**CHAPTER: 2**

**REVIEW OF LITERATURE**

**2. Antibiotic resistance:**

Antibiotic 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).

**Fig 1**: 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).

**2.1. 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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**Fig 2**: Molecular mechanisms of antibiotic resistance(Hayes and Wolf, 1996).

**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).

**2.3. 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).

 **2.4. 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).

**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).

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**Fig 3:** Mechanism of bacterial genetic transfer (Levy, 1998)

**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).

**2.7. 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).

**2.7.1. The emergence of resistance in human pathogenic bacteria**

In the past, bacteria were the most important cause of disease and mortality among humans. The introduction of antibiotics in human medicine has markedly reduced the impact of bacterial diseases on human mortality. Nevertheless, the extraordinary capacity for adaptation of bacteria soon allowed these organisms to develop mechanisms of resistance enabling them to overcome the toxic effects of antibiotics. A survey on enterobacterial isolates collected between 1917 and 1954 has demonstrated that bacteria were generally susceptible to antibiotics before these drugs became commonly available in human medicine (Hughes and Datta, 1983). However, other studies indicate that resistant bacteria were present at the time, although they were not prevalent in bacterial populations (Smith, 1967).Thus, it appears that the indiscriminate use of antibiotics has played a major role in the emergence of antibiotic resistance by exerting a selection in favors of resistant bacteria. The first case of penicillin resistance in *E. coli* was reported in the 1950’s. Since then, things have taken a turn for the worse. Today, antibiotic resistance represents an important problem in the therapy of various human pathogenic bacteria (Pathak *et al.,* 1993). Three bacterial species causing life-threatening infections (*Pseudomonas aeruginosa*, *Mycobacterium tubercolosis* and *Enterococcus faecalis)* can demonstrate resistance to any available antibiotic (Levy, 1998). Vancomycin is the only effective drug for treatment of infections caused by methicillin-resistant *Staphylococcus aureus*, but the occurrence of strains with reduced susceptibility to this antibiotic has already been reported (Hiramatsu *et al.,* 1997). Problems may also occur in the therapy of hospital infections caused by *Acinetobacter baumannii*, *Haemophilus influenzae*, *Klebsiella pneumoniae*, *Neisseria meningitidis* and *Streptococcus pneumoniae* (Levy, 1998). The problem of antibiotic resistance is of particular concern for immunosuppressed patients, such as those affected by HIV, cancer or chronic diseases, as antibiotic therapy represents the only way to overcome bacterial infections for these people. Serious problems may also occur in developing countries where the use of new and expensive drugs is limited by their cost and availability. In addition to the risks for human health, this situation incurs a worldwide increase in the cost of hospital care, including the use of new expensive drugs, increased costs for bacteriological analysis and prolonged hospitalization (Acar, 1997).

**2.7.2. The spread of resistance among environmental bacteria**

Antibiotic resistance is not only found in pathogenic bacteria but also in environmental organisms inhabiting terrestrial and aquatic habitats. The occurrence of resistant bacteria in nature may have originated from antibiotic producing organisms, as suggested by the evidence that in some cases the mechanisms and genes protecting these organisms from the antibiotics they produce are similar to those responsible for resistance in clinical isolates (Cundliffe, 1989).However, higher numbers of resistant bacteria occur in polluted habitats compared with unpolluted habitats (Baya *et al.,* 1986; Pathak *et al*., 1993), indicating that humans have contributed substantially to the increased proportion of resistant bacteria occurring in the environment. Possible mechanisms by which humans enhance the spread of antibiotic resistance among environmental bacteria include the deliberate or accidental introduction of antibiotics, resistant bacteria and resistance genes into the environment. The main risk for public health is that resistance genes are transferred from environmental bacteria to human pathogens. The ability of resistant bacteria and resistance genes to move from one ecosystem to another is documented by the various cases in which transmission of resistant bacteria has been demonstrated between animals and humans. The inclusion of certain growth promoters in animal feed has been recognized as a cause for the selection of resistance genes in the commensal microflora of animals and their transmission to humans *via* the food chain (Wegener *et al.,* 1999; Kruse, 1999). Similarly, drinking and bathing water could represent a source for the acquisition of resistant bacteria in humans. However, further studies are necessary to validate this hypothesis. The ecological consequences associated with the dissemination of resistant bacteria in the environment have been scarcely investigated. However, it(Baya *et al.,* 1986) appears evident that environmental contamination with antibiotics, resistant bacteria and resistance genes affects the biodiversity of natural ecosystems. Antibiotics are likely to determine a reduction in the levels of microbial diversity by the suppression of susceptible organisms, including bacteria, fungi, protozoa and algae. Resistant bacteria and genetic elements could find favorable conditions to become predominant in habitats contaminated by antibiotics, thereby, altering the original composition (balance) of natural microbial communities.

