CHAPTER-I

1.1. Introduction

Antimicrobial resistance (AMR) is a concern to human and animal health on a worldwide scale. In 2013, the World Economic Forum identified AMR as one of the biggest global risks and stated that: "While viruses may capture more headlines, arguably the greatest risk of hubris to human health comes in the form of antibiotic-resistant bacteria". The EU has also accounted the problem of AMR by establishing an EU-wide control strategy for antimicrobial resistance through particular action plans. Particularly worrisome is the widespread use of antibiotics in animal agriculture including aquaculture, which has the potential to create resistance in both animals and humans. The European Medicines Authority (EMA) releases an annual report on the sales of veterinary antimicrobial medications in 25 countries. Antimicrobial Resistance (AMR) diminishes the efficacy of antibiotics, making treatment more complicated, time-consuming, costly, and even impossible. It has been estimated that if proper actions are not taken to solve the difficulties, AMR would result in a worldwide disaster with 10 million fatalities each year, a terrible economic cost of 100 trillion USD, and an 11% decline in animal output by 2050 (O'Neill, 2016).

The World Health Organization (WHO) now recognizes it as a serious developing worldwide concern. Since 1987, there have been no successful discoveries of new antibiotic classes. There is a dearth of antibiotic discovery, and it is now commonly recognized that the development of new antimicrobial drugs is an immediate necessity. AMR is defined as the indifference of microorganisms to clinically relevant antimicrobial medicines at normal dosages (Ganguly *et al*., 2011).

Since the discovery of the first antibiotic, resistance to antimicrobials has been considered a natural process in which microorganisms adapt to resist the effects of medications (Annunziato *et al*., 2019). But AMR has progressively become a catastrophic setback in recent years due to the imbalance between the overuse of antimicrobials and the absence of new antibiotic development to combat these new resistance bacteria (Jackson *et al*., 2018).

Food-borne pathogens have been the paramount cause of illness and death in the world. As they affect the health and economy, the awareness on food-borne pathogens is increasing. Poultry and other meats also occupy one of the most important reservoirs for pathogenic bacteria. Normally, the meat of healthy animals contains very few or nil microorganisms, but contamination arise from slaughtering, transportation, and

processing. The most important food-borne bacteria transmitted through meat include *Salmonella, Shigella, Staphylococcus aureus (S. aureus), Escherichia coli (E. coli), Campylobacter jejuni (C. jejuni), Listeria monocytogenes (L. monocytogenes), Clostridium perfringens, Yersinia enterocolitica* and *Aeromonas hydrophila*. These bacteria usually cause self-limiting gastroenteritis; however, invasive diseases and various complexities also may occur (Kamana Bantawa *et al.*, 2019).

E. *coli* may cause bloody diarrhea and hemolytic uremic syndrome in humans, is a member of the Enterobacteriaceae family, is a common microbiological contamination of supermarket chicken meat. *E. coli* is also well-known as one of the most significant foodborne pathogens in humans, which has been linked to a variety of acute and invasive diseases in people, and it is easily dispersed throughout many habitats and transmits mainly through the food chain. It is a highly adaptable bacterial species that includes both non-pathogenic strains and many pathogenic variations capable of causing intestinal or extra intestinal illnesses. Some strains of poultry-derived *E. coli* can be opportunistic and pathogenic in nature. The majority of *E. coli* strains are non-pathogenic in humans (e.g., uncomplicated urinary tract infections) or exist as part of the indigenous flora, often contributing to the vital functions performed by the intestinal microflora (e.g., bloodstream infections). During handling, incorrect preparation, cleaning, and unclean meat-selling methods, chicken flesh is commonly infected with E. coli. Chicken flesh is a possible cause of *E. coli* infection in humans, either through direct contact during food preparation or by the intake of under-cooked or raw meat products. Although *E. coli* is heat sensitive at temperatures between 60 and 80^{0}C, certain strains of the bacteria have been observed to be very resistant to heat. Salmonella can cause systemic salmonellosis, whereas *S. aureus* causes food poisoning in humans (Kamana Bantawa *et al.*, 2019; Mst. Sonia Parvin *et al*., 2020). *S. aureus*, a Gram-positive and catalase-positive bacteria, is regarded as a significant source of food-borne infections characterized by a brief incubation period, weakness, vomiting, nausea, abdominal cramps, and toxic shock syndrome. In addition, it has been observed that *S. aureus* bacteria isolated from foods of animal origin have a high rate of resistance to a variety of antibiotics, including penicillins, cephalosporins, tetracyclines, aminoglycosides, macrolides, and fluoroquinolones. *S. aureus* has been identified in foods that are raw or undercooked (Zeinab Torki Baghbaderani *et al*., 2018).

The growth of *S. aureus* resistance to antibiotics poses a significant danger to world health and is a major cause for worry, posing a formidable challenge to the veterinary and public

health professionals as well as dairy cow farmers due to their harmful influence on treatment (Brouillette *et al*., 2005). Microbes have already developed resistance to several antimicrobial drugs, including aminoglycosides, macrolides, glycopeptides, fluoroquinolones, and tetracyclines (Rahi *et al.*, 2020). Multiple genes for antibiotic resistance have a role in *S. aureus*, such as macrolide resistance produced by the erm genes (*ermB, ermC*) for gentamicin and tetracycline (*tetK and tetM*) (Hasanpour *et al*., 2017; Momtaz *et al*., 2013; Qae *et al.*, 2015).

Multiple transmission vehicles, including food items, have been linked to the human transmission of *Campylobacter* species (Jorgensen *et al*., 2002). The most important documented risk factors were the ingestion and/or handling of raw or undercooked chicken or other meats, raw milk, and surface waters. Cross-contamination of ready-to-eat items during meal preparation and direct animal interaction have both been reported (Anonymous, 1994; Tompkin, 1994; Adak *et al*., 1995). Food animals may be asymptomatic *Campylobacter* intestinal carriers, and animal food items might get infected with this bacterium during slaughter and carcass preparation (Berndtson *et al*., 1996; Whyte *et al*., 2003). It is now generally acknowledged that campylobacteriosis is obtained mostly through the ingestion of contaminated foods (Anonymous, 1995). Wide differences in *Campylobacter* prevalence have been recorded in both live animals and foods derived from animals. *Campylobacter jejuni* is a common cause of diarrhea/dysentery in children, which is frequently associated with maintaining pets and consuming chicken meat, as well as drinking unclean water (Ali *et al.*, 2003). Previously reported infection rates in live broilers varied from zero to one hundred percent (Bryan and Doyle, 1995; Moore *et al*., 2003), whereas the frequency in cattle was as high as sixty percent (Orr *et al*., 1995; Neilson *et al*., 1997).

Prevalence of up to 100% has also been found on dressed chicken carcasses (Waldroup *et al*., 1992; Attanasova and Ring, 1999; Dominguez *et al*., 2002), although a much lower prevalence of the organism is often reported in beef carcasses (Kwiatek *et al*., 1990; Zanetti *et al.*, 1996; Madden *et al*., 2001). Additionally, *Campylobacter* has been isolated from raw milk and milk products (Rohrbach *et al*., 1992; Lacerc *et al*., 2002).

The need for food derived from animals is expanding rapidly. With this increasing demand, the worldwide market value of veterinary pharmaceuticals increased from 8.65 billion dollars in 1992 to 20 billion dollars in 2010 and is anticipated to surpass 42.9 billion dollars in 2018 (Haoetal., 2014). The development and risk of resistant bacterial strains being

transmitted from animals to humans might increase according to the increased use of antimicrobials and therefore, AMR in animals is the top concern (Loo *et al*., 2019).

In dairy cow production, drug-resistant strains of animal origin may transfer to humans via the food supply chain (meat and dairy products), direct animal contact, or environmental pathways (Lhermie *et al*., 2017). In addition, people may be exposed to resistant strains and genes by ingestion of contaminated food products, including meat, unpasteurized milk, and milk derivatives, or via environmental transmission, such as animal waste and run off water from agricultural locations (Loo *et al*., 2019; Ayukekbong *et al*., 2017).

The possible spread of multidrug-resistant (MDR) bacteria, which have demonstrated resistance to several antibiotics, is a further cause for concern. MDR bacteria can resist antibiotics through the acquisition of resistance genes, spontaneous mutation, and the dissemination of resistance genes via mobile genetic elements (i.e., plasmid, transposon, and insertion sequences). In order to boost the efficiency with which they collect and disseminate their resistance determinants to other bacteria, these bacteria produce an integron element, a specialized component. Despite the fact that integrons are not categorized as mobile genetic elements, they have been proven to be connected with other mobile genetic elements (e.g., plasmids) and to promote fast transmission across pathogenic and commensal bacteria. Although commensal bacteria may not directly cause disease, their significance as a reservoir of resistance genes is a cause for worry. Moreover, clonal spread is largely responsible for the spread of antibiotic-resistant bacteria. *Salmonella typhimurium*, definitive phage type 104 (DT 104) is a well-known case that originated in cattle in the 1990s and spread internationally (Chaiyaporn Chaisatit *et al*., 2012).

Considering the above facts, we aimed to identify the resistant bacteria, MDR bacteria and resistant genes in animal originated foods, particularly in meat and milk of different animal species.

1.2. Specific Objectives:

(1) To estimate the prevalence of different bacterial isolates from meat and milk.

(2) To determine the antimicrobial sensitivity/resistance pattern of each isolate.

(3) To Identify the resistance genes.

CHAPTER - II
REVIEW OF LITERATURE

2.1. Public Health Crisis

Resistance of bacteria to antimicrobials is a "public health crisis" and "one of our biggest health challenges" (U.S. Center for Disease Control and Prevention). The World Health Organization developed a Global Action Plan on Antimicrobial Resistance in response to growing concerns about antibiotic resistance. This plan explains how to monitor antibiotic use and improve it in the future. This is significant because the abuse or misuse of antibiotics in human and veterinary treatment can select for and promote the growth of resistant bacterial communities inside a host. Antibiotic resistance has existed for centuries (D'Costa, V. M., *et al*., 2011). However, data shows that an increase in treatment failures for bacterial infections and a rise in multidrug resistance (MDR) during the past fifty years may be attributable to the widespread use of antibiotics in contemporary medicine (Joint FAO/OIE/WHO Expert Workshop). Since the 1940s and 1950s, when the development of novel antibiotic therapies slowed to a crawl, multidrug-resistant infections and treatment failure have been a major worry for the medical and scientific communities. A multidrug-resistant bacterial strain is resistant to at least three kinds of antibiotics. "Pandrug" resistance (PDR) is resistance to all antimicrobial drugs. It is less common but still a big worry (Magiorakos, A.-P. *et al.,* 2012*).*

2.2. History of Antibiotics

Diseases, particularly infectious diseases, were responsible for increased morbidity and mortality around the turn of the twentieth century. Pregnancy was riskier, and infant mortality was higher than average (Blaskovich *et al*., 2018). Antibiotic compounds have been used for hundreds of years (Aminov, R. I., 2010). Smallpox, malaria, diphtheria, TB, and other ailments were common. The 1928 discovery of the first antibiotic by Sir Alexander Flaming sparked a medical revolution. In 1942, Ernst Chain and Howard Florey isolated penicillin G (Durand *et al*., 2019).However, the creation of Salvarsan, a medication designed to treat syphilis around the turn of the 20th century, marked the beginning of the modern age of antibiotics. By sheer luck, Alexander Fleming discovered penicillin in 1928 (Fleming, A., 1929), and it swiftly surpassed salvarsan as the most extensively used antibiotic in the world. Over half of the classes of antibiotics currently in use were found in the twenty years after Fleming's success, earning this period the

nickname "the golden era of antibiotic discovery" (Davies, J., 2006). With the discovery of streptomycin in 1944, isolated from the soil bacterium *Streptomyces griseus,* a worldwide search for further naturally occurring antibiotics was launched. To wit: Gould, K. (2016). Researchers have been clamoring for new methods to develop antibiotic medicines capable of fighting antibiotic-resistant illnesses ever since the initial boom in antibiotic discoveries from soil bacteria ceased. Antibiotics were first discovered using a method of assessing bacteria's resistance to antibiotics first used by Fleming in 1928. There is no denying the success of those methods, but they could only be used on germs that could be cultured. The development of culture-independent procedures has accelerated in recent years, and one of these approaches has led to the discovery of a novel antibiotic therapy used to battle resistant bacteria (Ling, L. *et al.*,2015*;* Hover, B. M. *et al.*, 2018*)*. Antibiotics may be discovered in the future, thanks to the effective application and refinement of culture-independent methods. Antibiotics revolutionized the world's approach to illness treatment. Developed nations demonstrated the most notable achievement. Noncommunicable illnesses, including cancer, stroke, and heart disease, are the leading causes of mortality in the United States (Banin *et al.*, 2017). But because of things like poverty, poor public health, bad sanitation, a sewage system and sanitation facility that aren't up to par, fewer people getting vaccinated, etc, antibiotics may not work as well as they should in every part of the world.

2.3. Persistence Versus Resistance

First, it's important to separate resistance from persistence before moving into the specifics of antimicrobial resistance. If a bacterium is resistant to a particular antimicrobial agent, it follows that its daughter cells are also resistant to this agent. Persistence, on the other hand, refers to drug-resistant bacterial cells that lack the necessary resistance genes. Some of the bacteria in a population may be in a latent, stationary growth phase, which makes them resistant to antimicrobials. Antimicrobials can't kill cells unless they're actively dividing and reproducing. The frequency of these persisted cells is around 1% in a stationary-phase culture. Figure 1 shows the difference between persistent and resistant bacterial cells.

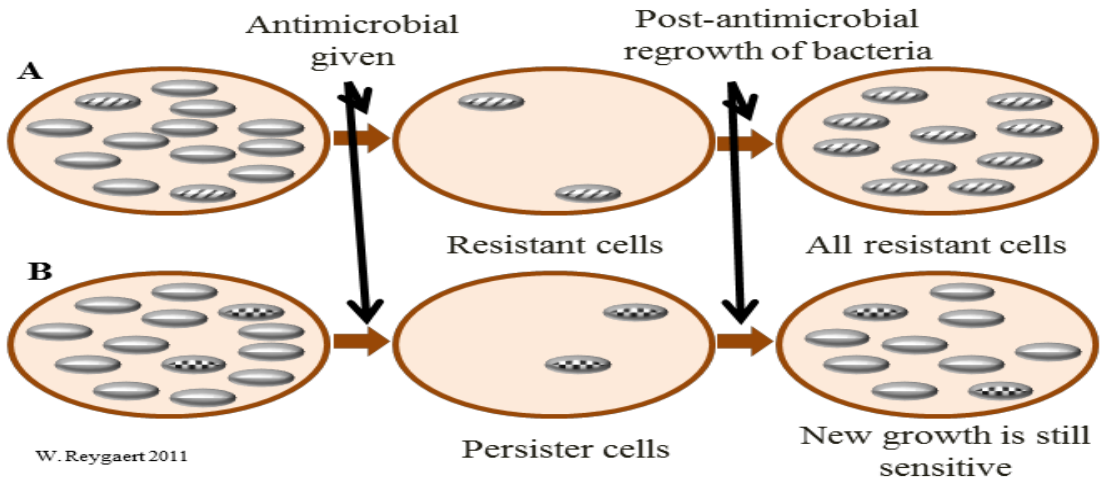


Figure 1: Resistance vs. Persistence

There are two different outcomes that occur when bacterial cells come into contact with an antibiotic substance (Figure:1). It's possible that some of the cells in the area are immune to the antibiotic treatment (A). In this process, only the resistant cells survive. The regrowth of resistant cells will result in a fully resistant culture. Another possibility is the presence of persister cells, which are essentially quiescent but not resistant to the infection (B). Only the persister cells survive when the non-persister cells are eradicated. The regrown population of persister cells will include some cells that are in a quiescent condition and those that are still vulnerable to the antibiotic. (Wanda., 2018)

2.4. Origins of resistance

As a result, bacteria of the same species or genus will not react the same way to different classes of antibiotics. Variable levels of resistance may be seen even among closely related bacterial species. The lowest concentration of medicine required to suppress bacterial growth is known as the MIC and is used to quantify susceptibility and resistance. This susceptibility range is comprised of the average minimum inhibitory concentrations (MICs) of a medicine tested against the same bacterial species. Species are thought to have inherent resistance to a medicine if their average MIC falls within the resistant section of the spectrum. To further complicate matters, bacteria can acquire resistance genes from closely related organisms, with varying degrees of protection depending on the species and the genes obtained. (Wanda., 2018)

2.5. Antibiotic Resistance

Significant turning event in human history, the discovery of antibiotics modernized medicine and numerous lives were saved. Unfortunately, widespread use of these drugs in human and veterinary treatment has been accompanied with a rise in resistant bacterial species (Davies, J., and D. Davies. 2010). While penicillin resistance was found in vitro years earlier to its widespread use in human medicine (Abraham E. P., Chain E. 1940), it did not receive much attention until penicillin-resistant infections and treatment failure began to emerge in the human population (Barber, M. 1948). *Escherichia coli* and *Salmonella enterica*, the most prevalent gram-negative bacteria, cause a variety of human and animal diseases. *E. coli* and *Salmonella* exhibited multi-drug resistance for the first time in the 1950s and 1960s (Levy, S. B., and B. Marshall.2004). In the past fifty years, a correlation between the exposure of specific microbes to antibiotics and the emergence of antibiotic resistance has been shown (Davies, J., and D. Davies. 2010). Several other examples of multidrug-resistant enteric bacteria have been reported after the discovery of resistance in *E. coli* and *Salmonella* (Fey, P. D. *et al.*, 2000; Malik, Y. S., Y *et al*., 2011; NARMS, 2018). On going antimicrobial resistance monitoring and surveillance is essential as the complexity of food production and distribution networks continues to grow. This is especially true because of the several antibiotic resistance routes.

2.5.1. Antimicrobial Mechanisms of Resistance

Antibiotics are employed to treat bacterial infections and are considered effective when they are able to cause cell death or inhibit cellular growth of the target pathogen by inhibiting DNA synthesis, RNA synthesis, cell wall production, or protein synthesis (Kohanski, M. A. *et al*., 2010). Antimicrobial-resistant bacteria are those that have developed a counter measure to antimicrobial agents. Frequently, the biochemical mechanism of resistance used by bacteria comprises antibiotic inactivation, target alteration, and or altered permeability (Kapoor, G., S. Saigal, and A. Elongavan, 2017). Due to enzymatic activity by beta-lactamases and aminoglycoside-modifying enzymes respectively, beta-lactams and aminoglycosides are most affected by antibiotic inactivation. Hydrolysis of ester and amide linkages, which are molecular structures that include penicillins, cephalosporins, monobactams, and carbapenems renders beta-lactamases inactive. It is believed that irresponsible usage of beta-lactams led to the formation of extended-spectrum beta-lactamases (ESBL) (Shaikh, S., J. Fatima *et al*., 2015). Co-resistance of ESBL-producing bacteria to numerous classes of antibiotics,

particularly third-generation cephalosporins, might pose significant therapeutic problems and increase the likelihood of significant medical treatment failure. Extended-spectrum beta-lactamases are often found in gastrointestinal infections, emphasizing the need for effective infection control methods in the agriculture sector (Tissera, S., and S. M. Lee, 2013). In addition, aminoglycoside-modifying enzymes inhibit the binding of antimicrobials to the 30S subunit and lower the affinity of bacterial surfaces for antimicrobial agents. These metabolic pathways result in a broader range of resistance to aminoglycosides and fluoroquinolones. Aminoglycoside-modifying enzymes have been found in *Staphylococcus aureus* isolates and have been shown to aggravate *Staphylococcus aureus* infections in humans (Courvalin, P., and M. Fiandt. 1980). In addition to antibiotic inactivation, another biological route of resistance is target alteration. Target modification, the alteration of antibiotic binding sites inside a bacterial cell, is a typical mechanism of antimicrobial resistance. Depending on the location of the mutation, a modest modification might impart antibiotic resistance by spontaneous mutation or selection of existing genes. Changes to the ribosomal subunit, for instance, might render ineffective antibiotic therapies that target protein synthesis. Moreover, modifications to the bacterial cell wall may influence antibiotics, such as beta-lactams, that target and inhibit cell wall formation (Blair, J. M. A., 2015). Similar to target site modification, minor alterations in the bacterial genome may result in target protection or the production of specific proteins that are capable of attaching to the active site and dislodging the antimicrobial agent. This is especially true for tetracycline resistance, which is frequently acquired via a mode of action involving the Tet(O) gene, because produced Tet(O) can attach directly to the 16S ribosomal subunit and dislodge bound tetracycline (Munita, J. M., and C. A. Arias. 2016). In addition to target alteration and drug inactivation, several bacteria have achieved antibiotic resistance via membrane permeability modifications (Delcour, A. H. 2009). Many antibiotics used to treat gram-negative bacterial infections, for instance, target cell components found inside the cytoplasm or cell membrane (Shown in table 2.1), altering the permeability of the cell. Antimicrobial compounds must pass through the outer cell wall and/or cytoplasmic membrane to reach their targets. Bacterial targets of antibiotics in clinical use shown in table 2.2.

Table 2.1- Antibiotics according to their mode of action (Wanda., 2018)

Mechanism of Action	Antimicrobial Groups
Inhibit Cell Wall Synthesis	β-Lactams Carbapenems Cephalosporins Monobactams Penicillins Glycopeptides
Depolarize Cell Membrane	Lipopeptides
Inhibit Protein Synthesis	Bind to 30S Ribosomal Subunit: Aminoglycosides Tetracyclines Bind to 50S Ribosomal Subunit: Chloramphenicol Lincosamides Macrolides Oxazolidinones Streptogramins
Inhibit Nucleic Acid Synthesis	Quinolones Fluoroquinolones
Inhibit Metabolic Pathways	Sulfonamides Trimethoprim

Table 2.2. Bacterial targets of antibiotics in clinical use (Chellat *et al*., 2016)

Target	Type of Antibiotic
Cell- wall Biosynthesis	Penicillin's, Cephalosporins, Carbapenems, Monobactams, Cyclomerize, Fosfomycin, Glycopeptides, Lipoglycopeptides
Protein Synthesis	Aminoglycosides, Tetracyclines *(Subunit 30S*) Oxazolidinones, Macrolides, Thiopeptides, Chloramphenicol, Fusaric Acid, Clindamycin *(Subunit50S)*
DNA Replication and Repair	Rifamycin, Annamycin, Actinomycin's, Miocamycin's (RNA polymerase) Fluoroquinolones, Aminocoumarins (DNA gyrase)
Folic Acid Metabolism	Sulfonamides, Trimethoprim
Membrane structure	Lipopeptides, Polymyxins

2.6. Critically Important Antibiotics

To treat a human illness that was either transferred from a non-human source or acquired resistance genes from a non-human source, an antibiotic belongs to the category of "critically important antimicrobials," as defined by the World Health Organization (WHO). Cephalosporins (especially those of the third, fourth, and fifth generations), glycopeptides, and macrolides are at the top of the World Health Organization's list of critically important antimicrobials. Third-generation cephalosporin resistance is particularly concerning, given the importance of this drug in combating enteric infections spread via food. Many cases of severe Salmonella and *E. coli* infections are treated with ceftriaxone, an acephalosporin. infection caused by *E. coli* in humans. There is mounting evidence that using third-generation cephalosporins favors the development of cephalosporin-resistant strains of *Salmonella* and *Escherichia coli.* animals with *E. coli* An estimated 1.2 million cases of non-typhoidal *Salmonella* and 265,000 cases of Shiga toxin-producing *Escherichia coli* are reported each year in the United States, according to the Centers for Disease Control and Prevention, because of coliform bacteria. Furthermore, the Centers for Disease Control and Prevention (CDC) predict that 6,200 cases of non-typhoidal *Salmonella* infections resistant to Ceftriaxone occur yearly (Medalla F. et al., 2017). Despite the alarming nature of these numbers, resistance to third-generation

cephalosporins represents just a fraction of the overall problem of antimicrobial resistance. Additionally, glycopeptides are a promising new class of antibacterial agents that warrants further study. The emergence of VRSA, a strain of *Staphylococcus aureus* resistant to the antibiotic vancomycin, has raised concerns about the inappropriate use of glycopeptides, particularly in healthcare facilities, where the spread of VRE and MRSA/VRSA by nosocomial transmission is frequent (Alsubaie, S., K. et al., 2012; Kurita, H., K. Kurashina, and T. Honda, 2006). Even though it's not as dangerous as *Staphylococcus* or *E. coli,* when it comes to the spread of AMR by horizontal gene transfer, enterococci like E. coli are of particular concern (Palmer, K. L., V. N. Kos, and M. S. Gilmore, 2010). On the other hand, macrolides, the third most significant antibiotic on the WHO list, are widely utilized in both cattle production and the treatment of intestinal diseases in humans. Since they are effective against both gram-negative and gram-positive bacteria, macrolides are considered a broad-spectrum class of antibiotics. As of today, seven distinct macrolides have been licensed by the FDA for use in animal husbandry; their first usage in food animal production dates back to the 1960s (U.S. Food and Drug Administration, 2016). More than 70 percent of cattle in feedlots with more than 1000 head of cattle are administered Tylosin (USDA. Feedlot 2011, Part IV, 2016). This use of macrolides in agriculture has been heavily criticized, with critics suggesting that the administration of Tylosin may cause birth defects in cattle. *Campylobacter* sp. is the most common bacterial cause of food poisoning across the globe. Antibiotics are often necessary for treating infections, making the emergence of strains that are resistant to these drugs particularly worrisome, especially when dealing with severe illnesses or those affecting people with impaired immune systems.

2.7. Natural resistance of antibiotic

This resistance may be intrinsic (the genes are present in the bacteria from the start) or induced (the genes are present in the bacteria from the start but are only expressed at resistant levels after exposure to an antibiotic) (González-Bello, 2017). Bacterial communities tend to have a similar set of intrinsic features, which are not affected by antibiotic selection or horizontal gene transfer. The most prevalent kind of intrinsic resistance is a decrease in the permeability of the outer membrane. Not only that, but they can also influence cellular efflux pumps (Xie et al., 2018).

Table 2.3. Organism and Resistance pattern

Organism	**Intrinsic Resistance**
Bacteroides (anaerobes)	Aminoglycosides, many β-lactams, quinolones
All Gram Positives	Aztreonam
Enterococci	Aminoglycosides, cephalosporins, Lincosamides
Listeria Monocytogenes	Cephalosporins
All Gram Negatives	Glycopeptides, lipopeptides
Escherichia Coli	Macrolides
Klebsiella spp.	Ampicillin
Serratia Marcescens	Macrolides
Pseudomonas Aeruginosa	Sulfonamides, ampicillin, 1st and 2nd generation cephalosporins, chloramphenicol, tetracycline
Stenotrophomonas Maltophilia	Aminoglycosides, β-lactams, carbapenems, quinolones
Acinetobacter spp.	Ampicillin, glycopeptides

2.8. Acquired resistance

Acquired resistance refers to the acquisition of resistant genetic elements by any of the three mechanisms—transformation, transposition, or conjugation. The most important of these three mechanisms is horizontal gene transfer, while chromosomal DNA mutation is also important. We don't know whether this resistance will be temporary or permanent. Plasmid-mediated transmission is the norm, whereas bacteriophage-mediated transmission is unusual. There are a variety of stresses (chemical and physical) and mutations (in genes) that may lead to resistance (substitution, deletion, etc.). The average bacterial cell undergoes a mutation once every 106–109 cell divisions, and these mutations are usually lethal. Certain genes, including those encoding drug targets, drug transporters, regulator genes, antibiotic-modifying enzymes, etc., have undergone mutations that enhance antimicrobial resistance (Aanen *et al*., 2019).

2.9. Mechanism of Antibiotic resistance

Mechanism of antimicrobial resistance mechanisms fall into four main groups:

- Limiting uptake of a drug
- Modifying a drug target
- Inactivating a drug
- Active drug efflux

A number of processes exist by which bacteria might develop resistance to antibacterial medicines. One class of antimicrobial medicines is ineffective against some bacterial strains because they have developed an intrinsic resistance to them. Of greater concern are cases of acquired resistance, in which initially susceptible populations of bacteria become resistant to an antibacterial agent and proliferate and spread under the selective pressure of the use of that agent. In such cases, all strains of that bacterial species are similarly resistant to all members of those antibacterial classes. Different types of bacteria may easily exchange antibiotic resistance mechanisms. To begin with, the organism could pick up genes for lactamases or other enzymes that neutralize the antibacterial drug before it can do any good. Second, the bacteria may develop efflux pumps that remove the antibiotic chemical from the cell before it can do any good. Third, bacteria can acquire mutations that reduce the amount of antimicrobial agent entering the intracellular target site by downregulating porin genes, or they can acquire several genes for a metabolic pathway that results in altered bacterial cell walls that no longer contain the binding site of the antimicrobial agent. (McManus M.C., 1997).

