**CHAPTER: 2**

**REVIEW OF LITERATURE**

**2.1.1 Enterobacteriaceae**

The large family Enterobacteriaceae includes gram-negative bacteria along with many harmless symbionts, many of the more familiar pathogens, such as *Salmonella, Escherichia* *coli, Yersinia pestis, Klebsiella* and *Shigella*. Other disease-causing bacteria in this family include *Proteus, Enterobacter, Serratia* and *Citrobacter*. This family is the only representative in the order Enterobacteriales of the class Gammaproteobacteria in the phylum Proteobacteria (George, 2005). Phylogenetically, in the Enterobacteriales, several peptidoglycan less insect endosymbionts from a sister clade to the Enterobacteriaceae, but as they are not validly described, this group is not officially a taxon; examples of these species are *Sodalis, Buchnera, Wigglesworthia, Baumannia* and *Blochmannia,* but not formers rickettsias (Williams *et al*., 2010). Members of the Enterobacteriaceae can be trivially referred to as Enterobacteria, as several members livein the intestines of animals. In fact, the etymology of the family is enterobacterium with the suffix to designate a family (aceae) not after the genus *Enterobacter* (which would be “Enterobacteriaceae”) and the type genus is *Escherichia*.

**2.1.2 *Escherichia coli***

The genera Escherichia diverged around 102 million years ago (credibility interval: 57-176 mya), which coincides with the divergens of their hosts: the former being found in mammals and the later in birds and reptiles (Battistuzzi *et al*., 2004). *Escherichia coli* were first described in 1885 by Theodor Escherich (Escherich, 1988). Escherich, a Bavarian pediatrician, had performed studies on the intestinal flora of infants and had discovered a normal microbial inhabitant in healthy individuals, which he named *Bacterium coli* *commune*. In 1919, the bacterium was renamed in his honor to *Escherichia coli* (Kaper, 2005).

**2.1.3 Taxonomy**

The taxonomy of *E.coli* is summarized below:

Domain: Bacteria

Kingdom: Eubacteria

Phylum: Proteobacteria

Class: Gammaproteobacteria

Order: Enterobacteriales

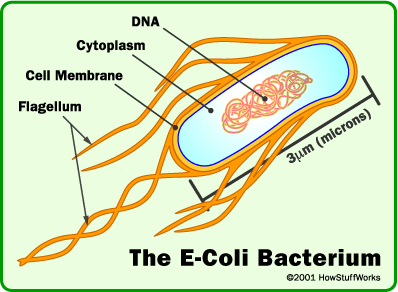
Family: Enterobacteriaceae

Genus: *Escherichia*

Species: *Escherichia coli* (VetBakt, 2007)

**2.1.4 Biology of *E. coli***

*E. coli* is [Gram-negative](http://en.wikipedia.org/wiki/Gram-negative_bacteria), oxidase-negative [facultative anaerobic](http://en.wikipedia.org/wiki/Facultative_anaerobic_organism) and [non-sporulating](http://en.wikipedia.org/wiki/Endospore) (Scheutz and Stockbine, 2005). Cells are typically rod-shaped, and are about 2.0 [micrometers](http://en.wikipedia.org/wiki/Micrometers) (μm) long and 0.25-1.0 μm in diameter, with a cell volume of 0.6–0.7 μm3 (Kubitschek, 1990). It can live on a wide variety of substrates. Optimal growth of *E. coli* occurs at 37 °C (98.6 °F) but some laboratory strains can multiply at temperatures of up to 49 °C (120 °F) (Fotadar *et al.,* 2005). Strains that possess [flagella](http://en.wikipedia.org/wiki/Flagellum) are [motile](http://en.wikipedia.org/wiki/Motility) or non motile. The flagella have a [peritrichous](http://en.wikipedia.org/wiki/Flagellum#Flagellar_arrangement_schemes) arrangement (Darnton *et al*.,2007) It is commonly found in the lower [intestine](http://en.wikipedia.org/wiki/Gastrointestinal_tract) of [warm-blooded](http://en.wikipedia.org/wiki/Warm-blooded) organisms (endotherms) (Singleton, 1999) Most *E. coli* [strains](http://en.wikipedia.org/wiki/Strain_%28biology%29) are harmless, but some [serotypes](http://en.wikipedia.org/wiki/Serotype) can cause serious [food poisoning](http://en.wikipedia.org/wiki/Foodborne_illness) in their hosts, and are occasionally responsible for [product recalls](http://en.wikipedia.org/wiki/Product_recall) due to [food contamination](http://en.wikipedia.org/wiki/Food_contamination) (Vogt *et al*., 2005). The harmless strains are part of the [normal flora](http://en.wikipedia.org/wiki/Human_flora) of the [gut](http://en.wikipedia.org/wiki/Gut_%28zoology%29), and can benefit their hosts by producing [vitamin K2](http://en.wikipedia.org/wiki/Vitamin_k) (Bentley *et al*., 1982) and preventing colonization of the intestine with [pathogenic](http://en.wikipedia.org/wiki/Pathogen) bacteria (Hudault *et al*., 2001 and Reid *et al*., 2001)

