	1(0.57%), (0.01%-3.16%)	-	-	-	1	-	-	-	-		
	AML, AMP,DO,TE,NE,SXT,FFC	1(0.57%), (0.01%-3.16%)	-	-	-	1	-	-	-	-		
	AML, AMP,DO,NE,SXT,CT,FFC	1(0.57%), (0.01%-3.16%)	-	-	-	1	-	-	-	-		
	AML, AMP,CL,KF,GM,SXT,FFC	1(0.57%), (0.01%-3.16%)	-	-	-	1	-	-	-	-		
	AML, AMP,GM,NE,SXT,CT,FFC	1(0.57%), (0.01%-3.16%)	-	-	-	1	-	-	-	-		
	AML, AMP,CL,KF,DO,SXT,FFC	1(0.57%), (0.01%-3.16%)	-	-	-	1	-	-	-	-		

	AML, AMP,CL,KF,DO,SXT,FFC	1(0.57%), (0.01%-3.16%)	-	1	-	-	-	-	-	-		
	AML, AMP,DO,TE,ENF,SXT,FFC	1(0.57%), (0.01%-3.16%)	-	1	-	-	-	-	-	-		
	AML, AMP,CL,DO,TE,SXT, CT	2(1.15%), (0.14%-4.09%)	2	-	-	-	-	-	-	-		
8	KF,DO,TE,GM,CT,SXT,CT,FFC	1(0.57%), (0.01%-3.16%)	-	-	-	1	-	-	-	-	0.4	
	AML, AMP,CL,KF,TE,NE,SXT, CT	1(0.57%), (0.01%-3.16%)	1	-	-	-	-	-	-	-		
	AML, AMP,CL,KF,DO,TE,SXT,FFC	1(0.57%), (0.01%-3.16%)	1	-	-	-	-	-	-	-		
	AML, AMP,CL,KF,TE,GM,SXT, FFC	1(0.57%), (0.01%-3.16%)	1	-	-	-	-	-	-	-		
	AML, AMP,CL,KF,TE,NE,ENF,SXT	2(1.15%), (0.14%-4.09%)	-	2	-	-	-	-	-	-		
	AMP,CL,KF,FOX,TE,NE,ENF, SXT	2(1.15%), (0.14%-4.09%)	-	-	2	-	-	-	-	-		
	AML,CL,FOX,TE,GM,CIP,ENF,SXT	1(0.57%), (0.01%-3.16%)	-	-	-	-	-	-	-	1		
	AML, AMP,CL,DO,TE,NE,SXT,FFC	1(0.57%), (0.01%-3.16%)	-	-	-	1	-	-	-	-		
	AML, AMP,CL,KF,DO,TE,ENF,SXT	1(0.57%), (0.01%-3.16%)	-	-	-	1	-	-	-	-		
	AML, AMP,CL,KF,TE,GM,SXT,FFC	1(0.57%), (0.01%-3.16%)	-	-	-	1	-	-	-	-		

	AML, AMP,CL,KF,DO,TE,SXT,FFC	1(0.57%), (0.01%-3.16%)	-	-	-	-	1	-	-	-		
	AML, AMP,CL,KF,NE,ENF,SXT,FFC	1(0.57%), (0.01%-3.16%)	-	-	-	-	1	-	-	-		
	AML, AMP,CL,KF,DO,TE,SXT,CT,FFC	1(0.57%), (0.01%-3.16%)	-	-	-	-	-	1	-	-		
	AML,AMP,CL,FOX,CTX,CIP,NOR,CT	1(0.57%), (0.01%-3.16%)	-	-	-	-	-	1	-	-		
	AML,AMP,CL,KF,ENF,SXT,CT,FFC	2(1.15%), (0.14%-4.09%)	-	-	-	-	-	-	2	-		
	AML, AMP,CL,KF,TE,ENF, SXT, CT	2(1.15%), (0.14%-4.09%)	-	-	-	-	-	-	1	1		
	AML, AMP,CL,KF,CTX,TE,ENF,SXT	1(0.57%), (0.01%-3.16%)	-	-	-	-	-	-	1	-		
	AML,AMP,KF,FOX,CIP,ENF,LEV, FFC	1(0.57%), (0.01%-3.16%)	-	-	-	-	-	-	1	-		
9	AML,CL,KF,TE,GM,NE,SXT,CT,FFC	1(0.57%), (0.01%-3.16%)	-	-	-	-	-	-	1	-	0.45	
	AML,AMP,CL,KF,DO,TE,ENF,SXT,CT	1(0.57%), (0.01%-3.16%)	-	-	-	-	-	1	-	-		
	AML, AMP,CL,KF,DO,TE,NE,SXT,FFC	2(1.15%), (0.14%-4.09%)	-	-	-	-	2	-	-	-		
	AML, AMP,CL,KF,TE,NE,SXT,CT,FFC	1(0.57%), (0.01%-3.16%)	-	-	-	1	-	-	-	-		
	AML, AMP,FOX,DO,TE,NE,CIP,ENF,CT	1(0.57%), (0.01%-3.16%)	-	-	-	1	-	-	-	-		

	AMP,CL,KF,FOX,TE,NE,CIP,ENF,SXT	1(0.57%), (0.01%-3.16%)	-	-	1	-	-	-	-	-		
	AML, AMP,CL,KF,FOX,DO,TE,CIP,SXT	2(1.15%), (0.14%-4.09%)	-	-	2	-	-	-	-	-		
	AML, AMP,CL,KF,DO,TE,SXT,CT, FFC	1(0.57%), (0.01%-3.16%)	-	1	-	-	-	-	-	-		
	AML, AMP,CL,KF,CTX,CAZ,DO,NOR,SXT	1(0.57%), (0.01%-3.16%)	-	-	-	-	-	-	-	1		
	AML,AMP,KF,FOX,CIP,ENF,LEV,SXT,FFC	1(0.57%), (0.01%-3.16%)	-	-	-	-	-	-	1	-		
	AML, AMP,CL,KF,CTX,CAZ,TE,SXT, CT	1(0.57%), (0.01%-3.16%)	1	-	-	-	-	-	-	-		
10	AML, AMP,CL,KF,DO,TE,NE,SXT,CT,FFC	1(0.57%), (0.01%-3.16%)	-	-	-	-	1	-	-	-	0.5	
	AML, AMP,CL,KF,TE,NE,ENF,SXT,CT,FFC	1(0.57%), (0.01%-3.16%)	-	-	-	-	1	-	-	-		
	AML, AMP,CL,KF,TE,GM,NE,SXT,CT,FFC	2(1.15%), (0.14%-4.09%)	-	-	-	-	-	-	2	-		
	AML,AMP,CL,KF,FOX,TE,GM,CIP,SXT,CT	1(0.57%), (0.01%-3.16%)	-	-	-	-	-	-	-	1		
	AML, AMP,CL,KF,DO,TE,NE,SXT, CT, FFC	3 (1.72%),(0.36%-4.96%)	2	-	-	1	-	-	-	-		
	AML, AMP,KF,FOX,DO,TE,NE,CIP,ENF, CT	1(0.57%), (0.01%-3.16%)	-	1	-	-	-	-	-	-		

	AML, AMP,CL,KF,DO,TE,NE,ENF,SXT, FFC	2(1.15%), (0.14%-4.09%)	-	1	-	1	-	-	-	-		
	AML, AMP,CL,KF,FOX,TE,NE,CIP,ENF,SXT	1(0.57%), (0.01%-3.16%)	-	1	-	-	-	-	-	-		
	AML, AMP,CL,KF,FOX,TE,NE,CIP,ENF,CT	1(0.57%), (0.01%-3.16%)	-	-	1	-	-	-	-	-		
	AML, AMP,FOX,DO,TE,NE,CIP,ENF,SXT,CT	1(0.57%), (0.01%-3.16%)	-	-	-	-	-	-	1	-		
11	AML, AMP,CL,KF,DO,TE,GM,NE,LEV,SXT,CT	1(0.57%), (0.01%-3.16%)	-	-	-	-	-	1	-	-	0.55	
	AML, AMP,CL,KF,FOX,TE,GM,CIP,SXT,CT,FFC	1(0.57%), (0.01%-3.16%)	-	-	-	1	-	-	-	-		
	AML, AMP,CL,KF,FOX,TE,NE,CIP,ENF,CT,FFC	3 (1.72%),(0.36%-4.96%)	-	-	-	-	-	-	-	3		
	AML, AMP,CL,KF,DO,TE,NE,ENF,SXT,CT,FFC	3 (1.72%),(0.36%-4.96%)	1	-	-	-	-	-	-	2		
	AML, AMP,CL,KF,DO,TE,GM,NE,SXT, CT, FFC	3 (1.72%),(0.36%-4.96%)	1	-	-	2	-	-	-	-		
	AML, AMP,CL,KF,TE,NE,CIP,ENF,LEV, SXT, CT	1(0.57%), (0.01%-3.16%)	1	-	-	-	-	-	-	-		
	AMP,KF,FOX,CTX,CAZ,TE,CIP,NOR,ENF, CT, FFC	1(0.57%), (0.01%-3.16%)	-	-	-	1	-	-	-	-		

	AML, AMP,CL,KF,CTX,CAZ,DO,NE,NOR,SXT,FFC	1(0.57%), (0.01%-3.16%)	-	-	-	1	-	-	-	-		
	AML, AMP,CL,KF,FOX,DO,TE,NE,CIP, SXT ,FFC	1(0.57%), (0.01%-3.16%)	1	-	-	-	-	-	-	-		
12	AML, AMP,CL,KF,CTX,CAZ,DO,TE,ENF,SXT, CT, FFC	1(0.57%), (0.01%-3.16%)	1	-	-	-	-	-	-	-	0.6	
	AML,AMP,CL,KF,FOX,TE,GM,NE,CIP,ENF,SXT,CT	1(0.57%), (0.01%-3.16%)	-	-	-	-	-	-	-	1		
	AMP,KF,CTX,CAZ,DO,TE,NE,CIP,NOR,ENF,SXT, CT	1(0.57%), (0.01%-3.16%)	-	-	-	-	-	1	-	-		
	AMP,KF,FOX,CTX,CAZ,TE,CIP,NOR,ENF,LEV, CT, FFC	1(0.57%), (0.01%-3.16%)	-	-	-	1	-	-	-	-		
13	AML, AMP,CL,KF,FOX,DO,TE,GM,NE,CIP,SXT,CT,FFC	3 (1.72%),(0.36%-4.96%)	-	-	-	1	-	-	-	2	0.65	
	AML, AMP,CL,KF,FOX,DO,TE,NE,CIP,NOR,ENF,SXT, CT	1(0.57%), (0.01%-3.16%)	1	-	-	-	-	-	-	-		
	AML, AMP,CL,KF,CTX,CAZ,TE,GM,NE,NOR,SXT, CT, FFC	1(0.57%), (0.01%-3.16%)	-	-	-	-	-	-	1	-		
	AML,AMP,KF,FOX,CTX,CAZ,GM,NE,CIP,NOR,ENF,LEV,FFC	1(0.57%), (0.01%-3.16%)	-	-	-	-	-	1	-	-		
	AML,AMP,FOX,CTX,CAZ,DO,NE,CIP,NOR,ENF,SXT, CT, FFC	1(0.57%), (0.01%-3.16%)	-	-	-	-	-	1	-	-		
	AML, AMP,CL,CTX,CTX-CV,CAZ,CAZ-CV,DO,TE,NE,SXT, CT, FFC	1(0.57%), (0.01%-3.16%)	1	-	-	-	-	-	-	-		