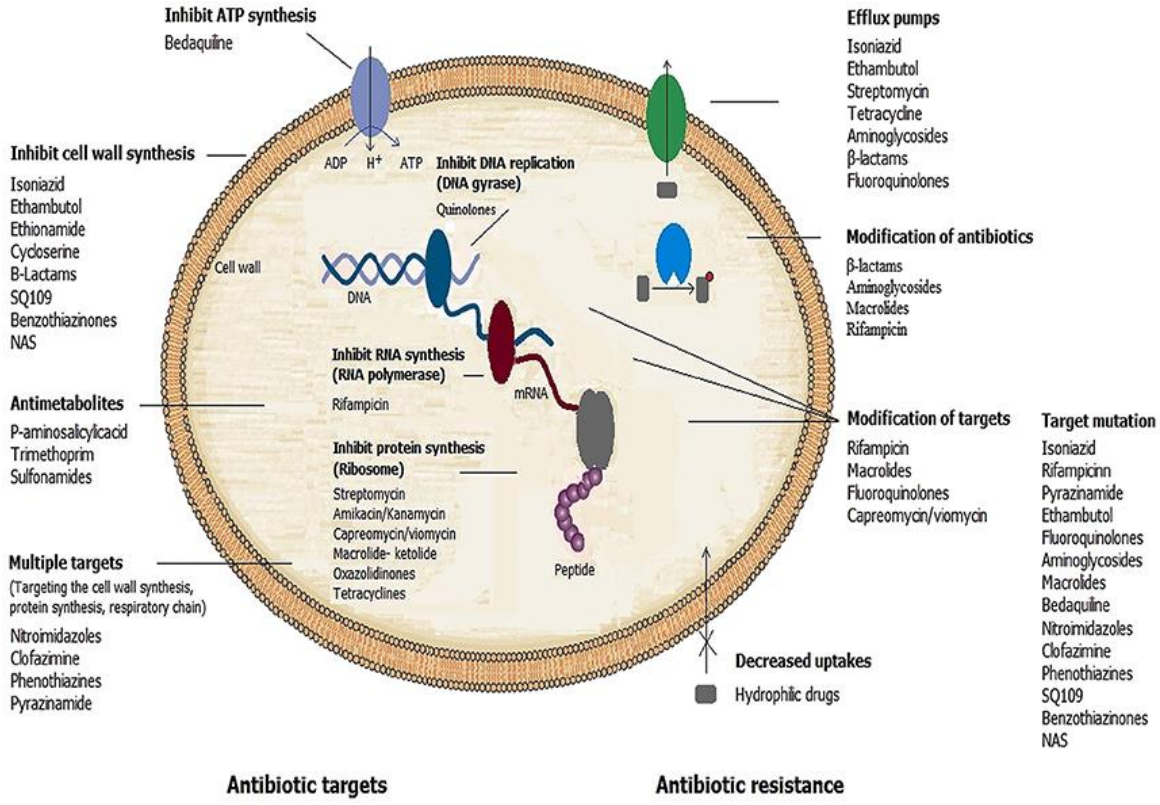


Figure 2.1: Antibiotics, effects and mechanisms of Drug Resistance Action (Haeili *et al*., 2017)

Thus, typically sensitive bacterial populations may acquire resistance to antimicrobial drugs via mutation and natural selection or by obtaining the genetic material that encodes resistance from other bacteria. The final event may take place through one of numerous genetic pathways, including transformation, conjugation, or transduction. Through mechanisms of genetic exchange, many bacteria have become resistant to multiple classes of antibacterial agents, and these bacteria with multidrug resistance (defined as resistance to three antibacterial drug classes) have become a cause for grave concern, especially in hospitals and other healthcare institutions where they tend to occur. As stated before, susceptible bacteria may gain antibiotic resistance via novel mutations. Such spontaneous mutations may result in resistance by: (1) modifying or removing the binding site of the target protein to which the antibacterial agent binds (e.g., change in penicillin-binding protein 2b in pneumococci, resulting in penicillin resistance); (2) upregulating the production of enzymes that inactivate the antimicrobial agent (e.g., erythromycin ribosomal methylase in staphylococci); or (3) downregulating (ef In each of these instances, strains of bacteria with resistance-conferring mutations are chosen by the application of antimicrobials, which kill the susceptible strains but permit the newly resistant strains to

live and proliferate. Vertical evolution refers to acquired resistance that arises as a result of chromosomal mutation and natural selection. Additionally, bacteria evolve resistance by acquiring additional genetic material from other resistant species. This is referred to as "horizontal evolution," and it may occur across strains of the same species or between distinct genera of bacteria. Conjugation, transduction, and transformation are the mechanisms of genetic exchange. For each of these steps, transposons may help with the transfer and integration of acquired resistance genes into the host's genome or plasmids. During conjugation, a gram-negative bacterium transmits plasmids encoding resistance genes to a neighboring bacterium, often through an extended proteinaceous structure known as a pilus that connects the two organisms. Conjugation among gram-positive bacteria is often triggered by the release of sex pheromones by the mating pair. These pheromones enhance the clumping of donor and recipient organisms, facilitating the transfer of DNA. During transduction, a bacteriophage transfers resistance genes from one bacterium to another (bacterial viruses). This is currently believed to be a very uncommon occurrence. Transformation, which is the process by which bacteria acquire and absorb DNA segments from other bacteria that have released their complement into the environment during cell lysis, may transfer resistance genes to previously vulnerable strains. Mutation, natural selection, and the processes of genetic exchange enable several bacterial species to rapidly adapt to the introduction of antibacterial drugs into their environment. Although a single mutation in a critical bacterial gene may only marginally diminish the sensitivity of the host bacterium to an antibacterial agent, it may be just enough to enable the bacteria to survive until it gains further mutations or genetic information resulting in full-fledged resistance. 18 Rarely, a single mutation may be sufficient to confer clinically relevant, high-level resistance to an organism (e.g., high-level rifampin resistance in S. aureus or high-level fluoroquinolone resistance in Campylobacter jejuni) (McManus M.C., 1997).

2.10. Some Leading Resistant Pathogens

Many types of microorganisms cause infection in humans and animals, so disease prevention and treatment strategies must be adapted to reflect infection risk factors and available treatment options. Over the past decades, most pathogenic species have developed resistance to one or more antimicrobials. Some of the species in which resistance is of greatest public health concern are listed below.

□

Escherichia coli

Staphylococcus aureus, including community-associated MRSA (Methicillin Resistant *S. aureus*)

- *Mycobacterium tuberculosis* (cause of tuberculosis)
- *Neisseria gonorrhoeae* (cause of gonorrhoea)
- *Salmonella* Typhi
- *Streptococcus pneumonia*
- Methicillin-resistant *Staphylococcus aureus* (MRSA) has become a focus of public health concern due to its increased virulence and resistance to an increasingly broad spectrum of antibiotics (Aliberti *et al*., 2012; Johnson *et al.,* 2011)

2.11. An overview on *E. coli* and *S. aureus, Campylobacter* and *Salmonella*; most frequently noticed bacteria in livestock products

2.11.1. Structure of *E. coli* and *S. aureus*

E. *coli* is an onsporulating bacterium and cells are typically rod-shaped, and are about 2.0 μm long and 0.25–1.0 μm in diameter, with a cell volume of 0.6–0.7 μm. Cell wall is composed of a thin peptidoglycan layer and an outer membrane. Strains that possess flagella are motile. The flagella have a peritrichous arrangement (Scheutz, 2005). It also attaches and effaces to the microvilli of the intestines via an adhesion molecule known as intimin. Pathogenic E. coli is divided into two major groups according to their infection sites namely extra intestinal pathogenic *E. coli* (ExPEC) and intestinal pathogenic *E. coli* (InPEC) (Leomil *et al*., 2005). ExPEC can cause diseases in urinary tract, meninges etc. but InPEC is subdivided into several categories such as enteropathogenic *E.coli* (EPEC), enterotoxigenic *E.coli* (ETEC), enterohemorrhagic *E. coli* (EHEC), enteroaggregative *E. coli* (EAEC), enteroinvasive *E. coli* (EIEC), and adherent-invasive *E. coli* (AIEC). That infection is occurred for both human and animals (Moriel *et al*., 2012). On the other hand the cell wall of *S. aureus* consists of a very thick peptidoglycan layer. They are 0.5-1.5 μm in diameter and spherical in shape with the absence of flagella moreover sometimes divide in more than one plane to form grape-like clusters (Braga *et al*., 2004).

2.12.2. Virulence Factors:

2.12.2.1. Virulence Factors of *E. coli* (Raeispour *et al*.,2018)

- Fimbrial adhesions (F2- F6, F17, F18, F41)
- Heat-stable (STa, STb) and heat-labile (LTp/h, LT-IIa, LT-IIb) enterotoxins
- Attaching and effacing (AE) lesion; type 4BFP fimbriae by typical (t) EPEC of humans (dogs, cats)
- Vero toxins (VTx), afimbrial and fimbrial adhesion
- Small fimbrial adhesions (AAF/Hda); toxins (Pet, EAST1, ShET1)

Transcriptional activator gene (aggR), Adhesions of the A Fimbrial Adhesion (AFA) Cytotoxic Necrotizing Factors (CNF) 1 or 2 and hemolysis (Hly) fimbrial (Pap/Prs, Sfa/F1C and /or F17) and/or afimbrial adhesions (AFAfamily); siderophores; resistance to complement.

2.12.2.2. Virulence factors of *S. aureus* (Lentz *et al*.,2018)

Enzymes such as coagulase, hyaluronidase, deoxyribonuclease, lipase, staphylokinase, beta-lactamase etc.

- Toxins such asTSST-1, and enterotoxin type B, Exfoliative toxins
- Toxins that act on cell membranes include alpha toxin, beta toxin, delta toxin, and several bicomponenttoxins
- Panton-Valentine leucocidin (PVL) and bacteriophage

2.13. Method of detecting *S. aureus*

2.13.1. Cultural characteristics of *Staphylococcus aureus* (Murray *et al.*,2013)

1. Staphylococci grow readily on most bacteriologic media under aerobic or microaerophilic conditions.
2. Colonies on solid media are round, smooth, and raised, and glistening.
3. *S. aureus* usually forms gray to deep golden yellow colonies.
4. **Mannitol Salt Agar:** circular, 2–3 mm in diameter, with a smooth, shiny surface; colonies appear opaque and are often pigmented golden yellow.
5. **Tryptic Soy Agar**: circular, convex, and entire margin.
6. **Blood Agar**: beta-hemolysis.
7. **Brain heart infusion agar:** Yellow pigmented colonies.

2.13.2. Biochemical characteristics of *Staphylococcus aureus* (Rusenova *et al*., 2017)

- Catalase positive
- Oxidase negative
- OF test –fermentative
- Coagulase positive: the presence of free and /or boundcoagulase
- Indole negative
- Gas negative
- Hydrogen sulfide negative
- Methyl red positive
- VP positive
- Nitrate reduction positive
- Gelatin hydrolysis positive
- Beta hemolysis on Bloodagar
- Citrate positive and Urease positive
- Motility negative
- PYR negative

2.13.3. Microscopic Feature

Microscopy is useful for pyogenic infections but not blood infections or toxinmediated infections. A direct smear for Gram staining may be performed as soon as the specimen is collected. The Gram stain showing typical Gram-positive cocci that occur singly and in pairs, tetrads, short chains, and irregular grapelike clusters can be suspected to be *S. aureus*.

2.13.4. Characteristics on growth medium (El-Jakee *et al.*,2008)

- The organism is isolated by streaking material from the clinical specimen (or from a blood culture) onto solid media such as blood agar, tryptic soy agar, or heart infusionagar.
- Specimens likely to be contaminated with other microorganisms can be plated on mannitol salt agar containing 7.5% sodium chloride, which allows the halo- tolerant staphylococci togrow.
- The inoculated plates should be incubated at 35°C to 37°C for 24 to 48hours.
- On Blood agar, growth occurs abundantly within 18 to 24 hours. Round, raised, opaque, yellow to golden yellow colonies of 1-2mm in diameter are seen with or without beta hemolysis.

On Mannitol Salt Agar (MSA), a selective media, S. *aureus* being a mannitol fermenting bacterium gives yellow or gold colonies.

An 18 h to 24 h culture can be used as the inoculum for additional tests.

Isolates should be subculture at least once on a nonselective medium after initial isolation before being used in a diagnostic test that requires pure culture or Heavy inoculum.

2.13.5. Presumptive Identification

Large mannitol fermenting colonies on MSA

Gram-positive cocci inclusters

Catalase-positiveorganisms

Coagulase-positiveorganisms

2.13.6. Confirmatory Tests

Confirmatory tests include biochemical tests, PCR, or mass spectrometry.

2.13.7. Identification of Toxins (Berube *et al*.,2013)

This is important for more severe cases like toxic shock syndrome and food poisoning.

Toxins produced by *S. aureus*, such as enterotoxins A to D and TSST-1 may be identified using agglutinationtests.

The tests are determined by the clumping of the latex particles by the toxins present in the samples.

Commercial latex agglutination tests are available for this purpose.

2.13.8. Nucleic acid amplification tests (Kateete *et al*.,2010)

Commercial nucleic acid amplification tests are available for the direct detection and identification of *S. aureus* in clinicalspecimens.

Whereas the earlier versions of these tests required manual extraction of bacterial DNA and testing multiple specimens in large batches, integrated processing of specimens (extraction, gene amplification, and target detection) is now performed on highly automated platforms with disposable reagent strips or cartridges.

They are useful for screening patients for carriage of methicillin-sensitive S. aureus (MSSA) and MRSA.

2.14. Different methods for detection of *E.coli*

2.14.1. Cultural characteristics of E. coli (Collee *et al.*,1996)

Nutrient Agar (NA)

- They appear large, circular, low convex, grayish, white, moist, smooth, andopaque.
- They are of 2 forms: Smooth (S) form and Rough (R) form. Smooth forms are emulsifiable insaline.
- Due to repeated subculture, there is smooth to rough variation (S-R variation).

Blood Agar (BA)

- Colonies are big, circular, gray andmoist.
- Beta (β) hemolytic colonies areformed.

MacConkey Agar (MAC)

- Colonies are circular, moist, smooth and of entiremargin.
- Colonies appear flat and pink.
- They are lactose fermentingcolonies.

Mueller Hinton Agar (MHA)

- Colonies are pale strawcolored.

Eosin Methylene Blue (EMB) Agar

- Green Metallic sheen colonies areformed. **m-ENDO Agar**
- Colonies are green metallicsheen.
- Metabolise lactose with the production of aldehyde andacid. **Violet Red Bile Agar (VRBA)**
- Red colonies (pink to red) areformed.
- Bluish fluorescence around are seen around colonies underUV. **Cysteine Lactose Electrolyte-Deficient (CLED) Agar**
- They give lactose positive yellowcolonies.

Characteristics on Liquid Media

- They show homogenous turbid growth within 12-18hours.
- R form agglutinates spontaneously, forming sediment on the bottom of the test tubes.
- After prolonged incubation (>72 hrs), pellicles are formed on the surface of liquidmedia.
- Heavy deposits are formed which disperses onshaking.

2.14.2. Other methods (Lindstedt et al., 2003; Frydendahl et al., 2002)

- Polymerase Chain Reaction (PCR)
- Biochemical profiling
- Bacteriophage typing
- DNA-fingerprinting methods
- Pulsed field gelelectrophoresis
- Targeted RFLP
- Typing of virulence factor encoding genes
- Multilocus variable-number tandem repeat analysis

2.14.3. Diseases caused by *S. aureus*

2.14.3.1 Human diseases caused by *S. aureus*

- Staphylococcal skin infections: The vast majority of cases of staphylococcal infection involve the skin. In the case of a superficial infection (furuncles and carbuncles), both nodular abscesses and nodular pustules (also known as impetigo) and vesicular pustules (also known as impetigo) may be seen. (Kwiatkowski *et al*,2017).
- Bacteremia is caused by Staphylococcus aureus due to the presence of a foreign body in the bloodstream, such as a catheter. Furthermore, there need not be a single proximal trigger (Holland *et al*., 2018). Staphylococcal neonatal infections: Neonatal infections include skin lesions with or without exfoliation, bacteremia, meningitis, and pneumonia. On the other hand, secondary pneumonia may arise in immunocompromised people who are infected with other viruses. Moreover, corticosteroid-treated people are sometimes more susceptible to respiratory infections (Cailes *et al.*, 2018).

S. aureus endocarditis is commonly associated with visceral abscesses, embolic manifestations, pericarditis, subungual petechial, subconjunctival hemorrhage, purpuric lesions, heart murmurs, perivalvular abscess, conduction abnormalities, and heart failure due to cardiac valve destruction (Liesenborghs *et al.*, 2019).

Staphylococcal toxic shock syndrome may be caused by the use of vaginal tampons or may worsen any sort of S. aureus infection (eg, postoperative wound infection, infection of a burn, skin infection). Although the majority of infections have been caused by methicillin-susceptible S. aureus (MSSA), MRSA is becoming more prevalent (Krogman *et al*., 2017).

Staphylococcal osteomyelitis is more prevalent in youngsters and is characterized by chills, fever, and bone discomfort. The overlaying soft tissue thereafter turns red and inflamed.

Articular infection is possible; it typically manifests as effusion, indicating septic arthritis as opposed to osteomyelitis (Kavanagh *et al*., 2018).

2.14.3.2. Diseases caused by *S. aureus* in Cattle

Along with Escherichia coli and other streptococcal species, including Streptococcus uberis and Streptococcus agalactiae, S. aureus is a leading cause of mastitis in dairy cows, which results in a substantial economic loss for the dairy sector. Leukocytes enter the udder as a result of mastitis, and several cutoff levels of leukocytes have been created to classify milk quality. Example: If there are more than 200,000 leukocytes per milliliter of cow's milk, we say that the milk is contaminated; in the European Union, if there are more than 400,000 cells per milliliter, we say that the milk is unfit for human consumption. As a result, raw milk products that have undergone fermentation might cause food poisoning if they are contaminated in quantity. (Martins *et al*., 2019). Animal microbiome has antibiotic resistance genes that may be acquired from their ecological niches and chosen for by the use of antibiotics in agriculture. (Sheppard *et al.*, 2018).

The propensity of some animal-adapted S. aureus strains to colonize and infect humans may lead to the emergence of novel epidemic clones with hitherto uncharacterized virulence. (Anjum *et al*., 2019). In addition, the frequency of bovine-to-human transfers has grown in recent years. Closer examination indicated the emergence of at least two CC97 subclades for human infection that arose from bovine-to-human host jumps and then expanded across the human population. (Haag *et al*., 2019). This offered more evidence that animals might serve as a reservoir for the spread of S. aureus clones that may spread swiftly from animals to humans and subsequently across the population. This offered more evidence that animals might serve as a reservoir for the spread of S. aureus clones that may spread swiftly from animals to humans and subsequently across the population. (Turner *et al*., 2019).

2.14.4. Diseases caused by *E. coli*

2.14.4.1. Human diseases caused by *E. coli*

□□Enterohemorrhagic: These strains (including serotype O157:H7 and others) produce several cytotoxins, neurotoxins, and enterotoxins, including Shiga toxin (verotoxin), and cause bloody diarrhea (Ahsan *et al*., 2020), hemolytic-uremic syndrome develops in 2 to 7% of cases (Loos *et al*., 2017). Such strains have most often been acquired from

undercooked ground beef but may also be acquired from infected people by the fecal-oral route when hygiene is inadequate. Enterotoxigenic: These strains can cause watery diarrhea, particularly in infants and travelers (traveler's diarrhea) (Mirhoseini *et al.*, 2018). Enteroinvasive: These strains can cause inflammatory diarrhea (Farajzadeh *et al.*, 2020). Enteropathogenic: These strains can cause watery diarrhea, particularly in infants (Moxley *et al*., 2010). Enteroaggregative: Some strains are emerging as potentially important causes of persistent diarrhea in patients with AIDS and in children in tropical areas (Kaur *et al*., 2010). Urinary tract infection which usually represent ascending infection (i.e., from the perineum via the urethra). *E. coli* may also cause prostatitis and pelvic inflammatory disease (PID) (Forsyth*et al*., 2020).

Extra intestinal infection if normal intestinal anatomic barriers are disrupted (e.g., by ischemia, inflammatory bowel disease, or trauma), in which case the organism may spread to adjacent structures or invade the bloodstream. Hepatobiliary, peritoneal, cutaneous, and pulmonary infections also occur. *E. coli* bacteremia may also occur without an evident portal of entry. In neonates, particularly preterm infants, *E. coli* bacteremia and meningitis (caused by strains with the K1 capsule, a marker for neuro invasiveness) are common (Russo *et al*.,2000)

2.14.4.2. Cattle diseases caused by *E. coli*

Post-mortem examination may reveal severe fecal soiling of the perineum, dehydration, and widespread muscle wasting in instances of diarrhea caused by enterotoxigenic E. coli (ETEC) (Das *et al*., 2013). The small and large intestines are bloated with fluid and gas, and the intestinal mucosa may be glossy. Upon histological examination, the intestinal mucosa normally seems normal. In extreme instances, lesions caused by verocytotoxin-producing Escherichia coli (VTEC) may spread from the colon to the small intestine. Lesions consist of edema, ulceration, and erosions in the large intestine mucosa, followed by localized and widespread hemorrhages in the intestinal lumen. There is widespread, multifocal bacterial colonization of the surface epithelium by a thin layer of darkly pigmented coccobacilli, which are often arranged in a palisade pattern. Microscopy with electrons may reveal the close attachment of bacteria to intestinal epithelial cells and the loss of microvilli. Petechial hemorrhages on the epicardium and serosal surfaces, as well as possible splenic enlargement, pulmonary edema, and pulmonary hemorrhage. (Fairbrother *et al*., 2006). Lesions in mastitis of cows are often difficult to exactly pinpoint due to the skin's color, temperature, and subcutaneous fat edema.

2.15. Classification and nomenclature of *salmonella*

Historically, Salmonella was named for the locations where it was first isolated, such as Salmonella London and Salmonella Indiana. This naming scheme was replaced by a categorization based on the sensitivity of isolates to various bacteriophages, generally known as phage typing. When the origin and characteristics of an epidemic must be established by distinguishing isolates of the same serotype, phage typing is often used. When using globally standard sets of typing phages, it is very repeatable. To far, more than 200 definite phage types (DT) have been identified. S. Typhimurium DT104, for instance, defines a specific phage type for Typhimurium isolates. (Hanes, 2003; Andrews and Baumler, 2005 and Pui *et al*., 2011).

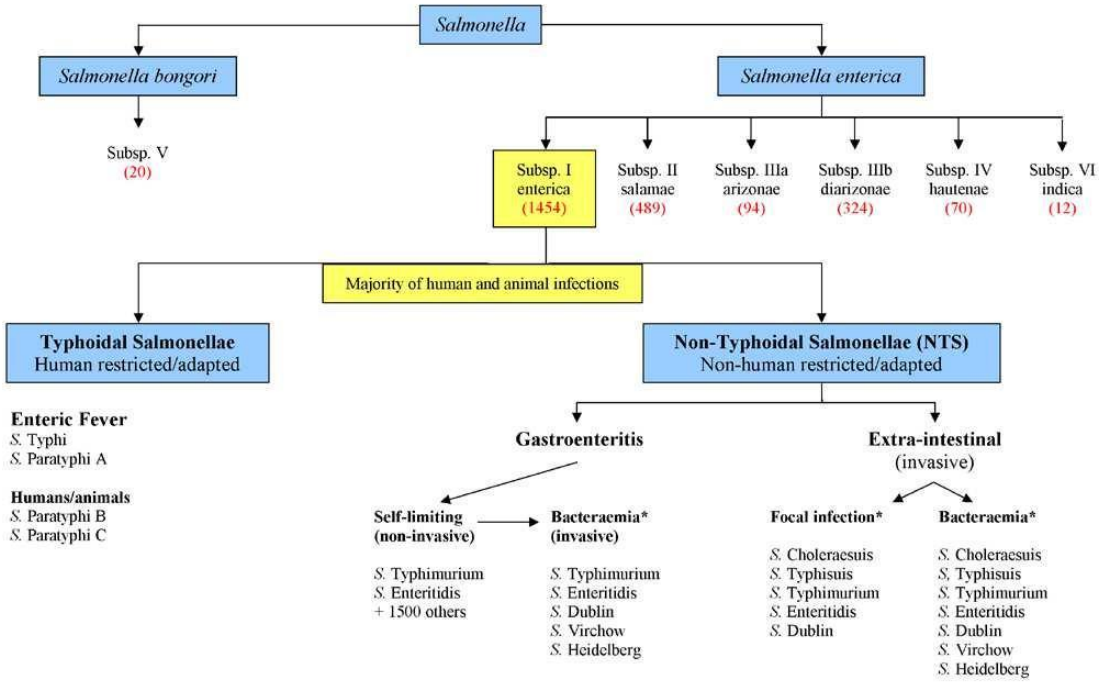
Epidemiologic classification of *Salmonella* is based on the host preferences.

- The first group includes host-restricted serotypes that infect only humans such as *S.* Typhi.
- The second group includes host-adapted serotypes which are associated with one host species but can cause disease in other hosts serotypes such as *S.* Pullorum in avian.
- The third group includes the remaining serotypes.
- Typically, *Salmonella* Enteritidis, *Salmonella* Typhimurium and *Salmonella* Heidelberg are the three most frequent serotypes recovered from humans each year (Gray and Fedorka-Cray, 2002 and Boyen *et al*., 2008).
- The genus consists of two species: the first is *S. enterica* which is divided into six subspecies (Figure 2.2):

- *S. enterica* subsp. *enterica*,
- *S. enterica* subsp. *salamae*,
- *S. enterica* subsp. *arizonae*,
- *S. enterica* subsp. *diarizonae*,
- *S. enterica* subsp. *houtenae* and
- *S. enterica* subsp. *indica*; and
- The second is *S. bongori* (formerly called *S. enterica* subsp. *bongori*)

Salmonella enterica subspecies I is primarily isolated from warm-blooded animals and accounts for more than 99% of clinical isolates, whereas the remaining subspecies and S. bongori are primarily isolated from cold-blooded animals and account for less than 1% of clinical isolates, according to WHO (2003). As an example, Salmonella Typhimurium is currently categorized as Salmonella enterica subspecies I serotype Typhimurium. Under

the contemporary nomenclature scheme, subspecies information is often deleted, and the culture is referred to as S. enterica serotype Typhimurium and afterwards as S. Typhimurium. This naming method is utilized now to provide consistency in reporting. (Andrews and Baumler, 2005 and Parry, 2006).



Source: Langridge *et al*., (2008)

Figure 2.2: Classification of the Genus *Salmonella*

Note: Numbers in brackets indicate the total number of serotypes included in each subspecies.

*Common serotypes are listed but other serotypes may cause bacteremia or focal infection; subsp = subspecies

Table 2.4: Scientific taxonomy of *E. coli, S. aureus, Campylobacter and Salmonella*

Taxonomy			
Escherichiacoli	*Staphylococcusaureus*	*Campylobacter*	*Salmonella*
Domain:	Domain:	Domain:	Domain:
Bacteria Phylum: Proteobacteria Class: Gammaproteobacteria Order: Enterobacteriales Family: Enterobacteriaceae Genus: Escheriahia Species: Escherichia coli	Bacteria Phylum: Firmicutes Class: Bacilli Order: Lactobacillales Family: Staphylococcacea Genus: Staphylococcus Species: Staphylococcus aureus	Bacteria Phylum: Campylobacteroa Class: Campylobacteria Order: Campylobacterales Family: Campylobacteriaceae. Genus: Campylobacter Species: C. jejuni	Bacteria Phylum: Psedomonadota Class: Gammaproteobacteria Order: Enterobacterales Family: Enterobacteriaceae Genus: Salmonella Species: Staphylococcus aureus

2.15.1. General Characteristics of *Salmonella*

Salmonella is a vast genus of gram-negative bacilli in the family Enterobacteriaceae, with over 2300 serotypes that are well adapted for growth in people and animals and can cause a broad range of diseases. The development of S. typhi and S. paratyphi is confined to human hosts, in whom they produce enteric fever (typhoid). Non-typhoidal Salmonella, the remaining Salmonella serotypes, may colonize the gastrointestinal tracts of a wide variety of species, including mammals, reptiles, birds, and insects. More than 200 of these serotypes are pathogenic to humans, causing gastroenteritis and/or local infections and/or bacteremia. (Fuaci and Jameson, 2005). Salmonella infections may be found in people, cattle, wild animals, reptiles, birds, and insects (Getenet, 2008) and can thrive in a wide range of nonhost environments. (Pui *et al*., 2011).

- *Salmonella*e are gram-negative, non-spore forming, facultative anaerobic bacilli, and 2 to 3 by 0.4 to 0.6 µm in size (Getenet, 2008).