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**Figure 1:** Diagrammatic figure of *Escherichia coli*

*E. coli* and other facultative anaerobes constitute about 0.1% of [gut flora](http://en.wikipedia.org/wiki/Gut_flora) (Eckburg *et al*., 2005) and [fecal–oral transmission](http://en.wikipedia.org/wiki/Fecal%E2%80%93oral_route) is the major route through which pathogenic strains of the bacterium cause disease. Cells are able to survive outside the body for a limited amount of time, which makes them ideal [indicator organisms](http://en.wikipedia.org/wiki/Indicator_organism) to test environmental samples for [fecal contamination](http://en.wikipedia.org/wiki/Feces) (Thompson, 2007).

*E. coli* and related bacteria possess the ability to transfer [DNA](http://en.wikipedia.org/wiki/DNA) via [bacterial conjugation](http://en.wikipedia.org/wiki/Bacterial_conjugation), [transduction](http://en.wikipedia.org/wiki/Transduction_%28genetics%29) or [transformation](http://en.wikipedia.org/wiki/Transformation_%28genetics%29), which allows genetic material to [spread horizontally](http://en.wikipedia.org/wiki/Horizontal_gene_transfer) through an existing population. This process led to the spread of the gene encoding [shiga toxin](http://en.wikipedia.org/wiki/Shiga_toxin) from [*Shigella*](http://en.wikipedia.org/wiki/Shigella) to [*E. coli* O157:H7](http://en.wikipedia.org/wiki/Escherichia_coli_O157:H7), carried by a [bacteriophage](http://en.wikipedia.org/wiki/Bacteriophage) and thus can produce antibiotic resistancy (Brüssow *et al.*, 2004).

**2.1.5 Isolation and identification of *E. coli***

**Cultural Characteristics**

1. Grows well on ordinary media: Colonies are 2-3 mm in diameter.
2. On nutrient agar: Colonies are circular, low convex, smooth and colorless.
3. MacConkey agar: Large pink colored colony.
4. Blood agar: Discoloration around the growth; may be hemolysis occurred.
5. Nutrient broth: Diffuse cloudiness, heavy sediment.
6. Eosine-Methylene Blue agar: The colonies have a metallic sheen, characteristic to *E.coli* (WHO, 2003).

**Biochemical Properties**

1. Indole test: Development of Cherry red colored ring on the top of the medium in the presence of Kovac’s reagent.

**Molecular Technique**

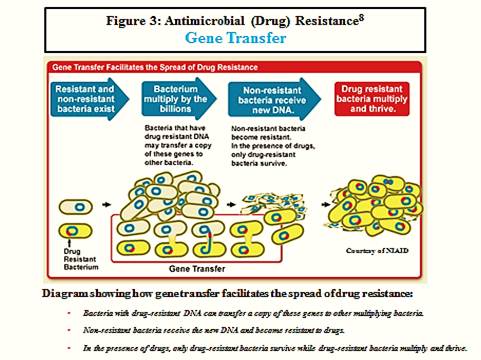
Polymerase Chain Reaction (PCR) of *E. coli* revealed the production of expected band at 585 bp when it is done using 16S rRNA primer (Wang *et al*., 1996).

