

# We are IntechOpen, the world's leading publisher of Open Access books Built by scientists, for scientists

6,900

Open access books available

185,000

International authors and editors

200M

Downloads

Our authors are among the

154

Countries delivered to

TOP 1%

most cited scientists

12.2%

Contributors from top 500 universities



WEB OF SCIENCE™

Selection of our books indexed in the Book Citation Index  
in Web of Science™ Core Collection (BKCI)

Interested in publishing with us?  
Contact [book.department@intechopen.com](mailto:book.department@intechopen.com)

Numbers displayed above are based on latest data collected.  
For more information visit [www.intechopen.com](http://www.intechopen.com)



---

## Resistance in Bacteria

---

S.O. Sadashiv and Basappa B. Kaliwal

Additional information is available at the end of the chapter

<http://dx.doi.org/10.5772/61479>

---

### Abstract

Resistance is the result of bacteria evolving new genes in response to the presence of pesticide and antibiotics. In our society day by day, a number of chemicals, pesticides, and antibiotics are introducing due to the result of resistance development of bacteria. Pesticides are added to the environment for the purpose of killing or injuring some form of life. Pesticide resistance describes the decreased susceptibility of a pest population to a pesticide that was previously effective at controlling the pest. Bacteria have been used extensively for bioremediation purposes. The ability of organisms to bioremediate pesticides is mainly based on their biodegradation activity. Methomyl and imidacloprid are widely using throughout the world as a pesticide. Many pesticide degradation genes present in soil bacteria have been shown to reside on plasmids or genome, a common location for other degradation genes. The excessive use of pesticides and antibiotic leads and promotes the development of resistance in the bacteria. An increase in the frequency of antibiotic resistance in bacteria since the 1950s has been observed for all major classes of antibiotics used to treat a wide variety of diseases. Development of resistance is a major concern for another reason of human and animal health. Antibiotic resistance profiles of the isolates must be done earlier to the use of antibiotics in both to choose appropriate antibiotic for treatment and prevention of the disease. Research into newer antibiotics continues, measures can and should be taken to reverse the practices that promote the development of antibiotic resistance in bacteria.

**Keywords:** Resistance, pesticide, methomyl, imidacloprid, antibiotic

---

## 1. Introduction

Resistance is the result of bacteria evolving new genes in response to the presence of pesticides and antibiotics, as a result the bacteria will be capable of remain in the surroundings. A number of xenobiotics and antibiotics are introducing due to the result of the resistance development of bacteria. The productive use of any therapeutic agent is conceded

by the potential development of tolerance or resistance to that compound from the time it is first applied. These remains very true for the agents are used in the treatment of parasitic and microbial infections and for the treatment of chronic disorders like cancer and diabetes, and it apply to conditions caused or suffered by any living organisms, including plants, insects, humans, animals, fish, etc. Many changes in physiological and biochemical mechanisms may be responsible for the incidence of resistance. In the case of antimicrobial agents and insecticides, the specific complexity of the developments that contributes to the emergence and spreading of resistance cannot be overemphasized, and also, sometime lack of basic information and knowledge on these issues is one of the major primary reasons that there has been very much slight significant achievement in the effective prevention and control of resistance development in the organisms [1]

## 2. Pesticides resistance in bacteria

Agriculture is the most essential of the Indian economy. It ensures food security for the biggest population with shrinking cultivable land resource necessitates use of high yielding variety of seeds, balanced use of fertilizers, and judicious use of quality pesticides. The indiscriminate and unplanned use of agrochemicals influences microbial processes that are an essential component of carbon, nitrogen, and sulfur cycles.

Pesticides are added to the environment for the purpose of killing or injuring the pests. Pesticides are the chemical substances that kill pests like fungi, insects, worms, nematodes, etc., which cause damage to field crops. It is almost impossible to control the spreading of pesticides effect. Even though it is applied in very much small area, it is dissolves in water, absorbed by soil, and spreads through the air. The contaminated water ultimately reaches much bigger and new area. These pesticides also frequently trickle into ground water, which we humans consume, and as a result, these pesticides poison us over a period of time. However, in addition to these, residual pesticides that remain on the plants are sometimes consumed by animals and also humans, leading to serious many disorders like cancer and even sometimes death. The excessive use of pesticides leads to an accumulation of a huge amount of pesticide residues in the food chain and drinking water environment that further leads to a substantial health hazard for the current and future generations due to uptake and accumulation of these toxic compounds [2]. Majority of agrochemicals devoid of mutagenic activity and induce their effects by genotoxic or non genotoxic modes of action. However, in some cases, the modes of action are known and they give a clear indication of the likely human hazards, but in many cases, the data are lacking or incomplete resulting in a more conventional approach toward human hazard and risk assessment [3].