- They do not require sodium chloride for growth but can grow in the presence of 0.4 to 4%.
- Most *Salmonella* serotypes grow at temperature range of 5 to 47°C with optimum temperature of 35 to 37°C but
- some can grow at temperature as low as 2 to 4°C or as high as 54°C.
- They are sensitive to heat and often killed at temperature of 70°C or above.
- *Salmonella* grows in a pH range of 4 to 9 with the optimum between 6.5 and 7.5.
- They require high water activity (aw) between 0.99 and 0.94 (pure water aw=1.0)
- yet can survive at water activity less than 0.2 such as in dried foods.
- Complete inhibition of growth occurs at temperatures less than 7°C, pH less than 3.8 or water activity less than 0.94 (Pui *et al*., 2011).

They, like other Enterobacteriaceae, ferment glucose to acetic acid, convert nitrates to nitrite, and don't make cytochrome oxidase. Furthermore, with the exception of *Salmonella typhi*, all *Salmonellae* are motile through peritrichous flagella and all save *Salmonella gallinarum-pullorum* generate gas (H2S) on sugar fermentation. (Fuaci and Jameson, 2005 and Getenet, 2008). *Salmonella* is non- capsulated except *S. Typhi*, *S. Paratyphi C* and some strain of *S*. Dublin (Getenet, 2008).

2.15.2. Diseases caused by *Salmonella*

Salmonella is a bacterium that may infect both humans and animals. Some of these infections cause sickness, but the vast majority likely result in asymptomatic carriers who sometimes shed *Salmonella* in their feces. Whether or whether a person becomes ill after ingesting *Salmonella* depends on a number of immunological parameters, both specific and non-specific. Gastrointestinal infections are often caused by *Salmonella* spp., including *S. typhimurium* and *S. enteritidis* (food poisoning). *Salmonella* is a common cause of foodborne illness, and most outbreaks occur when people eat food tainted with *Salmonella* either directly or indirectly from infected animals. In many cases, people get infected after coming into contact with contaminated animal corpses. (Quinn *et al*., 1999).

2.15.2.1. *Salmonella* Infections in Animals

Salmonella may infect several domestic and wild animals. The infection may or may not manifest clinically. The animal may have a latent infection and harbor the pathogen in its lymph nodes, or it may be a carrier and eliminate the agent in its feces momentarily, sporadically, or consistently in the subclinical form. Clinical enteritis caused by species-

adapted serotypes, such as *S. pullorum* or *S. abortusequi*, is common in domestic animals. Other clinically manifest or latent infections are produced by serotypes with multiple host species. (PAHO, 2001). Serotype *Dublin* and *S. Typhimurium* are the primary agents of clinical salmonellosis in cattle. Occasionally, other serotypes may be identified from ill animals. Salmonellosis is rare in adult cattle, but in calves it often assumes epizootic proportions.

Generally, the illness arises when stressors are present. Serotype *Dublin*, which is suited to cattle, has a geographical distribution center. Confirmed outbreaks have occurred in the western United States, Venezuela, Brazil, and Argentina in the Americas. Additionally, it is found throughout Europe and South Africa. The sickness starts with high fever and the formation of blood clots in the feces in adult cattle, followed by diarrhea and a return to normal body temperature. Extremely obvious signs of stomach discomfort are present. The sickness may be deadly within a few days, or the animal may recover, at which point it often becomes a carrier and additional instances emerge. Calves are more vulnerable to infection than adults, resulting in real epidemic outbreaks with significant fatality rates. In babies, septicemia and mortality are prevalent. The carrier condition is less common in young animals and more common in mature cattle. The illness is usually often transmitted by the feces of a cow that is shedding the agent, although it may also be transmitted through milk. (PAHO, 2001).

2.15.2.2. *Salmonella* Infections in Humans

Salmonella infections in people may vary from self-limiting gastroenteritis often caused by non-typhoidal *Salmonella* (NTS) to typhoidal fever with consequences such as deadly intestinal perforation. (OIÉ, 2000). Non-typhoidal *Salmonella* is a leading cause of foodborne illness, accounting for an estimated 1.3 billion yearly cases and 3 million annual fatalities (Torpdahl *et al*., 2007). Salmonellosis outbreaks have been documented for decades, but the last 25 years have seen a dramatic increase in the global prevalence of this illness. Disease tends to be more common in regions with widespread animal farming (OIÉ, 2000). Between 12 to 36 hours is a typical incubation time in humans, however this might vary. Diarrhea is the most common presenting symptom, however other symptoms, such as nausea and stomach discomfort, may be present. Vomiting is uncommon. Headaches and fevers are other possible side effects. However, bacteremia may develop rarely with more invasive *Salmonella* such as S. Virchow, despite the fact that the infection is often

self-limiting and does not need antibiotic treatment in the vast majority of cases. Human fatalities from this virus are quite uncommon (Gracey *et al*., 1999). *S. enterica* subsp. *typhimurium* and *S. enterica* subsp. *enteritidis* are the most frequent pathogens responsible for salmonellosis. Second, the bacteria Salmonella *enterica* subsp. *typhi* and *S. enterica* subsp. *paratyphi* are responsible for typhoid fever and paratyphoid fever, respectively. *Salmonella* is capable of replicating both within the vacuoles of host cells and in the wider environment. *Salmonella* are the number two most prevalent pathogens identified from people with gastrointestinal illness in high-income nations (Buncic, 2006). Livestock, like other animals, may have *Salmonella enteritidis* and *Salmonella typhimurium* in their gastrointestinal tracts. The illness resolves on its own, but it may be rather serious for small children, the elderly, or anybody with a damaged immune system. Epithelial cells in the ileum are invaded by *Salmonella*, which then multiply in the lamina propria, causing severe, watery diarrhea. The diarrhea-causing heat-labile enterotoxin is produced by certain isolates. Consequences include reiter's syndrome, systemic infection, and reactive arthritis after enteritis. Carrier status may last for up to six months in an individual. Growth of the pathogen in foods does not seem to be a role in all instances of foodborne salmonellosis, but it does appear to be a factor in some. The infectious dosage ranges from a few CFU to >105 CFU.

2.15.3. Diseases Caused by *Campylobacter*

2.15.3.1. *Campylobacter* Diseases in Human

Campylobacteriosis is the disease caused by the infection with *Campylobacter*:

- The onset of disease symptoms usually occurs 2 to 5 days after infection with the bacteria but can range from 1 to 10 days.
- The most common clinical symptoms of *Campylobacter* infections include diarrhoea (frequently bloody), abdominal pain, fever, headache, nausea, and/or vomiting. The symptoms typically last 3 to 6 days.
- Death from campylobacteriosis is rare and is usually confined to very young children or elderly patients, or to those already suffering from another serious disease such as AIDS. (Igwaran, A., & Okoh, A. I. 2019)
- Complications such as bacteraemia (presence of bacteria in the blood), hepatitis, pancreatitis (infections of liver and pancreas, respectively), and miscarriage have been reported with various degrees of frequency. Post-infection complications may

include reactive arthritis (painful inflammation of the joints which can last for several months) and neurological disorders such as Guillain-Barré syndrome, a polio-like form of paralysis that can result in respiratory and severe neurological dysfunction in a small number of cases. (WHO)

2.15.3.2. Diseases caused by *Campylobacter* in Animals

Campylobacter jejuni and *C. coli* are transferred by feces to the mouth. Poultry and other foods that are contaminated or undercooked are a cause of illness for carnivores such as pets and professionally farmed mink. Aborting sheep may also include *C. jejuni* in their vaginal secretions, aborted fetuses, and fetal membranes. As mechanical vectors, wild rodents and insects such as houseflies may exist. *Campylobacter fetus* subsp. fetus is spread to cattle, sheep, and goats by ingestion. Contact with excrement, vaginal secretions, aborted fetuses, or fetal membranes may infect animals. This pathogen and *C. fetus* subsp. venerealis are both sexually transmitted in cattle. Infections caused by *C. fetus* may be transmitted by fomites such as contaminated sperm, infected tools, and contaminated bedding. Several hours after mating with an infected cow, males may transmit *C. fetal*; some bulls may become lifelong carriers. Additionally, cows may become carriers for years. *Campylobacter* species can not resist drying or heat, however they may typically persist in damp settings for a period. *Campylobacter* can live in water at 4°C (39°F) for weeks, but just a few days at 15°C (59°F) or above. *C. jejuni* may stay viable in feces for up to 9 days, in milk for 3 days, and in water for between 2 and 5 days. *C. jejuni* and *C. coli* may stay infectious for extended durations in damp poultry litter. C. fetus may live for 24 hours in liquid manure and 20 days in soil. Enteritis is caused by *C. jejuni* and infrequently *C. coli* in dogs, cats, calves, lambs, mink, ferrets, poultry, pigs, and several species of laboratory animals. Younger animals, such as kittens, puppies, and calves, may have more severe clinical manifestations. Dogs may have diarrhea, appetite loss, vomiting, and sometimes fever. Typically, the feces are watery or bile-streaked, with mucous and even blood. The clinical indications typically persist between 3 and 7 days, however some animals may have sporadic diarrhea for weeks or even months. Typically, a thick, mucoid diarrhea with occasional blood specks is seen in calves with or without a fever. In addition to cats, primates, mink, ferrets, hamsters, guinea pigs, mice, and rats, mucoid, watery, and sometimes bloody diarrhea is seen in primates, mink, ferrets, hamsters, guinea pigs, mice, and rats Poultry has a very high colonization rate, despite the fact that the vast majority of birds show no indications of sickness. Reportedly, newly born chicks and poults may get

acute enteritis, characterized by quick onset of diarrhea and mortality; however, the illness has not been scientifically confirmed. *C. jejuni* has been isolated from ostriches with enteritis and has been linked to the demise of juvenile birds (Facciolà *et al.*, 2017)

2.15.4. Antimicrobial Resistance in food from animal origin

Because of the 23,000 yearly fatalities caused by antimicrobial resistance in commonly consumed foods (US Department of Health and Human Services), the inability of antibiotics to cure life-threatening infections is a major cause for concern. In addition, Salmonella and *Campylobacter* transmission via food intake is responsible for an estimated 410,000 antibiotic-resistant illnesses each year, according to the CDC. Concern concerning antimicrobial resistance (AMR) in meat products has increased as a result of the widespread use of antimicrobials in food-animal production. Consequently, there has been an effect on customer buying habits, with a resulting change in preference toward organic and organically derived food goods, including beef (U.S. Department of Agriculture, Economic Research Service). Despite this change in buying habits, there is no hard data on how different cattle production methods affect the prevalence of AMR in beef products. Although AMR has been studied at various points in the beef production process, very little research has been done on meat products at the retail level (Noyes, N. R., *et al.,* 2016). In 2017, the U.S. Department of Agriculture's Economic Research Service estimated that the average U.S. resident ate 25.8 kg of beef annually (U.S. Department of Agriculture, Economic Research Service). However, it is difficult to assess the effect of exposure, infection, and treatment failure owing to AMR bacteria via meat eating because of the existing void in the scientific literature. The methods currently employed to examine antibiotic resistance in food-borne isolates provide a particular difficulty. Various classes of antimicrobial medicines are on hand for the treatment of sick livestock, as well as for the prevention of infection and as a growth promoter. Antibiotics are often used to treat dairy cattle, with the most popular classes being tetracyclines, beta-lactams, sulphonamides, aminoglycosides, macrolides, and chloramphenicol (McGrane, 2000; Movassagh and Karami, 2010; Pecou and Diserens, 2011). Injectables, feed or water additions, topical applications, intramammary and intrauterine infusions, and oral administrations are only some of the ways antimicrobials may be given to animals (Mitchell *et al.,* 1998). According to Babapour (2012), antibiotics are commonly used in the food animal industry due to their low cost and wide availability. WHO (2001) defines

prudent (or optimal) use of antibiotics in food animal production as the use of antimicrobials that maximizes clinical therapeutic effect, minimizes drug-related toxicity, and minimizes the development of antimicrobial resistance. All therapeutic antibiotics should be provided by, or with a prescription from, a veterinary surgeon to ensure correct use of antibiotics in livestock, and the prescription, delivery, and record-keeping of antibiotics used in livestock should be under the supervision of the prescribing veterinarian. The current data demonstrate that barely half of the livestock caretakers acquired antibiotics for animal treatments with a veterinarian's prescription. The rest of the antibiotics are sold over the counter and delivered by inexperienced staff without a prescription. When farmers realized that the antibiotics they had been using weren't working, they switched to a different kind without consulting a veterinarian, and they also employed local botanicals. Antibiotic misuse, according to Komolafe (2003) and Carlos (2010), is a major contributor to the epidemic of antibiotic resistance. This involves giving the incorrect antibiotic, giving too much antibiotic, or giving antibiotics for conditions that antibiotics cannot cure. According to research by Karimuribo et al. (2005), this may be an especially pressing issue in LDCs where animal health services have been less than ideal, leading pet owners to increasingly keep medication in their homes and hire inexperienced individuals like farmers and animal attendants to care for their pets. Mmbando (2004) conducted research that indicated widespread drug misuse among livestock managers. This included not following the authorized therapeutic dosages, using improper methods of administration, randomly combining medications, and not respecting withdrawal periods (Iruka and Ojo 2010).

2.16. Transmission of antimicrobial resistance via livestock

Microorganisms expressing AMR genes propagate throughout the environment and infect mammals (Baker *at al.*, 2018). The visual display illustrates the spread of antibiotic-resistant microorganisms. The use of antimicrobial agents in veterinary procedures is regarded as one of the fundamental pathways of AMR and antibiotic resistance transmission. Some diseases follow a direct route to propagate resistance from animals to humans. Environment and flora serve as a reservoir of antibiotic resistance and as a source of the growth and transmission of antibiotic-resistant bacteria among people and animals. Due to the fact that antibiotic residues and bacteria are reintroduced into the environment through manure from food-animal production, they encourage the development of resistance. The use of animal waste as fertilizer and the abuse of antibiotics in aquaculture

are two significant ways that antibiotic resistance is disseminated (Magouras *et al.*, 2017). Antibiotics used to food-producing animals are comparable to those administered to humans and may select for antibiotic resistance in animals. Cross-transmission of resistant microorganisms and resistant genetic elements is also common (Tang *et al.*, 2017). In one of the experimental experiments conducted in the United States, the existence of gentamycin-resistance genes in *Enterococci* isolated from animals was verified, and the same genes were detected in the food products of the same animals. Similar resistance patterns were detected in *Enterococci* isolated from human and retail food samples from various areas (Donabedian et al., 2003). A Nigerian investigation shows the existence of resistant *E.coli* in chicken samples. The isolates included several resistance genes, including *blaTEM, sul2, sul3, aadA, tetA, tetB,* etc. These data demonstrate that animal production farms are significant antibiotic resistance gene (ARG) reservoirs (Adelowo et al., 2014).

2.16.1. Animal-to-human antibiotic resistance transmission pathways

2.16.1.1 Direct exposure

Farmworkers, veterinarians, slaughterhouse workers and animal food handlers who are under the direct exposure with animals and their biological substances, are at high risk of being colonized with antibiotic-resistant bacteria from animals and animal farms,which subsequently may provide an opportunity for the entry of ARGs/ARB sin local communities and health care settings (Marshall & Levy, 2011).Reynaga *et al*. Reported that the high prevalence(81/140,57.9%)of pig farm workers had been colonized or infected with livestock-associated clonal lineage

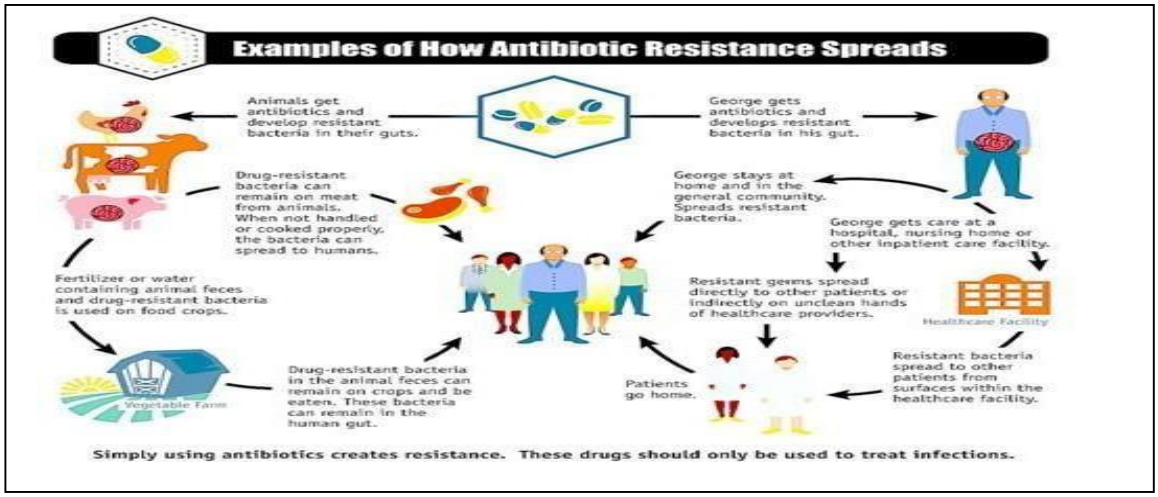


Figure 2.3. The U.S. Centers for Disease Control and Prevention; Transmission of Antimicrobial Resistance

ST398 of MRSA (Reynaga *et al*.,2016). *E. coli* strains identified from turkey and chicken producers in the Netherlands were found to be practically resistant to all tested antibiotics. These ARGs/ARBs linked with livestock might spread from farmers to their families and the local population through human-to-human transmissions, resulting in an increase in colonization and illnesses in humans with/without livestock interaction. (Larsen, 2017)

2.16.1.2. Food chain and food trade

Pathways for the transfer of AMR from animals to humans through food chains are extensive and intricate. Numerous studies have indicated that animal meals, including pig, poultry, cattle, and fish, have a significant concentration of ARGs/ARBs. (Founou *et al*., 2016). For example, in research on MRSA in retail meat, the contamination rate was greatest in turkey (35%), followed by chicken (16%), veal (15%), pig (10%), and beef (10%), and lowest in finished goods (3%) compared to meat during processing (4.2%). (de Boer et al., 2009). Another example is the mcr-1 gene, which transmitted from animals to people via food chains, as indicated by its greater detection rate in animal samples (21%) and raw meat (15%) than in clinical samples (1%). (Liu*et al*.,2016).

2.16.2. The horizontal gene transfer promotes the transmission

The horizontal transfer of ARGs may facilitate the transmission of ARGs from animals to humans. (Soucy*etal*.,2015; von Wintersdorffetal.,2016). As several environmental microorganisms, particularly aquatic bacteria from aquaculture, share a high number of MGEs, such as plasmids, integrative conjugative elements, integrons, and transposons, considerable genetic exchange and recombination may occur for a variety of reasons. (Elsas*etal*.,2003; Marti *et al*., 2014; von Wintersdorff *et al*., 2016). MGE-mediated HGT assembles tandem arrays of different ARGs into integrons, transposons, and plasmids, and then renders them mobile, as shown by the substantial link between the antibiotic resistome of soil habitats and human clinical diseases. (Forsberg *et al*., 2012). These mobile ARGs and bacteria may spread into the environment and move into our food chains (Zhu et al., 2017; Zhu et al., 2018). ARGs may be transferred to human pathogens by transduction, bacterial conjugation, and bacterial absorption of "free" DNA (Zhu et al., 2017; Zhu et al., 2018). Class 1 integrons, which are often physically connected to several antibiotic-resistance determinants, are thus postulated to be the most important and pervasive agents of ARGs and a viable surrogate for ARGs with anthropogenic origins, such as the animal-food producing business (Gaze et al., 2011; Gillings, 2018; Gillings et al., 2015). Conjugation is the transfer of DNA from a contributing cell to a receiving cell

through bacterial pili or adhesins, and it has been identified as having a greater impact on the spread of ARGs across bacterial populations than transformation and transduction (von Wintersdorff et al., 2016). ARGs are often connected with conjugative plasmids, integrons, and transposons in livestock systems, particularly aquaculture (Watts, 2017). Once ARG exchange events have happened in environmental bacteria, the ARGs may be disseminated further among local bacterial populations, including human diseases, and subsequently spread internationally through the international trade of food goods and global travelers (Cabello et al., 2016; Zhu et al., 2017; Zhu et al., 2018). Several studies suggest that livestock environments may have contributed to the emergence of the plasmid-encoded qnrA gene that confers low-level resistance to quinolones, and the qnrA gene is associated with the waterborne species Shewanella spp, which are widely dispersed in marine and freshwater environments (Poirel et al., 2005; Yan et al., 2017). Using high-throughput sequencing, Yang et al. analyzed the resistome in sand samples from a marine fish farm and discovered that some contigs including resistance genes (e.g., strAB, qnrA, and tetL) and transposons or plasmids were very similar (>90%) to those from human diseases (Yang *et al*., 2013). A further intriguing example suggests that plasmid-borne mcr genes may have evolved predominantly in aquatic systems due to aquaculture operations that carry mcr genes from aquatic bacteria to terrestrial bacteria (Cabello *et al*., 2017). Public Wellness Antimicrobial Resistance Importance Prior to the discovery and widespread use of antimicrobials, infectious illnesses were humanity's leading cause of death. In most of the poor world that lacks access to high-quality treatments, infections continue to be the leading cause of mortality, as do healthcare-associated diseases caused by resistant microbes in all nations (Jinadal et al., 2015; Ferri *et al*., 2017). Depending on the scenario, it is anticipated that if AMR is not addressed, the global population in 2050 would be between 11 million to 444 million less than it would be without AMR. The lower limit indicates a scenario in which resistance rates have been maintained at a reasonably low level, while the upper bound reflects a world without viable antimicrobial medications. (Taylor*et al*., 2014).

2.17. Antimicrobial resistance is an unusual public health threat

- Antimicrobial resistance is not a "disease". There is often little variation in illness severity between susceptible and resistant strains. Resistance is often not a disease pathology issue, but rather a consequence of restricted treatment alternatives.

- Our dependency on antimicrobials to treat infections is the core concern. If other means of treating illnesses were available, antibiotic resistance would still exist in the globe, but it would no longer be a public health problem.
- Antimicrobial resistance is a concern to public health caused by healthcare practices, namely the misuse of antimicrobials for illnesses in which they are ineffective.
- Furthermore, it has been estimated that AMR will result in a global catastrophe by causing 10 million deaths annually, a terrifying economic cost of 100 trillion USD, and an 11% decline in livestock productions by 2050 if adequate measures are not taken to address the challenges (O'Neill, J.,2016)
- Resistance is a feature of several microorganisms that cause various illnesses. Thus, containment measures must be tailored to the demands of certain disease prevention and treatment initiatives (Jinadal *et al*., 2015; Ferri *et al.*,2017).

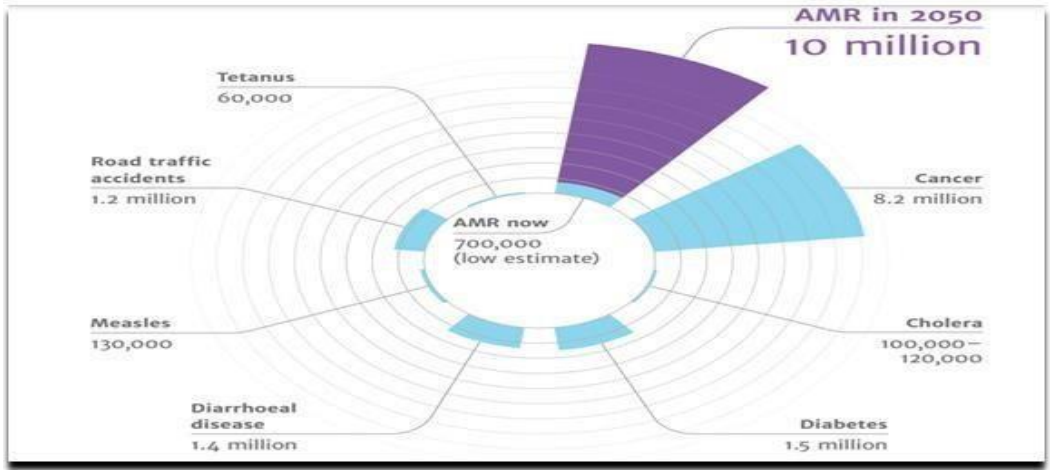


Figure 2.4. World wide economic loss due to AMR

2.17.1. Managing resistance in farm animals

Determining the levels of resistance in these groups is a crucial step in determining any danger to public health posed by AMR in agricultural animals. At the national level, AMR in agricultural animals is mainly reported via passive monitoring. Alternatives to passive monitoring have been suggested for AMR in people. As has been proposed in the context of developing zoonotic illnesses in general, these types of techniques may theoretically be used to farm animals. (Keusch *et al*., 2009)

2.17.2. Reducing antimicrobial usage in farm animals

As seen by the experience of the EU-wide ban on growth boosters, reducing the intake of antimicrobials by farm animals has proven difficult. Outside of Europe, the acceptance of voluntary codes and the formulation of recommendations for drug use, although commendable in and of itself, do not seem likely to significantly decrease drug usage. There may be possibilities for a more effective use of antimicrobials in farm animals, especially if this provided measurable cost savings or increased production. These include the same tactics recommended for human treatment, such as overkill, combination therapy, and medication reuse and recycling (Imamovicet al., 2013). As with humans, fast detection of bacterial infections and real-time profiling of resistance determinants using whole genome sequencing data would help identify treatment methods more swiftly and precisely (Gordon et al., 2014).

A total restriction on the use of antimicrobials in farm animals will certainly have devastating effects on animal health, welfare, and production, as well as food costs. However, reducing antibiotic usage in farm animals might be part of a sector-wide coordinated plan. (Davies *et al.,* 2013). Any adverse effects of this on the agricultural industry would beat least partially alleviated if viable alternatives to antimicrobials were available.

2.17.3. Alternatives to antimicrobials for farm animals

Several prebiotics and probiotics are already available; however, their efficiency is unknown and possibly vary. It has also been suggested to combine the two, a concept known as'synbiotics' Phage treatment may be successful, for instance against *Salmonella Typhimurium* in chicken and swine, but this needs prompt selection and delivery of the phage, as well as large bacterial burdens (Allen et al., 2013). It may be feasible to employ pure phage lysins directly as opposed to the phage, so preventing the unintentional transmission of genetic information from the phage. However, none of these options are near to becoming ready for worldwide commercial usage against the whole range of microbial diseases in farm animals.

Expanding the spectrum of vaccinations available for use in animals may be a more immediately applicable suggestion. Despite the availability of vaccinations against several of the most prevalent viral illnesses of cattle, the regular use of vaccines that protect against bacterial infection and disease is still restricted. Even when a vaccination is available, it is

not always embraced by producers; for instance, one study of a live oral Lawsonia vaccine in pigs resulted in 80% less oxytetracycline use and enhanced productivity (Bak et al., 2009), yet the vaccine is not commonly employed. As long as antibiotics remain accessible and effective, there is probably no financial motivation to employ current antibacterial vaccinations for agricultural animals or to create new ones.

Long-term goals for lowering antibiotic use in farm animals might include the use of cattle that are genetically immune to illness or disease, most likely via the use of genetic modification technology. The production of transgenic chicken's incapable of transmitting avian influenza is an example of early success in this area (Lyall et al.,2011) In general, however, it is evident that substantial investment in research and development would be required before any of the aforementioned methods to disease management in farm animals could serve as viable alternatives to antimicrobials.

2.18. One-Health approaches to check the AMR issue

The complicated epidemiological and socioeconomic determinants of AMR make this problem the archetypal One-Health concern. Trans sectoral and trans disciplinary methods are required to effectively combat AMR. Reducing the spread and transfer of resistant bacteria within and across animal and human populations is essential for combating antimicrobial resistance. It is difficult to explain with confidence the genesis of resistant bacteria strains due to the propensity of bacteria to propagate from one environment to another, often across huge geographic distances and among diverse populations. Therefore, the reservoirs and transmission channels of antimicrobial-resistant bacteria demand more research, preferably via a One-Health approach. Therefore, it is essential to increase our understanding of how animal contacts and trade (direct transmission), farm management, and the broader farm environment (indirect transmission) contribute to the spread of antimicrobial resistance and to identify potential countermeasures to this phenomenon.