**2.1.6 Clinical Significance**

Virulent strains of *E. coli* can cause [gastroenteritis](http://en.wikipedia.org/wiki/Gastroenteritis), [urinary tract infections](http://en.wikipedia.org/wiki/Urinary_tract_infection), and [neonatal](http://en.wikipedia.org/wiki/Neonatal) [meningitis](http://en.wikipedia.org/wiki/Meningitis). In rare cases, virulent strains are also responsible for [hemolytic-uremic syndrome](http://en.wikipedia.org/wiki/Hemolytic-uremic_syndrome), [peritonitis](http://en.wikipedia.org/wiki/Peritonitis), [mastitis](http://en.wikipedia.org/wiki/Mastitis), [septicemia](http://en.wikipedia.org/wiki/Septicemia) and Gram-negative [pneumonia](http://en.wikipedia.org/wiki/Pneumonia) (Todar, 2007). UPEC (uropathogenic *E. coli*) is one of the main causes of [urinary tract infections](http://en.wikipedia.org/wiki/Urinary_tract_infection) (Hilbert, 2013). It is part of the normal flora in the gut and can be introduced in many ways. In particular for females, the direction of wiping after defecation (wiping back to front) can lead to fecal contamination of the urogenital orifices. Anal intercourse can also introduce this bacteria into the male urethra, and in switching from anal to vaginal intercourse the male can also introduce UPEC to the female urogenital system (Hilbert, 2013). EHECs (enterohaemorrhagic *E.coli*) that induce bloody diarrhea lead to HUS (Hemolytic Uremic Syndrome) in 10% of cases. The clinical manifestations of postdiarrheal HUS include [acute renal failure](http://en.wikipedia.org/wiki/Acute_kidney_injury), [microangiopathic hemolytic anemia](http://en.wikipedia.org/wiki/Microangiopathic_hemolytic_anemia), and [thrombocytopenia](http://en.wikipedia.org/wiki/Thrombocytopenia) (FAO and WHO, 2011).

**2.2 Antimicrobial resistance**

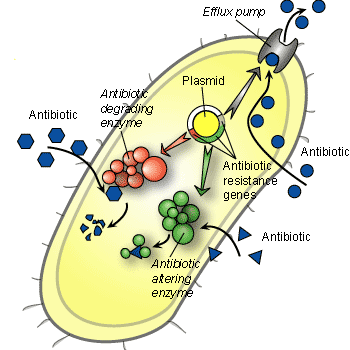
Antibiotic or antimicrobial resistance is a relatively new term. A bacterial strain can be defined resistant if it survives in the presence of higher antibiotic concentrations in comparison with phylogenetically related strains (Guardabassi, 1998). Antibiotic resistance is not a bacterial property that can be determined by studying a single strain, but only by comparison under identical conditions of two or more strains belonging to the same species. The above mentioned definition of antibiotic resistance refers to *in vitro* conditions. Under *in vivo* conditions, antibiotic resistance is a context dependent term as it depends on the location of the bacterium and the bioavailability of the drug. Bacteria are less susceptible to antibiotics when assembled in compared with the same organisms living separately (Guardabassi *et al.*, 1999). In aquatic environments, binding of the antibiotic molecule with ions or substances present in sediment strongly reduces both the activity of the drug and its absorption in the intestine (Guardabassi, 2000).

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**Fig 2**: Diagram showing the difference between non-resistant bacteria and drug resistant bacteria. Non-resistant bacteria multiply and upon drug treatment, the bacteria die. Drug resistant bacteria multiply as well, but upon drug treatment, the bacteria continue to spread   
(Credit NIAD, 2009).

**2.2.1 Molecular mechanisms**

Bacterial resistance to antibiotics can be caused by different molecular mechanisms (Guardabassi and Dalsgaard, 2000).The most common mechanisms include: reduced drug uptake; active drug efflux; drug deactivation, modification of the drug target; increased concentration of the drug target, or alternative pathways to elude the drug (Fig.2).

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**Figure 3:** Molecular mechanism of antibiotic resistant

**2.2.2 Natural and acquired resistance**

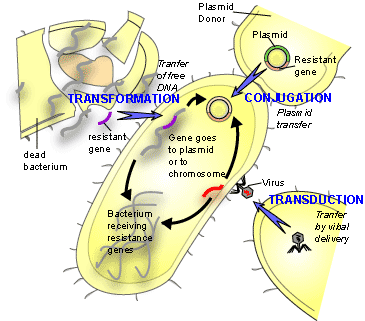
An important distinction should be made between natural and acquired resistance. Bacteria are termed naturally, intrinsically or constitutively resistant when resistance is due to characteristic features typical of the species. For example, *Pseudomonas aeruginosa* is naturally resistant to penicillins, due partly to the inability of the drug to diffuse through the outer membrane (Chopra and Ball, 1982) and partly to the deactivation of the drug by chromosomally encoded enzymes (Ohmori *et al.,* 1977). In contrast, acquired resistance emerges in a bacterial population that was previously susceptible, because of modifications of the bacterial DNA caused by either chromosomal mutation or horizontal gene transfer. Natural resistance results from a long process of genetic evolution, whereas, acquired resistance can arise within a short time (Hayes and Wolf, 1996).