**2.8. Spread of antibiotic resistance in sewage**

Sewage is waste matter resulting from the discharge into the sewers of human excreta and wastewater originating from the community and its industries. Sewage contains a high content of both organic and inorganic matter, as well as high densities of living organisms, including pathogenic, commensal and environmental bacteria. This characteristic composition makes sewage a particularly suitable ecological niche for the growth and spread of antibiotic resistance (Guardabassi and Dalsgaard, 2000).

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**2.8.1. Antibiotic selective pressure**

The acquisition of antibiotic resistance genes is generally independent of the presence of antibiotics. However, the exposure of bacteria to antibiotics confers an ecological advantage to resistant strains on susceptible strains, allowing them to become predominant in the bacterial population. This situation is commonly termed as antibiotic selective pressureand can occur in either the host *in vivo* as a consequence of chemotherapy or in the environment, for example when antibiotic residues are introduced in sewage. Residues of antibiotics administered to humans and animals reach the sewage systems in urine or feces, in the form of either parent compound or degraded metabolites depending on the pharmacology of the specific antibiotic. Furthermore, an unknown amount of antibiotics enter the sewers by waste derived from antibiotic production and disposal of a surplus of drugs. Indeed, various antibiotics have been found in municipal sewage, including fluoroquinolones, sulfonamides and erythromycin metabolites (Hartmann *et al.,* 1998;Raloff, 1998; Hirsh *et al.*, 1999; Hartig *et al*., 1999).The antibiotic concentrations found in sewage vary between 1 and 100μg per liter. Such concentrations are 100 to 1000 fold lower compared with those necessary to inhibit resistant bacteria, but are sufficient to affect susceptible bacteria (Al-Ahmad *et al.,* 1999; Backhaus and Grimme, 1999). Therefore, the occurrence of such antibiotic concentrations in sewage has the potential to select for antibiotic resistance. The fate of antibiotics in sewage depends on their chemical properties. Lipophilic and non-readily degradable substances are likely to be retained in the sludge, whereas, hydrophilic substances may be able to pass through treatment plants and end up in the natural recipients receiving treated sewage. It also appears that the solubility in water of drug metabolites is generally higher compared with the parent compounds (Halling-Sørensen *et al.,* 1998). Thus, it is likely that a large proportion of the antibiotic residues introduced into the sewage system can reach surface waters through municipal sewage effluent.