14	AML, AMP,CL,KF,CTX,CAZ,DO,TE,CIP,NOR,ENF,LEV,SXT,CT	1(0.57%), (0.01%-3.16%)	-	-	-	-	-	-	-	1	0.7	
	AMP,CL,KF,FOX,CTX,CAZ,DO,TE,NE,CIP,NOR,ENF, CT, FFC	1(0.57%), (0.01%-3.16%)	1	-	-	-	-	-	-	-		
	AML, AMP,CL,KF,FOX,DO,TE,CIP,NOR,ENF,LEV, SXT, CT, FFC	1(0.57%), (0.01%-3.16%)	1	-	-	-	-	-	-	-		
	AML, AMP,CL,FOX,CTX,CAZ,DO,TE,NE,CIP,NOR,ENF, CT, FFC	1(0.57%), (0.01%-3.16%)	1	-	-	-	-	-	-	-		
	AML, AMP,FOX,CTX,CAZ,DO,TE,NE,CIP,NOR,ENF,SXT, CT, FFC	1(0.57%), (0.01%-3.16%)	-	-	-	-	-	1	-	-		
	AMP,CL,KF,FOX,CTX,CAZ,DO,TE,NE,CIP,NOR,ENF, SXT, CT	1(0.57%), (0.01%-3.16%)	-	1	-	-	-	-	-	-		
	AML, AMP,FOX,CTX,CAZ,TE,GM,NE,CIP,NOR,ENF,LEV, SXT, FFC	1(0.57%), (0.01%-3.16%)	-	-	-	-	-	-	1	-		
	AML, AMP,CL,KF,FOX,CTX,CAZ,GM,NE,CIP,NOR,ENF,CT,FFC	1(0.57%), (0.01%-3.16%)	-	1	-	-	-	-	-	-		
	AML, AMP,CL,KF,FOX,CTX,TE,GM,NE,CIP,ENF, SXT, CT, FFC	1(0.57%), (0.01%-3.16%)	-	-	-	-	-	-	1	-		
	AML, AMP,CL,KF,FOX,CTX,CAZ,GM,NE,CIP,NOR,ENF,CT, FFC	1(0.57%), (0.01%-3.16%)	-	-	-	-	-	1	-	-		
	AML, AMP,CL,KF,CTX,CTX-CV,CAZ,CAZ-CV,TE,GM,NOR,ESXT, FFC	1(0.57%), (0.01%-3.16%)	-	-	-	1	-	-	-	-		
15	AML, AMP,CL,KF,FOX,CTX,CAZ,TE,GM,NE,CIP,NOR,ENF,SXT,CT	1(0.57%), (0.01%-3.16%)	-	-	-	1	-	-	-	-	0.75	
	AML, AMP,CL,KF,FOX,TE,GM,NE,CIP,NOR,ENF,LEV, SXT, CT, FFC	1(0.57%), (0.01%-3.16%)	1	-	-	-	-	-	1	-		

	AML,AMP,FOX,CTX,CAZ,DO,TE,GM,NE,CIP,NOR,ENF,LEV, SXT, FFC	1(0.57%), (0.01%-3.16%)	-	-	-	-	-	-	-	-		
	AML, AMP,CL,KF,CTX,CTX-CV,CAZ,CAZ-CV,DO,TE,GM,NE,ENF,SXT, CT	1(0.57%), (0.01%-3.16%)	1	-	-	-	-	-	-	-		
	AMP,CL,KF,FOX,CTX,CTX-CV,CAZ,CAZ-CV,TE,CIP,NOR,ENF,LEV,CT,FFC	1(0.57%), (0.01%-3.16%)	-	-	-	-	-	-	1	-		
	AML, AMP,KF,FOX,CTX,CTX-CV,CAZ,CAZ-CV,GM,NE,CIP,NOR,ENF,LEV,FFC	3 (1.72%), (0.36%-4.96%)	-	-	-	-	-	-	3	-		
16	AMP,CL,KF,FOX,CTX,CAZ,DO,TE,NE,CIP,NOR,ENF,LEV, SXT, CT, FFC	1(0.57%), (0.01%-3.16%)	1	-	-	-	-	-	-	-	0.80	
	AML, AMP,CL,KF,FOX,CTX,CAZ,DO,TE,CIP,NOR,ENF,LEV, SXT, CT, FFC	1(0.57%), (0.01%-3.16%)	1	-	-	-	-	-	-	-		
	AML,AMP,CL,FOX,CAZ,DO,TE,GM,NE,CIP,NOR,ENF,LEV, SXT, CT, FFC	1(0.57%), (0.01%-3.16%)	-	-	-	-	-	-	1	-		
	AML, AMP,CL,KF,FOX,CTX,CAZ,TE,GM,CIP,NOR,ENF,LEV,SXT,CT, FFC	1(0.57%), (0.01%-3.16%)	-	-	-	1	-	-	-	-		
	AML,AMP,KF,FOX,CTX,CTX-CV,CAZ,CAZ-CV,DO,TE,NE,CIP,NOR,ENF,SXT,CT	1(0.57%), (0.01%-3.16%)	-	-	-	-	-	1	-	-		
	AML,AMP,KF,FOX,CTX,CTX-CV,CAZ,CAZ-CV,TE,GM,NE,CIP,NOR,ENF,CT, FFC	1(0.57%), (0.01%-3.16%)	-	-	-	1	-	-	-	-		
17	AML, AMP,CL,KF,FOX,CTX,CAZ,DO,TE,NE,CIP,NOR,ENF,LEV, SXT, CT, FFC	1(0.57%), (0.01%-3.16%)	1	-	-	-	-	-	-	-	0.85	
	AML, AMP,CL,KF,FOX,CTX,CTX-CV,CAZ,CAZ-CV,TE,GM,NE,CIP,ENF,SXT,CT,FFC	1(0.57%), (0.01%-3.16%)	-	-	-	-	-	-	1	-		

	AML, AMP,CL,KF,FOX,CTX,CTX-CV,CAZ,CAZ-CV,DO,TE,CIP,NOR,ENF,SXT, CT, FFC	1(0.57%), (0.01%-3.16%)	1	-	-	-	-	-	-	-		
	AML, AMP,CL,KF,FOX,CTX,CTX-CV,CAZ,CAZ-CV,TE,NE,CIP,NOR,ENF,SXT, CT, FFC	1(0.57%), (0.01%-3.16%)	-	-	-	-	-	1	-	-		
18	AML, AMP,CL,KF,FOX,CTX,CTX-CV,CAZ,CAZ-CV,DO,TE,NE,CIP,NOR,ENF, SXT, CT, FFC	1(0.57%), (0.01%-3.16%)	-	-	-	-	-	-	1	-	0.90	
	AML,AMP,KF,FOX,CTX,CTX-CV,CAZ,CAZ-CV,DO,GM,NE,CIP,NOR,ENF,LEV, SXT, CT, FFC	1(0.57%), (0.01%-3.16%)	-	-	-	-	1	-	-	-		
	AML,AMP,KF,FOX,CTX,CTX-CV,CAZ,CAZ-CV,TE,GM,NE,CIP,NOR,ENF,LEV, SXT, CT, FFC	1(0.57%), (0.01%-3.16%)	-	-	-	-	-	1	-	-		
	AML, AMP,CL,KF,FOX,CTX,CAZ,DO,TE,GM,NE,CIP,NOR,ENF,LEV, SXT, CT, FFC	3 (1.72%),(0.36%-4.96%)	2	-	-	1	-	-	-	-		
	AML, AMP,CL,KF,FOX,CTX,CTX-CV,CAZ,CAZ-CV,TE,GM,NE,CIP,NOR,ENF, SXT, CT, FFC	1(0.57%), (0.01%-3.16%)	-	-	-	-	-	-	1	-		
	AML, AMP, CL, KF, FOX, CTX, CTX-CV, CAZ, CAZ-CV, DO, TE, NE, CIP, NOR, ENF, SXT, CT, FFC	2(1.15%), (0.14%-4.09%)	1	-	-	1	-	-	-	-		
19	AML, AMP, KF, FOX, CTX,CTX-CV,CAZ,CAZ-CV,DO,TE,GM,NE,CIP,NOR,ENF,LEV, SXT, CT, FFC	1(0.57%), (0.01%-3.16%)	-	-	-	-	-	-	1	-	0.95	
	AML, AMP, CL,KF,FOX,CTX,CTX-CV,CAZ,CAZ-CV,DO,TE,NE,CIP,NOR,ENF,LEV, SXT, CT, FFC	1(0.57%), (0.01%-3.16%)	1	-	-	-	-	-	-	-		
20	AML, AMP, CL,KF,FOX,CTX,CTX-CV,CAZ,CAZ-CV,DO,TE,GM,NE,CIP,NOR,ENF,LEV, SXT, CT, FFC	1(0.57%), (0.01%-3.16%)	1	-	-	-	-	-	-	-	1.00	
	Total Number of observed patterns	**174**										

4.4.2 Multidrug-Resistance Patterns of *E. coli* isolates at wet market level

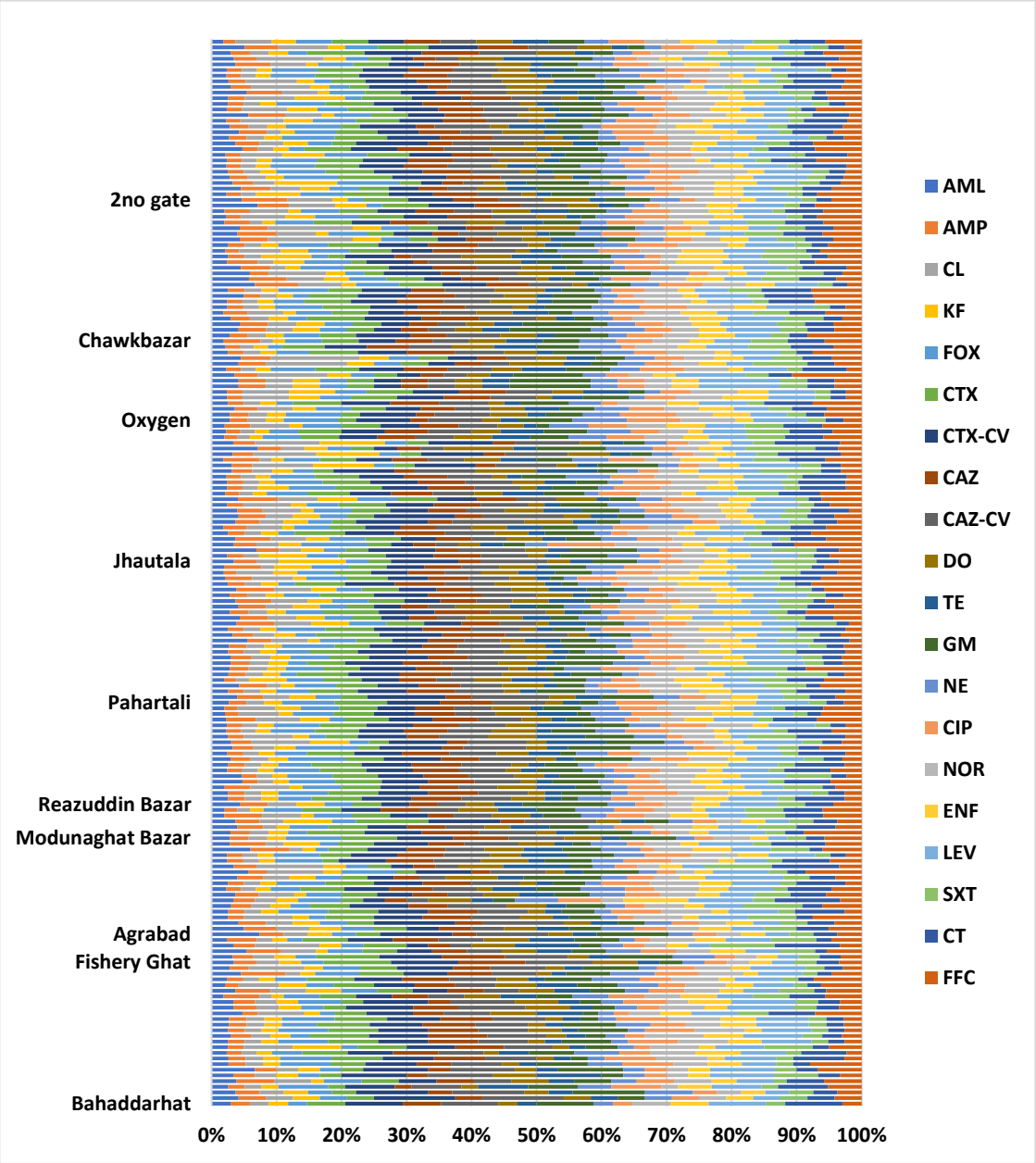


Figure-4.10. Multidrug-Resistance Patterns at Wet Market Level

Figure-4.10, portraying Multidrug- Resistance Patterns of *E. coli* isolates at wet market level of all tested antimicrobials. Color code of all antibiotics tested are labeled in left.

4.5 Detection of Antimicrobial Resistant (AMR) Genes in *E. coli* isolates from fish samples

All of the phenotypically resistant isolates (n=188) weretested to detect the presence of antimicrobial resistant genes i.e., bla_{TEM}, bla_{SHV}, bla_{CTX}, $bla_{OXA\text{-}1\ like\ group}$, $bla_{OXA\text{-}2\ like\ group}$, $bla_{CMY\text{-}1\ like\ group}$, $bla_{CMY\text{-}2\ like\ group}$, $bla_{ACC\text{-}1}$, P_{ampC}, *tet-A, tet-B, tet-C, tet-D, Sul-1* & *Sul-2* via uniplex, duplex and multiplex PCR assays (Figure 4.11 to Figure 4.16). A heatmap is portrayed based on all the resistant genes detected in Figure 4.16.