**2.2.3 Acquisition by chromosomal mutations**

Mutation is a heritable change in the sequence of the DNA occurring due to errors during DNA replication (Snyder and Champness, 1997). The frequencies of chromosomal mutations leading to antibiotic resistance depend on the specific antibiotic. For example, mutation frequencies are high for compounds like nalidixic acid, rifampicin and streptomycin, low for erythromycin and are not known to occur for vancomycin and polymixin-B. For antibiotics like streptomycin, a single mutation can determine a 1000-fold increase in the resistance levels (Prescott and Baggot, 1994). In contrast, for other drugs the acquisition of resistance is a gradual, step-wise process in which different mutations are involved (Everett *et al.,* 1996).

**2.2.4 Acquisition by horizontal gene transfer**

Horizontal gene transfer is the relocation of genetic material from one bacterial cell (donor) to another (recipient). Such a transfer may occur directly by physical contact or indirectly, using the surrounding medium or bacteriophage as vectors (Brock and Madigan, 1999) (Fig: 3).Bacterial transfer of antibiotic resistance has been demonstrated to occur in various natural habitats, including water, sediment, soil, plants and animals (Davison, 1999). The DNA transferred from the donor to the recipient may be contained in mobile genetic elements called plasmids, structures of circular DNA that reproduce independently from the chromosome (Brock and Madigan, 1999). Functions that are of importance under particular conditions, such as antibiotic resistance, heavy metal resistance, metabolic functions, or production of antibiotics, toxins and virulence factors (Snyder and Champness, 1997).

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**Figure 4:** Mechanism of horizontal gene transfer in bacteria.

**2.2.5 Intracellular migration of resistance genes**

Antibiotic resistance genes can migrate from one site to another on the bacterial genome using small vectors called transposons (Mahillon, 1998)and integrons (Sundström, 1998).These genetic elements containing antibiotic resistance genes are able to move between different sites of the bacterial genome without any requirement of DNA homology. This process is known as non-homologous recombination and differs from the normal process of genetic recombination, which requires a high degree of DNA homology (Brock and Madigan, 1999). Both transposons and integrons make it possible for new antibiotic resistance genes to be acquired by plasmids and subsequently spread in the bacterial population by mechanisms of horizontal gene transfer, as suggested by the frequent recovery of these genetic elements as part of broad host plasmids (Bennett, 1999).

**2.2.6 Measurement of resistance in bacterial populations**

The value of the term "measurement of antibiotic resistance" in environmental microbiology generally differs from that in clinical studies. The main concern for environmental microbiologists is to investigate the distribution of antibiotic resistance in bacterial populations rather than the level of resistance in individual strains. Unfortunately, culture methods are not efficient enough to determine the actual prevalence of antibiotic resistance in a bacterial population. In fact, only a small proportion of the aquatic bacterial flora (<1%) can be cultured on laboratory media (Pickup *et al*., 1999). The method traditionally used for the measurement of antibiotic resistance at the population level consists in standard bacteriological counts on media containing specific concentrations of antibiotics. The main drawback of this method is the use of a single breakpoint for the determination of antibiotic resistance. In fact, the use of a single breakpoint, corresponding to the amount of antibiotic agent added to the medium, does not take into account the variability in the levels of antibiotic resistance existing among different bacterial species. Consequently, bacteria characterized by intermediate levels of resistance can be classified either as resistant or susceptible depending on the concentration of antibiotic added to the medium resistance (Cundliffe, 1989). An alternative approach is to use a group of phylogenetically related organisms as bacterial indicators of antibiotic resistance. This method is based on the principle that spatial and temporal differences observed in the levels of antibiotic resistance of the bacterial indicator are indicative of the selective pressure to which the entire bacterial population is exposed. Thus, this method does not aim to determine the exact prevalence of antibiotic resistance in the bacterial population under study, but rather to detect the effect of potential sources of antibiotic resistance on the bacterial population (Cundliffe, 1989).