**2.8.2. Non-antibiotic selective pressure**

Among the multitude of substances occurring in sewage, there are some that have the potential to select for antibiotic resistance, even though they are not antibiotics. Heavy metals and biocides are two important groups of non-antibiotic substances showing this property. Heavy metals are widespread in sewage as a consequence of industrial pollution. Biocides are introduced into sewage by hospitals, farms, slaughterhouses and food-processing establishments; where these agents are used for the disinfection of environments and utensils, or by the community, due to the presence of these agents in house-hold products, such as soaps and dishwashing detergents (Guardabassi and Dalsgaard, 2002). There are two possible ways by which heavy metals and biocides can select for antibiotic resistance. The genes encoding resistance to heavy metals and biocides can be located together with antibiotic resistance genes on either the same genetic structure, or different genetic structures within the same bacterial strain. Alternatively, bacteria can have unspecific mechanisms of resistance to different substances, including heavy metals, biocides and antibiotics. In both cases, exposure to one substance results in the selection of bacterial strains able also able to resist the other substance. Genes encoding resistance due to heavy metals and antibiotics often co-exist on plasmids (Foster, 1983). In addition, unspecific mechanisms conferring resistance to both heavy metals and antibiotics are known to exist in some bacterial species. The co-selective property of heavy metals is confirmed by the indirect evidence that bacteria isolated from heavy metal-polluted marine sediment are significantly more resistant to antibiotics compared with bacteria isolated from unpolluted sites (Rasmussen and Sørensen, 1998). Although genes encoding resistance to biocides have been found on plasmids and integrons (McDonell and Russel, 1999), these substances are more likely to select for antibiotic resistance by induction of unspecific mechanisms of multiple resistance. Laboratory experiments have shown that biocides such as triclosan and pine oil can select for resistance to different antibiotics when bacteria are exposed to low concentrations of biocide (Moken *et al.,* 1997; McMurry *et al.,* 1998). Accordingly, the co-selective effect of biocides for antibiotic resistance could be particularly marked when these substances are dispersed in the environment, because of dilution and formation of concentration gradients.

**2.8.3. Optimal condition for horizontal gene transfer**

Sewage is a suitable habitat for the transfer of resistance genes across different groups of bacteria. In this habitat, environmental bacteria meet resistant bacteria selected by use of antibiotics in human and veterinary medicine. Consequently, resistance genes occurring in bacteria of human and animal origin can be transferred to environmental bacteria, contributing to the formation of an environmental pool of resistant bacteria and resistance genes. The high concentrations of bacteria, nutrients and suspended solids in sewage are all factors enhancing horizontal gene transfer (Saye *et al*., 1989;Lorenz and Wackernagel, 1994; Ripp and Miller, 1995). High bacterial concentrations increase the chance that donor and recipient cells come in contact. Nutrients are more likely to have an indirect influence on the occurrence of gene transfer by increasing the concentration and the metabolic activity of bacteria. Suspended solids provide ideal surfaces on which the various components contributing to the process of horizontal gene transfer are concentrated(Kruse, 1999). Plasmids and transposons harboring antibiotic resistance genes are widespread in the bacterial flora of sewage (Bell *et al.,* 1981). Multiple-resistant bacteria isolated from sewage can transfer plasmid-mediated antibiotic resistance at high frequencies in the laboratory (Alcaide *et al.,* 1986). Experiments performed using membrane chambers immersed in sewage have shown that high frequencies of transfer may also occur under real conditions (Mach and Grimes, 1982; Marcinek *et al.,* 1998).

**2.8.4. 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).

**Fig 4:** Diagrammatic comparison of the five families of efflux pumps. (Courtesy of Melissa Brown; reproduced by kind permission).

Murti *et al.,* (1962) reported that resistance of Salmonella typhi to chloramphenicol has not been reported so far except in strains made resistant in the laboratory. While examining 52 smooth strains of S. typhi and three smooth strains of S. paratyphi A 10 strains of S. typhi were found to be resistant to 50 to 500μg chloramphenicol. Of these 10 strains, eight appeared to be tolerant of the antibiotic, but the remaining two strains appeared to produce a substance that antagonizes or destroys chloramphenicol.

Everett, (1974) reported that a high incidence of penicillin resistance among hospital staphylococci is widely recognized, but some believe the same does not occur in general practice. This is not so. In the casualty department, that no-man's land between hospital and general practice, the incidence of penicillin-resistant staphylococci is recorded as 50 % (Price *et al.*, 1968) and 73 % (Rutherford *et al*., 1970). In true general practice the incidence has been recorded as 23 % (Roodyn, 1954)and 25 %, 45 % (Kay, 1962), 38 % (Harris and Wise, 1969)and 53 %. These figures alone suggest that treatment by penicillin is an unwise first choice, and this view was voiced ten years ago by Kay, (1962) who cast doubt on the advisability of using penicillin for the treatment of staphylococcal infection in general practice.