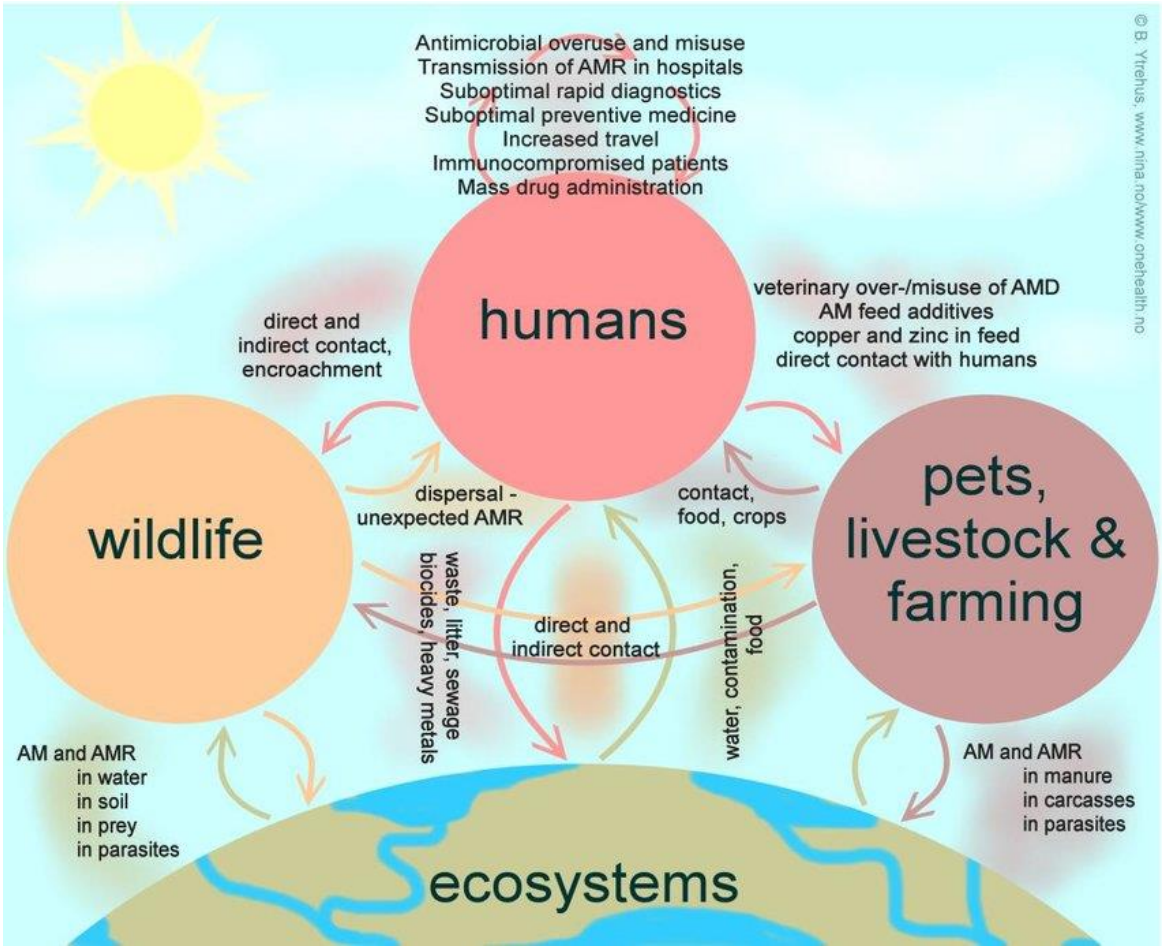


Figure 2.5. One health approaches to AMR

Farm management studies may include all practices that may facilitate the spread of resistant bacteria within and between farms and from farms to the environment, including farm hygiene and biosecurity, animal waste management, structure (and construction material) of holdings, and animal production intensity. One-Health techniques should always be supported by molecular epidemiological data, which may offer information on the relationships between resistance genes identified in diverse samples, such as those from animals of different origins. Not only should resistance genes be researched in animal samples, but also in the larger farm environment, including farmers, other livestock species, farm pets, wildlife, waste, and water. These ecological data may offer the molecular connection necessary to describe reservoirs of resistant bacteria and might enable investigations on transmission channels across animal populations, as well as between animals and humans. Source attribution may aid in shedding insight on the impact of AMR from cattle to the public health resistance burden. In addition, it may be a crucial piece of evidence in the development of tailored therapies against AMR. In addition,

genomic data may give further insight into the evolution of bacteria during transmission within the examined populations. In addition, molecular epidemiology data may give information on the proportion of the resistance reservoir that can be attributable to the transmission of resistant bacteria or de novo emergence as a result of AMU selection pressure in the examined farms.

2.19. Metagenomics of Meat ("Meatagenomics")

Prior to the introduction of DNA sequencing, microbiological study was not conceivable with the use of modern molecular tools. In the late 1970s, Carl Woese originally advocated using ribosomal RNA genes as molecular markers for phylogenetic categorization (Escobar-Zepeda, *et al.*, 2015). Together with the introduction of Sanger sequencing and other methods like as PCR, this concept had a profound influence on molecular biology and the characterisation of microbial communities. Since the earliest versions of these technologies, several advancements have been achieved, allowing scientists to investigate the metagenome of a range of ecological and environmental materials. The metagenome has been defined in a variety of ways, but generally includes "individual genome-level characterization of a community or its members, high-throughput gene-level studies of communities with methods borrowed from genomics or other 'omics' studies that aim to understand transorganismal behaviors and the biosphere at the genomic level" (Woese, C. R., and G. E. Fox., 1977). Continues to give information on the symbiotic interaction between microbial populations and their surroundings. Despite the fact that metagenomics may be undertaken using a range of methodologies - each tailored to the research subject at hand - 16S rRNA gene sequencing and shotgun sequencing are two of the most often used methods for characterizing ecological materials.

2.19.1. Metagenomics Used in Research

Prior to the advent of genetic sequencing technology, microbial species and communities were exclusively studied via culture approaches. In combination with culture techniques, metagenomic approaches, such as 16S rRNA gene sequencing and shotgun sequencing, may be used. However, they are often used independently. 16S rRNA gene sequencing, sometimes referred to as amplicon sequencing, employs a hypervariable, highly conserved area inside the 16S ribosomal RNA of a bacterial cell to identify an isolate or define an entire microbial community within aspecified ecological niche. Typically, DNA is extracted from samples using DNA extraction kits, and the V4 region of the 16S rRNA

gene is amplified using primers and PCR. Typically, the V4 area is utilized to evaluate the phylogeny of bacterial communities, since research comparing the nine variable sections of the 16S gene have shown that the V4 region is the most dependable in reflecting the whole length of the 16S rRNA gene in subsequent phylogenetic analysis (National Research Council, 2007). Following DNA isolation, shotgun sequencing employs a differentmethod. Instead of amplifying a particular gene area, the DNA is sheared into minute pieces that are then read. When analyzing the microbiome of an ecological sample, amplicon sequencing offers several benefits over shotgun sequencing. Nonetheless, shotgun sequencing offers several additional complimentary benefits that cannot be overlooked, such as its capacity to sequence genomic areas outside of the 16s gene (Yang, B., Y. Wang, and P.-Y. Qian., 2016). Following the commencement of the NIH's Human Microbiome Project (HMP) - a follow-up tothe Human Genome Project done in the late 1990s and early 2000s - amplicon sequencing was widely used in microbiome research. The HMP investigated the interaction between people and the many microbial habitats inside and on the human body's surface (Tessler, M. et al.,2017). In addition, the Human Microbiota Project stimulated several research focusing on the significance of the human gut microbiome and its effect on human health, particularly gastrointestinal health. Onesuch research examined the impact of a fecal microbiota transplant from a healthy donor to a person afflicted with Clostridium difficile using 16S rRNA gene sequencing. The 16S study revealed that the fecal transplant changed the gut microbiota enough to eradicate the illness without the significant side effects seen with antibiotic treatment (Turnbaugh, P. J et al., 2007). Since then, fecal microbiota transplants have gained widespread acceptance in the medical community because they remove the need for antibiotics to treat a common gastrointestinal disorder. Shotgun sequencing, like amplicon sequencing, may define microbial communitieswithin an ecological sample. Instead of amplifying a specified section of the genome and matching sequenced results to a reference database, shotgun sequencing produces reads that can be assembled de novo. This technique has led to the discovery of novel bacteria and the identification of certain bacterial genetic components (Gupta, S., E et al., 2016; Siegl, A. et al.,2011). In addition to examining the phylogenetic categorization of microbial communities, shotgun sequencing has been widely used to characterize other essential genetic components Of bacteria. In addition, shotgun sequencing has significantly contributed to our understanding of the pathogenicity of deadly bacteria. Prior to the advent of next-generation sequencing, little was understood about the pathogenic genetic components of

bacteria that cause human illness. Modern sequencing technologies have offered new tools for investigating pathogenicity mechanisms. Comparing the genomes of pathogenic Escherichia coli O157:H7 and nonpathogenic Escherichia coli K12 using high-throughput sequencing led to the identification of E. coli O157:H7 pathogenicity islands (O-islands) that code for production of shiga toxins and other proteins that aid in infection of a host (Lasken, R. S., and J. S. McLean., 2014) In addition to virulence factors, shotgun sequencing has been widely used in the study of antibiotic resistance. As antimicrobial resistance continues to be a top public health concern among global health agencies such as the World Health Organization and the Centers for Disease Control and Prevention of the United States, there has been an increase in research into the mechanisms and transmission of antimicrobial resistance. Antimicrobial resistance has been studied and described in different biological niches, such as ocean water (Perna, N. T. et al.,2001), soil microorganisms (Hatosy, S. M., and A. C. Martiny., 2015), and the human gut (Kozhevin, P. A et al., 2015). Studies have also examined changes in antimicrobial resistance across an entire production system, beginning with feedlot entry and continuing with carcass fabrication after slaughter. The variety of antibiotic resistance research demonstrates the influence that shotgun sequencing has had on the scientific community (Noyes, N. R. et al., 2016). While scientists Will continue exploratory research in an effort to better comprehend antimicrobial resistance, the creation of new antibiotics will be essential to addressing the growth of antimicrobial-resistant bacterial illnesses. Previously, Song and colleagues analyzed the genomes of several pathogens that had been sequenced using shotgun sequencing to find more than 200 genes critical to the proliferation of gram-positive bacteria (van Schaik, W. 2015) Similarly, E. coli has 27 growth-required genes. (Song, J.H. et al., 2005) Identification of such genes throws information on probable processes of antibiotic resistance and provides targets for future antibiotic development, particularly when next-generation sequencing and bio-informatics approaches are used.

CHAPTER III

MATERIALS AND METHODS

3.1. Study area

A total of 130 milk and meat samples were collected from different farms of Chattogram Metropolitan area (CMA) where buffalo and goat milk was collected from different areas of Chattogram division (Bahaddarhat, Bashkhali, Anowara) and meat sample was collected from different farms of CMA.

3.2. Sample collection duration

The samples were collected spanning the time between November 2021 to April 2022.

3.3 Study population

A total of 130 meat and milk samples including 40poultry products (broiler & layer chicken meat) and 90 large (buffalo and cattle) and small (goat) animal products were collected from the study population. The distribution of samples collected from different animals are shown in table 3.1.

Table 3.1: Samples Collected from different sources

Type of sample	Species	Number of samples	Total sample	Collection Area
a. Poultry Products				
Meat	Broiler	20	40	**From 10 different markets of CMA**
	Layer	20		
b. Large/Small Animal				
Meat	Beef	20	50	**From 10 different markets of CMA**
	Goat	20		
	Buffalo	10		
Milk	Goat	25	40	**From 3 goat farms**
	Buffalo	15		**From 3 buffalo farms**

3.4. Study design

We followed a cross-sectional design. A purposive, convenient sampling was done.

3.4.1 Sample size

The sample size was determined using the single proportion formula: where (n) is the required sample size, $Z = Z$ value for a given confidence level, $p =$ expected prevalence, and $d =$ allowable error of estimation. The confidence level was assumed to be 95% with an allowable error of 0.1, and thus, Z was 1.96. Prevalence of 50% was used in the calculation, which resulted in $n = 97$ as the minimum sample size.

3.5. Sample collection procedure

3.5.1. Milk sample:

The samples were collected aseptically in clean sterile 15 ml labelled falcon tubes from the individual bucket full of milk used for individual animal. Soon after collection, samples were kept into a cool box with ice for ceasing the growth. The samples were then shipped to the clinical pathology laboratory of Chattogram Veterinary and Animal Sciences University (CVASU), where they were kept at 4℃ until investigation but not exceeding 6 hours.

3.5.2. Meat sample:

40 samples from broiler and layer and 50 samples from cattle, buffalo and goats were collected from traditional markets, live bird markets (LBMs) and super shops (SS) in the Chattogram metropolitan area **(figure 3.1).** The meat samples were transported to the laboratory in an icebox. Five grams of samples (breast muscle, thigh muscle, liver and gizzard) were collected aseptically using sterile scissors and placed in separate Falcon tubes containing 45 mL BPW (Oxoid™ Ltd., Basingstoke, UK). All samples were kept at 4°C (max 24 h) and subsequently used for bacteriological investigation.

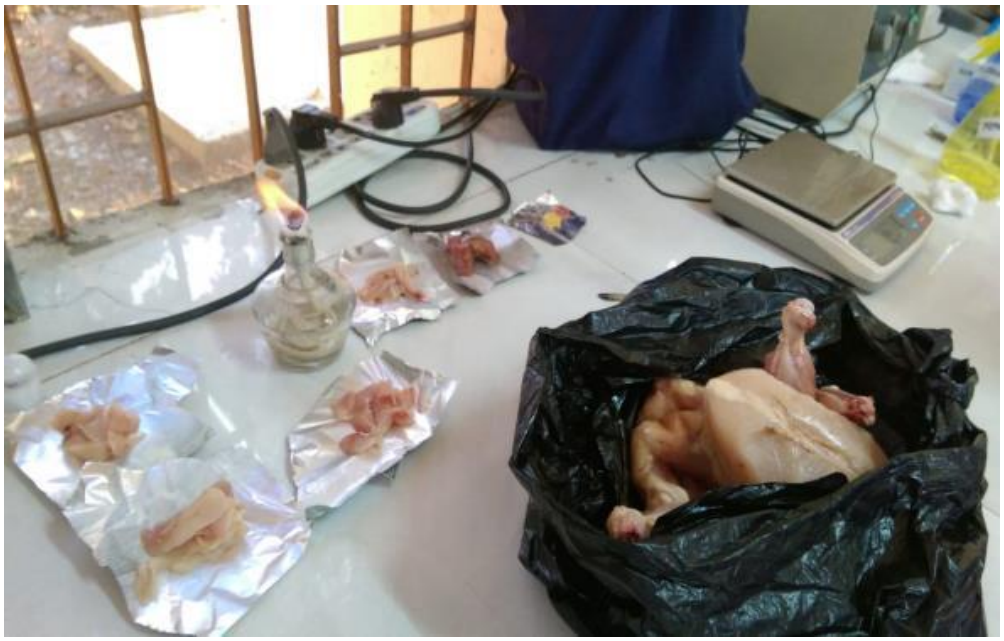


Fig 3.1: Meat sample collected from different market.

3.5.3. Transportation:

For microbiological study, meat and milk samples were collected in nutrient broth. After collection samples were transported to the respective laboratory in transport box maintaining aseptic conditions and refrigerator temperature.

3.6. Bacteriological investigation:

(Isolation, identification of bacteria from collected samples)

3.6.1. *Staphylococcus aureus*

3.6.1.1 Isolation of *Staphylococcus aureus*

Selective enrichment of samples was performed in Muller Hinton Broth (Oxoid, Basingstoke, Hampshire, UK) with 6.5% NaCl at 37°C overnight incubation and then inoculated onto Mannitol salt agar (Oxoid, Basingstoke, Hampshire, UK), where S. aureus produced bright yellow-coloured colonies after incubation of 24 hrs at 37°C.The presumptive positive colonies were identified based on the colony characteristics on MSA. The presumptive positive colonies (bright yellow color) were sub cultured onto blood agar and incubated at 37ºC for 24 hours to detect characteristics appearance on blood agar and the haemolytic properties of organism (Rana et al., 2020).

3.6.1.2. Identification of *Staphylococcus Aureus* by Coagulase test

Suspected colonies were biochemically confirmed by coagulase test. To conduct the coagulase test, whole blood from horse was collected into commercially available EDTA -treated lavender tops. Then blood was centrifuged at 2600 rpm for 10 minutes using a refrigerated centrifuge. The resulting supernatant, the plasma was then immediately transferred to a sterile 1.5 ml eppendorf tube using a sterile micropipette. The plasma was then stored at - 20°C for future use. All the positive samples were subjected to coagulase tests for biochemical confirmation of *Staphylococcus* sp. as previously described (Monica, 1991). For this, few colonies were picked up and transferred to a10ml test tube containing 5ml of BHIB which was prepared according to the instructions of manufacturer (Oxoid ltd, Basingstoke, Hampshire, UK), incubated at 37 °C for6 h. On the otherhand, whole blood from horse was collected into commercially available sterile tubes containing Ethylene Diamine Tetra Acetic Acid (EDTA). Blood was then centrifuged at 2600 rpm for 10 minutes at 4°C. Resulting supernatant, the plasma was immediately transferred to the sterile 1.5 ml Eppendorf tube using sterile tip and stored at -20ºC for further analysis. Fifty microliters of cultivated samples containing BHIB was transferred to the sterile tubes containing 50 µL of horse plasma and incubated at 37ºC for 6 hours. The presence of coagulates were considered when large organized coagulation of all the contents of the tube occurred which do not come off when inverted (Brasil,2003). A control tube without horse plasma also is placed to validate the result.

Catalase test

This test demonstrates the presence of catalase, an enzyme that catalyses the release of oxygen from hydrogen peroxide (H2O2). It is used to differentiate bacteria produces an enzyme catalase, *staphylococci*, from non-catalase producing bacteria such as *streptococci*. Normally 3% H2O2 is used for the routine culture.The enzyme catalase mediates the breakdown of hydrogen peroxide into oxygen and water. The presence of the enzyme in a bacterial isolate is evident when a small inoculum is introduced into hydrogen peroxide, and the rapid elaboration of oxygen bubbles occurs. The lack of catalase is evident by a lack of or weak bubble production. The culture should not be more than 24 hours old.

3.6.2. *Escherichia coli*

3.6.2.1. Isolation of *Escherichia coli*

Escherichia coli sample was selectively enriched in MacConkey (Oxoid, Basingstoke, Hampshire, UK) at 37°C overnight. After enrichment, sample Was inoculated onto MacConkey agar (Oxoid, Basingstoke, Hampshire, UK), where *E. coli* produces large pink colour colony after incubation of 24 hrs at 37°C.The suspected large colour colony was inoculated onto Eosin Methylene Blue (EMB) (Oxoid ltd, Basingstoke, Hampshire, UK) agar and incubated for 24 hours at 37ºC to verify whether such population produced colonies with metallic sheen, a diagnostic criterion for *E. coli* (Dyes Eosin Y and Methylene Blue react with products released by *E. coli* from lactose or sucrose as carbon and energy source, forming metallic green sheen).

3.6.2.2 Identification of *Escherichia coli*

Typical metallic sheen colony was sub-cultured onto Blood agar and finally tested for standard biochemical tests for *E. coli*, e.g Catalase test. Indole, Methyl red, Voges-Proskauer test, Nitrate reduction, Urease production, Simmon's citrate agar, and various sugar fermentation tests (**Table 3.2**).

Table 3.2: Typical biochemical reactions shown by any isolate belonging to *E.coli*

BiochemicalTest	**Reaction**
Lactose fermentation	+ve
Catalase	+ve
Simmon'sCitrate	-ve
IndoleProduction	+ve
NitrateReduction	+ve
MethylRed	+ve
Voges-Proskauer	-ve
Urease	-ve
Acid from Sugar	
Glucose	+ve
Mannitol	+ve
Lactose	-ve
Salicin&Sucrose	+ve

3.6.3. *Campylobacter jejuni*

3.6.3.1. Isolation of *Campylobacter jejuni*

Standard bacteriological approaches followed by molecular techniques were applied for isolation and identification of *C. jejuni* from the collected samples. Briefly, all samples were directly inoculated on selective *campylobacter* base agar (OxoidLtd, UK) containing antibiotics and 5-7% sheep blood (Vanderzant&Splittstroesser, 2001). The plates were incubated in an anaerobic jar (Oxoid™ AnaeroJar™ 2.5L) under the microaerophilic conditions with a CO2 sachet (Thermo ScientificTM Oxoid Anaero Gen2.5 Lsachet) (10% CO2, 95% humidity) in 42° C for three days.

3.6.3.2. Identification of *Campylobacter jejuni*

After 72 hours, single characteristic (small, round, creamy-gray, or whitish) colonies from each plate were selected and inoculated in tryptic soy broth (OxoidLtd, UK) and incubated 37°C for three days under microaerophilic condition. The presumptive *Campylobacter* isolates were subjected to microscopic examination to observe the seagull appearance of *C. jejuni* with Gram staining (Vandamme et al., 2008). The isolates were then stored at -80°C in brain heart infusion broth (Oxoid Ltd, UK) containing 50% glycerol for further validation using molecular method.

3.6.4. *Salmonella spp.*

3.6.4.1. Isolation of *Salmonella spp.*

For pre-enrichment, samples were diluted 1:9 (w/v) with buffered peptone water (BPW; Oxoid, England) contained in a blue capped bottle. A vortex machine was used to make the mixture homogenous. Then the bottles were incubated at 37°C for 18hours and100µl of the overnight culture, divided into three separate and equally-spaced drops, was inoculated on to the surface of Modified Semisolid Rappaport Vassiliadis (MSRV) medium (HiMedia,India) supplemented with novobiocin (HiMedia,India) (Figure 4) and incubated at 41.5° C for 24-36 hours. Any swarming growth observed on the MSRV plates was transferred to brilliant-green agar (Oxoid Ltd., England) by dipping an inoculating loop into the swarmed zone and incubated overnight at 37°C to obtain isolated colonies.

3.6.4.2. Identification of *Salmonella spp.*

The presumptive *Salmonella* isolates were identified by two confirmatory biochemical tests, triple-sugar-iron (TSI) agar test and the urease test. The presumptive *Salmonella* colonies were directly stabbed into the TSI agar slant.

Inoculated samples were incubated with loosened caps for 24 h at 35^{O}C For the urease test, 2 loopful of pure and well isolated *Salmonella* colonies were inoculated into the urea broth. The inoculated tubes were shaken gently and incubated with loosened caps for 48 h at 35^{O}C in an incubator. The TSI agar was checked for the production of hydrogen sulphide (H2S) gas and the alkalinity, while the urease test was checked for the degradation of urea in urea broth.

3.7 Preservation of isolates

3.7.1 *Staphylococcus aureus, E. coli* and *Campylobacter jejuni*

All positive isolates of *E coli* and *Staphylococcus aureus* were inoculated in Brain Heart infusion (BHI)broth (Oxoidltd, Basingstoke, Hampshire, UK) and incubated overnight at 37°C. 700µl BHI broth culture and 300µl of 50% glycerol were added in a 1.5 ml Eppendorf tube for each isolate. Finally, the tubes were properly labelled and stored at -80°C for further investigation.

3.7.2 *Salmonella*

The Salmonella colonies that were hydrogen sulphide gas positive on TSI agar, urease negative was subcultured onto fresh SS agar plates. Then a single colony was transferred into 20 mL of nutrient broth (enrichment media) and incubated for 18 h at 35^{O}C for further studies.

3.8. Screening of antimicrobial pattern of *S. aureus, E. coli, Campylobacter and Salmonella spp.*

Bacterial isolates were screened for antimicrobial susceptibility against a panel of antimicrobials using Kirby-Bauer disc diffusion method. Seven antimicrobials of six different groups of drugs having public health significance were selected for the cultural susceptibility (CS) testing. To interpret the result of CS test, it was compared with the CLSI standards. We screened the isolates against 6 groups of unrelated antimicrobials namely: β-lactam antibiotics, tetracyclines, polymyxins, aminoglycosides, quinolones and sulfonamides. The following anti-microbial agents (with respective disc potencies) were

used: CAZ: Cefatazidime(30µg), E: Erythromycin(15µg), S: Streptomycin (10µg), DO: Doxycycline(30µg), CRO: Ceftriaxone (30µg), AMC: Amoxicillin+Clavulinic acid (10µg), TE: Tetracycline (30µg), CN: Gentamycin(10µg), AMP: Ampicillin(10µg), CTX: Cefotaxime(30µg), OT: Oxytetracycline(30µg), SXT: Sulfamethoxazole-trimethoprim (23.75µg+1.25µg), CIP: Ciprofloxacin (5µg), MEM: Meropenem(10µg), OX: Oxacillin(1µg).

3.8.1. Procedure of cultural sensitivity test (CS) test

At first sub-culturing of the preserved organism was done on blood agar and incubated at 37°C for 24hours to obtain a pure growth. Using sterile inoculating loop 3 or 4 individual colonies from the blood agar were transferred into a tube containing 3ml of sterile phosphate buffer saline solution (0.85% w/v NaCl solution). Emulsification of the inoculums was done to avoid clumping of the cells inside test tube using vortex machine. Then the bacterial suspension was adjusted to the turbidity of 0.5 McFarland standard (equivalent to growth of 1-2×108 CFU/ml). Within 15minutes of preparing the inoculums, a pre-sterile cotton swab was dipped into the inoculums and rotated against the side of the tube with firm pressure to remove excess fluid. Then the swab was streaked over the entire dry surface of Mueller Hinton agar for three times rotating the plate approximately at 60 degrees. After 15 minutes of inoculation the discs were placed on the agar surface using a sterile forceps. After dispensing all the discs, the agar plates were incubated at 37°C for 18 hours. After incubation the size of zone of inhibition (in mm) around a disc including the diameter of the disc was measured using a ruler and the result was interpreted according to CLSI, 2011.

Table 3.3: Concentrations and diffusion zone breakpoints for resistance against antimicrobials standard for isolates (CLSI, 2011).

Antimicrobial Agent	Disc Conc.	Diffusion Zone Break point (diameter in mm)											
		Staphylococcus aureus			***Escherichia coli***			***Campylobacter***			***Salmonella***		
		R	**I**	**S**	**R**	**I**	**S**	**R**	**I**	**S**	**R**	**I**	**S**
Cefatazidime (CAZ)	30µg	≤14	15-17	≥18	≤17	18-20	≥21	≤14	15-17	≥18	-	-	-
Erythromycin(E)	15µg	≤13	14-22	≥23	≤13	14-22	≥23	≤13	14-22	≥23	≤13	14-22	≥23
Streptomycin(S)	10µg	-	-	-	≤11	12-14	≥15	≤11	12-14	≥15	≤11	12-14	≥15
Doxycycline (DO)	30µg	≤12	13-15	≥16	≤10	11-13	≥14	≤12	13-15	≥16	-	-	-
Ceftriaxone (CRO)	30µg	≤13	14-20	≥21	≤19	20-22	≥23	≤13	-	≥21	≤13	14-20	≥21
Amoxicillin+Clavulinic acid (AMC)	10µg	≤19	-	≥20	≤13	14-17	≥18	-	-	-	-	-	-
Gentamycin (CN)	10µg	≤12	13-14	≥15	≤12	13-14	≥15	≤12	13-14	≥15	≤12	13-14	≥15
Cefotaxime (CTX)	30µg	≤14	15-22	≥23	≤22	23-25	≥26	≤14	15-22	≥23	-	-	-
Oxytetracilin (OT)	30µg	≤14	15-18	≥19	≤11	12-14	≥15	-	-	-	-	-	-
Ciprofloxacin (CIP)	15µg	≤15	16-20	≥21	≤15	16-20	≥21	≤15	16-20	≥21	≤15	16-20	≥21
Meropenem (MEM)	10µg	≤13	14-15	≥16	≤19	20-22	≥23	-	-	-	-	-	-
Trimethoprim/Sulfamethoxazole (SXT)	25µg	≤10	11-15	≥16	≤10	11-15	≥16	≤10	11-15	≥16	≤10	11-15	≥16
Imepenem (IMP)	10µg	≤13	14-15	≥16	≤19	20-22	≥23	-	-	-	-	-	-
Tetracycline (TE)	30µg	≤14	15-18	≥19	≤11	12-14	≥15	≤14	15-18	≥19	≤14	15-18	≥19
Oxacillin (OX)	1µg	≤10	11-12	≥13	-	-	-	-	-	-	-	-	-
Ampicillin (AMP)	10µg	≤28	-	≥29	≤13	14-16	≤17	≤28	-	≥29	≤13	14-16	≤17
Azithromycin (AZM)	15µg	-	-	-	-	-	-	-3	-		-	-	-
Aztreonam (ATM)	30µg	-	-	-	-	-	-	-	-	-	≤17	-	≥21
Colistin (CT)	10µg	-	-	-	-	-	-	-	-	-	≤22	-	≥26

AMP: Ampicillin, E: Erythromycin, S: Streptomycin, CN: Gentamycin, CIP: Ciprofloxacin, SXT: Sulphamethoxazole-Trimethoprim, TE: Tetracycline, CRO: Ceftriaxone, CAZ: Cefatazidime, CTX: Cefotaxime, ATM: Aztreonam, CT: Colistin sulphate, ERE: Erythromycin

3.9. Molecular detection of bacterial isolates

Polymerase chain reaction was performed for molecular detection of *Staphylococcus aureus, E. Coli, Campylobacter jejuni, Salmonella spp.by nuc*, *16SrRNA, stx* genes respectively as described earlier (Dashti et al., 2009; Khal et al., 2005).

3.9.1. Sub-culturing on blood agar

The preserved isolates were removed from the freezer and thawed at room temperature. There after, the isolates were inoculated on blood agar and incubated at 37°C for 24 hours. After completion of incubation period colonies from blood agar were used for DNA extraction to be used for polymerase chain reaction (PCR).