**2.2.7 The microbial threat**

In the last decades, bacterial resistance to antibiotics has assumed an increasing importance with regard to its impact on both public health and ecology. Obviously, the primary problem is represented by the emergence of antibiotic resistance among bacteria pathogenic to humans and animals, which makes difficult the treatment of some life-threatening infections. However, independent from the risks for human health, is the spread of antibiotic resistance and the problems rose in ecological nature. In fact, the introduction and selection of resistant bacteria in the environment can lead to structural changes in the composition of microbial communities, with possible deleterious effects on the balance of natural ecosystems (Dalsgaard and Guardabassi, 2001).

**2.2.8 Multidrug resistance efflux pumps in bacteria**

Efflux is the pumping of a solute out of a cell. Efflux pump genes and proteins are present in both antibiotic-susceptible and antibiotic-resistant bacteria. Some systems can be induced by their substrates so that an apparently susceptible strain can overproduce a pump and become resistant. Antimicrobial resistance in an efflux mutant is due to one of two mechanisms: either (i) expression of the efflux pump protein is increased or (ii) the protein contains an amino acid substitution(s) that makes the protein more efficient at export. In either case, the intracellular concentration of the substrate antimicrobial is lowered and the organism becomes less susceptible to that agent. Efflux pumps may be specific for one substrate or may transport a range of structurally dissimilar compounds; such pumps can be associated with multiple drug resistance(Laura and Piddock, 2006).

Luaibi *et al.* (2013)deals with isolation, identification and characterization of *E. coli* isolated from fecal samples of young lions, dogs and sheep and the antibiotic resistance pattern of *E. coli* was determined by means of disc diffusion assay. The resistance pattern determined streptomycin, amoxicillin, ciprofloxacin, ceftriaxone amoxicillin/clavulanic acid, cefepimeandazteronam. *E. coli* were isolates reported as resistant to more than five antibiotics (multidrug-resistant). This might result from expansion of the antibiotic resistance image among animals and from the animals to human living in close contact with them.

Tadesse *et al.* (2012) conducted a retrospective study of Escherichia coli isolates recovered from human and food animal samples during 1950-2002 to assess historical changes in antimicrobial drug resistance. A total of 1,729 E. coli isolates (983 from humans, 323 from cattle, 138 from chickens, and 285 from pigs) were tested for susceptibility to 15 antimicrobial drugs. A significant upward trend in resistance was observed for ampicillin (*p<0.01*), sulfonamide (*p<0.01*), and tetracycline (*p<0.01*). Animal strains showed increased resistance to 11/15 antimicrobial agents, including ampicillin (*p<0.01*), sulfonamide (*p<0.01*), and (*p<0.01*). Multidrug resistance (≥3 antimicrobial drug classes) in E. coli increased from 7.2% during the 1950s to 63.6% during the 2000s. The most frequent co-resistant phenotype observed was to tetracycline and streptomycin (29.7%), followed by tetracycline and sulfonamide (29.0%). These data describe the evolution of resistance after introduction of new antimicrobial agents into clinical medicine and help explain the range of resistance in modern E. coli isolates. Shrestha, (2013) reported that the resistance pattern for the isolates of E. coli from poultry farm fecal waste was tetracycline (100%), penicillin (100%), erythromycin (100%), amoxicillin (90%) and chloramphenicol (60%).

Hoffmann *et al.* (2011) reported that antibiotic resistance is an increasing challenge for health care services worldwide. While up to 90% of antibiotics are being prescribed in the outpatient sector recommendations for the treatment of community acquired infections are usually based on resistance findings from hospitalized patients. For *Escherichia coli* e.g. the highest antibiotic resistance rates can be seen with fluoroquinolones (19%) and trimethoprim/sulfamethoxazole (27%). Ibekwe *et al.,* (2011) reported that eight antibiotics were used for susceptibility tests of E. coli isolates from water sample of Cypress channel. E. coli isolates were resistant to rifampicin (100%), tetracycline (74.4%), erythromycin (36.3%), ampicillin (11.7%), streptomycin (5.8%), cephalothin (11.7%) and amoxicillin (0%) Resistance to the remaining antimicrobials was minimal (<7).