Duncan *et al.,* (1981) reported that a survey was made of the frequency of resistance to amikacin, gentamicin 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 gentamicin, 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.

Ward and Rowe, (1990) reported that each year from 1981 through to 1988 the most common serotypes isolated from man in England and Wales and identified at the Division of Enteric Pathogens were *S. typhimurium*, *S. enteritidis,* and *S. virchow*. In 1981 these three serotypes accounted for 45%, 12%, and 7% of isolations. The remaining 35% comprised strains belonging to a further 188 different serotypes, none of which accounted for more than 1% of the total. In 1988 *S. typhimurium* accounted for 24% of isolations, *S. enteritidis* 57%, and *S. virchow* 4%. The remaining 15% comprised strains of a further 184 serotypes. The resistances to the common antimicrobial drugs in non-typhoidal *Salmonellas* isolated in England and Wales in 1981 and 1988 were reported with particular reference to resistance to four or more antimicrobial drugs (multiple resistances). For *S. typhimurium* the overall percentage of resistant strains varied little, but multiple resistances more than doubled from 5% to 12%; in *S. enteritidis* the incidence remained the same. In *S. virchow* the percentages of strains resistant to all the antimicrobial drugs and in particular, to chloramphenicol, streptomycin, trimethoprim and furazolidone, rose from 0.2% to 10.4%.

[Arvanitidou](http://www.sciencedirect.com/science/article/pii/S0043135496003405) *et al.,* (1997)reported that resistance to 20 antimicrobials was tested in 79 Salmonella strains isolated from river and lake waters in northern Greece. Of the strains, 19 (24.1%) exhibited resistance to one or more of the antibiotics while single, double and multiple resistances were observed in 12.7%, 6.3% and 5.1% of the isolates, respectively. Streptomycin resistance was the most common and nine different antibiotic resistance patterns were recorded in total. All of the strains were susceptible to amoxycillin-clavulanate, cefuroxime, ciprofloxacin, colistin, amikacin and apramycin. Among the resistant *Salmonellas*, five (26.3%) were able to transfer R factors to the Escherichia coli recipient.

[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.*

Boonmar *et al.,* (1998) reported that a total of 1715 Salmonella strains, including 600 S. enteritidis, 290 S. derby, 257 S. weltevreden, 122 S. 1,4,5,12:i:-, 235 S. anatum, and 211 S. typhimurium, originating from 1308 human beings and 407 frozen chicken meat specimens collected in 1993 and 1994 were tested for antibiotic resistance. The disk diffusion method was used with nine disks of chloramphenicol, ceftriaxone, amikacin, kanamycin, ampicillin, sulfamethoxazole plus trimethoprim, nalidixic acid, gentamicin and ofloxacin. The resistance rates of human beings isolates in 1994 to ceftriaxone, amikacin and kanamycin were, respectively, 10.7%, 8.6%, 17.8% in S. enteritidis; 23.1%, 17.3% 33.0% in S. derby; 30.9%, 40.2%. 60.4% in S. weltevreden; 16.1%, 17.7%, 70.9% in S. 1, 4, 5, 12:i:-; 25.7%, 21.6%, 24.7% in S. anatum; 18.9%, 15.7% 37.8% in S. typhimurium, while those isolates in 1993 to the same three antibiotics were, respectively, 1.8%, 0.6%, 3.7% in S. enteritidis; 0.8%, 0%, 9.1% in S. derby; 1.8%, 2.7%, 10.8% in S. weltevreden; 3.8%, 0%, 23.0% in S.1, 4, 5, 12:i:-; 2.2%, 2.2%, 6.7% in S. anatum; 4.5%, 1.5%, 10.6% in S. typhimurium. It was shown that the resistance rates in 1994 were significantly higher than those in 1993. All isolates were susceptible to ofloxacin with the exception of one isolate.