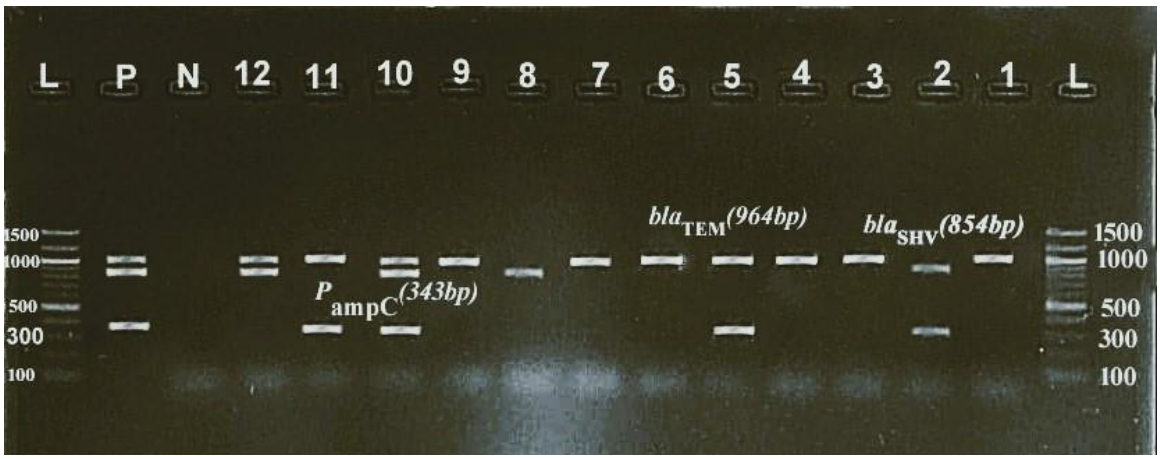


Figure-4.11. Presence of bla_{TEM} (964bp), bla_{SHV} (854bp) &P_{ampC} (343bp) genes with MDR *E. coli* isolates in multiplex PCR assay of agarose gel electrophoresis. Lane L: DNA ladder; Lane P: Positive control; Lane N: Negative control.

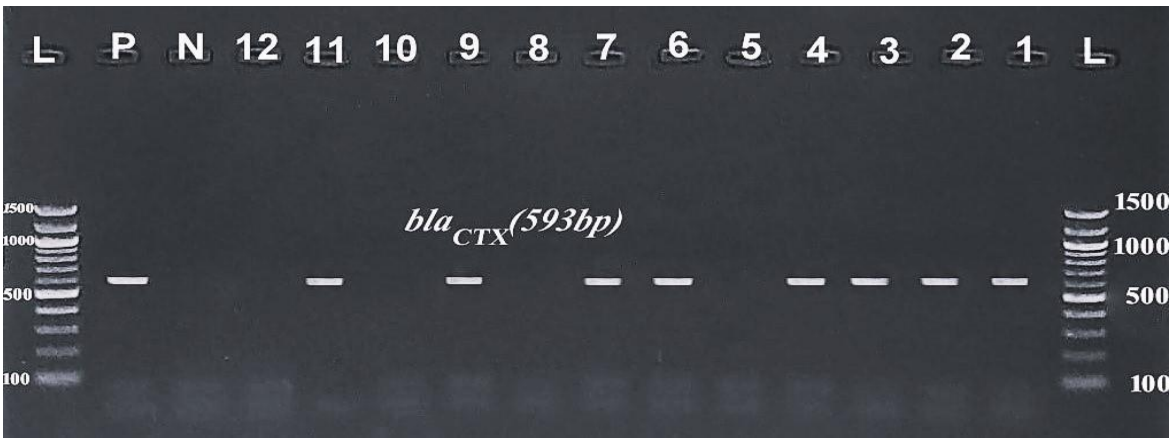


Figure-4.12. Presence of bla_{CTX} (593bp) with MDR *E. coli* isolates in uniplex PCR assay of agarose gel electrophoresis. Lane L: DNA ladder; Lane P: Positive control; Lane N: Negative control.

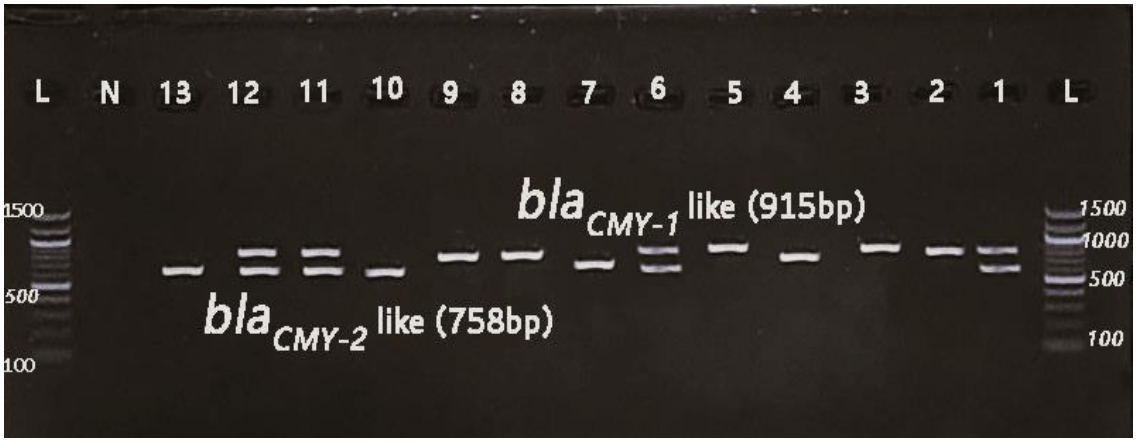


Figure-4.13. Presence of *bla*$_{CMY-1}$ like group (915bp) &*bla*$_{CMY-2}$ like group(758bp) genes with MDR *E. coli* isolates in Duplex PCR assay of agarose gel electrophoresis. Lane L: DNA ladder; Lane P: Positive control; Lane N: Negative control.

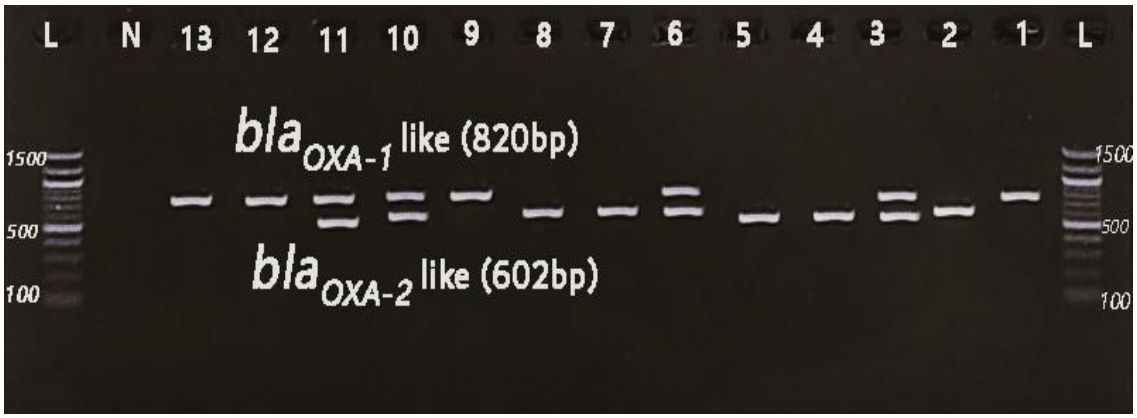


Figure-4.14. Presence of *bla*$_{OXA-1}$ like group (820bp) & *bla*$_{OXA-1}$ like group (602bp) genes with MDR *E. coli* isolates in Duplex PCR assay of agarose gel electrophoresis. Lane L: DNA ladder; Lane P: Positive control; Lane N: Negative control.

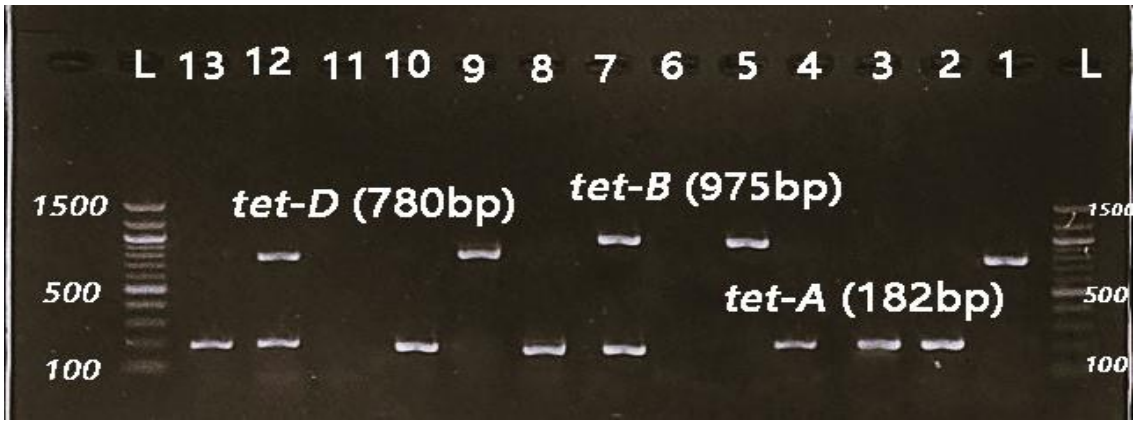


Figure-4.15. Presence of *tet-A* (182bp), *tet-B* (975bp), and *tet-D* (780bp) with MDR *E. coli* isolates in multiplex PCR assay of agarose gel electrophoresis. Lane L: DNA ladder; Lane P: Positive control; Lane N: Negative control.

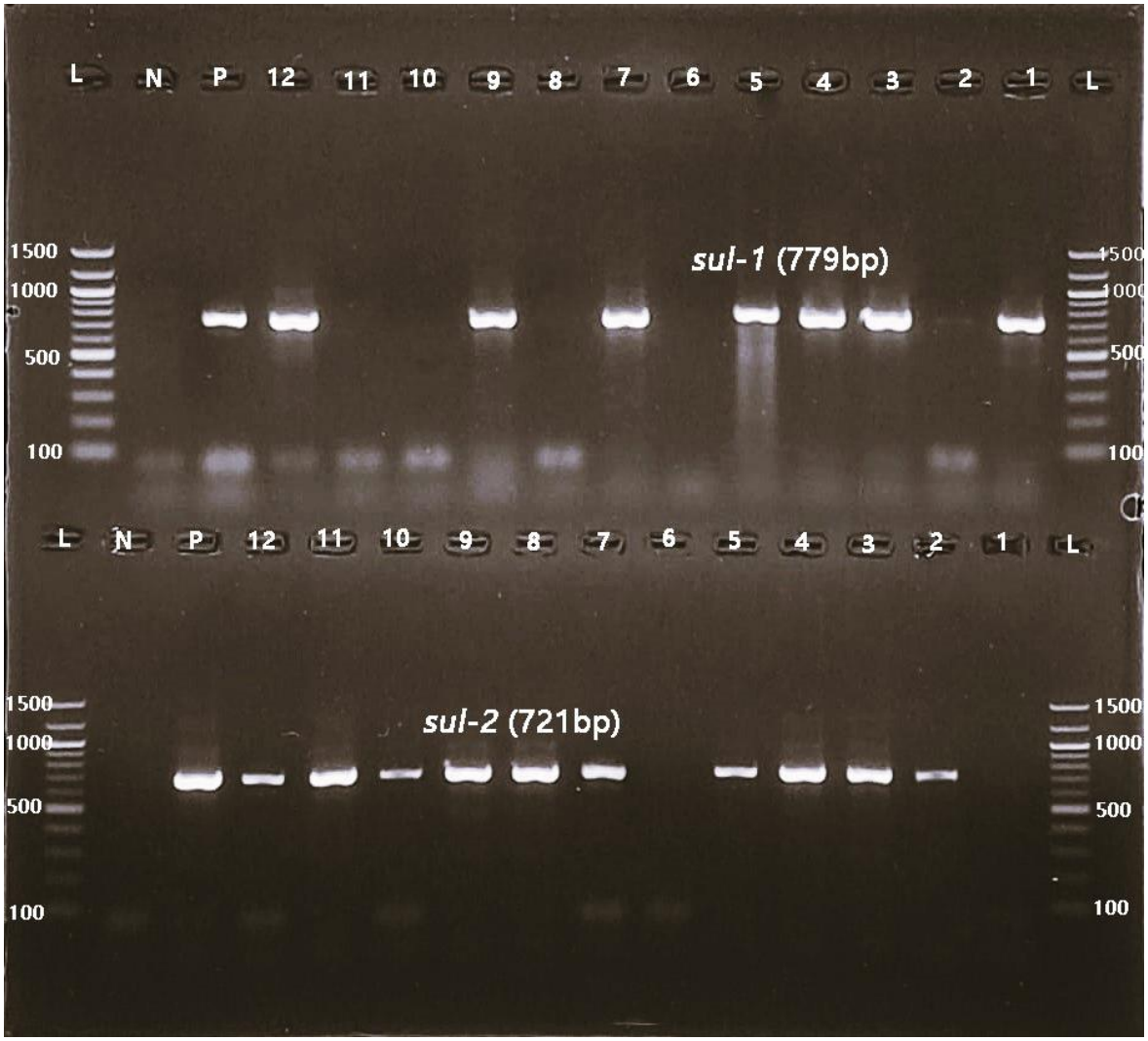


Figure-4.16. Presence of sul-1 (779bp) & sul-2 (721bp) genes with MDR E. coli isolates in simplex PCR assay of agarose gel electrophoresis. Lane L: DNA ladder; Lane P: Positive control; Lane N: Negative control.