Danishta *et al.* (2010 )reported that antibiotic resistance of *Escherichia coli* isolates from environmental and waste water samples in Mauritiusfound thatmost prevalent resistance were to erythromycin (100%), neomycin (100%), penicillin (100%) followed by tetracycline and sulphamethoxazole/trimethoprim (42.1%). The low prevalence was to streptomycin (31.6%), tetracycline (31.6%), amoxicillin/clavulanic acid (21.5%), cefpodoxime (10.5%), ceftazidime and cefpodoxime (10.5%), fosfomycin, enrofloxacin Baytril and cefotaxime (5.3%).

Nam et al. (2010) conducted a study where a total of 628 *Escherichia coli* isolates recovered from 877 intestinal samples of stray pet dogs (n¼565) and hospitalized pet dogs (n¼312) in Korea were analyzed for resistance to 15 antimicrobial agents. Most common resistance observed in E. coli isolated from both groups of dogs was to tetracycline (52.4–53.6%), streptomycin (35.8–41.7%), ampicillin (32.9–47.1%), nalidixic acid (21.6–37.4%), and trimethoprim=sulfamethoxazole (19.7–36.4%). Resistance to chloramphenicol, , and ciprofloxacin was observed in 19.4% (17.1–24.3%), 18% (16.1–21.8%), and 16.1% (13.5–21.4%) of the isolates, respectively. No *E. coli* isolated from hospitalized dogs showed resistance to imipenem and cefepime, whereas three (0.7%) isolates from stray dogs were resistant to cefepime. Some of the isolates from both groups showed resistance to cefotaxime (2.4–3.9%) and amikacin (0.5–1.5%). In general, the frequency of resistance tended to be higher in isolates from hospitalized dogs than isolates from stray dogs against most antimicrobials tested. Around 39% (162=422) and 27% (55=206) of *E. coli* isolates from stray dogs and hospitalized dogs were susceptible to all antimicrobials tested, respectively. Multiresistance (3 subclasses of antimicrobials) was observed in 32% and 48% of *E. coli* isolates from stray dogs and hospitalized dogs, respectively.

Akond *et al.* (2009) reported that isolation and identification of *Escherichia coli* were made from poultry sources of different poultry markets in the capital city of Bangladesh and found 13 antimicrobial agents to check their susceptibility. 88%, 82%, 80%, 76%, 70%, 68%, 64%, 58%, 52%, and 20% of the tested *Escherichia coli* strains from poultry sources were found resistant respectively to Penicillin, Ciprofloxacin, Riphampicin, Kanamycin, Streptomycin, Cefixine, Erythromycin, Ampicillin, Tetracycline, and Chloramphenicol and Neomycin. None of the strains showed resistance to Norfloxacin and . Sensitivity was recorded in case of 86%, 80%, 60%, 36%, 30%, and 26% of the strains to Norfloxacin, and Chloramphenicol, Neomycin, Tetracycline, Streptomycin and Ampicillin, respectively. Intermediate susceptibility to various antibiotics was observed for 12-36% *Escherichia coli* strains. Both, resistance and susceptibility were exhibited against Chloramphenicol, Ampicillin, , Neomycin, Tetracycline, Streptomycin and Norfloxacin.

*E. coli* isolated from dogs in Trinidad were tested for their susceptibility to antimicrobial agents using the disk diffusion method by Seepersadsing *et al*. (2007). Antimicrobial agents and concentrations included cephalothin (KF, 30 μg), ampicillin (AMP, 10μg), kanamycin (K, 30 μg), neomycin (N, 30 μg), (CN, 10 μg), sulphamethoxazole/trimethoprim (SXT, 23.25 μg/1.75 μg), nalidixic acid (NA, 30 μg) and norfloxacin (NOR, 10 μg). The overall prevalence of resistance to one or more antimicrobial agents for *E. coli* isolated from dogs was 47.9%. The difference in prevalence across the various sources of the isolates from dogs was statistically significant (P<0.001; χ2). Overall, resistance was highest to cephalothin (30.1%). A total of 45 resistance patterns were observed from dogs from all sources and the predominant pattern was KF (25.6%).

Alam *et al*. (2006) reported about the *E. coli* from the aquatic sources in Bangladesh. Hereported that Resistance was commonly observed against Penicillin-G (94%), Tetracycline(65%), Ampicillin (75%) and Trimethoprim-sulfamethoxazole (49%). On the other hand,most of the strains were sensitive to Ciprofloxacin (76%), Chloramphenicol (70%),Ceftazidime (92%) and 97%. Eighty-eight percent of the tetracycline-resistantstrains were also resistant to penicillin-G and ampicillin. Sixty-nine percent of the strainswere resistant to more than four drugs and 24% were resistant to more than seven drugs.