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.Swann, (1969) reported the practice of feeding animals sub-therapeutic doses of antibioticsand the possible effect on human health because of the emergence of cross-resistant humanpathogens.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.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 gentamicin 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.Conly, (2001) stated that clinically important pathogens such as methicillin resistant *Staphylococcus aureus*, vancomycin-resistant enterococci, penicillin-resistant *Pneumococci* and a host of other pathogens are placing an increasing economic, operational and social burden on health care facilities and communities around the world. To date, Canada has an enviable record of relatively low levels of resistance, although some regions of the country are experiencing rapidly rising rates of resistance.

Hakanen *et al.,* (2001)stated thatduring 1995 to 1999, we collected 1,210 *Salmonella* isolates; 629 were from Finnish travelers returning from abroad. These isolates were tested for susceptibility by determining MICs to ciprofloxacin, nalidixic acid, and seven additional antimicrobial agents. The annual proportion of reduced ciprofloxacin susceptibility (MIC > 0.125μg/ml) among all travelers isolates increased from 3.9% to 23.5% (*p<0.01*) during the period of 1995 to 1999. The increasing trend was outstanding among the isolates from Southeast Asia; isolates from Thailand alone increased from 5.6% to 50.0% (*p<0.01*). The reduced fluoroquinolone susceptibility was non-clonal in character and significantly associated with multidrug resistance. A point mutation in the quinolone resistance-determining region of *gyrA* was present in all isolates with reduced susceptibility. These data provide further evidence for the rapid spread of multidrug-resistant pathogens from one continent to another.

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.

Molla *et al.,* (2003) reported that fifty-one (63.7%) of the 80 *Salmonella* strains were resistant to one or more antimicrobials of which 42 (52.5%) displayed multiple-drug resistance. Among the strains, 51.2% were resistant to sulfisoxazole, 46.2% to spectinomycin, 45% to amoxicillin-clavulanic acid and ampicillin, 41.2% to tetracycline and 30% to chloramphenicol. Less than 27.5% of the strains showed resistance to florfenicol, streptomycin, cotrimoxazole and to trimethoprim. *S. typhimurium* var. Copenhagen (100%), *S. anatum* (62.5%), *S. typhimurium* (33.3%) and *S. braenderup* (34.3%) showed multiple antimicrobial resistance to up to eight antimicrobials. None of the strains were resistant to amikacin, apramycin, gentamicin, kanamycin, neomycin, tobramycin, quinolones, cephalosporins and nitrofurantoin. They also indicated the potential importance of chickens as source of multiple antimicrobial-resistant *Salmonella* for human infections.

Orrett, (2004)reported that 554 *Pseudomonas aeruginosa* isolates was recovered from various clinical sources throughout Trinidad, and their resistance patterns to anti-pseudomonal antimicrobial agents were determined. Of the 554 *P. aeruginosa* isolates, 20.6% (114/554) were community isolates, 17.3% (96/554) from the intensive care unit, 10.1% (56/554) from the nursery, and the remaining 52% (288/554) were from other hospital inpatient services. Respiratory tract infections were the predominant source of *P. aeruginosa* isolates from the ICU 46.9% (45/96) and nursery 21.4% (12/56), whereas wounds were the principal source of *P. aeruginosa* from the surgical services 77.0% (141/183). Community isolates of *P. aeruginosa* were predominantly from ear 100% (51/51) and urinary tract infections 35.5%, (33/93). The overall prevalence of resistance was low for both hospital isolates (13.9%) and community isolates (3.8%). All community isolates were fully sensitive to four of the nine antimicrobials tested. Resistance rates among community strains ranged from 2.6% (ciprofloxacin and ceftazidime) to 12.3% for piperacillin.