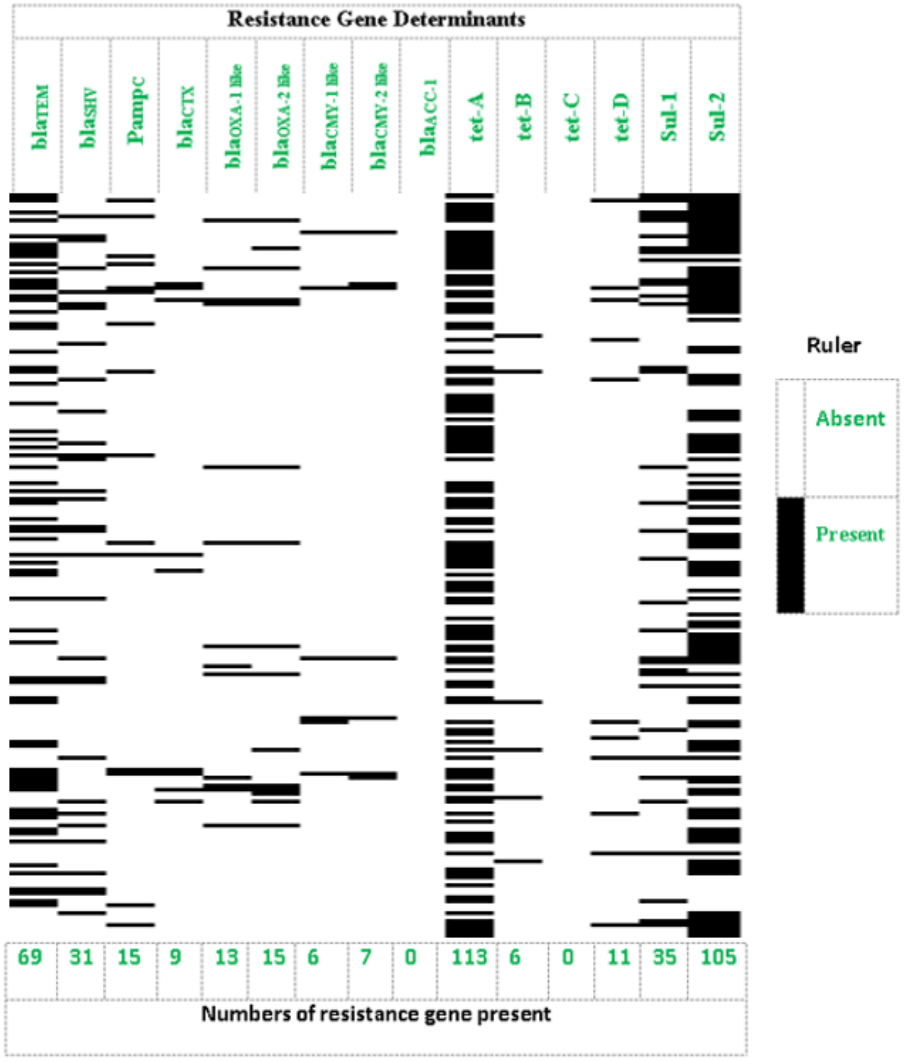


Figure-4.17. Heatmap representing multidrug -resistance gene detection in *E. coli* isolates.

*A ruler wasused to interpret the detection of the resistance genes in the heatmap, whereas color code black represents the presence of resistance genes and the color code white denotes absence of resistance genes. Name of all tested resistance genes (above) and total number of each gene detected (below) are also included in the map. Distribution of resistant genes detected among all positive *E. coli* isolates are enlisted in **Table-4.8.** Among all 188 Phenotypically resistant isolates 157 (83.51% **95% CI** 77.42%- 88.51%) isolates were characterized with the presence of antimicrobial resistance genes and no gene was traced in remaining 31 (16.49% **95% CI** 11.49%-22.58%) isolates.

Amid the prevalence of ampicillin resistant genes of β-lactam resistance determinants (n=85, 45.21%, **95% CI** 37.96% - 52.62%) highest share was contributed by *bla$_{TEM}$* gene (n=54, 63.53%, **95% CI** 52.38% - 73.71%).

Within the Cefoxitin resistant genes of cephalosporin resistance determinants (n=15, 7.98%, **95% CI** 4.53%- 12.82%) entire gene was *P$_{ampC}$*(n=15,100%, **95% CI** 78.20% - 100%). Similarly, *bla$_{CTX}$*(n=9,100%, **95% CI** 66.37% - 100%) gene was responsible for the Cefotaxime resistant genes of broad-spectrum cephalosporin resistance determinants (n=9, 4.79%, **95% CI** 2.21% - 8.89%). Among the ESBLs resistance determinants both *bla$_{OXA-1}$ like group &bla$_{OXA-2}$ like group,* (n=11, 52.38% **95% CI** 29.78% - 74.29%) genes was the utmost and no *bla$_{ACC-1}$* gene wasidentified.

What's more, in non β-lactam resistance determinants *tet-A*, (n=103, 85.83%, **95% CI**78.29% - 91.53%) was detected utmost following *sul-2* (n=82, 70.09%, **95% CI** 60.93% - 78.20%) while no *tet-C* gene was detected.

Table-4.8. Prevalence of antimicrobial resistance gene detected in phenotypically resistant isolates

Antibiotic (code)	**Overall Prevalence; % (n), 95%CI [Phenotypically resistant isolates]**	**Overall Prevalence, % (n), 95%CI [Genotypically resistant isolates]**	**Resistant gene, % (n), 95%CI**
Ampicillin (AMP)	82.98% (156), (76.83% - 88.06%)	45.21% (85), (37.96% - 52.62%)	*bla$_{TEM,}$* 63.53% (54), (52.38% - 73.71%)
			*bla$_{SHV,}$*18.82% (16), (11.16% - 28.76%)
			Bla$_{TEM}$ + *bla$_{SHV,}$* 17.65% (15), (10.23% - 27.43%)
Cefoxitin (FOX)	36.70% (69), (29.81- 44.02%)	7.98% (15), (4.53%- 12.82%)	*P$_{ampC,}$*100% (15), (78.20% - 100%)
Cefotaxime (CTX)	26.60% (50), (20.43% - 33.52%)	4.79% (9), (2.21% - 8.89%)	*bla$_{CTX,}$*100% (9), (66.37% - 100%)
Cefotaxime+ Clavulanic acid (CTX-CV)	11.17% (21), (7.05% - 16.57%)	11.17% (21), (7.05% - 16.57%)	*bla$_{OXA-1\ LIKE}$*, 9.52% (2), (1.17% - 30.38%)
			bla$_{OXA-2\ LIKE,}$ 19.05% (4),

			(5.45% - 41.91%)
			bla$_{OXA\text{-}1\ LIKE}$ + *bla*$_{OXA\text{-}2\ LIKE}$, 52.38% (11), (29.78% - 74.29%)
Ceftazidime+ Clavulanic acid (CAZ-CV)			*bla*$_{CMY\text{-}1\ LIKE}$, 4.76% (1), (0.12% - 23.82%)
			bla$_{CMY\text{-}2\ LIKE}$, 9.52% (2), (11.75% - 30.38%)
			bla$_{CMY\text{-}1\ LIKE}$ + *bla*$_{CMY\text{-}2\ LIKE}$, 23.81% (5), (8.22% - 47.17%)
			bla$_{ACC\text{-}1}$, 0.00% (0), (0.00% - 16.11%)
Tetracycline (TE)	68.62% (129), (61.46% - 75.17%)	63.83% (120), (56.52% - 70.70%)	*tetA*, 85.83% (103), (78.29% - 91.53%)
			tetB, 2.50% (3), (0.52% - 7.13%)
			tetC, 0.00% (0), (0.00% - 3.03%)
			tetD, 3.33% (4), (0.92% - 8.31%)
			tetA+*tetB*,2.50% (3), (0.52% - 7.13%)
			tetA+*tetD*, 5.83% (7), (2.38% - 11.65%)
Trimethoprim/ Sulfamethoxazole (SXT)	67.55% (127), (60.36% - 74.19%)	62.23% (117), (54.89% - 69.19%)	*Sul-1*,10.26% (12), (5.41% - 17.23%)
			Sul-2,70.09% (82), (60.93% - 78.20%)
			Sul-1+ *Sul-2*, 19.66% (23), (12.89% - 28.02%)

4.5.1 Antibiotic resistant genotypic patterns of *E. coli* isolates from fish samples

Diverse genotypic patterns were detected are shown in **Table 4.9.** Worthy of note is that majority of the genotypes occurred uniquely. Amongst all the 157 isolates harboring resistant genes, 35 isolates (22.30%, **95%CI** 16.05% -29.62%) harboring single gene, 31 isolates (19.75%, 95%CI 13.83% -26.84%) harboring three gene, 26 isolates (16.56%, **95%CI** 11.11% -23.32%) harboring four genes, 13 isolates (8.28%, **95%CI** 4.48% -13.74%) harboring five genes. Highest seven genes were harboring by only 1 isolate (0.64%, **95%CI** 0.02% -3.50%) following six genes were harboring by seven isolates (4.46%, **95%CI** 1.81% -8.97%). However, Highest number of isolates i.e., 44 (28.03% **95%CI** 21.16% -35.74%) were harboring double genes.

Further, among all the 157 isolates, 56 (35.67% **95%CI** 28.19% 43.70%) isolates harboring β-lactam encoding genes. Within the β-lactamases, the ESBLs assayed and detected in 35 (22.29% **95%CI** 16.05% -29.62%) isolates. Additionally, 14 (8.91% **95% CI** 4.96% -14.51%) isolates harboring pAmpC genes and utmost 111(70.70% **95%CI** 62.92% - 77.68%) isolates harboring non-βlactam encoding genes

Table-4.9. Multidrug resistance patterns of antimicrobial classes from *E. coli* isolates of fish samples

Antibiotic resistance genotypes patterns	**No. of β-lactam encoding genes**	**No. of ESBL genes**	**No. of pAmpC genes**	**No. of non-β-lactam encoding genes**	**No. of the genotype patterns observed, n (%), 95%CI**	**Sample Type**								***P- value***
						Catfish	**Koi**	**Loitta**	**Pangus**	**Poa**	**Pabda**	**Prawn**	**Tilapia**	
tet-A	0	0	0	1	13(8.28%), (4.48%-13.74%)	-	-	4	1	3	-	2	3	
tet-B	0	0	0	1	1(0.64%), (0.02%-3.50%)	-	1	-	-	-	-	-	-	
tet-D	0	0	0	1	1(0.64%), (0.02%-3.50%)	-	-	-	-	-	-	1	-	
sul-1	0	0	0	1	1(0.64%), (0.02%-3.50%)	-	-	-	-	-	1	-	-	
sul-2	0	0	0	1	15(9.55%), (5.45%-15.27%)	2	3	1	3	3	1	1	1	0.485

bla_{SHV}	1	0	0	0	2 (1.27%), (0.15%-4.53%)	-	1	-	-	-	-	-	1	
blaOXA-1 like	0	1	0	0	1(0.64%), (0.02%-3.50%)	-	-	-	-	-	1	-	-	
blaCMY-2 like	0	1	0	0	1(0.64%), (0.02%-3.50%)	-	-	-	-	-	-	-	1	
tet-A+ tet-B	0	0	0	2	1(0.64%), (0.02%-3.50%)	-	-	-	-	-	-	1	-	
tet-A + tet-D	0	0	0	2	1(0.64%), (0.02%-3.50%)	-	1	-	-	-	-	-	-	
tet-A+sul-1	0	0	0	2	4 (2.55%), (0.70%-6.39%)	-	-	-	1	2	-	1	-	
tet-A+sul-2	0	0	0	2	17(10.83%), (6.44%-16.77%)	3	-	2	5	1	3	-	3	
tet-B+sul-2	1	0	0	1	1(0.64%), (0.02%-3.50%)	-	-	-	-	-	-	-	1	
blaTEM + tet-A	1	0	0	1	3(1.91%), (0.40%-5.48%)	-	-	2	-	-	1	-	-	
blaTEM + sul-1	1	0	0	1	1(0.64%), (0.02%-3.50%)	-	-	-	-	-	-	-	1	
blaTEM + sul-2	1	0	0	1	9(7.53%), (2.65%-10.60%)	1	-	-	1	-	1	4	2	
blaTEM +blaSHV	2	0	0	0	5 (3.18%),	-	-	-	1	-	2	-	2	