3.9.2. DNA extraction from the isolates

For the extraction of DNA from the recovered isolates boiling method was used. Briefly the procedure is mentioned below:

i. A loop full of fresh colonies (about 3-4) was picked from each blood agar and transferred to 1.5ml Eppendorf tubes containing 100µl deionized water. The tubes were then vortexed to make a homogenous cell suspension. A ventilation hole was made on the lid of each tube.

ii. Then the tubes were boiled at 99°C for 15 minutes in water bath. Immediately after boiling the tubes were placed into the ice pack for 5 minutes. The process of high temperature boiling and immediate cooling allowed the cell wall to breakdown to releae (Repeat this step for S. aureus due to its double cell wall)

iii. DNA from the bacterial cell.

Iv. Finally, the tubes with the suspension were centrifuged at 15000 rpm for 5 minutes.

V. Then 50 µl of supernatant containing bacterial DNA from each tube was collected in another sterile eppendorf tubes and preserved at-20°C until used.

3.9.3 PCR reactions for the identification of species

The primer sequences used for the PCR are shown in **Table 3.4**.

Table 3.4: Oligonucleotide primer sequence of nuc, *16sr RNA* and *ST11/15*genes

Organisms	Gene	Primer Sequences (5′-3′)	Temp.	Amplicon size (bp)	Ref.
aureus	***nuc***	ATATGTATGGCAATCGTTTCAAT	56^{o}C	395	Kahletal., 2005
		GTAAATGCACTTGCTTCAGGAC			
E. coli	***16s rRNA***	GACCTCGGTTTAGTTCACAGA	58°C	585	Dashtiet al.,2009
		CACACGCTGACGCTGACCA			
jejuni	***16s rRNA***	ATCTAATGGCTTAACCATTAAAC	58°C	857	(Lintonet al.1997)
		GGACGGTAACTAGTTTAGTATT			
Salmonella spp.	***ST11 ST15***	AGCCAACCATTGCTAAATTGGCGCA	56°C	429	Aabo *et al*.,1993
		TGGTAGAAATTCCCAGCGGGTACTG			

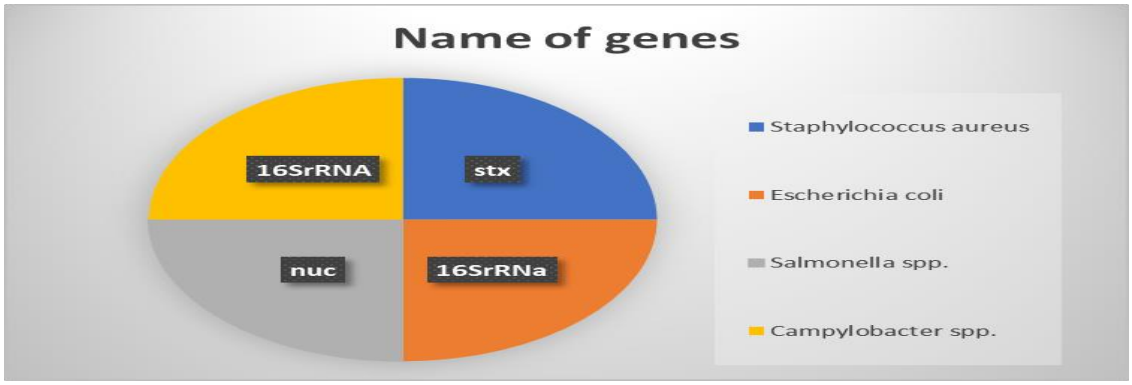


Figure 3.2: Confirmatory gene for identification of different pathogens

3.9.4 Molecular detection of all oligonucleotide primer

Polymerase chain reaction (PCR) assay was conducted for the final confirmation of the suspected isolates by conventional PCR using genus-specific primer.

PCR reactions were conducted with a final volume of 15 µl using 20 picomoles of each primer concentration. PCR reaction mixture contained 9.5 µl of nuclease free water, 12.5 µl dreamtaq master mix, 0.5 µl of each primer and 2µl of DNA template previously isolated positive strain and Nuclease-free water were used as positive and negative control, respectively. Run on a thermo cycler (Applied Biosystem, 2720 thermal cycler, Singapore) following the manufacturer recommended cycling conditions. PCR products (amplicons) were stored at 4°C until analyzed by electrophoresis in 1% agarose containing Ethidium Bromide.

Table 3.5: Resistant gene of different bacterial isolates targeted in the present study

S. aureus	*Erm (B)*	*Erm (C)*	*Tet (K)*	*Tet(M)*	*mecA*	*blaz*			
E. coli	*blaSHV*	*blaCMY*	*Sul1*	*Tet (A)*	*tet(B)*	*tet(C)*	*BlaTem*	*aac(3)IV*	*ere(A)*
Salmonella	*blaTEM*	*blaSHV*	*blaCMY*	*Sul1*	*Sul2*	*Sul-Gka*	*Tet (A)*	*Tet(B)*	*tet(C)*
Campylobacter	*blaSHV*	*blaCMY-2*	*tet (A)*	*Tet (B)*	*tet(C)*	*blaTem*	*ermB-2*		

3.10. Identification of resistant gene

3.10.1. Resistant genes in *S. aureus*

Table 3.6: Primers used in multiplex polymerase chain reaction systems for the detection of related antibiotic resistance genes in *S. aureus*.

Gene	Primer (5′–3′)	PCR condition	Product size (bp)	Ref.
blaZ	AAGAGATTTGCCTATGCTTC	Initial denaturation at 95◦C for 5 min, 38 cycles of denaturation at 95◦C for 30 secs, annealing at 50◦C for 35 secs, extension at 65◦C for 1.5 min, and final extension at 65◦C for 10 min.	517	Gao *et al.* 2011
	GCTTGACCACTTTTATCAGC			
erm (B)	ACGACGAAACTGGCTAA		409	
	TGGTATGGCGGGTAA			
erm (C)	CTTGTTGATCACGATAATTTCC		190	
	ATCTTTTAGCAAACCCGTATTC			
tet (K)	TCGATAGGAACAGCAGTA		169	
	CAGCAGATCCTACTCCTT			
tet (M)	CCGCACCCTCTACTACAA		351	
	CATTCCACTTCCCAACG			
mecA	TCCAGATTACAACTTCACCAGG	Initial denaturation at 94◦C for 4 min, 30 cycles of denaturation at 94◦C for 30 secs, annealing at 53◦C for 30 secs, extension at 72◦C for 1 min, and final extension at 72◦C for 4 min	162	Liveiraet al.,2002

3.10.2 Polymerase chain reaction (PCR) to test for the presence of resistant genes in *E. coli*

Genes against b -lactams, TE, E, and quinolones were detected using 4 different PCRs (Table 9). The b-lactamase genes *blaTEM*, *blaSHV*, and *blaCMY-2* and the primary genes for TE *tetA*, *tetB*, and *tetC* were tested by multiplex PCRs using the primers and conditions described by Kozaketal. (2009) and Lanzetal. (2003). A single gene (ermB) was analyzed to determine the against E, according to Chenetal. (2007).

Table 3.7: Primers used for the multiplex polymerase chain reaction for the identification of *E. coli* and related antibiotic gene

Antibiotic	Gene	Primer sequence (5´-3´)	Product size (bp)	Ref.
Tetracycline	*tet(A)*	GGTTCACTCGAACGACGTCA	577	Momtaz et al., 2012
		CTGTCCGACAAGTTGCATGA		
	tet(B)	CCTCAGCTTCTCAACGCGTG	634	Momtaz et al., 2012
		GCACCTTGCTGATGACTCTT		
	tet(C)	AACAATGCGCTCATCGT	1138	Kim et al., 2013
		GGAGGCAGACAAGGT AT		
Gentamycin	*Aac* (3)-IV	CTTCAGGATGGCAAGTTGG T	286	Momtaz et al., 2012
		TCATCTCGTTCTCCGCTCAT		
Ampicillin	*bla*TEM	ATAAAATTCTTGAAGAC	1073	Kim et al., 2013
		TTACCAATGCTTAAT CA		
Ceftriaxone	*blaS HV*	TCGCCTGTGTATTATCTCCC	768	Momtaz et al., 2012
		CGCAGATAAATCACCACAATG		
	blaC MY	TGGCCAGAACTGACAGGCAAA	462	Momtaz et al., 2012
		TTTCTCCTGAACGTGGCTGGC		
Streptomycin	*aadA 1*	TATCCAGCTAAGCGCGAACT	447	Momtaz et al., 2012
		ATTTGCCGACTACCTTGGTC		
Sulfonamide	*sul1*	TTCGGCATTCTGAATCTCAC	822	Momtaz et al., 2012
		ATGATCTAACCCTCGGTCTC		
Erythromycin	*ere(A)*	GCCGGTGCTCATGAACTTGAG	419	Momtaz et al., 2012
		CGACTCTATTCGATCAGAG GC		

3.10.3. PCR for resistant genes in *C. jejuni*

Resistance genes against b -lactams, TE, E, and quinolones were detected using 4 different PCRs **(Table 3.8)**. The b-lactamase genes *blaTEM*, *blaSHV*, and blaCMY-2 and the primary resistance genes for TE *tetA*, *tetB*, and *tetC* were tested by multiplex PCRs using the primers and conditions described by Kozaketal. (2009) and Lanzetal. (2003). A single gene (ermB) was analyzed to determine the resistance against E, according to Chenetal.

(2007). *Escherichia coli* PCR positive amplicons for each gene belonging to previous studies were used as positive controls (Fenollar et al., 2019).

Determination of AMR was carried out by a standard disc diffusion assay (Antimicrobial Susceptibility Test Disc, Oxoid Ltd.) in Mueller-Hinton Agar medium (Mueller-Hinton Broth, Scharlau, Barcelona, Spain) enriched with 5% defibrinated sheep blood (Oxoid Ltd.). An inoculum of each isolate was diluted in a 0.9% saline solution (Scharlau) and adjusted to a concentration of 2.0 on the McFarland scale. Incubation conditions were 41.5± 1°C during 44±4 h, specific for thermophilic Campylobacter, under a micro-aerobic atmosphere (84% N_2, 10%CO_2, and6%O_2) (CampyGen, OxoidLtd.). The measurement and interpretation of the results were carried out following the guidelines of the European Committee on Antimicrobial Susceptibility Testing (ECDC, 2016). Isolates were considered multidrug resistant (MDR) when the isolate was resistant to at least 2 antimicrobial classes (ECDC,2016). Resistance level were classified based on the values indicated by European Food Safety Authority and ECDC (2018a, b): sporadic, 0.1%;very low0.1 to 1.0%; low.1.0 to 10.0%; moderate.10.0 to 20.0%; elevated.20.0 to 50.0%; very high 50.0 to 70.0%; and extremely high. 70.0%

Table 3.8: Primers sequences and product size in the PCRs used for detection of resistance genes in ***Campylobacter jejuni***

Genes	Primer sequences (5'-3')	Product	Reference
blaTEM	TTAACTGGCGAACTACTTAC	247bp	Kozak et al.(2009)
	GTCTATTTCGTTCATCCATA		
blaSHV	AGGATTGACTGCCTTTTTG	393bp	Colom et al.(2003)
	ATTTGCTGATTTCGCTCG		
bla CMY-2	GACAGCCTCTTTCTCCACA	1,000bp	Kozak et al.(2009)
	TGGACACGAAGGCTACGTA		
tetA	GGCGGTCTTCTTCATCATGC	502bp	Lanz et al.(2003)
	GGCAGGCAGAGCAAGTAGA		
tetB	CGCCCAGTGCTGTTGTTGTC	173bp	Goswami et al. (2008)
	GCGTTGAGAAGCTGAGGTG		
tetC	GCTGTAGGCATAGGCTTGGT	888bp	Lanz et al.(2003)
	CCGGAAGCGAGAAGAATCA		
ermB-2	GATACCGTTTACGAAATTGG	364bp	Chen et al.(2007)
	GAATCGAGACTTGAGTGTGC		

3.10.4. PCR for resistant genes in *Salmonella spp.*

PCR using the set of specific primers used for each gene described in **Table 3.9.**

Table 3.9: Oligonucleotide primer sequences used to detect the selected antimicrobial genes in the *Salmonella spp* isolates

Antibiotic	Genes	Primer sequence (5′-3′)	Product size(bp)	Ref.
Tetracycline	*tet(A)*	GGTTCACTCGAACGACGTCA CTGTCCGACAAGTTGCATGA	577	Momtaz et al., 2012
	tet(B)	CCTCAGCTTCTCAACGCGTG GCACCTTGCTGATGACTCTT	634	Momtaz et al., 2012
	tet(C)	AACAATGCGCTCATCGT GGAGGCAGACAAGGT AT	1138	Kim et al., 2013
Gentamycin	*aac*(3)-IV	CTTCAGGATGGCAAGTTGGT TCATCTCGTTCTCCGCTCAT	286	Momtaz et al., 2012
Ampicillin	*blaTEM*	ATAAAATTCTTGAAGAC TTACCAATGCTTAATCA	1073	Kim et al.,2013
Ceftriaxone	*blaSHV*	TCGCCTGTGTATTATCTCCC CGCAGATAAATCACCACAATG	768	Momtaz et al., 2012
	blaCMY	TGGCCAGAACTGACAGGCAAA TTTCTCCTGAACGTGGCTGGC	462	Momtaz et al., 2012
Streptomycin	*aadA1*	TATCCAGCTAAGCGCGAACT ATTTGCCGACTACCTTGGTC	447	Momtaz et al., 2012
Sulfonamide	*sul1*	TTCGGCATTCTGAATCTCAC ATGATCTAACCCTCGGTCTC	822	Momtaz et al., 2012
Erythromycin	*ere(A)*	GCCGGTGCTCATGAACTTGAG CGACTCTATTCGATCAGAGGC	419	Momtaz et al., 2012

3.11. Statistical analysis

Epidemiological data were entered in to a spread sheet program (Microsoft Office Excel and transferred to STATA-13 software for data summary and analysis. Descriptive analysis was done using frequency and percentage. Data presentation was done by tables and graphs.

CHAPTER-IV

4. RESULTS

4.1. *S. aureus* isolated from meat and milk

A total of 130 milk and meat samples were collected and among those, seven *staphyloccus aureus* had been found, four in broiler meat (20% ,95% CI 5.73%-43.6%), one in beef meat (5% ,95% CI 0.12%- 24.8%), one in buffalo meat (10% , 95% CI 0.25%- 44.5%), one in Goat meat (5% , 95% CI 0.12%- 24.8%). Isolates were confirmed as *S. aureus* based on the PCR assay, with characteristic growth of *S.aureus* strain on Mannitol Salt agar plate and β hemolysis on Blood agar plate (Figure4.1 and Figure4.2, respectively), and the result of Catalase and Coagulase test as well as Gram's staining property of it are displayed in Figure 4.3, Figure 4.4 and Figure 4.5, respectively. There results of PCR assay of some of the isolates after gel electrophoresis for the detection of the *spa* gene in these isolates are Displayed in Figure 4.6.

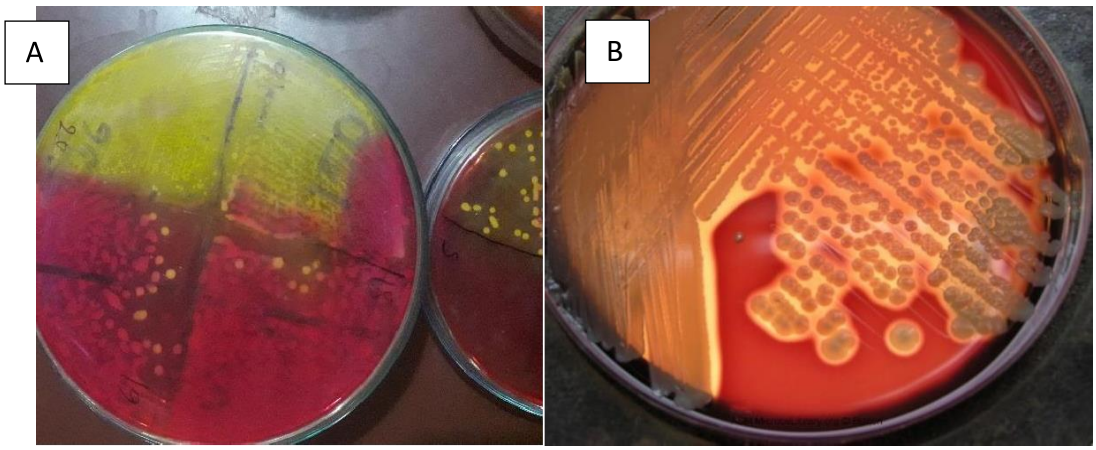


Figure 4:(4.1)A *S. aureus* on Mannitol salt agar plates; (4.2)B *S. aureus* on blood agar plates (β hemolysis)

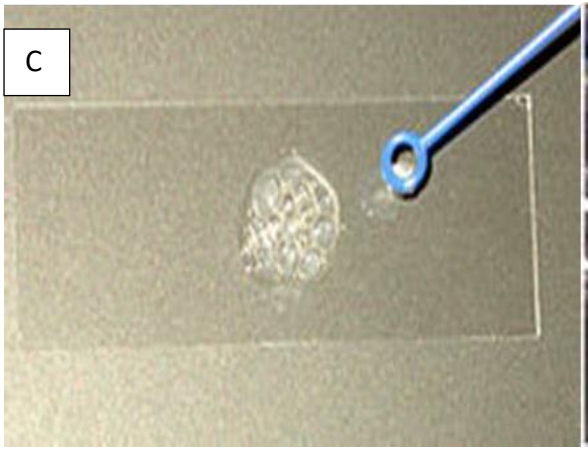


Figure (4.3) C Catalase positive test. **(4.4) D Coagulase positive test**

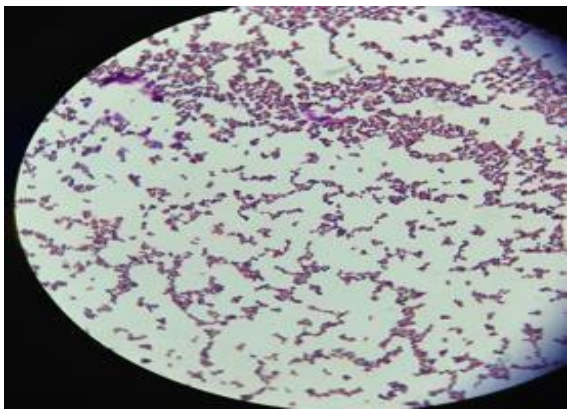


Figure 4.5: Gram's staining properties of *Staphylococcus aureus.*

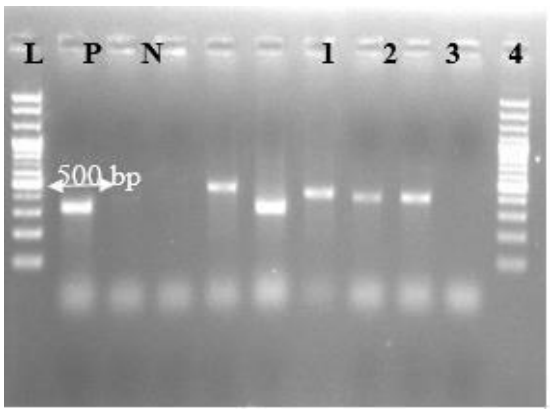


Figure 4.6: Result of PCR assay for the detection of the *spa* gene (variable bp)

Table 4.1: Prevalence of resistance genes in *Staphylococcus aureus* from different milk and meat samples

Prevalence of resistant genes									
Sample name	**Sample size N**	**Total number of *S. aureus* (n)**	**Prevalence of S. aureus**	**ESBL producing gene**	**Erythromycin**		**Tetracycycline**		
				blaz(n)	*Erm (B)*(n)	*Erm (C)*(n)	*Tet (K)*(n)	*Tet(M)(n)*	*mec(A)(n)*
Layer Meat	**20**	**0**	0%	0	0	0	0	0	0
Broiler Meat	**20**	**4**	20%	50%(2)	25%(1)	25%(1)	75%(3)	25%(1)	50%(2)
Beef Meat	**20**	**1**	5%	100%(1)	0	0	0	0	0
Buffalo Meat	**10**	**1**	10%	0 (0)	100%(1)	100%(1)	100%(1)	0	0
Goat Meat	**20**	**1**	5%	100% (1)	0	100%(1)	0	0	100%(1)
Goat Milk	**25**	**0**	0%	0	0	0	0	0	0
Buffalo Milk	**15**	**0**	0%	0 (0)	0	0	0	0	0

4.1.1. Antimicrobial resistance profile of *S. aureus*

All the *S. aureus* (n=7) isolates were found to be resistant to at least one type of selected antimicrobials phenotypically. Isolates showed sensitivity and resistance to different antimicrobials is shown in **Figure 4.7** and McFarland Standard in **4.8**. Antimicrobial susceptibility patterns of the isolates were interpreted following the guidelines of Clinical and Laboratory Standard Institute (CLSI). The susceptibility patterns of the isolates are shown in **Table 4.2.**

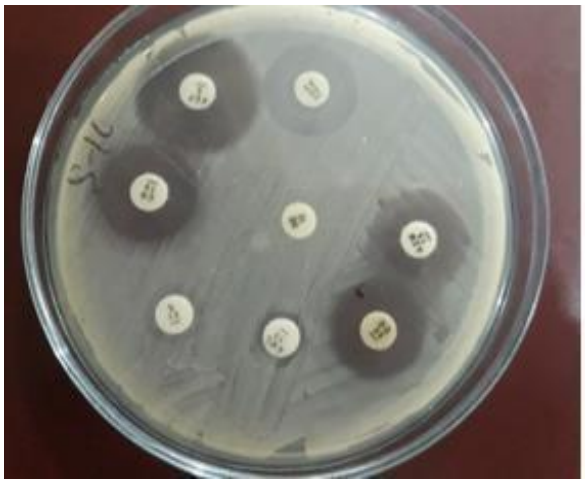


Figure4.7: Bacterial zone of inhibition

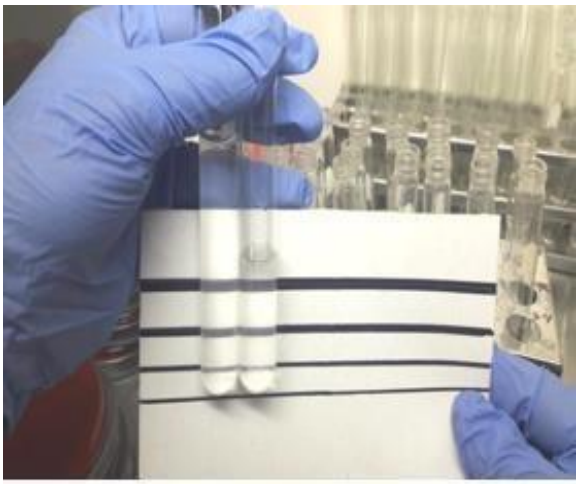


Figure4.8: Comparing inoculum with McFarland Standard

Table 4.2: Antimicrobial resistance pattern of *S.aureus* isolates

Name of Antibiotics	**Broiler meat** (n=4)				**Buffalo Meat** (n=1)	**Goat Meat** (n=1)	**Cattle meat** (n=1)	Percentage of overall resistance (%)
	isolate-1	isolate-2	isolate-3	isolate-4				
(CRO 30µg)	**R**	S	**R**	S	S	**R**	S	42.85
(CN, 10µg)	I	S	**R**	I	I	**R**	**R**	42.85
(SXT, 25µg)	I	**R**	**R**	I	I	**R**	**R**	57.14
(CIP, 5µg)	**R**	S	**R**	S	**R**	**R**	I	57.14
(S, 10µg)	**R**	S	**R**	I	**R**	**R**	**R**	71.42
(ERE, 15µg)	S	**R**	S	S	**R**	**R**	I	42.85
(TE, 10µg)	**R**	**R**	**R**	**R**	**R**	**R**	S	85.71
(MEM, 10µg)	S	S	S	S	S	S	S	14.28
(OT, 30µg)	**R**	**R**	I	**R**	S	**R**	S	57.14
(AMP 10µg)	**R**	**R**	S	**R**	**R**	**R**	**R**	85.71
(OX, 1µg)	**R**	S	I	**R**	S	**R**	S	42.85
(AMC, 10µg)	**R**	**R**	S	I	S	**R**	**R**	57.14
(DO, 30µg)	**R**	**R**	S	S	I	**R**	S	42.85
MDR	MDR	MDR	MDR	MDR	MDR	MDR	MDR	

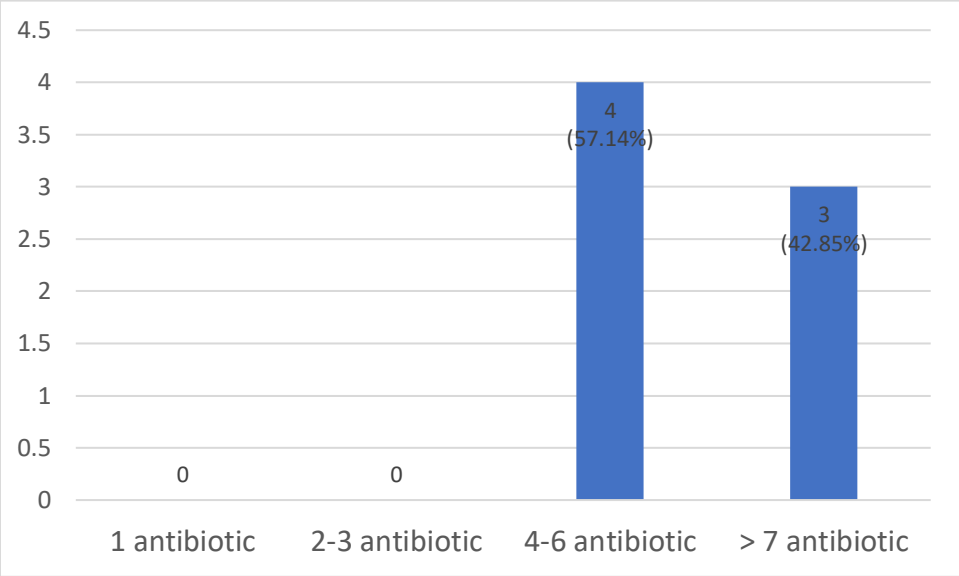


Figure 4.9: MDR pattern of *Staphylococcus aureus* isolates in Chattogram division

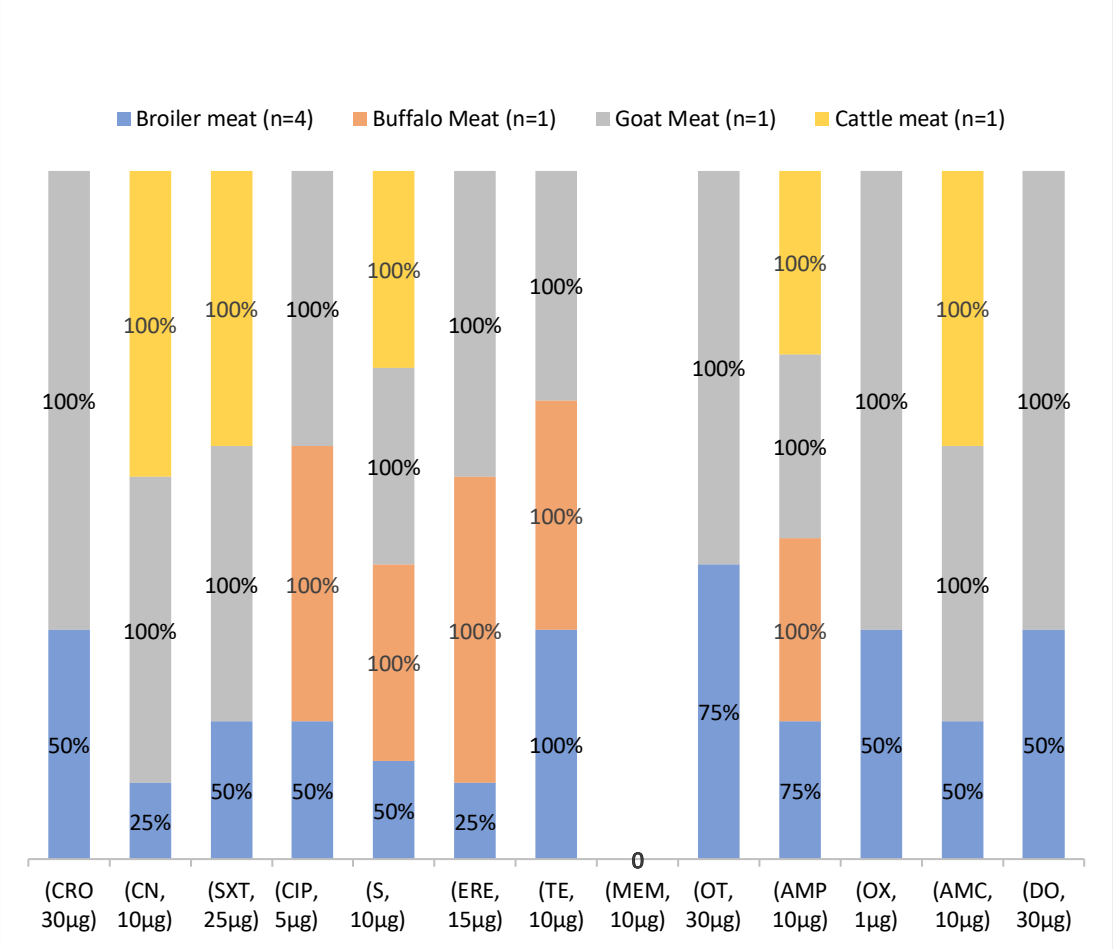


Figure 4.10: Antibiotic resistance pattern of *Staphylococcus aureus* in meat sample

S.aureus showed resistance against all tested antibiotics except Meropenem(MEM). Highest number of isolates (100%) in broiler meat was resistant against Tetracycline (TE), followed by 75% to Oxytetracycline (OT) and 50% to Ceftriaxone (CRO), Oxacillin (OX) and others. Also showing lowest resistance to Erythromycin (ERE), and Gentamycin (CN) is 25%. In buffalo meat 100% resistance showed against Ciprofloxacin (CIP), Streptomycin(S), Tetracycline (TE), Erythromycin (ERE) and Ampicillin (AMP). In goat meat 100% resistance showed against all antibiotics except Meropenem. In cattle meat 100% resistance showed against Gentamycin (CN), Sulphamethoxazole-Trimethoprim (SXT), Streptomycin(S), Ampicillin (AMP) and Amoxycillin/ Clavulanic Acid (AMC) **(Figure 4.10)**. All *S. aureus* isolates were multi drug resistant, 57.14 % of the isolates had resistance against 4 to 6 tested antimicrobials and 42.85% had resistance to more than 7 antimicrobials (**Figure 4.9**).