[Ardic](http://www.sciencedirect.com/science/article/pii/S0924857905001743) *et al.,* (2005) reported that erythromycin [erm(A) and erm(C)] and tetracycline [tet(K) and tet(M)] resistance genes were investigated by multiplex polymerase chain reaction (PCR) in a total of 56 methicillin-resistant (mecA+) staphylococcal hospital isolates, 28 of which were determined to be Staphylococcus aureus (MRSA) and the other 28 were coagulase-negative *Staphylococci* (MRCNS). The resistance rates for tetracycline and erythromycin were 57.1% and 78.6%, respectively.

[Shakespeare](http://www.ncbi.nlm.nih.gov/pubmed/?term=Shakespeare%20WA%5Bauth%5D) *et al.,* (2005) stated that Salmonella enterica serotype Typhi presenting as a primary psoas abscess. The isolate tested susceptible to ciprofloxacin but resistant to nalidixic acid *in vitro*, a pattern associated with fluoroquinolone therapeutic failures.

Vo *et al.,* (2006) investigated that antimicrobial resistance patterns, integron characteristics and gene cassettes as well as the presence of *Salmonella* genomic island 1 (SGI1) in non-typhoidal *Salmonella* (NTS) isolates from human and animal origin. Epidemiologically unrelated Dutch NTS strains (n=237) originating from food-producing animals and human cases of salmonellosis were tested for their susceptibility to 15 antimicrobial agents. Resistance to 14 of these antimicrobials, including the third-generation cephalosporins was detected. Resistance to sulphonamides, ampicillin, tetracycline, streptomycin, trimethoprim and nalidixic acid was common (>/=10% of the strains were resistant). Resistance against three or more antimicrobials was observed in 57 isolates.

Perron, (2008) stated that multidrug-resistance in *Salmonella typhimurium isolated* from swine shown resistance to ampicillin (96%), chloramphenicol (88%), neomycin (72%), tetracycline (90%). No resistance to enrofloxacin or gentamicin was found. The distribution of resistance to the different antibiotics was largely dependent on the serotype identity. More precisely, resistance to ampicillin (w2 = 105.0; Po0.0001), chloramphenicol (w2 = 107.0; Po0.0001), neomycin (w2 = 69.8; Po0.0001), tetracycline (w2 = 95.8; Po0.0001) and trimethoprim-sulfas (w2 = 246.0; Po0.0001) was significantly associated with serotype Typhimurium. On the other hand, resistance to coamoxiclav (w2 = 53.0; Po 0.0001), cefoxitin (w2 = 22.5; Po0.0001), ceftiotur (w2 = 15.1; Po0.01) and cefalotin (w2 = 53.1; Po0.0001) was significantly associated to serotype Heidelberg.

Fu *et al.,* (2008) reported that resistance to ciprofloxacin was detected in 111 (48.1%) isolates of *Klebsiella pneumoniae* from China. *GyrA* alterations were identified in the ciprofloxacin-resistant and ciprofloxacin susceptible isolates. The results, including previously published data, indicate that the single substitution *Ser833Ile* and three types of double mutations at *Ser83* and *Asp87* were required for ciprofloxacin resistance (*P* < *0.05*).

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 Gentamicin. Sensitivity was recorded in case of 86%, 80%, 60%, 36%, 30%, and 26% of the strains to Norfloxacin, Gentamicin 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, Gentamicin, Neomycin, Tetracycline, Streptomycin and Norfloxacin.

[Akoachere](http://www.ncbi.nlm.nih.gov/pubmed/?term=Akoachere%20JF%5Bauth%5D), (2009) stated that cattle and pigs slaughtered in Buea as reservoirs of Salmonella Typhimurium and the susceptibility of isolates to antibiotics. In total, 230 specimens (comprising 50 each from the rectum, ileum, and gall bladder of cattle; and 10 each from same anatomical sites of pigs and 50 from abattoir drains) were analyzed for Salmonella using the standard microbiological, biochemical and serological techniques. Antibiotic susceptibility of the isolates was determined by the Kirby-Bauer disc-diffusion test. The most active drugs were ciprofloxacin (98.6%), ofloxacin (93.3%), amikacin (90.6%), and gentamicin (84%). All the isolates (100%) were resistant to tetracycline and ampicillin.