					(1.04%-7.28%)									
blaTEM + PampC	1	0	1	0	1(0.64%), (0.02%-3.50%)	-	-	-	-	-	-	-	1	
blaCMY-1 like+blaCMY-2 like	0	2	0	0	1(0.64%), (0.02%-3.50%)	-	-	-	-	-	-	1	-	
tet-A+ sul-1+sul-2	0	0	0	3	4 (2.55%), (0.70%-6.39%)	1	-	-	-	-	2	-	1	
tet-A + tet-D +sul-2	0	0	0	3	1(0.64%), (0.02%-3.50%)	-	-	-	-	-	-	1	-	
blaTEM +tet-A+sul-1	1	0	1	1	1(0.64%), (0.02%-3.50%)	-	1	-	-	-	-	-	1	
blaTEM +tet-A+sul-2	1	0	0	2	11(7.01%), (3.55%-12.19%)	1	2	1	4	-	-	2	1	
blaSHV + tet-A+sul-2	1	0	0	2	7(4.46%), (1.81%-8.97%)	2	-	1	3	-	-	-	1	
blaTEM+blaCTX+sul-2	2	0	0	1	1(0.64%), (0.02%-3.50%)	-	-	-	1	-	-	-	-	
blaTEM+PampC+ tet-A	1	0	1	1	3(1.91%), (0.40%-5.48%)	2	1	-	-	-	-	-	-	
blaTEM + blaSHV+PampC	2	0	1	0	1(0.64%), (0.02%-3.50%)	-	-	-	1	-	-	-	-	
blaOXA-1 like+blaOXA-2 like+ sul-2	0	2	0	1	1(0.64%), (0.02%-3.50%)	-	-	-	-	-	-	1	-	
blaSHV +blaOXA-1 like+blaOXA-2 like	1	2	0	0	1(0.64%),	-	-	-	-	-	-	1	-	

					(0.02%-3.50%)									
tet-A+ tet-D+ sul-1+sul-2	0	0	0	4	1(0.64%), (0.02%-3.50%)	-	-	-	-	-	-	-	1	
blaSHV+tet-A+tet-D+sul-2	1	0	0	3	1(0.64%), (0.02%-3.50%)	-	1	-	-	-	-	-	-	
blaSHV+PampC+tet-A+sul-2	1	0	1	3	1(0.64%), (0.02%-3.50%)	1	-	-	-	-	-	-	-	
blaTEM + tet-A+ tet-B +sul-2	1	0	0	3	1(0.64%), (0.02%-3.50%)	-	-	-	-	-	-	1	-	
blaTEM + tet-A+sul-1+sul-2	1	0	0	3	9(7.53%), (2.65%-10.60%)	7	-	-	1	-	-	1	-	
blaTEM +blaSHV + tet-A+sul-1	2	0	0	2	1(0.64%), (0.02%-3.50%)	-	-	-	1	-	-	-	-	
blaTEM +blaSHV + tet-A+sul-2	2	0	0	2	5 (3.18%), (1.04%-7.28%)	-	-	-	2	1	-	-	2	
blaTEM+PampC+blaCTX+ tet-A	2	0	1	1	1(0.64%), (0.02%-3.50%)	-	-	-	-	-	-	1	-	
blaOXA-2 like+tet-A+ tet-B+sul-2	0	1	0	3	1(0.64%), (0.02%-3.50%)	-	-	-	-	-	-	1	-	
blaOXA-1 like+blaOXA-2 like+tet-A+sul-2	2	0	0	2	2 (1.27%), (0.15%-4.53%)	1	-	-	-	-	1	-	-	
blaOXA-1 like+blaOXA-2 like+ sul-1+sul-2	0	2	0	2	1(0.64%), (0.02%-3.50%)	-	-	-	-	-	1	-	-	
blaTEM+blaOXA-1 like+blaOXA-2 like+tet-A	1	2	0	1	1(0.64%),	-	-	-	-	-	-	1	-	

					(0.02%-3.50%)									
blaTEM +blaOXA-1 like+blaOXA-2 like+sul-1	1	2	0	1	1(0.64%), (0.02%-3.50%)	-	-	-	1	-	-	-	-	
PampC+ tet-A+ tet-D+ sul-1+sul-2	0	0	1	4	1(0.64%), (0.02%-3.50%)	-	-	-	-	-	-	-	1	
blaSHV+tet-A+ tet-D+ sul-1+sul-2	1	0	0	4	2 (1.27%), (0.15%-4.53%)	-	-	-	-	-	-	1	1	
blaTEM+blaSHV+ tet-A+sul-1+sul-2	2	0	0	3	1(0.64%), (0.02%-3.50%)	1	-	-	-	-	-	-	-	
blaSHV+PampC +tet-A+sul-1+sul-2	1	0	1	3	1(0.64%), (0.02%-3.50%)	1	-	-	-	-	-	-	-	
blaTEM +PampC + tet-A+tet-B+sul-1	1	0	1	3	1(0.64%), (0.02%-3.50%)	-	1	-	-	-	-	-	-	
blaTEM + blaSHV+tet-A+ tet-D+ sul-2	2	0	0	3	1(0.64%), (0.02%-3.50%)	-	-	-	-	-	-	1	-	
blaTEM +PampC + tet-D+sul-1+sul-2	1	0	1	3	1(0.64%), (0.02%-3.50%)	1	-	-	-	-	-	-	-	
blaTEM+ blaSHV +PampC+blaCTX+ tet-A	3	0	1	1	1(0.64%), (0.02%-3.50%)	-	-	-	1	-	-	-	-	
blaTEM+blaOXA-2 like+tet-A+sul-1+sul-2	1	1	0	3	1(0.64%), (0.02%-3.50%)	1	-	-	-	-	-	-	-	
blaSHV+ blaCTX + blaOXA-2 like + tet-A+ sul-1	2	1	0	2	1(0.64%), (0.02%-3.50%)	-	-	-	-	-	-	1	-	
blaCMY-1 like+blaCMY-2 like+tet-A+sul-1+sul-2	0	2	0	3	1(0.64%), (0.02%-3.50%)	1	-	-	-	-	-	-	-	

PampC +blaOXA-1 like+blaOXA-2 like+ tet-A+sul-2	0	2	1	2	1(0.64%), (0.02%-3.50%)	-	-	-	1	-	-	-	-	
blaSHV +blaOXA-1 like+blaOXA-2 like+tet-A+ sul-1+sul-2	1	2	0	3	1(0.64%), (0.02%-3.50%)	1	-	-	-	-	-	-	-	
blaTEM+blaOXA-1 like+blaOXA-2 like+tet-A+ sul-1+sul-2	1	2	0	3	1(0.64%), (0.02%-3.50%)	1	-	-	-	-	-	-	-	
blaSHV +blaCMY-1 like+blaCMY-2 like+ tet-A+ sul-1+sul-2	1	2	0	3	1(0.64%), (0.02%-3.50%)	-	-	-	-	-	1	-	-	
blaTEM+blaCTX+blaOXA-1 like+blaOXA-2 like + tet-D+sul-2	2	2	0	2	2 (1.27%), (0.15%-4.53%)	1	-	-	-	-	-	-	-	
blaTEM+PampC+blaCTX+blaCMY-1 like+blaCMY-2 like++tet-A	2	2	1	1	1(0.64%), (0.02%-3.50%)	-	-	-	-	-	-	1	-	
blaTEM+blaCTX+blaOXA-1 like+blaOXA-2 like+tet-A + sul-2	2	2	0	2	1(0.64%), (0.02%-3.50%)	-	-	-	-	-	-	1	-	
blaTEM+PampC+blaCTX+blaCMY-1 like+blaCMY-2 like+tet-D+sul-2	2	2	1	2	1(0.64%), (0.02%-3.50%)	1	-	-	-	-	-	-	-	
Total number of genotype patterns observed					**157**									

Chapter-5: Discussion

This research work was performed based on the formulated hypothesis in the introduction section. Firstly, the prevalence of *E. coli* in fish samples sold at wet markets was assessed thoroughly along with their associated factors.

E. coli is frequently used as a marker for fecal contamination water, fish, shellfish and others (Akande et al., 2019; Dutta, 2016; Khan et al., 2021) since *E. coli* is not a normal resident of the aquatic environment (Odumosu et al., 2021). The findings from the present study provide the overall prevalence of *E. coli* in fish samples is 41.78% including commercial fish, shrimp and seafood samples. The result is close to the previous findings of *E. coli* prevalence in fish samples, 44.1% in Nigeria (Odumosu et al., 2021) and 48% in Assam (Sivaraman et al., 2020). Although, previous study reported notably lower *E. coli* prevalence in fish samples of 6.7% in Seoul, Korea (Ryu et al., 2012) and 38% in the Chhattisgarh State of India (Khan et al., 2021). On the other hand, much higher results of 80.70% fish *E. coli* prevalence in Kolkata, West Bengal (Dutta, 2016) and 69.3% fish *E. coli* prevalence in Africa (Adenaike et al., 2016) also reported. Though, in this study commercial fish samples contribute significantly on the overall *E. coli* prevalence but shrimp samples represent the highest 66% prevalence, corresponding with previous study records of 64 % in Switzerland (Boss et al., 2016) & 85% in Kolkata, West Bengal (Dutta, 2016). Additionally, the present study indicates that *E. coli* contamination in seafood is quite common resembling the previous study in Chhattisgarh State (Khan et al., 2021).

The outcome of present study is an indicative of the poor hygienic and sanitation quality of the wet markets, landing centers, the handling, transportation, processing storage which exacerbate the problem of poor hygiene as well as consumer and associated handlers safety of fish (Akande et al., 2019; Akter et al., 2022; Dutta, 2016). Quality of fish is also depending on the quality of waters from where the fishes are captured (Dutta, 2016). Further, inadequately cleaned and disinfected boat decks and fish containers used to catch fish are known to contaminate the catch with *E. coli*. Contamination can also occur from ice, unclean workers and unhygienic handling after catching of fish and shellfishes (Khan et al., 2021) might represent post-harvest cross contamination (Dutta, 2016) .

Secondly, to ascertain the antimicrobial sensitivity, all of the *E. coli* isolates were subjected to twenty antimicrobial agents and sensitivity result was assessed thoroughly to determine the association among multidrug- resistance profiles, MAR index of the isolates with their associative factors. Southeast Asian countries, such as Bangladesh, are at the greatest risk of AMR among all the regions monitored by the WHO (Jain et al., 2021).

The AST results of Ampicillin 82.98%, Cefotaxime 26.60% and Cefoxitin 36.70% of this study is much lower than previous work findings of Ampicillin 100% and Cefotaxime 93.3% resistance reported in Saudi Arabia (Elhadi& Alsamman, 2015) and Cefoxitin77.1% in Nigeria (Akande et al., 2019). But, Cephalothin (80.32%) resistance findings in this research work is comparatively higher than previous finding 11.7% reported in Seoul, Korea (Ryu et al., 2012). Also, Amoxicillin 79.79%, Trimethoprim-Sulfamethoxazole 67.55%, Gentamycin 57.98% and Colistin Sulfate 59.04% resistance of this work are relatively higher than previous findings of 51.4%, 42.9%, 30.6% and 40%44.4% reported in Nigeria (Adenaike et al., 2016; Odumosu et al., 2021). Ciprofloxacin resistance in our findings is 38.83% which is close to previous finding of 30.60% in Nigeria (Odumosu et al., 2021). In addition, Ceftazidime resistance findings of this work is 25% which contradicts with much higher 57% (Khan et al., 2021) and lower 6.7% resistance findings in India (Sekhar et al., 2017). Similarly, as tetracycline 68.62% resistance comparison with previous findings range from 30.7% to 100% (Elhadi & Alsamman, 2015; Odumosu et al., 2021; Ryu et al., 2012).

The presence of antimicrobial resistant of *E. coli* isolates in fish samples suggests that they can serve as a source for the transfer of resistant genes among pathogens. It is important to note the resistance of isolates to new-generation cephalosporins from this study results. In addition to developing resistance to β-lactams (>79%), more than 80% of isolates exhibited AMR to first-generation cephalosporins with cephalothin ranked the topmost resistant one. While other new generation antibiotics from the 2nd and 3rd generation of cephalosporins were effective against most tested bacteria, around 36% of isolates showed resistance to these classes. Additionally, the fact that 3rd generation of cephalosporins especially cefotaxime is an antibiotic used in clinical settings makes its presence even more concerning and also corroborates the assumption that contamination is due to discharge from anthropogenic sources (Harwalkar et al., 2013).