A study was conducted by Rantala *et al*. (2004) with the aim of evaluating antimicrobial resistance in canine staphylococci, *Escherichia coli* and enterococci, which were isolated from 22 dogs with pyoderma and a history of previous antibiotic treatment, compared to bacterial isolates from 56 non-treated control dogs. Two isolates of each bacterial species per dog were investigated, if detected. Staphylococcal isolates from dogs with pyoderma (35 isolates) were more resistant to sulphatrimethoprim than the isolates from controls (56 isolates) (57% vs. 25%, p<0.004). Multiresistance in staphylococci was also more common in dogs with pyoderma (29% vs. 9%, p=0.02). A similar trend among isolates of *E. coli* was detected (24 and 74 isolates from treated and control dogs, respectively), but the differences were not significant. Resistance for macrolide-lincosamides was approximately 20% among staphylococci in both groups. Resistance to ampicillin among enterococci was 4%-7%. The age of the dogs might have an impact on resistance: multiresistance among staphylococcal isolates from younger dogs (≤5 years) was more common than in older dogs (≥6 years) (24%, vs. 0%, 63 and 27 isolates, respectively, p=0.02). Staphylococci in younger dogs were more resistant to tetracycline (48% vs. 11%, p<0.001) and sulphatrimethoprim (48% vs. 15%, p<0.01) than those in older dogs. In contrast, the isolates of *E. coli* from older dogs tended to be more resistant, although a significant difference was detected only in resistance to tetracycline (13% vs. 2% of 40 and 50 isolates respectively, p=0.04)). The results of this small study indicate that resistance in canine staphylococci in the capital area of Finland is comparable with many other countries in Europe. Resistance in indicator bacteria, *E. coli* and enterococci, was low.

Odusanya, (2002)observed thatfive hundred and fifty-one samples from urine, wound, reproductive tract and other body fluids were analyzed. The most frequently isolated pathogens (n=586) were *Staphylococcus aureus*, *Escherichia coli* and *Klebsiella pneumoniae*. Most of the organisms were sensitive to ciprofloxacin (92.3%), perfloxacin (80.8%), cefuroxime (80.1%), ceftriaxone (77.6%) and azithromycin (82.1%) but were resistant to ampicillin (79.5%), cotrimoxazole (100%) and penicillin (94.90%). *Pseudomonas aeruginosa* was multi resistant. The susceptibility pattern obtained at this hospital is similar to what obtains in teaching hospitals in Nigeria.

Biswas *et al.* (2001) reported that 100% of his poultry *E. coli* isolates were resistant totetracycline but 72% isolates were found to susceptible to Gentamycin but 20% were foundresistant to Gentamycin.

Barton, (2000) reported that *E coli* strains showed widespread resistance to tetracycline andmoderately common resistance (30-60%) to ampicillin and sulphadiazine. Resistances tomore than one antibiotic were common. Barton also reported in 2000 that the development ofantibiotic resistance in bacteria has been linked to the use of antibiotics in agriculture inoverseas studies, particularly for intensively housed species such as pigs, poultry and feedlotcattle.

[Threlfall](http://jcp.bmj.com/search?author1=E+J+Threlfall&sortspec=date&submit=Submit) *et al*. (1997)reported thatin 1996, 6% of *Escherichia coli* from extra intestinal infections were resistant to ciprofloxacin with minimum inhibitory concentrations (MICs) > or =2mg/l (high level resistance). Low level resistance (MIC 0.125-1mg/l) was also identified in 7% of *Salmonella typhi*, 4% of *S. paratyphi A*, and 4% of non-typhoidal *Salmonellas*. However, resistance to ciprofloxacin was rarely identified in *Shigella.*

Duncan *et al.* (1981) reported that a survey was made of the frequency of resistance to amikacin, and tobramycin among aerobic gram-negative bacilli isolated over a 4-week period in 1979 at six large, geographically separated Canadian hospitals. In the entire series of 4407 isolates the frequency of resistance was 2.5% to amikacin, 8.1% to , 5.9% to tobramycin and 1.7% to all three. Most (81%) of the resistant bacteria were acquired by the patients after admission to hospital. The frequency of resistance to the three aminoglycosides antibiotics in each hospital largely reflected the local rate of cross-infection by endemic strains of resistant bacteria.