4.1.2. Resistance gene in *Staphylococcus aureus*

Staphylococcus aureus isolates had ESBL producing 2 *blaz* gene in broiler meat, 1 in cattle meat and 1 in goat meat and Erythromycin producing 1 *Erm (B*) gene in broiler meat and 1 in buffalo meat. Whereas, 1 *Erm (C)* gene in broiler meat, 1 in buffalo meat and 1 in goat meat was identified. Tetracycline producing 3 *Tet (K)* gene found in broiler meat and 1 in buffalo meat. 1 isolate had *Tet(M*) in broiler meat. 2 *mecA* gene found in broiler meat and 1 in goat meat **(Figure 4.11).**

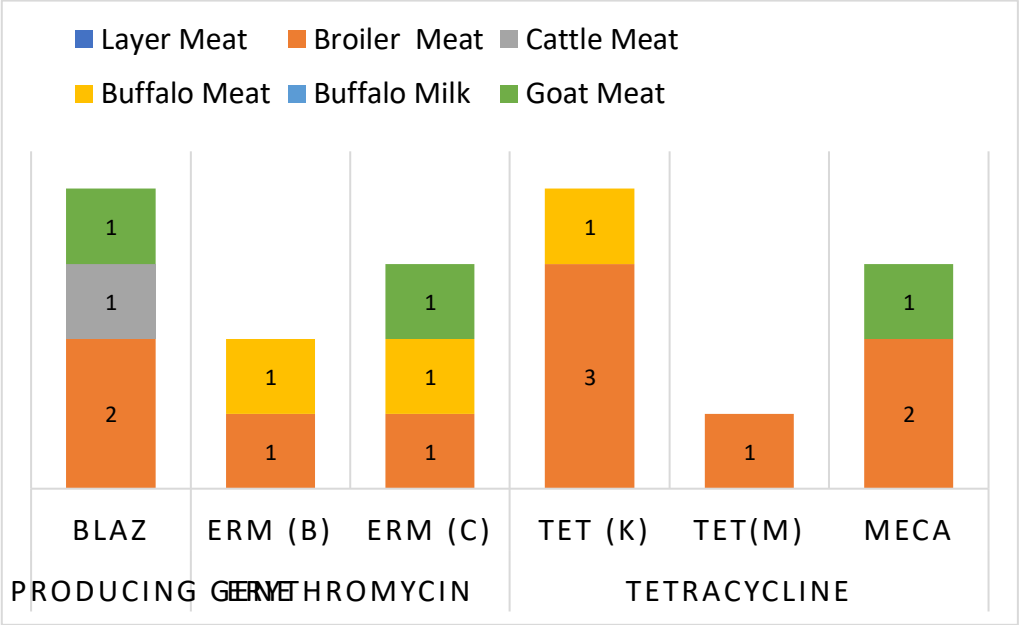


Figure 4.11: Resistant gene in staphylococcus aureus

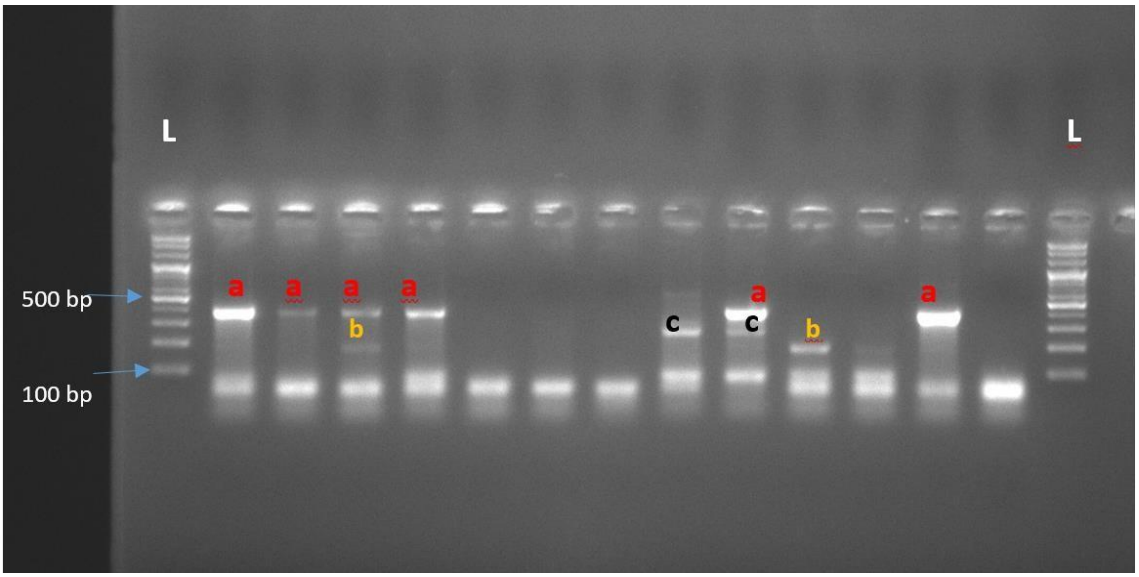


Figure 4.12: PCR assay for the detection of the genes in *S. aureus*; LaneL: 1kbplusDNAladder; Lanea: *nucgene*(395bp); Laneb*: ermC*(109bp); Lanec: *tetM* (351 bp) amplicon**.**

4.2. *E. coli* isolated from milk and meat samples

A total of 130 milk and meat samples were collected and among those 47 isolates were confirmed as *E. coli.* Five in broiler meat (25% , 95% CI 8.65%- 49.1%), nine in beef meat (45% ,95%CI 23.05%- 68.45), four in buffalo meat (40%, 95% CI 12.15% - 73.76%), thirteen in goat meat (65%, 95%CI 40.78%- 84.60%), six in goat milk (24%, 95%CI 9.35%- 45.12%), seven in buffalo milk (46.67%, 95% CI 21.26%-73.41%), three in layer meat (15% , 95% CI 3.2%- 37.8%). Characteristic growth of *E. coli* strain on MacConkey agar plates and on EMB agar plate are shown in **Figure 4.13** And **Figure 4.14** respectively, and the result of indole test and Gram's staining property of it are displayed in **Figure 4.15** and **Figure 4.16** respectively. PCR assay of some of the isolates after gel electrophoresis for the detection of 16s rRNA gene in those are displayed in **Figure 4.17.**

Figure 4.13: *Ecoli* producing large pink colour growth on McConkey

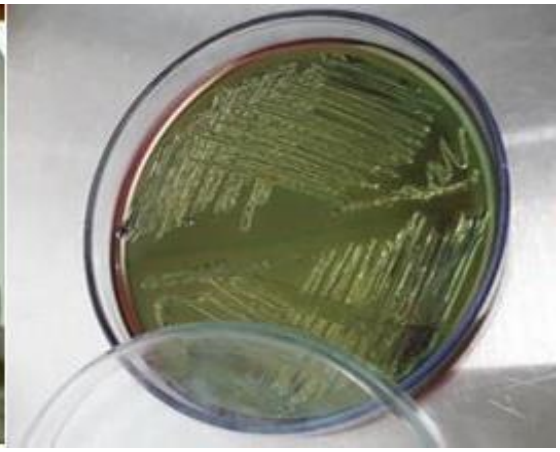


Figure 4.14: Metalic green sheen on EMB agar

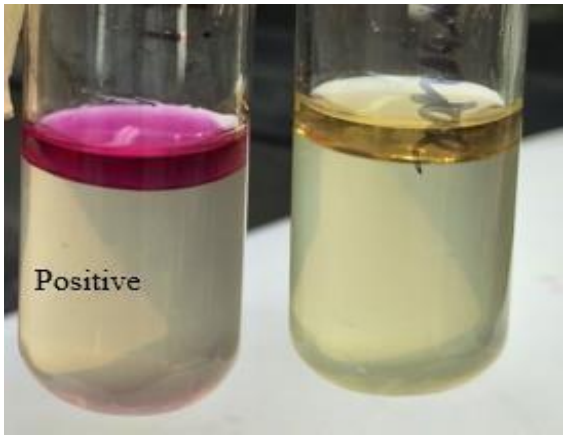


Figure 4.15:*E.coli*;Indole positive

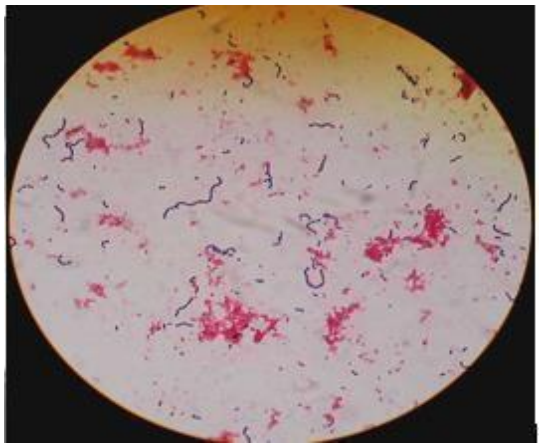


Figure 4.16: Gram's Staining property of *E.coli*

Figure: 4.13(A) *E. coli* producing large pink colour growth on McConkey; (B) 4.14 Metalic green sheen on EMB agar (C)4.15 Indole positive *E. coli; (*D) 4.16 Gram staining property of *E. coli*

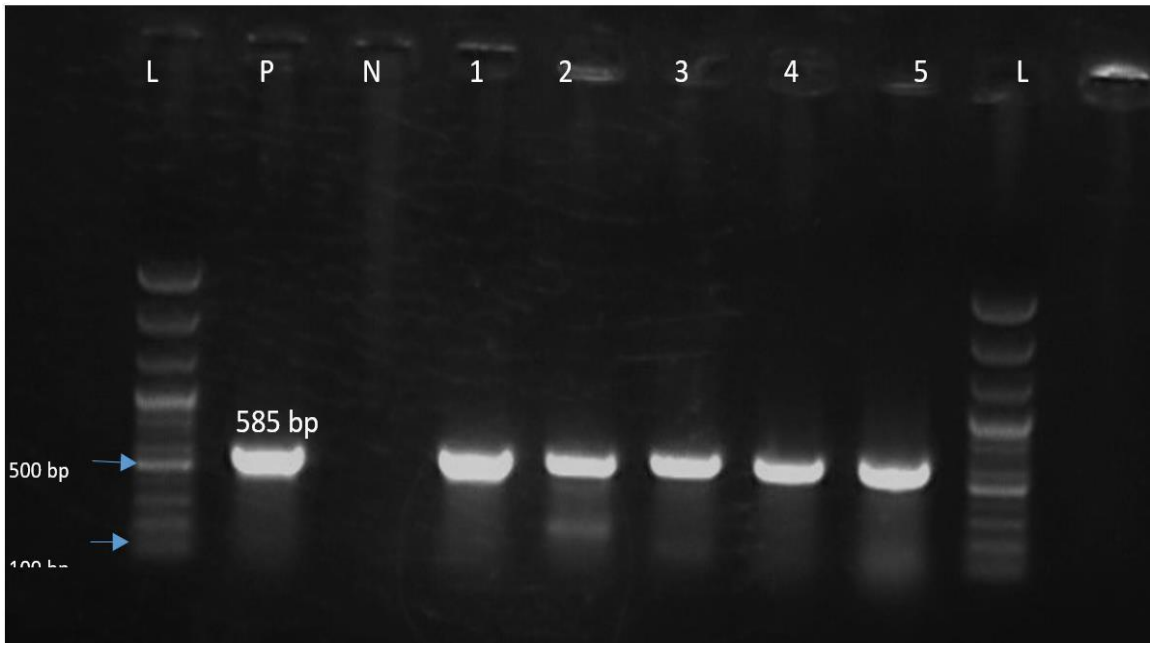


Figure 4.17: PCR assay for the detection of the 16s rRNA gene in *E. coli*; Lane L:plus DNAladder; LaneP:Positive control; LaneN:Negative control; Lane1-5:gene-16s rRNA sized (585 bp) amplicon

Table 4.3: Prevalence of resistance genes in *E. coli* from different Milk and Meat Samples

Prevalence of resistant genes												
Sample name	**Sample size N**	**Total number of *E.coli* (n)**	**Prevalence of *E.coli* %**	***blaSHV***	***blaCMY***	***Sul1***	***Tet (A)***	***tet(B)***	***tet(C)***	***BlaTem***	***aac(3)IV***	***ere(A)***
Layer Meat	**20**	**3**	15%	0	0	33.33% (1)	0	33.33% (1)	0	0	0	0
Broiler Meat	**20**	**5**	25%	0	0	40%(2)	0	40%(2)	0	0	20%(1)	40%(2)
Beef Meat	**20**	**9**	45%	0	33.33% (3)	11.11% (1)	0	22.22% (2)	0	0	33.33% (3)	11.11% (1)
Buffalo Meat	**10**	**4**	40%	0	0	100%(4)	0	50%(2)	0	0	25%(1)	0
Goat Meat	**20**	**13**	65%	0	0	7.69%(1)	0	7.69% (1)	0	0	7.69% (1)	0
Goat Milk	**25**	**6**	24%	0	0	66.66% (4)	0	50%(3)	33.33% (2)	0	16.66% (1)	0
Buffalo Milk	**15**	**7**	46.66%	0	0	50%(2)	0	25%(1)	0	0	25%(1)	0

4.2.1 Antimicrobial resistance pattern of *Escherichia coli*

All the *E. coli* (47) isolates were found to be resistant to at least one type of selected antimicrobials phenotypically. Isolate showing sensitivity and resistance to different antimicrobials is shown in **Figure4.7** and McFarland Standard in Figure **4.8** respectively. Antimicrobial susceptibility patterns of the isolates were interpreted following the guidelines of Clinical and Laboratory Standard Institute (CLSI).The susceptibility patterns of the isolates are shown in **Table 4.4.**

Table4.4: Antimicrobial resistance pattern of *E. coli* isolates

Name of Antibiotics	Buffolo milk (n=7)			Goat milk (n=6)			Broiler meat (n=5)			layer meat (n=3)			Cattle meat (n=9)			Buffolo meat (n=4)			Goat meat (n=13)			Percentage(%) of resistance
	S	**I**	**R**	**S**	**I**	**R**	**S**	**I**	**R**	**S**	**I**	**R**	**S**	**I**	**R**	**S**	**I**	**R**	**S**	**I**	**R**	
(CRO 30µg)	6	0	**1**	6	0	**0**	4	0	**1**	3	0	**0**	7	0	**2**	4	0	**0**	10	0	**3**	14.89
(CN, 10µg)	2	1	**4**	1	1	**4**	1	1	**3**	1	0	**2**	3	0	**6**	2	0	**2**	4	3	**6**	57.44
(SXT, 25µg)	0	0	**7**	0	0	**6**	0	0	**5**	0	0	**3**	0	0	**9**	1	0	**3**	0	0	**3**	97.87
(CIP, 5µg)	1	0	**6**	0	0	**6**	1	0	**4**	0	1	**2**	1	0	**8**	0	0	**4**	1	0	**2**	89.36
(ERE, 15µg)	0	0	7	0	0	**6**	0	0	**5**	0	0	**3**	0	0	**9**	0	1	**3**	0	0	**3**	97.87
(TE, 10µg)	0	0	7	0	0	**6**	0	0	**5**	0	0	**3**	0	0	**9**	1	0	**3**	0	0	**3**	97.87
(AMP 10µg)	0	0	7	0	0	**6**	0	0	**5**	0	0	**3**	0	0	**9**	0	0	**4**	0	0	**3**	100
(N, neomycin 30µg)	2	1	**4**	1	0	**5**	2	1	**2**	1	0	**2**	0	4	**5**	0	1	**3**	4	2	**7**	59.57
	MDR			MDR			MDR			MDR			MDR			MDR			MDR			MDR

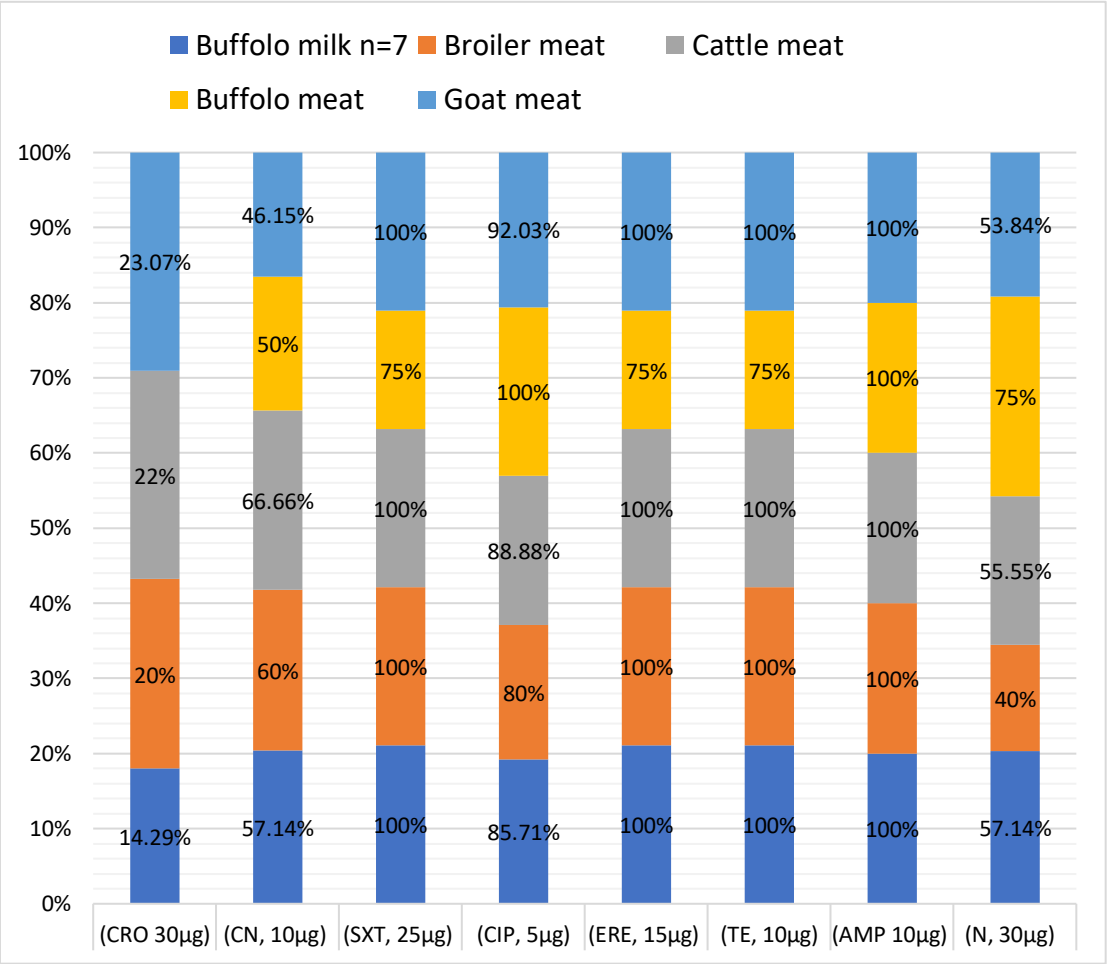


Figure 4.18: Antibiotic resistant pattern of *E. coli* isolates in Chattogram division

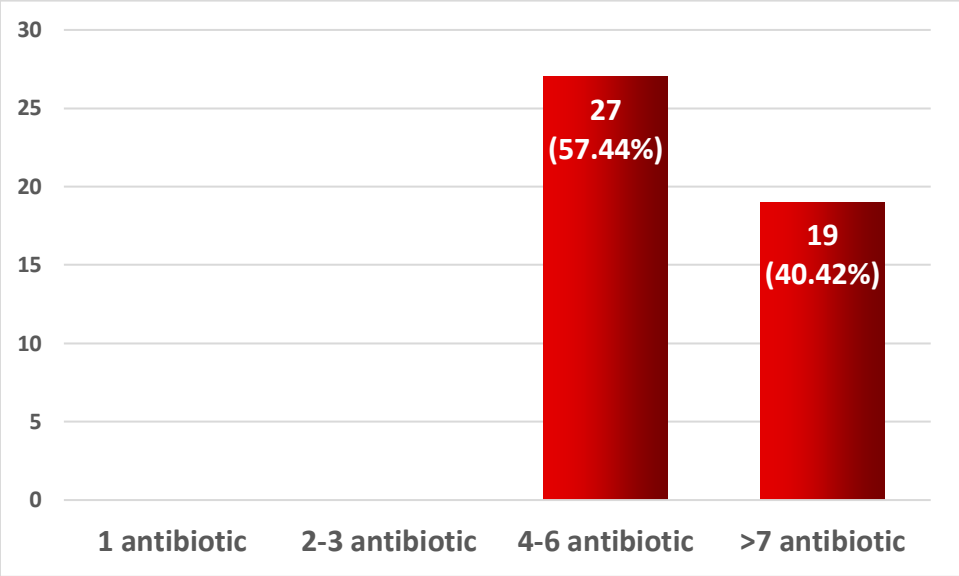


Figure 4.19: MDR pattern of *E. coli* isolates in Chattogram division

Alarmingly in buffalo milk, broiler meat, cattle meat and goat meat, 100% *E. coli* isolates of CMA showed resistance to Sulphamethoxazole-trimethoprim (SXT), Erythromycin (ERE), Tetracycline (TE) and Ampicillin (AMP), followed by in buffalo milk 85.71% against Ciprofloxacin (CIP), 57.14% against Gentamycin (CN), and Neomycin (N). In broiler meat, 80%, 40%, 60%, 20% against Ciprofloxacin (CIP), Gentamycin (CN), Neomycin (N) and Ceftriaxone (CRO). Moreover, in cattle meat, 88.88% against Ciprofloxacin (CIP), 66.66% against Gentamycin (CN). In buffalo meat, 100% resistance showed only against Ciprofloxacin (CIP) and Ampicillin (AMP), Whereas 75% against Sulphamethoxazole-trimethoprim (SXT), Erythromycin (ERE), Tetracycline (TE) and Neomycin (N) **(Figure 4.18).** All 47 E. coli isolates were multi drug resistant. 57.44% isolates were resistant to 4-6 antimicrobials and 40.42% were resistant to more than 7 tested drugs **(Figure 4.19).**

4.2.2 Resistance gene in *Escherichia coli* isolates

4 *Sul1* gene(sulfonamide) gene and 1*Tet (B)*(tetracycline) and 1 *AAC(IV)* gene were identified in goat meat and 2 *Sul1*, 1*Tet (B)* and1 *AAC(IV)* gene were identified in buffalo milk. Also found 3 *blaCMY* gene, 4 *Sul1*, *2Tet (B)*, *3AAC(IV)* and 1 *ERE* gene in **c**attle meat. In buffalo meat, only 1 *Sul1*, *2Tet (B)* gene were found and in broiler meat,2 *Sul1*, *2Tet (B)* and1 *AAC(IV)* and 2*ERE* gene were found. In layer meat, only 1*Sul1* and 1*Tet (B)* gene found. In goat milk, 2 *Sul1*, 3*Tet (B)*, 2 *Tet (C)* and 1 *AAC(IV)* gene were identified in *E. coli* isolates of CMA, respectively (**Figure 4.20**).