Infections caused by multidrug-resistant (MDR) *E. coli* are now more well understood to be serious and to be linked to higher morbidity and mortality rates. The World Health

Organization claims that resistant Gram-negative bacteria, particularly *E. coli*, have become a significant and quickly spreading issue internationally (Adebowale et al., 2022; Mohamed et al., 2022). The antimicrobial sensitivity tests of this research work revealed that, 38.67 % prevalence of total study fish samples (174 out of 450) and 92.55% of total fish *E. coli* isolates (174 out of 188) were multidrug-resistant (MDR). The result of this findings is supported by previous work reported in Dhaka, exposed as 100% fish *E. coli* are MDR (Akter et al., 2022). This study suggests that the presence of MDR *E. coli* in fish may result from excessive use of antimicrobials during harvesting and post-harvest cross-contamination through factors such as contact with infected handlers, use of unclean vessels, during transportation and processing and exposure to contaminated water at fishery outlets.

Multiple antibiotic resistant phenotypes revealed the developments of resistance towards most of the antibiotic tested of all the classes. Resistance patterns towards highest number of antibiotics was observed frequently in commercial fish (Catfish, Tilapia, Koi, Pangus, Pabda) and shrimp isolates rather than isolates origin from seafoods (loitta, Poa). A MAR index of 0.2 or above suggests high risk contamination sources, while a MAR index of 0.4 or higher implies a human fecal source of contamination. It further adds that MAR index values greater than 0.2 indicate the presence of an isolate from a high-risk contaminated source with frequent antibiotic use, and values less than 0.2 suggest pathogens from a source with less antibiotic use (Adenaike et al., 2016). To calculate the MAR index, isolates displaying all MAR phenotypic resistance patterns (n=174) to the antimicrobial agents were investigated. The majority of the observed phenotypes were unique. Maximal 13.22% of strains demonstrated resistance to seven different antibiotics following 12.64% showed resistance to eight different antibiotics yet 8.62% showed resistance to ten and eleven different antibiotics.

The MARI value obtained in this research work ranged from 0.15 to 1.00 (average 0.50), wherein 89.36% (168 out of 174) MDR isolates which significantly exceeds the maximum MARI value benchmarked at 0.2 or above. Highest MARI value of 1.00 was revealed in a *E. coli* isolates of Catfish sample. To support the MARI calculated in this research work, MDR *E. coli* isolates, indicating the worrisome issue that the isolates were originated from environments of the high-risk source of contamination and high antimicrobial exposure (Adebowale et al., 2022; Akande et al., 2019; Akter et al., 2022; Krumperman, 1983). Though it is challenging to forecast the origin and distribution of contamination until a thorough investigation is carried out to ascertain the existence and spread of MDR *E. coli.*

Thereafter, overall resistant phenotypic and genotypic prevalence and the genotypic resistant patterns of MDR *E. coli* isolates of the study samples was evaluated thoroughly. Study findings revealed that most of the genotypes occurred uniquely, indicating the possibility of the isolates acquiring unique and differing antimicrobial resistance genes.

Multi-resistant strains of *E. coli* that produce extended-spectrum b-lactamases (ESBLs) and/or plasmid-mediated AmpC (p_{AmpC}) are an important reason for therapy failure with β-lactam antibiotics (Almeida et al., 2017; Moremi et al., 2016a). Though in regard of p_{AmpC} genes, there is no CLSI guidelines for phenotypic methods to screen and detect AmpC activity in *E. coli*. Several methods have been developed for detection the p_{AmpC} β-*lactamases* (Harwalkar et al., 2013; Yilmaz et al., 2013). Reduced sensitivity to cefoxitin (FOX) in the Enterobacteriaceae may be an indicator of *AmpC* activity. Unfortunately, FOX resistance is not only due to *pAmpC* β-*lactamase* production, but may also due to alterations to outer membrane permeability. AST of phenotypic detection result of this research work showed that 36.70% (69 of the 188) *E. coli* isolates were FOX resistant isolates were only 7.98% (15 isolates) were confirmed as *AmpC* β-*lactamase* positive by molecular confirmatory test PCR. The cause of cefoxitin (FOX) resistance phenotypes in the remaining isolates probably due to nonenzymatic-resistance mechanisms such as altered permeability (Yilmaz et al., 2013).

In recent years, besides nosocomial infection ESBL-producing *E. coli* also been reported in healthy food-producing animals as well as in feral animalsand in the environment, e.g., in aquatic systems like rivers and lakes (Zurfluh et al., 2015). The genotypic techniques enable us to validate the existence of the ESBL-producing genes. Numerous genes encoded on both chromosomal and plasmid DNA regulate the production of ESBL in bacteria. Multiplex PCR has been used to simultaneously detect two or more genes in a single isolate that were responsible for producing ESBLs. The fact that the majority of ESBL-producing bacteria frequently exhibit cross-resistance to other classes of antimicrobial drugs is cause for concern. This is because the *bla* ESBL genes conferring resistance to other antibiotic classes, like quinolones and aminoglycosides, are frequently found on conjugative plasmids and associated with horizontal gene transfer (Khan et al., 2021; Zurfluh et al., 2015).

Among all phenotypically detected isolates of *β-lactamases*, extended-spectrum β-lactamases (ESBLs) and ESBLs with inhibitor, highest prevalence of *β-lactamases* was 63.53% (n=54) revealed for bla_{TEM} genes, following bla_{SHV}gene prevalence is 18.82% (n=16) and both for bla_{TEM} and bla_{SHV} gene prevalence is 17.65% (n=15). This study revealed the

detection of bla_{TEM} gene is much higher than bla_{SHV} gene corresponding with the previous study findings in Andhra Pradesh and Chhattisgarh State (Khan et al., 2021; Sekhar et al., 2017). Also, this study reported the prevalence of p_{AmpC} *β-lactamases* for cefoxitin is 7.98% (n=15). Almost close findings of 12.5% prevalence was reported in Andhra Pradesh (Sekhar et al., 2017). The Prevalence of bla_{CTX} gene is 4.79% (n=9 isolates) is also supported by similar findings of prior studies reported in Andhra Pradesh and Burgundy, French (Bollache et al., 2019; Sekhar et al., 2017), but much higher prevalence also reported by research works performed in Assam and Eastern Province of Saudi Arabia (Elhadi & Alsamman, 2015; Sivaraman et al., 2020).The bla_{OXA-1} like, bla_{OXA-2} like, bla_{CMY-1} like, bla_{CMY-2} like and bla_{ACC-1} genes are frequently associated with genes encoding extended-spectrum β-lactamases (*ESBLs*) and suggested imparting resistance to beta -lactam or ESBLs with inhibitor combinations. Out of the 11.17 % (n=25) isolates, prevalence of bla_{OXA-1} like is 9.52 % (n=2), prevalence of bla_{OXA-2} like is 19.05 % (n=4) and prevalence of both bla_{OXA-1} like and bla_{OXA-2} like is 52.38 % (n=11). Molecular findings of the bla_{OXA} genes associated this study is supported by corresponding results revealed in Pune and Andhra Pradesh (Marathe et al., 2016; Sekhar et al., 2017). Additionally, this study revealed bla_{CMY-1} like gene prevalence is 4.76% (n=1 isolates), bla_{CMY-2} like gene prevalence is 9.52% (n=2 isolates) prevalence of both bla_{CMY-1} like and bla_{CMY-2} like gene is 23.81 % (n=5). Whereas, no amplification was detected for bla_{ACC-1} gene.

A high rate of detection of the non-β-lactam encoding genes was also observed in this study. All the genes in this category detected occurred in combination with the *β-lactamase* resistance determinants. From figure 4.19 and table 4.8, the most prevalent *non-β-lactam* resistance gene detected was *tet-A* gene in this study. Out of 63.86% (n=120) tetracyclineresistant isolates, 85.83% (n=103) isolates detected to harbored only *tet-A* gene, 2.50% (n=3) isolates detected to harbored *tet-B* gene, 3.33% (n=4) isolates detected to harbored *tet-D* gene, 2.50% (n=3) isolates detected to harbor both *tet-A*&*tet-B* genes and 5.83% (n=7) isolates detected to harbor both *tet-A*& *tet-D* genes. This study revealed no amplification of *tet-C* gene, which is corroborated by no detection of *tet-C* gene in Korea (Ryu et al., 2012). The result of *tet-A*&*tet-B* is supported by previous study performed in Assam, where *tet-A* also detected higher in rate than *tet-B* (Sivaraman et al., 2020). At the same time, a complete controversy also observed in both *tet-A* & *tet-D* genes detection, *tet-D* was detected at almost half in the rate of *tet-B* detection is Seoul, Korea (Ryu et al., 2012). But the scenario of our study, revealed presence of *tet-D* is almost double than that of *tet-D*. This may

be due to variation in geographical location, misuse or overuse of tetracyclines in fish farms, flow of antibiotic resistance genes (ARGs) and/or mutation of resistant genes in the aquatic environments (Thongkao & Sudjaroen, 2019).The widespread use of tetracycline as a first-line therapy for disease in the farming industry, including poultry and aquaculture, has been partially blamed for the pattern of high tetracycline resistance by pathogens over the world (Odumosu et al., 2021). Following the trends of *non-β-lactam*s resistance genes, *sul-2* detected in 70.09% (n=82) isolates as the second most prevalent *non-β-lactams* resistance genes following both *sul-1*&*sul-2* genes was detected in 19.66% (n=23 isolates), whereas *sul-1* genes was detected only in 10.26% (n=12) isolates out of 62.23% (n=117) sulfonamides phenotypically resistant isolates.

Genotypic resistance patterns revealed the coexistence of resistance genes of *β-lactam* encoding genes, $P_{ampC,}$ *ESBLs, non-β-lactams* encoding genes in same or altered fish *E. coli* isolates. Maximum resistant patters were unique. Highest seven resistance gene including bla_{TEM}, P_{ampC}, bla_{CTX}, bla_{CMY-1} like, bla_{CMY-2} like, *tet-D, sul-2* noticed in one isolate following six resistance genes detected in six isolates. Genotypic resistance patterns observed most frequently in shrimp and commercial fish rather than seafoods. Finally, all these observation makes a conclude with accomplishing this research work hypothesis.

However, MDR *E. coli* in fish samples may beattributed due to the extensive use of various antibiotics to treat and prevent diseases in aquaculture (Elhadi & Alsamman, 2015; Preena et al., 2020a). The emergence of *E. coli* strains resistant to fluoroquinolones and cephalosporins, which arecommonly used to treat bacterial infections in humans, could pose a significant challenge to both animal and human treatment efforts and have serious public health implications (Preena et al., 2020b). Finally, apropos to effectively address the issues regular tracking of antibiotic resistance concerning MDR *E. coli* in fish, proper patterns, and efforts to reduce empirical monitoring and prudent use of antibiotics, antibiotic therapy are all crucial.

Chapter-6: Conclusions and Recommendations

This research work, which was conducted for the first time in Bangladesh, presents recent information about multidrug resistance and antimicrobial-resistant genotypes in MDR *E. coli* isolates from fish samples at wet market level, despite the fact that the study site limited only to Chattogram.

This research work revealed a significant prevalence of *E. coli* isolates is 41.78% and MDR *E. coli* isolates is 38.67% wherein 83.51 % isolates characterized with the presence of antimicrobial resistance genes in fish samples. The multi-drug resistance pattern represents the co-resistance towards three or more antimicrobial groups interpreting 92.55% *E. coli* isolates as MDR. The high rates of MARI of this research work, averaging a worrisome index value of 0.50 in fish *E. coli* isolates also suggest the overuse of antimicrobials in fish production and aquaculture.

Antibiotic resistance genotype patterns highlight the co-existence of antibiotic resistance genes including β-lactam encoding genes, $P_{ampC,}$ ESBLs, non-β-lactam encoding genes in same or altered isolates, as highest coexistence of seven antibiotic resistance genes was detected in one isolate. Maximum seven resistance gene including bla_{TEM}, P_{ampC}, bla_{CTX}, bla_{CMY-1} like group, bla_{CMY-2} like group, *tet-D, sul-2* was noticedin one isolate. Among antibiotic resistance gene prevalence of non-βlactam encoding gene *tet-A* is highest 85.83%, following *sul-2* is 70.09%. Merely, bla_{TEM} is most prevailing among all β-lactam encoding gene with a prevalence of 63.53% following the prevalence of co-existence of the bla_{OXA-1} like group & bla_{OXA-2} like group is 52.38 % detected as ESBLs. Though, no amplification of *tet-C* and bla_{ACC-1} detected.

Therefore, efforts areneeded to prevent the widespread, intensive and unregulated use of antimicrobial agents in the aquaculture zone. National and international cooperation from organizations like the WHO and FAO is alsorequired to educate farmers, establish capacity and implementation of MDR preventive measures in fish farming and aquaculture. The use of contaminated water sources for aquaculture should be avoided through adequate treatment. Hygienic procedures should be promoted among workers of fish farms and fish handlers in wet markets while loading-unloading, handling, shipping, transportation and processing of fish to lessen the risk of cross-contamination. Governmental organizations must also build up hazard analysis and crucial control point systems to continuously monitor the quality of fish provided to the community. Another suggestion is that antibiotic surveillance programs must beensured by

policymakers and appropriate management practices must be implemented in aquaculture settings. AMR stewardship programs may also beimplemented through campaigns and training programs. This will help to mitigate the potential risks associated with the spread of MDR *E. coli* in aquatic environments and food-fish chains, and to ensure food safety and public health.