Li *et al.,* (2009)reported that the highest prevalence of resistance among β-lactam antibiotics in wastewater, downstream water and upstream water was always against ampicillin (92.2%, 78.5% and 22.7% respectively), followed by oxytetracycline (90.5%, 77.9% and 20% respectively), while the lowest resistance were against cefotaxime (67.0%, 36.8% and 0% respectively) and ceftazidime (75.4%, 56.4% and 1.3% respectively).

Lindgren *et al.,* (2009) reported that the fluoroquinolone susceptibility of 499 Salmonella enterica isolates collected from travelers returning to Finland during 2003–2007. Among isolates from travelers to Thailand and Malaysia, reduced fluoroquinolone susceptibility decreased from 65% to 22% (p =0.002). All isolates showing nonclassical quinolone resistance were from travelers to these 2 countries**.**

Rahman *et al.,* (2009) reported that 150 *Salmonella* isolates were 100% sensitive to Gentamycin followed by Amoxicillin (90%), Colistin (70%), Co-trimoxazole (60%) and Furazolidone (40%) but the isolates were highly resistant to Norfloxacin, Flumequine, Ciprofloxacin and Enrofoxacin. The study demonstrated that the *Salmonella gallinarum* were more sensitive to Gentamycin than Amoxycillin or Colistin.

Samra *et al.,* (2009) reported that the prevalence of kanamycin resistant bacteria in drinking water from a residential area was 70.58%, 42.85% from college drinking water and 12.5% from hospital drinking water. The prevalence of ampicillin resistant bacteria in drinking water from a residential area was 54.70%, 73.75% in hospital drinking water and 27.14% in drinking water samples from colleges.

Zhang *et al.,* (2009) reported that 366 strains of *Acinetobacter spp*. were isolated from five different sites, three in a wastewater treatment plant (raw influent, second effluent, and final effluent) and two in the receiving. The antibiotic susceptibility phenotypes were determined by the disc diffusion method for 8 antibiotics, amoxicillin/clavulanic acid, chloramphenicol, ciprofloxacin, colistin, gentamicin, rifampin, sulfisoxazole, and trimethoprim. The prevalence of antibiotic resistance in *Acinetobacter* isolates to AMC, CHL, RA, and multi-drug (three antibiotics or more) significantly increased (pb0.01) from the raw influent samples (AMC, 8.7%; CHL, 25.2%; RA, 63.1%; multi-drug, 33.0%) to the final effluent samples (AMC, 37.9%; CHL, 69.0%; RA, 84.5%; multi-drug, 72.4%), and was significantly higher (p <0.05) in the downstream samples (AMC, 25.8%; CHL, 48.4%; RA, 85.5%; multi-drug, 56.5%) than in the upstream samples (AMC, 9.5%; CHL, 27.0%; RA, 65.1%; multi-drug, 28.6%).

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%).

Virdis *et al.,* (2010)reported thatantimicrobial resistance patterns and gene coding for methicillin resistance (*mecA*) were determined in 25 *S. aureus* and 75 Coagulase Negative *Staphylococci* (CNS) strains isolates from half-udder milk samples collected from goats with subclinical mastitis. Fourteen (56.0%) *S. aureus* and thirty-one (41.3%) CNS isolates were resistant to one or more antimicrobial agents. *S. aureus* showed the highest resistance rate against kanamycin (28.0%), oxytetracycline (16.0%), and ampicillin (12.0%). The CNS tested was more frequently resistant to ampicillin (36.0%) and kanamycin (6.7%). Multiple antimicrobial resistances were observed in eight isolates, and one *Staphylococcus epidermidis* was found to be resistant to six antibiotics. The *mecA* gene was not found in any of the tested isolates. Single resistance against *β*-lactamics or aminoglycosides is the most common trait observed while multi resistance is less frequent.