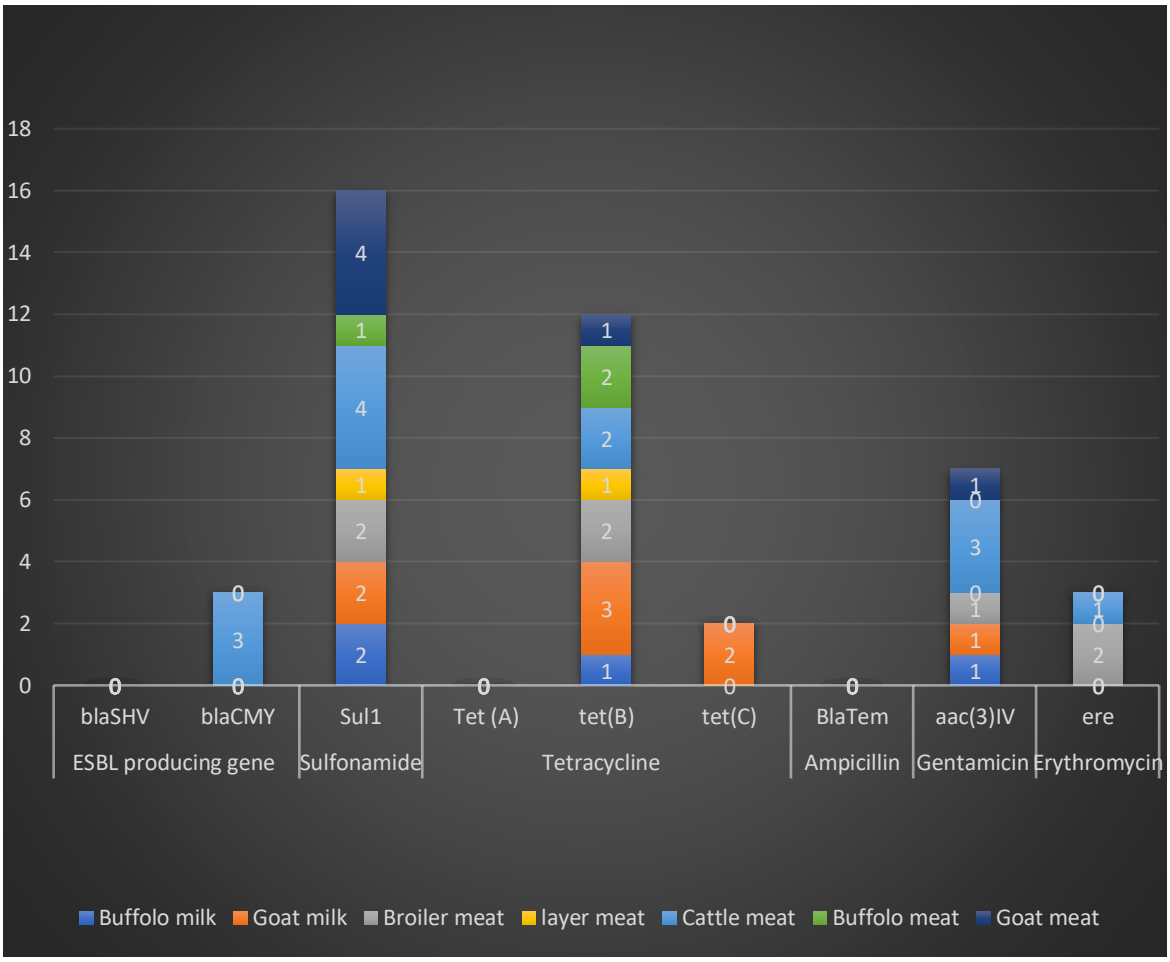


Figure 4.20: Presence of resistant gene in *E. coli* spp isolates in Chattogram area

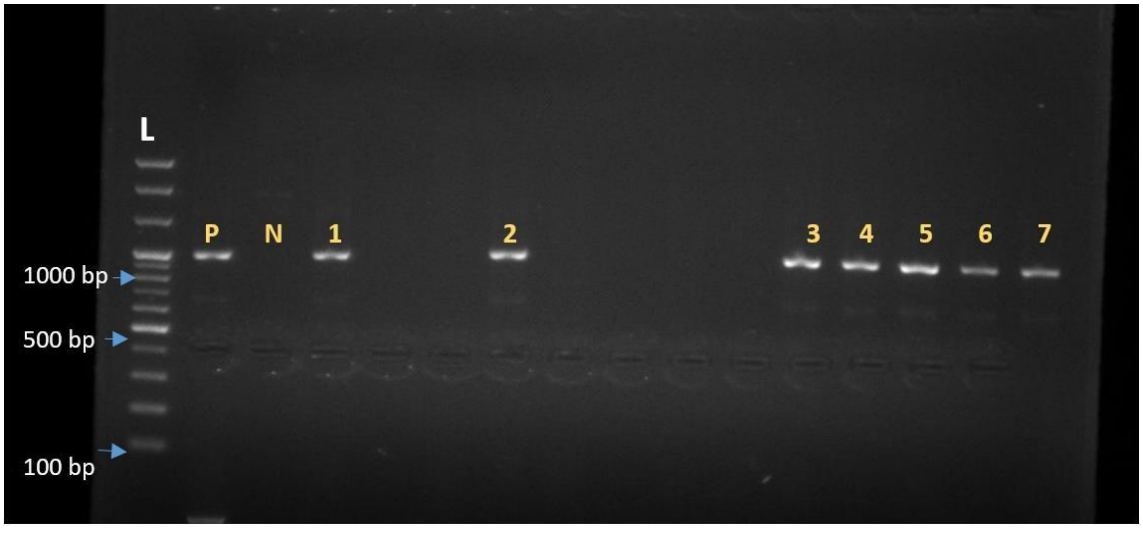


Figure 4.21: PCR assay for the detection of the *blaTEM* gene (964 bp) in *E. coli*; Lane L: 1kb plus DNA ladder; Lane P: Positive control; Lane N: Negative control; Lane 1-7: *blaTEM* gene

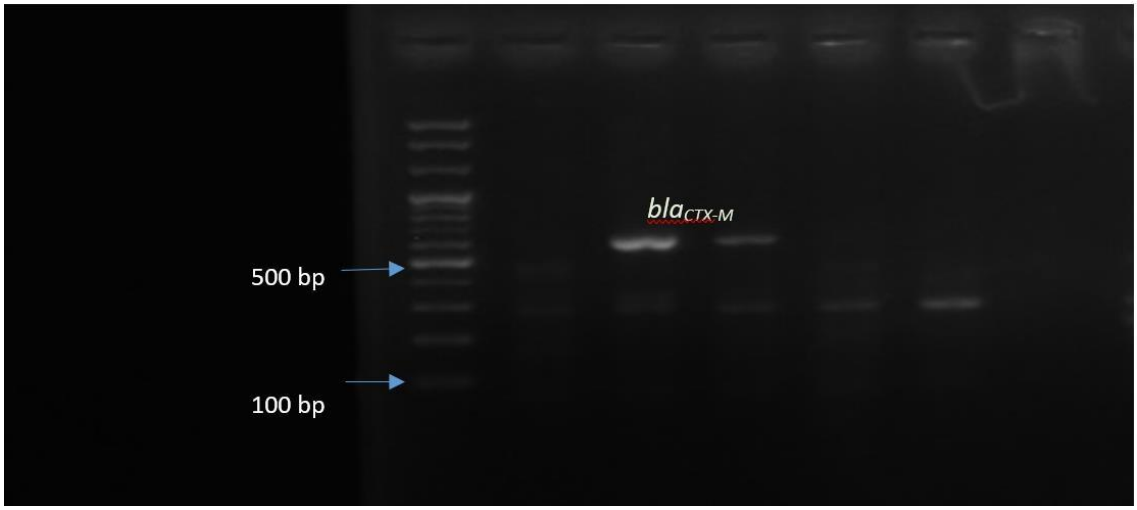


Figure 4.22: PCR assay for the detection of the *blaCTX-M* gene (557 bp) in E. coli

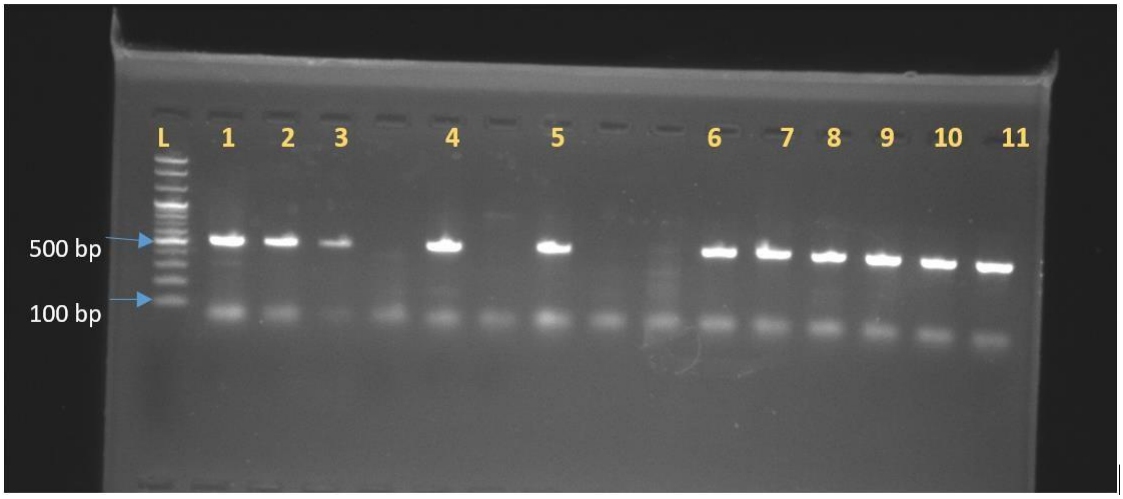


Figure 4.23: PCR assay for the detection of the *tetK* gene(502bp)in *E.coli*;LaneL:1kb plus DNA ladder; Lane 1-11:*tetK* gene

4.3 *Campylobacter* isolated from milk and meat:

A total of twenty-one isolates were confirmed as *Campylobacter* strain out of 130 samples by PCR; Nine in layer meat (45% ,95% CI 23.05%-68.47%), twelve in broiler meat (60% ,95% CI 36.05%- 80.88%). Characteristic growth of *Campylobacter* strain on anaerobic jar with CO2 sachet and gram staining characteristics are shown in **Figure 4.24** and **Figure 4.25**, and good luxuriant growth of C*ampylobacter spp* on culture at **Figure 4.26** and **Figure 4.27**, respectively. PCR assay of some of the isolates after gel electrophoresis for the detection of 16*s rRNA* gene in those are displayed in **Figure 4.28 and Figure 4.29.**

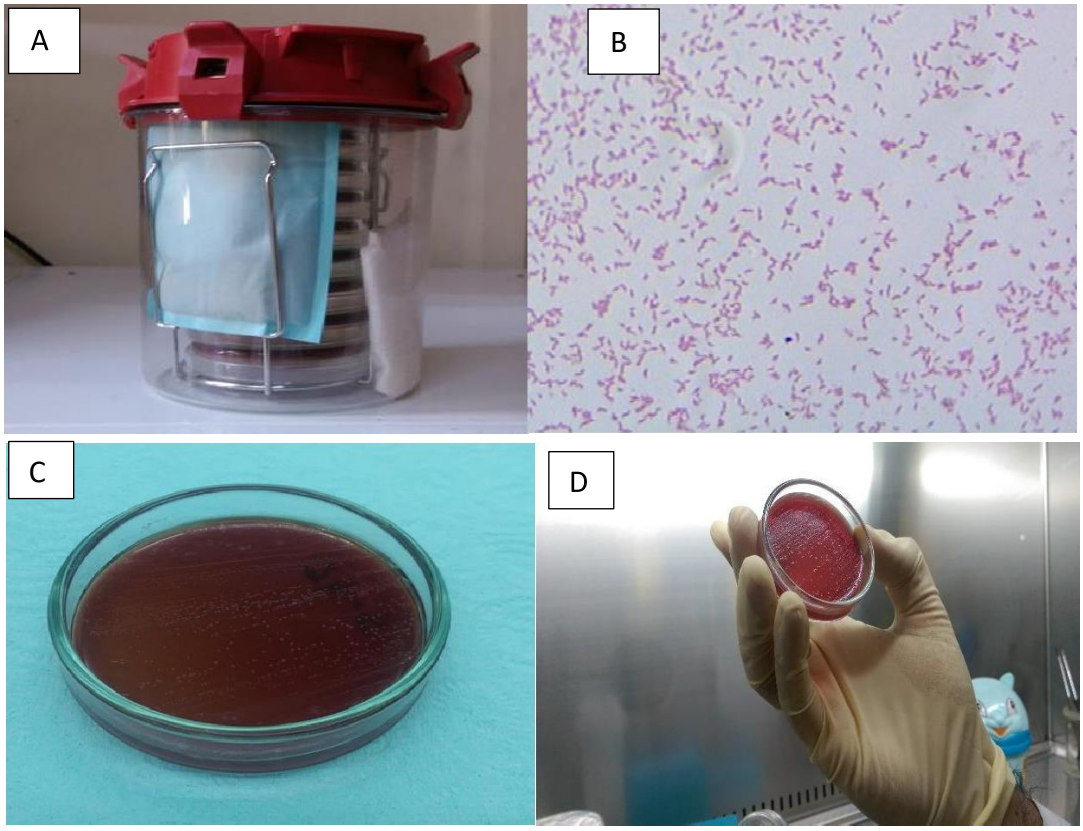


Figure (A) 4.24 Anaerobic jar with CO2 sachet; (B) **4.25** Gram staining property of *Campylobacter spp* **4.26** and **4.27**(C to D) Cultural Response: Good-luxuriant growth of *Campylobacter spp***.**

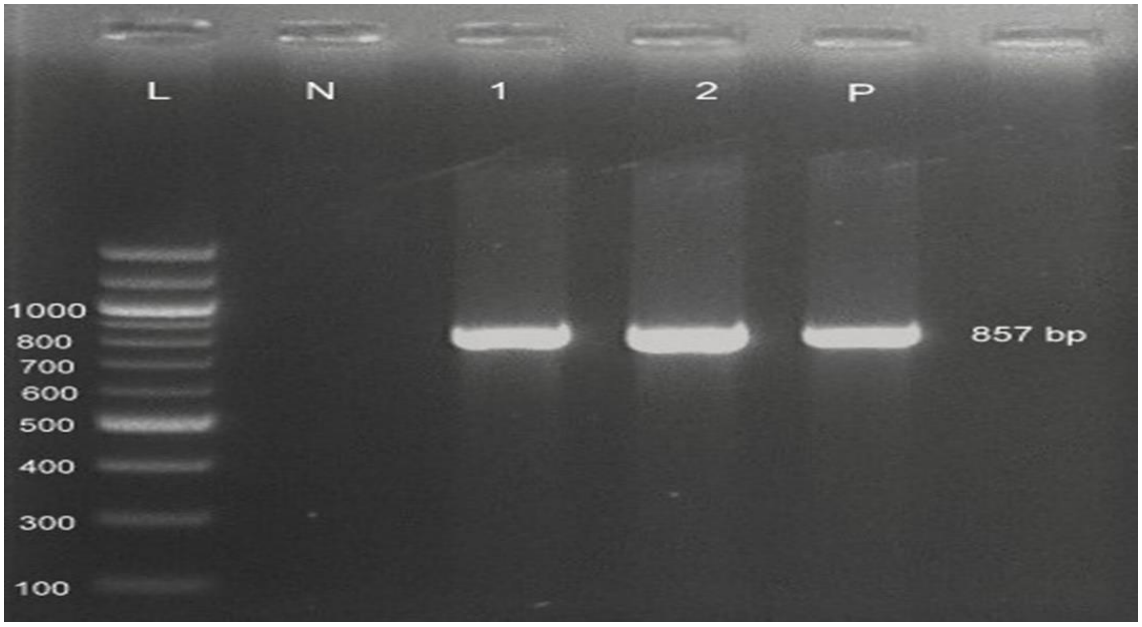


Figure 4:4.28 Result of PCR assay for *16SrRNA* gene of *Campylobacter spp* isolates; Lane L:1kb plus DNA ladder; LaneP: Positive control; LaneN: Negative control; Lane1 and 2: *16S rRNA* gene-sized (857 bp) amplicon

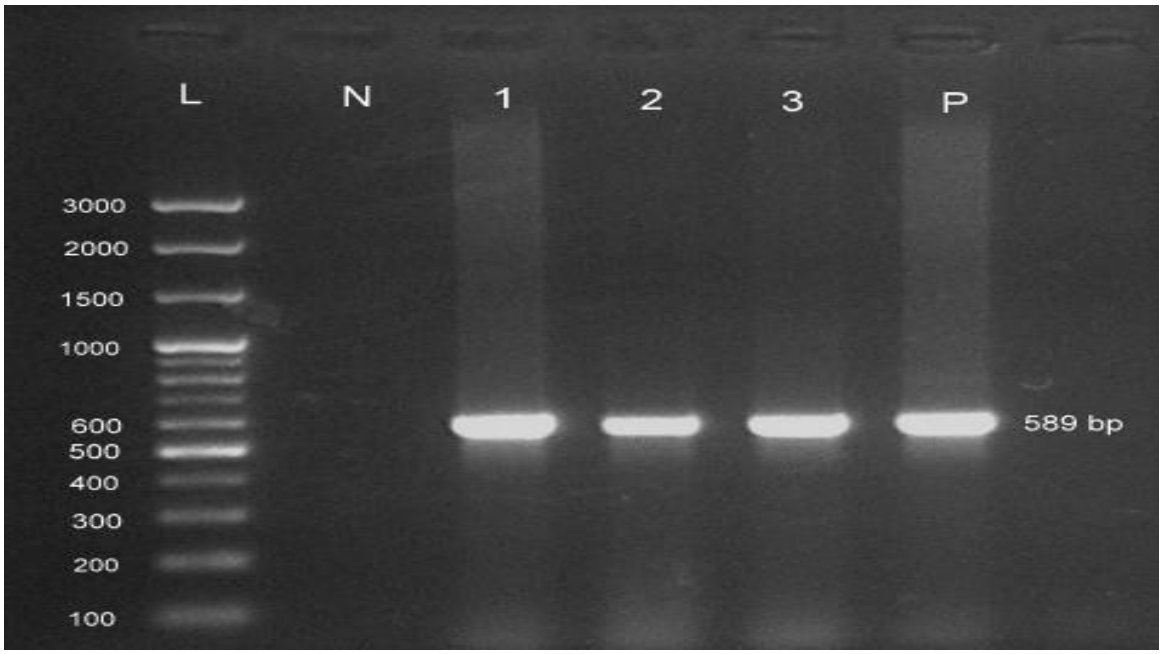


Figure 4.29: Result of PCR assay for *mapA* gene of *C. jejuni* isolates; Lane L: 1kb plus DNA ladder; Lane P: Positive control; Lane N: Negative control; Lane 1, 2 and 3: *mapA*gene-sized (589 bp) amplicon.

Table 4.5: Prevalence of resistance genes in *Campylobacter* from different milk and meat samples

				ESBL producing gene		Tetracycline			Ampicillin	Erythromycin
Sample name	**Sample size N**	**Total number of *Campylobacter* (n)**	**Prevalence of *Campylobacter***	*blaSHV*	*blaCMY-2*	*tet(A)*	*tet(B)*	*tet(C)*	*blaTem*	*ermB-2*
Layer Meat	**20**	9	45%	0	11.11 %(1)	0	0	0	33.33 %(3)	11.11 %(1)
Broiler Meat	**20**	12	60%	0	0	0	0	2	25%(3)	0
Beef Meat	**20**	0	0	0	0	0	0	0	0	0
Buffalo Meat	**10**	0	0	0	0	0	0	0	0	0
Goat Meat	**20**	0	0	0	0	0	0	0	0	0
Goat Milk	**25**	0	0	0	0	0	0	0	0	0
Buffalo Milk	**15**	0	0	0	0	0	0	0	0	0

Table 4.7: Antimicrobial resistance pattern of *Campylobacter* isolates

Name of Antibiotics	**Broiler meat (n=12)**			**Layer meat (n=9)**			**Percentage of Resistance (%)**
	S	I	**R**	S	I	**R**	
(CRO 30µg)	4	0	**8**	2	0	**7**	**71.42**
(CN, 10µg)	6	0	**6**	5	0	**4**	**47.61**
(SXT, 25µg)	2	0	**10**	1	0	**8**	**85.71**
(CIP, 5µg)	2	0	**10**	1	0	**8**	**85.71**
(ERE, 15µg)	0	0	**12**	0	0	**9**	**84**
(TE, 10µg)	4	0	**8**	3	1	**5**	**61.90**
(AMP 10µg)	4	0	**8**	4	0	**5**	**61.90**
(AZM, 15µg)	0	0	**12**	0	0	**9**	**84**
(DO, 30µg)	9	3	**0**	6	3	**0**	**0**
(S, 10µg)	0	0	**12**	0	0	**9**	**84**
(CTX, 30µg)	4	0	**8**	2	0	**7**	**71.42**
(CAZ, 30µg)	0	3	**9**	0	2	**7**	**76.19**
	MDR			**MDR**			

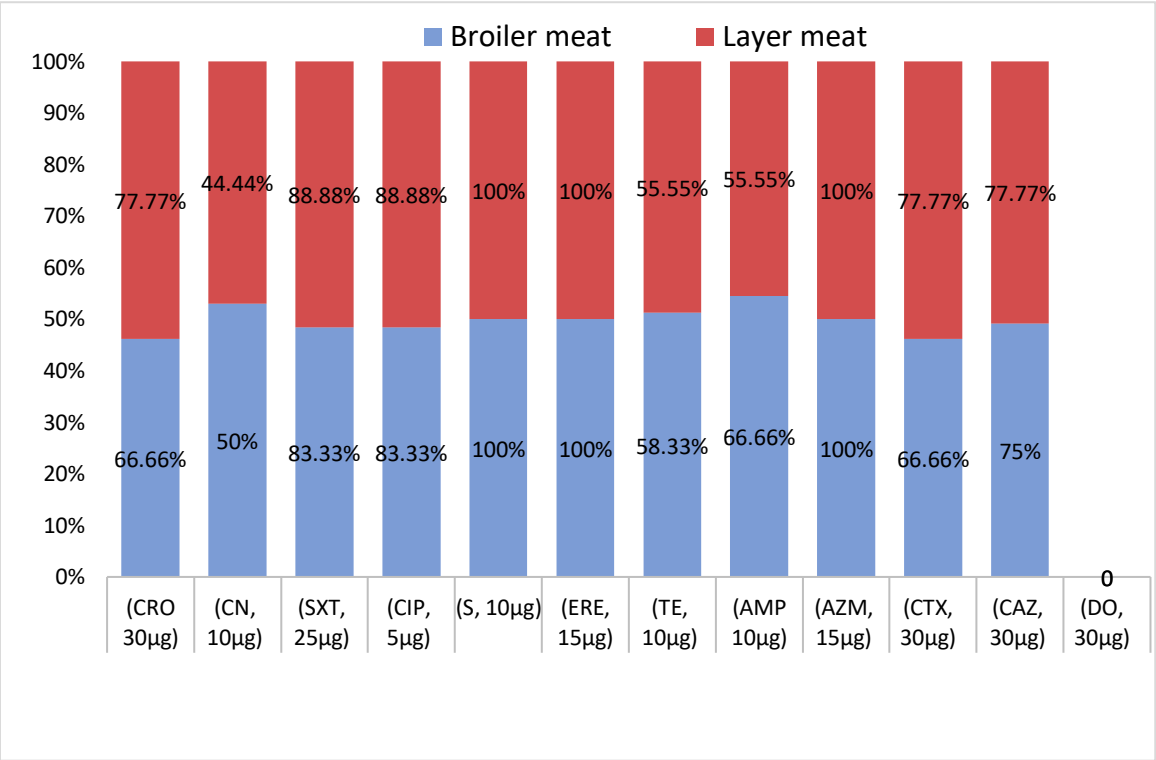


Figure 4.30: Antibiotic resistant pattern of *Campylobacter* isolates in Chattogram division

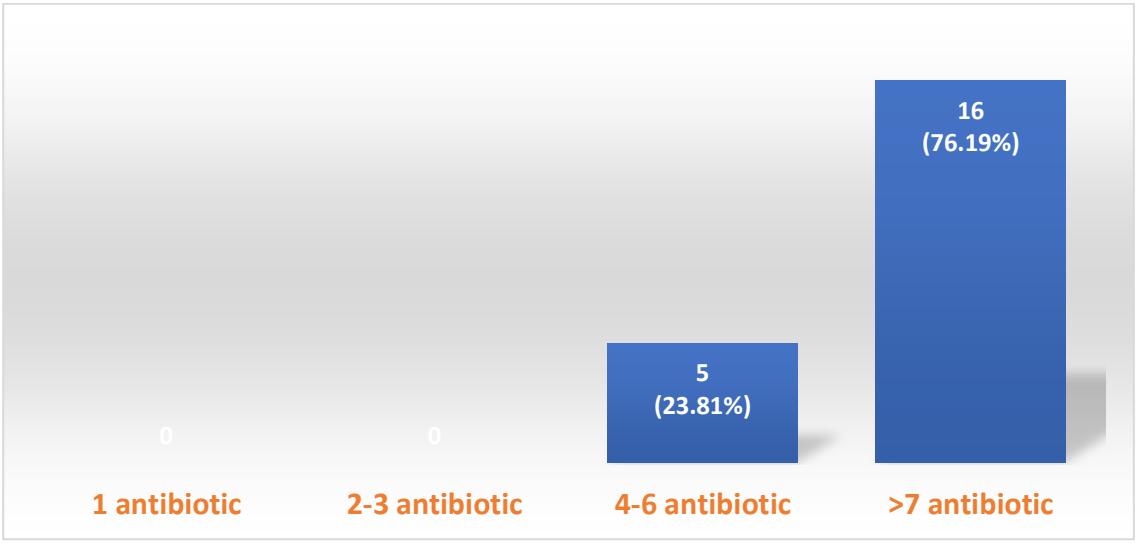


Figure 4.31: MDR pattern of *Campylobacter spp.* isolates in Chattogram division

In Layer meat 100% *Campylobacter* isolates of Chattogram area showed resistance to Erythromycin (ERE), Streptomycin (S) and Azythromycin (AZM) followed by 88.88% against Ciprofloxacin (CIP) and Sulphamethoxazole-trimethoprim (SXT), following 77.77% resistance against Cafotaxime (CTX), Ceftriaxone (CRO) and Cefotaxime (CAZ). In broiler meat,100% *Campylobacter* isolates showed resistance to Erythromycin (ERE), Streptomycin (S) and Azythromycin (AZM) followed by 83.33% against Ciprofloxacin (CIP) and Sulphamethoxazole-trimethoprim (SXT)(Figure 4.30). All 21 *Campylobacter* isolates were multi drug resistant. 23.81% isolates were resistant to 4-6 antimicrobials and 76.19% were resistant to more than 7 tested drugs (Figure 4.31).

4.3.1 Resistance gene in *Campylobacter* isolates

Very few *Campylobacters* isolates of Chattogram area was identified with antimicrobial resistant gene. 3 isolates had *blaTem* (ampicillin) gene both in broiler and layer meat. 2 isolates in broiler meat had *Tet (C)* gene (Figure 4.32).

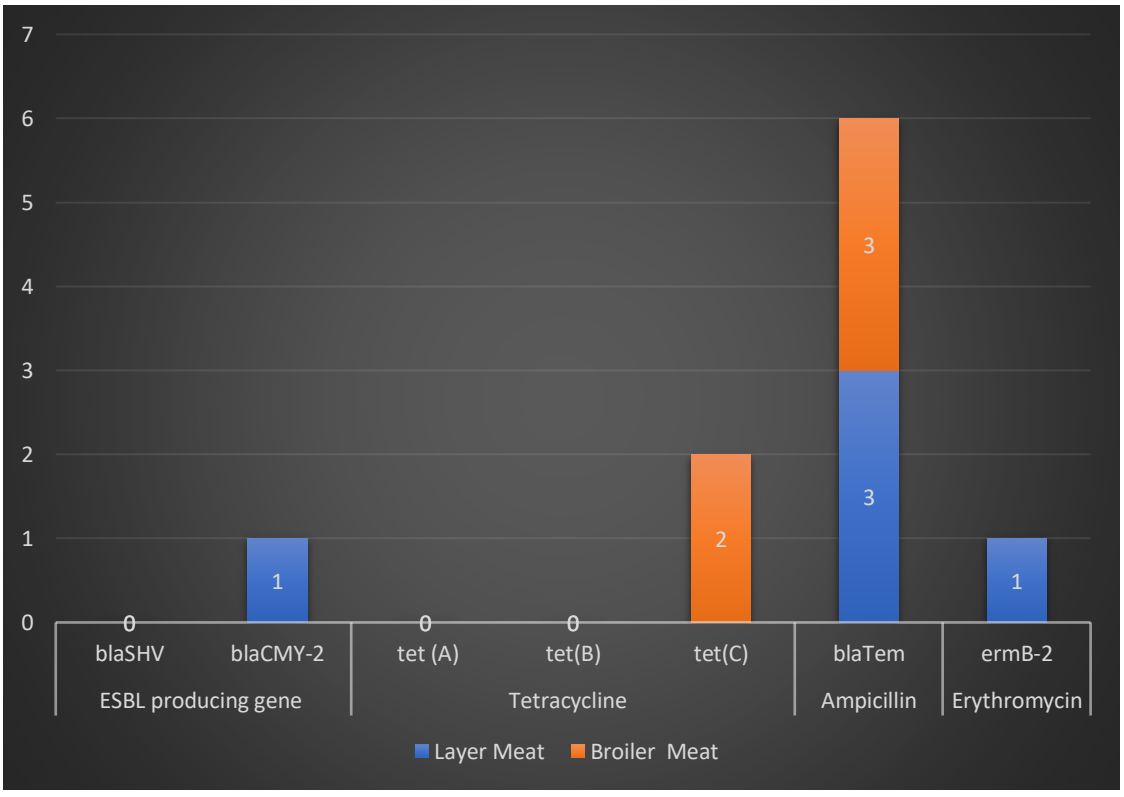


Figure 4.32: Presence of resistant gene in *Campylobacter* spp isolates in Chattogram division

4.4 *Salmonella* isolated from milk and meat:

A total of 5 isolates were confirmed as *Salmonella* strain out of 130 meat and milk samples by PCR. One in beef meat (5%, 95% CI 0.12%-24.8%), four in buffalo milk (26.67% , 95% CI 7.78%- 55.10%). Characteristic growth of S*almonella* strain on Blood Agar: *S. typhi and S. paratyphi* produced non-hemolytic smooth white colonies in **figure 4.33.** On MacConkey Agar, non-lactose fermenting smooth colonies i.e. pale colonies is shown in **figure 4.34.** On Xylose Lysine Deoxycholate media (XLD), red colonies, some with black centers in **figure 4.35** and gram staining characteristics are shown in **Figure 4.36.** PCR assay of some of the isolates after gel electrophoresis for the detection of ***stx*** gene in those are displayed in **Figure 4.37.**

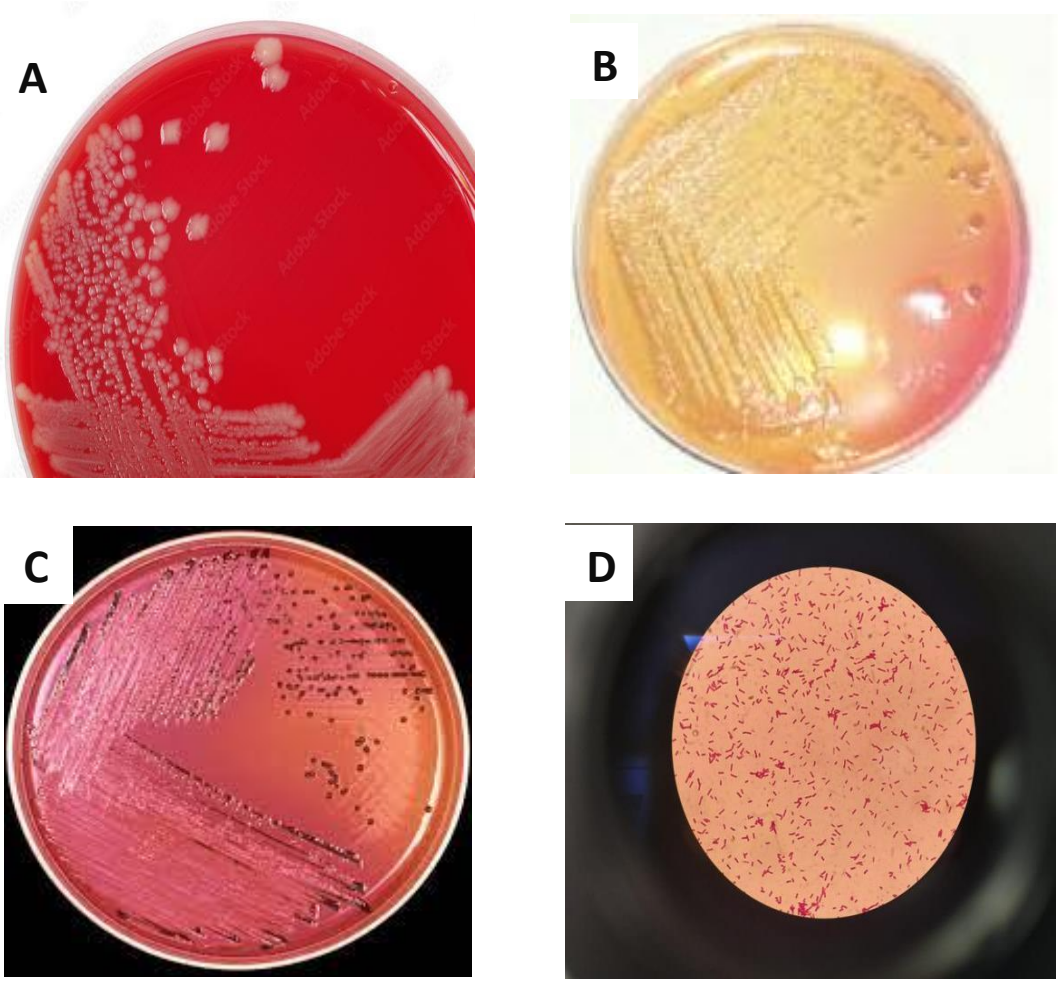


Figure (A) 4.33 Characteristic growth of S*almonella* strain on Blood Agar: *S. typhi and S. paratyphi* produce non-hemolytic smooth white colonies;(B)4.34 On MacConkey Agar: Non-lactose fermenting smooth colonies i.e. pale colonies*;* (C) 4.35 on On XyloseLysine Deoxycholate media (XLD) : red colonies, some with black center.(D) Gram staining showing gram negative bacilli in Fig 4.36.