Once for all, the findings of this study strongly remark that an effective, consistent and unified approach based on the public health ethics to combat MDR *E. coli* emergence in fish population is the global appalling challenge of One-Health interest.

Chapter-7: Limitations

Leading drawback of studying the epidemiology and molecular characterization of MDR *E. coli* in fish is the lack of available data and research on this topic. This makes it difficult to draw conclusions and make informed decisions about the prevalence and impact of MDR *E. coli* in fish populations. Also, Due to resource constraints we were unable to undertake further genotyping tests such as sequencing and typing the isolates may be required for better understanding of the genetic relatedness and molecular epidemiology of resistant genes of our interest.

Chapter-8: Appendix

Appendix-A

8.1 Buffered peptone water (Himedia)

Composition	Gm./Liter
Proteose peptone	10.0
Sodium chloride	5.0
Disodium hydrogen phosphate	3.5
Potassium hydrogen phosphate	1.5

*****Formula adjusted with a final pH of 7.2±0.2 (at 25°C)

Suspend 20.0 grams in 1000 ml purified/distilled water. Heat if necessary to dissolve the medium completely. Dispense in 50 ml amounts into tubes or flasks or as desired. Sterilize by autoclaving at 15lbs pressure (121°C) for 15 minutes.

8.2 Culture media for isolation of *E. coli*

8.2.1 Composition of MacConkey agar (Himedia)

Composition	Gm. /Liter
Peptone	20.00
Lactose	10.0
Bile Salts	5.0
Sodium chloride	5.0
Neutral red	0.070
Agar	15.00

*****Formula adjusted with a final pH of 7.5±0.2 (at 25°C)

Suspend 55.07 grams in 1000 ml distilled water. Heat to boiling with gentle swirling to dissolve the medium completely. Sterilize by autoclaving at 15 lbs pressure (121°C) for

15 minutes. Avoid overheating. Cool to 45-50°C and pour into sterile Petri plates. The surface of the medium should be dry when inoculated.

8.2.2 Composition of Eosin-Methylene Blue (EMB) agar (Oxoid)

Composition	Gram / Liter
Peptone	10.0
Lactose	10.0
Dipotassium hydrogen phosphate	2.0
Eosin Y	0.4
Methylene blue	0.065
Agar	15.0

*****Formula adjusted with a final pH of 7.1±0.2 (at 25°C)

Suspend 37.5g in 1 liter of distilled water. Bring to the boil to dissolve completely. Sterilize by autoclaving at 121°C for 15 minutes. Cool to 60°C and shake the medium in order to oxidize the methylene blue (i.e. restore its blue color) and to suspend the precipitate which is an essential part of the medium**.**

8.2.3 Composition of Violet Red Bile (VRB) agar (Himedia)

Composition	Gram / Liter
Peptone	7.00
Yeast Extract	3.00
Lactose	10.00
Bile Salt Mixture	1.50
Sodium Chloride	5.00
Neutral Red	0.030

Crystal Violet	0.002
agar	15.00

*****Formula adjusted with a final pH of 7.4±0.2 (at 25°C)

Suspend 41.53 grams in 1 liter of distilled water. Bring to the boil to dissolve completely. Sterilize by autoclaving at 121°C at 15lbs for 15 minutes. Cool to 45°C and pour with inoculum (for colony counting) or without inoculum for striking suspected colony on solid media surface. VRB agar relies on the use selective inhibitory components crystals violet and bile salts, along with the indicator system lactose and neutral red.

8.2.4 Composition of blood agar medium (Himedia)

Composition	Gm. /Liter
HM Peptone B#	10.0
Tryptose	10.0
Sodium chloride	5.0
Agar	15.0

*****Formula adjusted with a final pH of 7.3±0.2 (at 25°C) # Equivalent to beef heart extract.

Suspend 40grams of grams in 1 liter of purified/distilled water Heat to boiling to dissolve the medium completely. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes. Cool to 45-50°C and aseptically add 5% v/v sterile defibrinated blood. Mix well and pour into sterile Petri plates. After that, allowed to solidify and sterile blood agar medium plates were kept at 4°C till further use.

8.3 Broth used for preservation of *E. coli* isolates

8.3.1 Composition of Brain Heart Infusion (BHI) Broth

Composition	Gram / Liter

HM infusion powder #	12.50
BHI powder	5.00
Proteose peptone	10.00
Dextrose (Glucose)	2.00
Sodium chloride	5.00
Disodium phosphate	2.50

*****Formula adjusted with a final pH of 7.4±0.2 (at 25°C)

Equivalent to Calf brain infusion from.

Suspend 37.0 grams in 1000 ml distilled water. Dispense into bottles or tubes and sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes. For best results, the medium should be used on the day it is prepared, otherwise, it should be boiled or steamed for a few minutes and then cooled before use.

8.4 Reagents used in antimicrobial sensitivity testing

8.4.1 0.5 McFarland Standard

A McFarland Standard is a chemical solution of 1% barium chloride (BaCl2) and 1% sulfuric acid (H2SO4) Solution in appropriate proportion; the reaction between these two chemicals results in the turbid solution which is due to the production of a fine precipitate of barium sulfate (BaSO4). Thus, prepared turbid solution is used as a standard solution to which the cultures bacterial suspensions are compared and standardized.

Most commonly 0.5 McFarland Solution is used as a standard for Antibiotic sensitivity test (AST) which is prepared by mixing 0.05 ml (or 50 µl) BaCl2 in 9.95 ml of 1% H2SO4 solution. Mix well the prepared solution to form a turbid suspension.

8.4.1.1 Procedure for the preparation of 0.5 McFarland Standard

⇒ Prepare a 1% Barium Chloride (BaCl2) solution by mixing 1gram of anhydrous barium chloride (BaCl2) in 100 ml Distilled water.

⇒ Also, prepare a 1% Sulfuric Acid (H2SO4) solution by mixing 1 ml of concentrated H2SO4 in 99 ml of Distilled water.

⇒ Now, mix well the 1% Barium chloride (BaCl2) & 1% Sulfuric acid (H2SO4) in an appropriate proportion as per the concentration required.

8.4.2 Composition of Muller Hinton agar (Himedia)

Composition	Gram / Liter
HM infusion solids B #	2.00
Acicase ##	17.50
Starch	1.50
Agar	17.00

*****Formula adjusted with a final pH of 7.4±0.1 (at 25°C)

Equivalent to Beef heart infusion

Equivalent to Casein acid hydrolysate

Suspend 38.0 grams in 1000 ml purified / distilled water. Heat to boiling to dissolve the medium completely. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes. Cool to 45-50°C. Mix well and pour into sterile Petri plates.

8.5 Reagents used for molecular characterization of *E. coli*

8.5.1 Ethidium bromide (0.625 mg/ml)

Ready to use, 5ml (294.32g/mole) dropped bottle of "Thermo Scientific" was used. The bottle was stored at room temperature in a supplied amber colored container.

8.5.2 Composition of Tris-Acetate-EDTA (TAE), stock solution (50X)

Tris base	121.0 g
Glacial acetic acid	28.5 ml
EDTA (0.5 M, pH 8.0)	50.0 ml
Distilled water (DW)	500 ml

For working solution (1X), stock solution was diluted fifty times in distilled water. Both working and loading solution stored at room temperature until use.

8.5.3 Composition of Loading Dye (6X)

Sucrose	40% w/v in DW
Bromophenol blue	0.25% w/v in DW
Xylene cyanole	0.25% w/v in DW

For working dye (1X), was prepared by proportionate mixing of 1 part of 6X loading dye: 1part of gene ruler :4 parts of nuclease free water. Both working and loading dye stored at 4°C until use.

Appendix-B

Table-4.5. Multidrug resistance patterns of antimicrobial classes from *E. coli* isolates of fish samples

No. of resistance antimicrobial class	MDR Pattern	n (%), 95%CI	*P*
3	β-lactams,1st gen cephalosporins, Quinolones and fluoroquinolones	1 (0.76%), (0.02%-4.18%)	0.000
4	1st gen cephalosporins, Tetracyclines, Aminoglycosides, Polymyxins	1 (0.76%), (0.02%-4.18%)	
	1st gen cephalosporins, Tetracyclines, Aminoglycosides, Sulfonamides	1 (0.76%), (0.02%-4.18%)	
	1st gen cephalosporins,2nd gen cephalosporins, Quinolones and fluoroquinolones, Polymyxins	1 (0.76%), (0.02%-4.18%)	
	β-lactams,1st gen cephalosporins, Tetracyclines, Aminoglycosides,	1 (0.76%), (0.02%-4.18%)	
	β-lactams,1st gen cephalosporins, Tetracyclines, Sulfonamides	3(2.29%), (0.47%-6.55%)	
	β-lactams,1st gen cephalosporins, Aminoglycosides, Quinolones and fluoroquinolones	1 (0.76%), (0.02%-4.18%)	
	β-lactams, Tetracyclines, Sulfonamides, Amphenicols	1 (0.76%), (0.02%-4.18%)	
	β-lactams, Tetracyclines, Aminoglycosides, Polymyxins	1 (0.76%), (0.02%-4.18%)	

	β-lactams,1st gen cephalosporins,2nd gen cephalosporins, Quinolones and fluoroquinolones	1 (0.76%), (0.02%-4.18%)	
5	β-lactams,2nd gen cephalosporins, Tetracyclines, Quinolones and fluoroquinolones, Sulfonamides	1 (0.76%), (0.02%-4.18%)	
	1st gen cephalosporins, Tetracyclines, Aminoglycosides, Sulfonamides, Amphenicols	1 (0.76%), (0.02%-4.18%)	
	β-lactams,1st gen cephalosporins, Tetracyclines, Aminoglycosides, Polymyxins	1 (0.76%), (0.02%-4.18%)	
	β-lactams,1st gen cephalosporins,2nd gen cephalosporins, aminoglycosides, Quinolones and fluoroquinolones	1 (0.76%), (0.02%-4.18%)	
	β-lactams,1st gen cephalosporins, Aminoglycosides, Sulfonamides, Polymyxins	2 (1.53%), (0.19%-5.41%)	
	1st gen cephalosporins, Tetracyclines, Quinolones and fluoroquinolones, Sulfonamides, Polymyxins	1 (0.76%), (0.02%-4.18%)	
	β-lactams,1st gen cephalosporins,Tetracyclines,Aminoglycosides,Sulfonamides	1 (0.76%), (0.02%-4.18%)	
	β-lactams,1st gen cephalosporins,Tetracyclines,Quinolones and fluoroquinolones,Sulfonamides	2 (1.53%), (0.19%-5.41%)	
	β-lactams,1st gen cephalosporins,Quinolones and fluoroquinolones,Sulfonamides,Amphenicols	1 (0.76%), (0.02%-4.18%)	

	β-lactams,1st gen cephalosporins,Sulfonamides,Polymyxins, Amphenicols	1 (0.76%), (0.02%-4.18%)	
	β-lactams,1st gen cephalosporins, Tetracyclines,Aminoglycosides,Quinolones and fluoroquinolones	1 (0.76%), (0.02%-4.18%)	
	β-lactams,1st gen cephalosporins,Tetracyclines,Sulfonamides,Polymyxins	2 (1.53%), (0.19%-5.41%)	
	β-lactams, Tetracyclines,Aminoglycosides,Sulfonamides,Amphenicols	1 (0.76%), (0.02%-4.18%)	
	β-lactams,1st gen cephalosporins,Aminoglycosides,Sulfonamides,Amphenicols	1 (0.76%), (0.02%-4.18%)	
	β-lactams, Aminoglycosides,Sulfonamides,Polymyxins,Amphenicols	1 (0.76%), (0.02%-4.18%)	
	β-lactams,1st gen cephalosporins,Tetracyclines,Sulfonamides,Amphenicols	3(2.29%), (0.47%-6.55%)	
	β-lactams,Tetracyclines,Quinolones and fluoroquinolones,Sulfonamides,Amphenicols	1 (0.76%), (0.02%-4.18%)	
	β-lactams,1st gen cephalosporins,2nd gen cephalosporins,Quinolones and fluoroquinolones,Amphenicols	1 (0.76%), (0.02%-4.18%)	
6	2nd gen cephalosporins,Tetracyclines,Aminoglycosides,Quinolones and fluoroquinolones,Sulfonamides,Polymyxins	1 (0.76%), (0.02%-4.18%)	
	1st gen cephalosporins, Tetracyclines,Aminoglycosides,Sulfonamides,Polymyxins,Amphenicols	1 (0.76%), (0.02%-4.18%)	