Anastasiou and Schmitt, (2011) investigated that hospital effluent as possible source of antibiotic resistant bacteria in the environment. Molecular epidemiological studies of human and animal derived *Enterococcus faecium* strains had previously shown that *E. faecium* from outbreaks and infections worldwide are characterized by ampicillin resistance and a high prevalence of the *esp* gene. Absolute concentrations of hospital-associated *E. faecium* were much lower in surface water (<1- 200 CFU/100ml) than in the effluent (102-103 CFU/100ml). Further characterization of the obtained isolates with multiple locus variant analysis showed a high frequency of MLVA type 159 and 12, again suggesting that antibiotic resistant *E. faecium* typical for hospital outbreaks might spread from the hospital into the environment. Hospital association was also suggested by multi locus sequence typing of selected isolates. The detection of hospital-associated *E. faecium* in surface water shows that exposure to water contaminated by sewage effluent might represent a transmission route for community acquisition of this bacterium.

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).

Dalhoff, (2012) demonstrated that fluoroquinolone resistance rates increased in the past years in almost all bacterial species except *S. pneumoniae* and *H. influenzae*, causing community acquired respiratory tract infections. However, 10 to 30% of these isolates harbored first-step mutations conferring low level fluoroquinolone resistance. Fluoroquinolone resistance increased in Enterobacteriaceae causing community acquired or healthcare associated urinary tract infections and intra abdominal infections, exceeding 50% in some parts of the world, particularly in Asia. One to two-thirds of Enterobacteriaceae producing extended spectrum *β*-lactamases was fluoroquinolone resistant too. Furthermore, fluoroquinolones select for methicillin resistance in *Staphylococci*. *Neisseria gonorrhoeae* acquired fluoroquinolone resistance rapidly; actual resistance rates are highly variable and can be as high as almost 100%, particularly in Asia, whereas resistance rates in Europe and North America range from *<*10% in rural areas to *>*30% in established sexual networks.

Gu et al., (2012) stated that Shigella is becoming an increasing public health problem due to development of multiple antimicrobial resistances, frequently resulting in treatment failure. In the area of Asia-Africa, resistance rates to nalidixic acid and ciprofloxacin were 33.6% and 5.0% respectively, 10.5 and 16.7 times those of Europe-America. Moreover, resistance to nalidixic acid and ciprofloxacin in Asia-Africa progressively increased each year, reaching 64.5% and 29.1% respectively, in 2007-2009, whilst isolates in Europe-America remained at low levels of resistance (<5.0% and <1.0%, respectively). All Shigella flexneri strains showed higher resistance than Shigella sonnei in Europe-America: overall, 3.5% vs. 2.6% resistant to nalidixic acid and 1.0% vs. 0.1% resistant to ciprofloxacin. In Asia-Africa, a similar trend was found for ciprofloxacin 3.0% vs. 0.5%, whereas the trend was reversed for nalidixic acid 32.6% vs. 44.3%. In conclusion, quinolone resistance in Shigella has increased at an alarming speed, reinforcing the importance of continuous monitoring of antimicrobial resistance in Shigella.

Shanthi *et al.,* (2012)deals with isolation, identification and characterization of isolated from tannery effluent collected in and around Erode, South India. A total of 60 isolates were screened from tannery effluent. Antibiotic sensitivity pattern was studied using disk diffusion method. Most of the bacterial strains were sensitive to cotrimoxazole, gentamycin, kanamycin, nalidixic acid, ampicillin, and Penicillin showed resistance against bacteria. *Staphylococcus aureus* was 50% resistant to amikacin, 49% resistant to gentamycin and norfloxacin. *Bacillus spp*. demonstrated higher resistance for Ciprofloxacin similarly *E.coli* had 49.7% resistance to clindamycin. *P. aeruginosa* exhibited very high resistance for colistin and norfloxacin. The two major dominant isolates were *Pseudomonas spp*. and *Bacillus spp., Staphylococcus aureus* was found to be in subdominant form.

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 gentamicin (*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%).