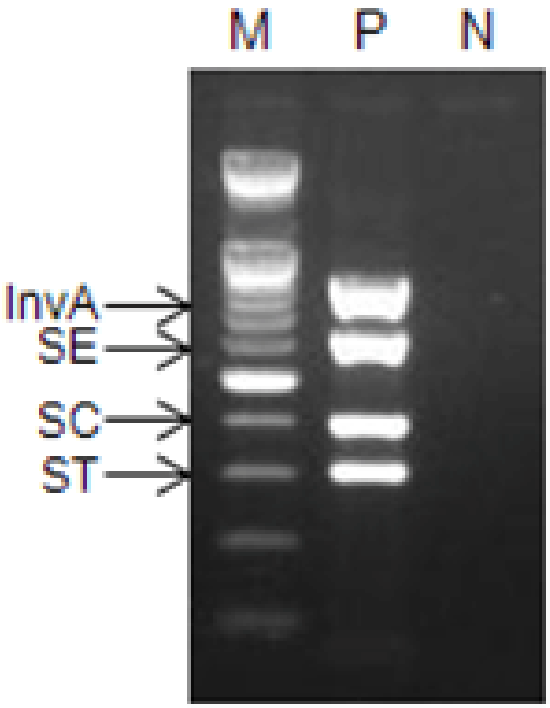


Figure 4.37: Result of PCR assay for *stx gene* of *salmonella* isolates; Lane M: 1kb plus DNA ladder; Lane P: Positive control; Lane N: Negative control;

Table 4.8: Prevalence of Resistant genes in *Salmonella* spp from different meat and milk samples

Sample name	**Sample size N**	**Total number (n)**	**Prevelance of salmonella**	**ESBL producing gene**			**Sulfonamide**			**Tetracycline**		
				blaTEM	***blaSHV***	***blaCMY***	***Sul1***	***Sul2***	***Sul-Gka***	***Tet (A)***	**tet(B)**	***tet(C)***
Layer Meat	**20**	0	0	0	0	0	0	0	0	0	0	0
Broiler Meat	**20**	0	0	0	0	0	0	0	0	0	0	0
Beef Meat	**20**	1	5%	0	100 (1)%	100 (1)%	100 (1)%	0	0	0	0	0
Buffalo Meat	**10**	0	0	0	0	0	0	0	0	0	0	0
Goat Meat	**20**	0	0	0	0	0	0	0	0	0	0	0
Goat Milk	**25**	0	0	0	0	0	0	0	0	0	0	0
Buffalo Milk	**15**	4	26.66%	0	0	0	50% (2)	0	0	25% (1)	0	0

Table 4.9 Antimicrobial resistance pattern of *Salmonella spp.* Isolates.

Name of Antibiotics	**Buffalo milk** (n=4)			**Cattle Meat** (n=1)			**Percentage (%)**
	S	I	**R**	S	I	**R**	
(CRO 30µg)	4	0	0	1	0	0	**0**
(ATM 30µg)	4	0	0	1	0	0	**0**
(CT, 10µg)	3	1	0	1	0	0	**0**
(C, 30µg)	4	0	0	1	0	0	**0**
(CN, 10µg)	1	2	**1**	0	0	**1**	**40**
(SXT, 25µg)	0	1	**3**	1	0	0	**60**
(CIP, 5µg)	0	0	**4**	0	0	**1**	**100**
(S, 10µg)	1	0	**3**	0	0	**1**	**80**
(ERE, 15µg)	2	1	**1**	1	0	0	**20**
(TE, 10µg)	1	0	**3**	1	0	0	**60**
(AMP 10µg)	2	0	**2**	0	0	**1**	**60**
MDR	MDR			MDR			

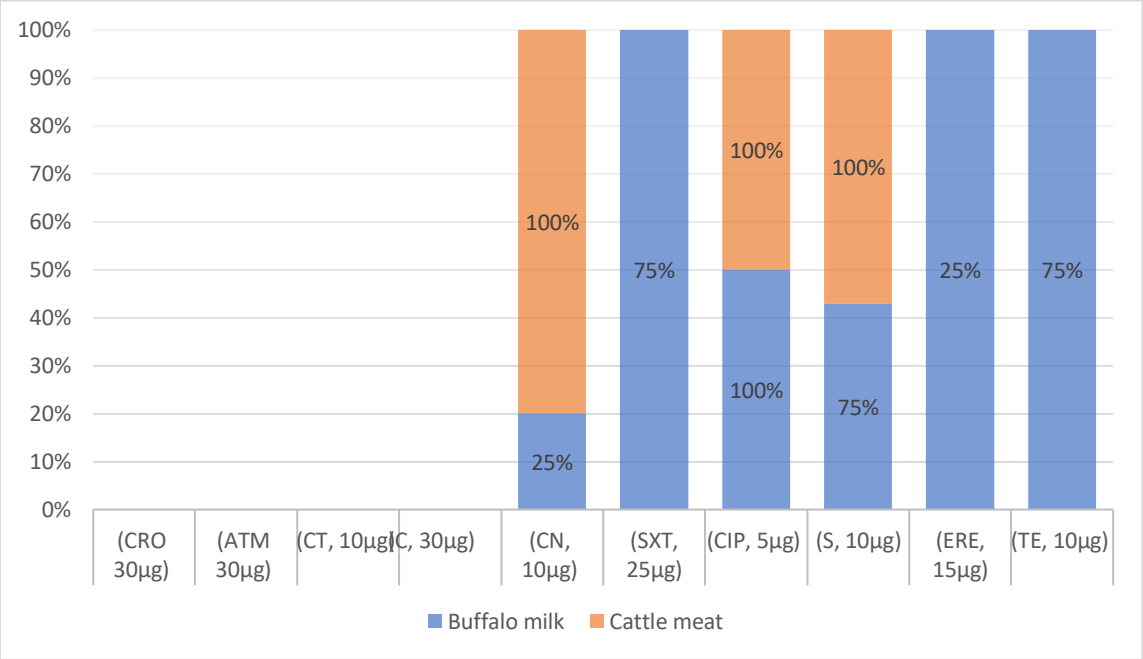


Figure 4.38: Antibiotic Resistance Pattern of *Salmonella* isolates in Chattogram division.

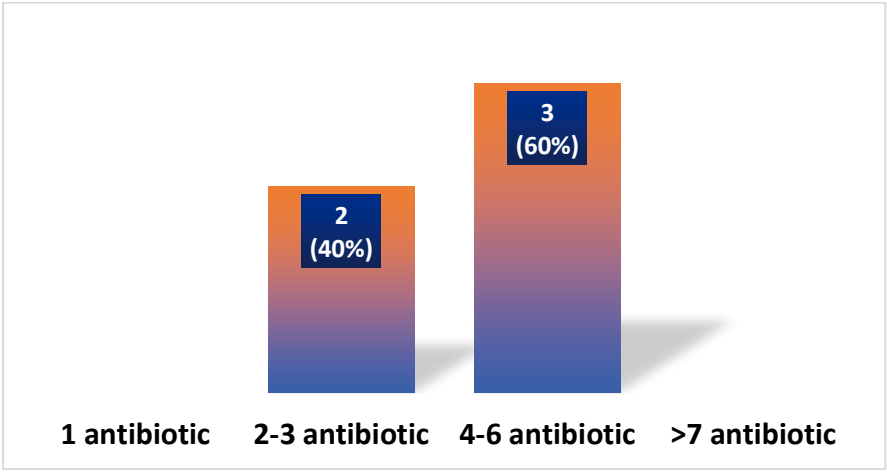


Figure 4.39: MDR pattern of *Salmonella spp.* isolates in Chattogram division

4.4.1. Antimicrobial resistant pattern in *Salmonella*

Salmonella spp. isolates of the study area from buffalo milk showed 100% resistance against Ciprofloxacin (CIP), followed by 75% against Tetracycline (TE), Sulphamethoxazole-Trimethoprim (STX), Streptomycin and 25% against Erythromycin (ERE) and Gentamycin (CN). In Cattle meat, 100% resistance showed against Gentamycin (CN), Ciprofloxacin (CIP) and Streptomycin(S) **(Figure 4.38)**. All 5 *Salmonella* isolates were multi drug resistant. 40 % isolates were resistant to 2-3 antimicrobials and 60% were resistant to more than 4-6 tested drugs **(Figure 4.39).**

4.4.2 Resistance gene in *Salmonella* isolates

2 *Sul1* gene(sulfonamide) and 1 *Tet(A)* gene was identified in *Salmonella* spp. isolates in buffalo milk whereas 1 *blaSHV,1 blaCMY* and 1 *Sul1* identified in cattle meat (Figure 4.40).

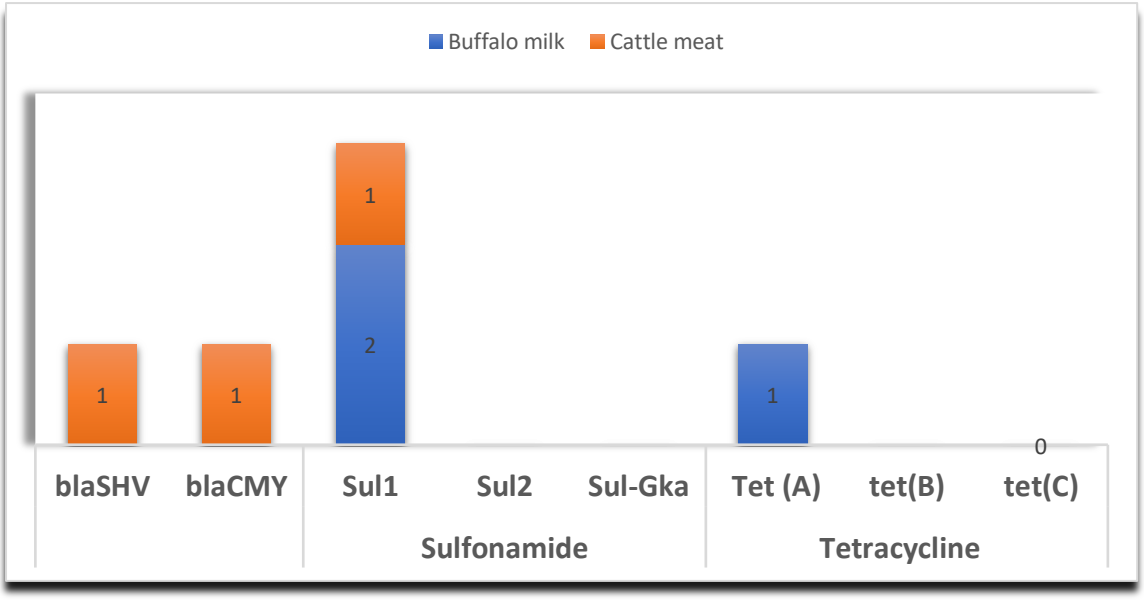


Figure 4.40: Presence of resistant genes in *Salmonella* isolates in Chattogram division

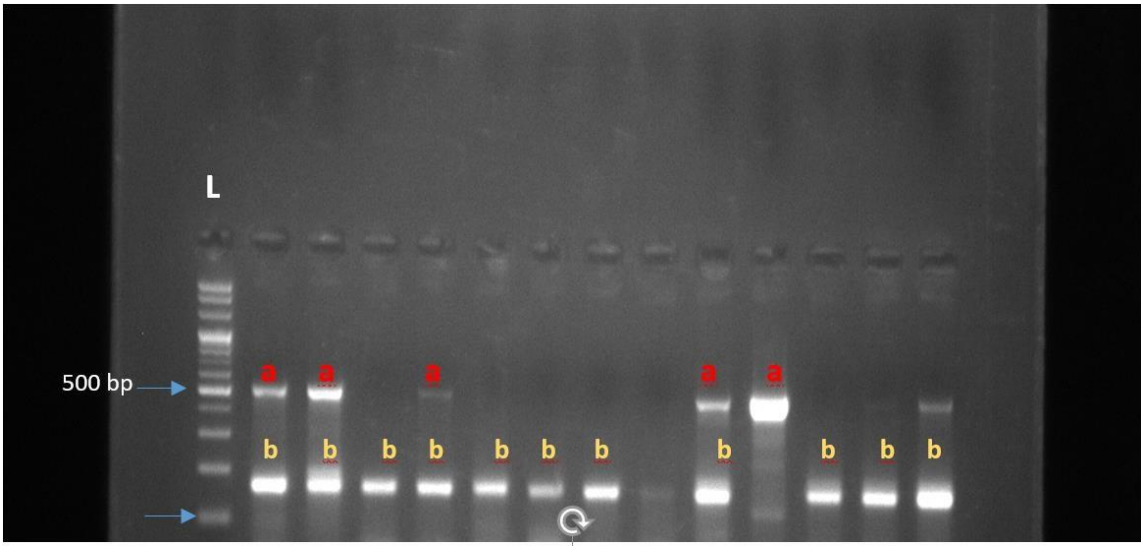


Figure 4.41: PCR assay for the detection of the salmonella genes; Lane L: 1kb plus DNA ladder; Lane a: *blaZ* gene (517bp); Lane b: *tetK* (169 bp) amplicon

CHAPTER-V

Discussions

Emergence and dissemination of antimicrobial resistance is on the increasing trend among enteric bacteria (Sawant AA *et al*, 2007). The overall prevalence of *S. aureus, E. coli, C. jejuni and Salmonella spp.*in meat and milk were assessed in the present study. The isolates showed antimicrobial-resistant attributes phenotypically were selected to identify antimicrobial resistant (AMR) genes, responsible for Multidrug-Resistant (MDR) features.

5.1. *S. aureus:*

The overall prevalence of *S. aureus* in broiler meat was 20% (4 out of 20 samples) in this study which is consistent with Rortana *et al.*, (2021) found an overall 29.1% were positive for *S. aureus.* However, a much higher prevalence in Beijing, China (35%) was reported by Wu *et al*. (2018). Meanwhile 5% prevalence seen in the beef meat (4 out of 20 samples). Similarly, Bissong *et al.*, 2017 found the overall prevalence of *S. aureus* in beef was 11.1% which was slightly higher. On the other hand, 10% buffalo meat (1 out of 10 samples) was found positive for *S. aureus* in this study. Similar finding described by Z.T *et al.1996 put the year)* that was16%. On the other hand, Likhita *et al.* (2022) found higher prevalence of *S. aureus* in cara beef (buffalo meat) which was 28%. 5% staphylococcus aureus was found in goat meat (1 out of 20 samples) in this study. Whereas, Yemisi*et al*. (2011) found near about 12% prevalence in goat meat.

In this study the cultural sensitivity test of 4 *S. aureus* isolates in broiler meat showed highest resistance against (100%) Tetracycline (TE), followed by 75% to Oxytetracycline (OT) and 50% to Ceftriaxone (CRO),Oxacillin (OX) and others. Also showing lowest resistance to Erythromycin (ERE), and Gentamycin (CN) is 25%. Similar findings were observed by Momtaz *et al.* (2013); *S. aureus* strains had the highest antibiotic resistance to Tetracycline (TE) (97.56%), and lowest to Gentamycin (CN) (29.26%).

According to Bantawa *et al*. (2019) higher resistance to Ampicillin (100%) and Tetracycline (93%) was observed in buffalo meat, whereas in our study100% resistance showed against Ciprofloxacin (CIP), Streptomycin (S), Tetracycline (TE), Erythromycin (ERE) and Ampicillin (AMP).

In observation ofBaghbaderani *et al. (*2020), *S. aureus* bacteria isolated from retail goat and cattle meat samples had a high incidence of resistance towards Ampicillin (100%), Ceftriaxone (80.00%), Amoxicillin–clavulanic acid (50.00%), Lincomycin (61.20%), Tetracycline (55.00%), Gatifloxacin (96.80%), Minocycline (51.20%), Cotrimoxazole (45.60%), Clindamycin (54.30%), Azithromycin (48.10%), Erythromycin (37.50%),

Oxacillin (76.20%), and Penicillin (100%). Meanwhile, in our study, we found 100% resistance against all antibiotics except Meropenem and in cattle meat 100% resistance showed against Gentamycin (CN), Sulphamethoxazole-Trimethoprim (SXT), Streptomycin (S), Ampicillin (AMP) and Amoxycillin/Clavulanic Acid (AMC).

Tsehayneh *et al*. (2021) revealed that about 98% of *S. aureus* isolates had high resistance to two or more drugs due to the fact that there is frequent irrational antimicrobial use and misuse behavior in the country. Similarly, here all *S. aureus* isolates were multi drug resistant, 57.14 % of the isolates had resistance against 4 to 6 tested antimicrobials and 42.85% had resistance to more than 7 antimicrobials.

In the study of Seedy *et al.* (2017), *blaz* gene was detected in all (100%) the tested isolates of broiler meat whereas in our study it was 50%. Similarly, *tet*K, *erm*B, *tet*M**,** *erm*C, *mec*A was detected in all the tested isolates of our study with a percentage of 75%, 25%, 25%, 50%, respectively. In Biomolecules 2019, the result was quite different where 87% isolates were detected with *erm*B, ermC genes and 94.4% isolates were positive for genes *tet*K, *tet*M, respectively. This huge difference may be due to smaller sample size of our study. On the other hand, in beef meat or cattle meat 100% ESBL producing *blaz* gene found in all isolates which is concordant with Seedy *et al. (*2017). In buffalo meat we found *tet*K, *erm*B, *erm*C genes in 100% isolates which is nearly comparable to the result of biomolecules 2019; 87% and 94.4% isolates were positive for these genes.

*Blaz erm*C, *mec*A gene was detected in all (100%) the tested isolates of goat meat in our study. However, in Mahdavi *et al.* (2019), the incidence of the *blaz erm*B, *erm*C and *mec*A genes were detected in 14%, 64%, 12%, and 26%, respectively.

5.2. *E. coli*

The overall prevalence of *E. coli* in broiler meat was 25% (5 out of 20 samples), consistent with Akbar *et al*. (2014) which was also 25%. But lower than Rahman *et al*. In Pakistan (2018), where the overall prevalence of *E. coli* in broiler meat was 38.8%. Meanwhile 45% prevalence seen in beef meat (9 out of 20 samples) in this study; whereas Farhoumand *et al.* (2020) revealed high contamination in beef meat with *E. coli* (68.89%). On the other hand, 40% (4 out of 10 samples) buffalo meat was contaminated with *E. coli* in this study which was nearly consistent with Singh *et al*. (2017) that was 35 %. Thirteen out of twenty isolates of *E. coli* were found in goat meat**;** 65% in this study. Prevalence *E. coli* in goat milk was estimated as 24% (6 out of 25 samples) in this study, which was consistent with Sultana *et al.* (2021) who found 26% in her study. Side by side Ibrahim *et al.* (2022) also found 28%

prevalence of *E. coli* from goat milk. In our study, in buffalo milk we observed 46.66% (7out of 15 samples) prevalence which is concordant with Saleh *et al*. (2019); also reported 46% prevalence. In layer meat 15% *E. coli* (3 out of 20) was isolated. While Rahman *et al.* (2017) found 49% which was higher than our study finding.

Regarding antibiotic sensitivity of *E. coli*, alarmingly in buffalo milk, broiler meat, cattle meat and goat meat 100% isolates of Chattogram area showed resistance to Sulphamethoxazole-trimethoprim (SXT), Erythromycin (ERE), Tetracycline (TE) and Ampicillin (AMP), whereas Rahman *et al*. (2017) found it 86% which was nearly similar. In our study all 47 *E. coli* isolates were multi drug resistant. 57.44% isolates were resistant to 4-6 antimicrobials and 40.42% were resistant to more than 7 tested drugs. According to Ibrahim *et al*. (2012) prevalence of MDR *E. coli* was 92.2% which wasnearly similar to our study. In our study *blaCMY* gene found in 33.33% isolates of beef meat whereas Messele *et al.* (2017) observed 65.1%, much higher than ours; might be due to variation in sample size and sampling technique. Variation in methodology of isolation and identification of microorganism might also attribute variable results.

5.3 *Campylobacter spp.*

In our study we estimated the prevalence of *Campylobacter spp.* as 45% in layer meat and 60 % in broiler meat. The finding was quite higher than the findings of Neogi et al. (2020) where the prevalence was 32% in broiler farms and LBMs of Bangladesh, and Tang et al. (2020) found prevalence in broiler meat was about 30.3% in Eastern China. However, the variation might be because of difference in sample type; we collected meat where as other collected cloacal swabs.

The antimicrobial susceptibility testing revealed high resistance rates against ciprofloxacin, azithromycin and sulfamethoxazole-trimethoprim combination with a percentage more than 80% which is in harmony with the findings of Gharbi et al. (2022) where the percentage ranges from 35.5% to 100% to the mentioned antibiotics.

Among all *Campylobacter spp*. isolated from layer meat, 11.1% had ESBL-producing *blaCMY-2* gene, 11.1% *ermB-2* and 33.33% *blaTem.* In the study of Marín et al. (2020), 34.8% bla_{TEM} gene was detected in Eastern Spain. The highest number of isolates having resistant gene was *blaTEM* (93%) according to Khan et al. (2020). Resistant gene *ermB* (18.29%) was found in Eastern Cape Province, South Africa (Igwaran et al., 2020).

5.4 *Salmonella spp.*

Salmonella spp. was present in beef meat at a prevalence of 5% in our investigation, which is consistent with the results of Gebremedhin et al., (2021), in Tunisia where they found 5.7% prevalence. On the other hand, 26.67% isolates were positive to Salmonella *spp.* isolated from buffalo milk in our study.

In this study all the isolates were resistant to ciprofloxacin and 80% *Salmonella* isolates were resistant to Streptomycin. According to Peruzy et al. (2020), almost 47.2% isolates were resistant to ciprofloxacin indicated worldwide resistant pattern load.

In our study in beef meat, we found 100% ESBL producing resistance gene against *blaSHV, blaCMY, Sul1* whereas Mąka and Popowska, (2016) detected 80.0% *sul1* isolateswhich is closer to our study. According to Giuriatti *et al.* (*2*017) most prevalent gene was *blaCMY* gene with 38.88% prevalence which was much lower than our study. In Rafiq et al. (2022), the prevalence of antibiotic-resistant genes *sul1* and *tetA* was 19.7% and 18.1%; relatively lower than our study.

Antimicrobial resistivity is affecting the global population, resulting in health and financial losses. The 'One Health' concept is supported by the 'World Organization for Animal Health' and WHO, under which suitable approaches can be developed and implemented to control AMR. Currently, the major focuses are on antimicrobial residues in food that may occur due to the indiscriminate use of antibiotics in agriculture. Two major steps need to be monitored to overcome or stop the risk of Antimicrobial resistant bacteria (ARMB) in the food chain, i.e., antimicrobial use in foods and AMRB originating from agricultural practices. The developed approaches should be policy-based, enforced for all countries and entirely backed by government regulations. No action taken by a single country will resolve the AMR problems facing the global food supply, but a collective global approach will surely do so. Understanding the attitude and knowledge of farmers and veterinarians toward AMU and AMR is a crucial step for the design of strategies to combat this public health threat. The food-borne infections associated with AMR are foremost among key public health concerns. Infections caused by AMRB substantially increase the morbidity and mortality rates, especially in the developing world, while in developed nations, the therapeutic costs increase due to these infections. The WHO created a **'Strategic and Technical Advisory Group'** on AMR and endorsed that the WHO should be a primary party in forming the action plan. The FAO launched its Plan for Antimicrobial Resistivity to support WHO's global action plan in food and agricultural regions. The One Health approach was proposed by international bodies to control AMR risks, forming an

association between WHO, FAO, Environment and OIE as a **'Quadripartite alliance'**. WHO also initiated a plan to stabilize this worldwide issue in association with tripartite partners and issued a **'Global Action Plan'** on AMR. The lack of detailed AMU data impacts our ability to interpret surveillance data on AMR and to design efficient interventions. Therefore, monitoring systems to fill this knowledge gap should be prioritized. Finally, the ecology of AMR should be addressed with a holistic, One Health approach combining expertise from different disciplines.

CHAPTER-VI

Conclusion

In this study, presence of *S. aureus, E. coli, Campylobacter* and *Salmonella* in dairy products and meat revealed significant prevalence in different type of Sample, characterized with the presence of AMR genes indicating a complex phenomenon. Understanding the attitude and knowledge of farmers and veterinarians toward AMU and AMR is a crucial step for the design of strategies to combat this public health threat. The lack of detailed AMU data impacts us ability to interpret surveillance data on AMR and to design efficient interventions. Therefore, monitoring systems to fill this knowledge gap should be prioritized. In this study, presence of *S. aureus, E. coli, Campylobacter* and *Salmonella* in dairy products and meat had significant level of prevalence in different type of samples. *Staphylococcus aureus* found in beef meat, broiler meat, goat meat, buffalo meat was resistant to at least one type of selected antimicrobials phenotypically. *E. coli* was isolated from all types of samples and were found to be MDR.

Our findings showed high resistance to sulfamethoxazole-trimethoprim and Tetracycline, indicating the uncontrolled use of antimicrobials in animals and poultry farms. The higher prevalence of *E. coli* in milk, chicken meat and beef indicates unhygienic production and processing of these foods. Presence of multi-drug resistant *E. coli* in these foods may pose serious public health threats. The antibiogram profile of the isolates may help in therapeutic decision making in cattle and poultry practice in Bangladesh. Resistance of *Salmonella* spp. in food might be linked to the use of antimicrobials in food animals. The practice of herd treatment of such animals (e.g., broiler chickens) with antimicrobials, might lead to their higher exposure to these compounds and consequently promotes the increase in antibiotic resistance. Based on the findings of this study indicate the importance of considering the potential public health risk associated with *Campylobacter* in the poultry food system. Therefore, implementation of good hygienic practices at the farm and retail level can minimize the *Campylobacter* contamination. Antimicrobial resistance in *Salmonella* spp. is a growing problem for food safety. As highlighted in this review, resistant *Salmonella* spp. are becoming more frequent in food in many countries situated in different regions of the world. Resistance of *Salmonella* spp. in food is linked to the use of antimicrobials in food animals. If current farming practices are not changed, the development and spread of antibiotic resistance will undoubtedly continue.

CHAPTER-VII

Limitations

The study has following limitations:

1.Due to time and resource limitation the study was conducted in small scale. In future, the study can be conducted involving a higher sample size.

2.Minimum inhibitory concentration (MIC) of any of the resistant isolates was not performed due to time and resource limitation.

3. Sequencing of the described genes could have provided better understanding on their source of origin and spread.

Chapter -VIII

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CHAPTER-IX

BIOGRAPHY

Ayesha Ahmed Khan passed Secondary School Certificate (SSC) examination from Riyadh international school at 2003 and then Higher Secondary School Certificate (HSC) examination from Govt. Chittagong College, Chittagong in 2005. She completed MBBS from University of science and technology Chattogram, Bangladesh in 2011. She has been studying Masters of Public Health at the One Health Institute of Chittagong Veterinary and Animal Sciences University, Chittagong, Bangladesh. She has great interest to work in One Health field.