	1st gen cephalosporins,2nd gen cephalosporins,Tetracyclines,Quinolones and fluoroquinolones,Sulfonamides,Polymyxins	1 (0.76%), (0.02%-4.18%)	
	β-lactams,1st gen cephalosporins,2nd gen cephalosporins,Aminoglycosides,Quinolones and fluoroquinolones,Polymyxins	1 (0.76%), (0.02%-4.18%)	
	β-lactams,1st gen cephalosporins,ESBLs,Tetracyclines,Quinolones and fluoroquinolones,Polymyxins	1 (0.76%), (0.02%-4.18%)	
	β-lactams, Tetracyclines, Aminoglycosides,Sulfonamides,Polymyxins,Amphenicols	1 (0.76%), (0.02%-4.18%)	
	1st gen cephalosporins, Tetracyclines,Polymyxins,Sulfonamides,Polymyxins,Amphenicols	1 (0.76%), (0.02%-4.18%)	
	β-lactams,1st gen cephalosporins, Tetracyclines,Aminoglycosides,Sulfonamides,Polymyxins,	2 (1.53%), (0.19%-5.41%)	
	β-lactams,1st gen cephalosporins,Tetracyclines,Aminoglycosides,Quinolones and fluoroquinolones,Sulfonamides	1 (0.76%), (0.02%-4.18%)	
	β-lactams,1st gen cephalosporins,Tetracyclines,Aminoglycosides,Sulfonamides,Amphenicols	4(3.05%), (0.84%-7.63%)	
	β-lactams,1st gen cephalosporins,Aminoglycosides,Quinolones and fluoroquinolones,Sulfonamides,Amphenicols	1 (0.76%), (0.02%-4.18%)	

	β-lactams,1st gen cephalosporins, Tetracyclines,Sulfonamides,Polymyxins,Amphenicols	2 (1.53%), (0.19%-5.41%)	
	β-lactams,1st gen cephalosporins,2nd gen cephalosporins,ESBLs,Quinolones and fluoroquinolones,Polymyxins	1 (0.76%), (0.02%-4.18%)	
	β-lactams,1st gen cephalosporins,Quinolones and fluoroquinolones,Sulfonamides,Polymyxins,Amphenicols	1 (0.76%), (0.02%-4.18%)	
	β-lactams, 1st gen cephalosporins,Tetracyclines,Quinolones and fluoroquinolones, Sulfonamides,Polymyxins,	2 (1.53%), (0.19%-5.41%)	
	β-lactams,2nd gen cephalosporins,Tetracyclines,Aminoglycosides,Quinolones and fluoroquinolones,Polymyxins	1 (0.76%), (0.02%-4.18%)	
	β-lactams,1st gen cephalosporins,2nd gen cephalosporins,Tetracyclines,Quinolones and fluoroquinolones,Sulfonamides	1 (0.76%), (0.02%-4.18%)	
	β-lactams,1st gen cephalosporins,ESBLs,Tetracyclines,Quinolones and fluoroquinolones,Sulfonamides	2 (1.53%), (0.19%-5.41%)	
	β-lactams,1st gen cephalosporins,2nd gen cephalosporins,Quinolones and fluoroquinolones,Sulfonamides,Amphenicols	1 (0.76%), (0.02%-4.18%)	
	β-lactams, 1st gen cephalosporins,ESBLs,Tetracyclines,Sulfonamides,Polymyxins,	1 (0.76%), (0.02%-4.18%)	
7	β-lactams,1st gen cephalosporins,2nd gen cephalosporins,Tetracyclines,Aminoglycosides,Quinolones and fluoroquinolones,Sulfonamides	2 (1.53%), (0.19%-5.41%)	

	β-lactams,1st gen cephalosporins,Tetracyclines,Aminoglycosides,Sulfonamides,Polymyxins,Amphenicols	6 (4.58%), (1.70%-9.70%)	
	β-lactams, 1st gen cephalosporins,2nd gen cephalosporins,Tetracyclines,Aminoglycosides,Quinolones and fluoroquinolones,Polymyxins	2 (1.53%), (0.19%-5.41%)	
	β-lactams,1st gen cephalosporins,Tetracyclines,Aminoglycosides,Quinolones and fluoroquinolones,Sulfonamides, Amphenicols	1 (0.76%), (0.02%-4.18%)	
	β-lactams,1st gen cephalosporins,2nd gen cephalosporins,Tetracyclines,Aminoglycosides,Quinolones and fluoroquinolones,Sulfonamides	2 (1.53%), (0.19%-5.41%)	
	β-lactams, 2nd gen cephalosporins,Tetracyclines,Aminoglycosides,Quinolones and fluoroquinolones,Sulfonamides,Polymyxins	1 (0.76%), (0.02%-4.18%)	
	β-lactams, 1st gen cephalosporins,Tetracyclines,Aminoglycosides,Quinolones and fluoroquinolones, Sulfonamides,Polymyxins,	1 (0.76%), (0.02%-4.18%)	
	β-lactams,1st gen cephalosporins,2nd gen cephalosporins,ESBLs,Aminoglycosides,Quinolones and fluoroquinolones,Amphenicols	1 (0.76%), (0.02%-4.18%)	
8	β-lactams,1st gen cephalosporins,Tetracyclines,Aminoglycosides,Quinolones and fluoroquinolones,Sulfonamides,Polymyxins,Amphenicols	3(2.29%), (0.47%-6.55%)	
	β-lactams,1st gen cephalosporins,2nd gen cephalosporins,Tetracyclines,Aminoglycosides,Quinolones and fluoroquinolones,Sulfonamides,Polymyxins	2 (1.53%), (0.19%-5.41%)	

	β-lactams,1st gen cephalosporins,2nd gen cephalosporins,ESBLs,Tetracyclines,Quinolones and fluoroquinolones,Polymyxins, Amphenicols	1 (0.76%), (0.02%-4.18%)	
	β-lactams, 1st gen cephalosporins,ESBLs,Tetracyclines,Aminoglycosides,Quinolones and fluoroquinolones,Sulfonamides,Amphenicols	1 (0.76%), (0.02%-4.18%)	
	β-lactams,1st gen cephalosporins,2nd gen cephalosporins,Tetracyclines,Aminoglycosides,Quinolones and fluoroquinolones,Polymyxins, Amphenicols	1 (0.76%), (0.02%-4.18%)	
	β-lactams,1st gen cephalosporins,ESBLs,Tetracyclines,Quinolones and fluoroquinolones,Sulfonamides,Polymyxins, Amphenicols	1 (0.76%),(0.02%-4.18%)	
	β-lactams,1st gen cephalosporins,2nd gen cephalosporins,Tetracyclines,Aminoglycosides,Quinolones and fluoroquinolones,Sulfonamides,Polymyxins	1 (0.76%), (0.02%-4.18%)	
	β-lactams,1st gen cephalosporins,ESBLs,Tetracyclines,Aminoglycosides,Quinolones and fluoroquinolones,Sulfonamides,Polymyxins,	1 (0.76%), (0.02%-4.18%)	
	β-lactams,1st gen cephalosporins,2nd gen cephalosporins,ESBLs,Tetracyclines,Quinolones and fluoroquinolones,Polymyxins, Amphenicols	1 (0.76%), (0.02%-4.18%)	
	β-lactams,1st gen cephalosporins,2nd gen cephalosporins,Tetracyclines,Quinolones and fluoroquinolones,Sulfonamides,Polymyxins, Amphenicols	1 (0.76%), (0.02%-4.18%)	

	β-lactams, 2nd gen cephalosporins,ESBLs,Tetracyclines,Aminoglycosides,Quinolones and fluoroquinolones,Sulfonamides, Amphenicols	2 (1.53%), (0.19%-5.41%)	
	β-lactams, 1st gen cephalosporins,2nd gen cephalosporins,ESBLs,Aminoglycosides,Quinolones and fluoroquinolones,Polymyxins, Amphenicols	2 (1.53%), (0.19%-5.41%)	
	β-lactams,1st gen cephalosporins,2nd gen cephalosporins,ESBLs,ESBLs+ inhibitor,Aminoglycosides,Quinolones and fluoroquinolones,Amphenicols	1 (0.76%), (0.02%-4.18%)	
	β-lactams,1st gen cephalosporins,2nd gen cephalosporins,Tetracyclines,Aminoglycosides,Quinolones and fluoroquinolones, Sulfonamides ,Amphenicols	1 (0.76%), (0.02%-4.18%)	
9	β-lactams,1st gen cephalosporins,2nd gen cephalosporins,Tetracyclines,Aminoglycosides,Quinolones and fluoroquinolones,Sulfonamides,Polymyxins,Amphenicols	3(2.29%), (0.47%-6.55%)	
	β-lactams,1st gen cephalosporins,ESBLs,Tetracyclines,Aminoglycosides,Quinolones and fluoroquinolones,Sulfonamides,Polymyxins, Amphenicols	1 (0.76%), (0.02%-4.18%)	
	β-lactams,2nd gen cephalosporins,ESBLs,Tetracyclines,Aminoglycosides,Quinolones and fluoroquinolones,Sulfonamides,Polymyxins, Amphenicols	2 (1.53%), (0.19%-5.41%)	
	β-lactams,1st gen cephalosporins,2nd gen cephalosporins,ESBLs,Tetracyclines,Aminoglycosides,Quinolones and fluoroquinolones,Polymyxins, Amphenicols	3 (1.53%), (0.19%-5.41%)	

	β-lactams,1st gen cephalosporins,2nd gen cephalosporins,ESBLs,Tetracyclines,Aminoglycosides,Quinolones and fluoroquinolones,Sulfonamides,Polymyxins,	4 (1.53%), (0.19%-5.41%)	
	β-lactams, 1st gen cephalosporins,2nd gen cephalosporins,Tetracyclines,Aminoglycosides,Quinolones and fluoroquinolones, Sulfonamides,Polymyxins, Amphenicols	1 (0.76%), (0.02%-4.18%)	
	β-lactams, 1st gen cephalosporins,ESBLs,ESBLs+ inhibitor,Tetracyclines,Aminoglycosides,Quinolones and fluoroquinolones,Sulfonamides,Polymyxins,	1 (0.76%), (0.02%-4.18%)	
	β-lactams, 1st gen cephalosporins,2nd gen cephalosporins,ESBLs,Tetracyclines,Quinolones and fluoroquinolones,Sulfonamides,Polymyxins, Amphenicols	1 (0.76%), (0.02%-4.18%)	
	β-lactams,1st gen cephalosporins,2nd gen cephalosporins,ESBLs,ESBLs+ inhibitor,Tetracyclines,Quinolones and fluoroquinolones,Polymyxins,Amphenicols	1 (0.76%), (0.02%-4.18%)	
	β-lactams,1st gen cephalosporins,2nd gen cephalosporins,ESBLs,Tetracyclines,Aminoglycosides,Quinolones and fluoroquinolones,Sulfonamides,Polymyxins, Amphenicols	1 (0.76%), (0.02%-4.18%)	
	β-lactams, 1st gen cephalosporins,ESBLs,ESBLs+ inhibitor,Tetracyclines,Aminoglycosides,Quinolones and fluoroquinolones, Sulfonamides, Amphenicols	1 (0.76%), (0.02%-4.18%)	

10	β-lactams,1st gen cephalosporins,ESBLs,ESBLs+ inhibitor,Tetracyclines,Aminoglycosides,Sulfonamides,Polymyxins, Amphenicols	1 (0.76%), (0.02%-4.18%)	
	β-lactams,1st gen cephalosporins,2nd gen cephalosporins,ESBLs,ESBLs+ inhibitor,Tetracyclines,Aminoglycosides,Quinolones and fluoroquinolones,Polymyxins, Amphenicols	1 (0.76%), (0.02%-4.18%)	
	β-lactams,1st gen cephalosporins,2nd gen cephalosporins,ESBLs,ESBLs+ inhibitor,Tetracyclines,Aminoglycosides,Quinolones and fluoroquinolones,Sulfonamides,Polymyxins	1 (0.76%), (0.02%-4.18%)	
	β-lactams, 1st gen cephalosporins,2nd gen cephalosporins,ESBLs,Tetracyclines,Aminoglycosides,Quinolones and fluoroquinolones, Sulfonamides,Polymyxins, Amphenicols	4(3.05%), (0.84%-7.63%)	
	β-lactams, 1st gen cephalosporins,2nd gen cephalosporins,ESBLs,ESBLs+ inhibitor,Tetracyclines,Quinolones and fluoroquinolones,Sulfonamides,Polymyxins, Amphenicols	2 (1.53%), (0.19%-5.41%)	
11	β-lactams, 1st gen cephalosporins,2nd gen cephalosporins,ESBLs,ESBLs+ inhibitor,Tetracyclines,Aminoglycosides,Quinolones and fluoroquinolones,Sulfonamides,Polymyxins,Amphenicols	9 (6.87%), (3.19%-12.64%)	
	Total number of MDR Patterns observed	**131**	

Chapter-9: References

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Chapter-10: Brief Biography

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