Pesticides can be classified according to their toxicity, chemical group, environmental persistence, target organism, or other features. Classes of organic pesticides (consisting of organic molecules) include organochlorine, organophosphate, organometallic, pyrethroids, and carbamates among others [4]. The use of explosives, refrigerants, pesticides, solvents, and many dyes in urban, industrial, and agricultural applications results into the release of

xenobiotic compounds into the environment, and the problem of such toxic waste disposal has become enormous due to the proliferation of these xenobiotic compounds. Many xenobiotic compounds, particularly those used as insecticides, are toxic. Insecticides have been reported to affect the microbial populations by controlling the survival and reproduction of species.

Biodegradation and bioremediation are identical processes to an extent that both of these are based on the conversion or metabolism of many chemicals and pesticides by microorganisms. Here, the difference is that biodegradation is a natural process while bioremediation is a technology. A successful bioremediation technique requires an efficient bacterial strain that can degrade largest pollutant to minimum level [5]. Soil microorganisms play a key role in biodegradation. Soil microorganisms are of great concern for using in biotechnology because they are able to metabolize and degrade many of pollutants and pesticides. Simultaneously, on the other hand, microbial degradation can lead to formation of more toxic and persistent metabolites. Although the soil microbial population are characterized by their adaptability to the changed environmental condition, fast flexibility and the application of pesticides in long term as well as in short term can cause significant irreversible changes in their population.

Bioremediation constitutes an attractive alternative to physicochemical methods of remediation, as it is less expensive and can selectively achieve complete destruction of organic pollutants. In bioremediation, microbes that can degrade the pesticides *in-situ* are used. For a successful bioremediation technique, an efficient bacterial strain that can degrade largest pollutant to minimum level is required. In predicting the persistence of synthetic chemicals in soil, sediment, and natural water, it is necessary to determine the role of endogenous microorganisms in the overall degradation process.

The ability of organisms to bioremediate pesticides is mainly based on their biodegradation activity. Although bioremediation has been firstly achieved using microorganisms (bacteria or fungi), other organisms like plants or algae can be used. To eradicate undesirable effects of pollutants from the environment the strategy of bioremediations are used. However, this method is not always possible and sometimes it would be appropriate to eliminate pollutants, although some organisms could restrict or immobilize them. For an instance, the used organisms can accumulate contaminants and to some extent decrease their presence and their environmental effect, but they do not completely eliminate them from the environment. Those organisms able to bioremediate would be called bioremediators. Traditionally, bioremediation has been achieved by using microorganisms [6].

Pesticide resistance describes the decreased susceptibility of a pest population to a pesticide that was previously effective at controlling the pest. Pest species develop pesticide resistance via natural selection; as a result, the highest resistant varieties survive and continuously transfer their genetic traits to their next generation [7]. Many times, when pesticide degradation happens, it usually involves more than one microorganism, i.e., each microorganism contributes to the process of biodegradation reactions on pesticides. However, there is no evidence, and an example of mineralization by a single strain has been described. It shows that the presence of different microorganisms is essential for an adequate and significant biodegradation. Reported micro-biodegraders belong to Basidiomycetes or to bacterial classes: gamma-proteobacteria (*Pseudomonas*, *Aerobacter*, *Acinetobacter*, *Moraxella*, and *Plesiomonas*), beta-

proteobacteria (*Burkholderia* and *Neisseria*), alpha-proteobacteria (*Sphingomonas*), actinobacteria (*Micrococcus*), and flavobacteria (*Flavobacterium*) [6].

Many studies have focused on the employment of bacteria, consortia or on the search for biotransformation enzymes. Bacteria have been used extensively for bioremediation purposes due to their fast growth, easy handling, and low cost, making them suitable for bioremediation. However, some disadvantages are there, such as the pathogenicity of bacteria, disposal of bacterial biomass, and bioactivation, among others. Bacteria can be found in the environment everywhere such as soil, water, or even in particles dispersed in air. Unfortunately, only a small segment of bacteria (<10% from soil) can be cultured in laboratory conditions. Because of this, a number of studies about pesticide biodegradation mechanisms are less than those about biodegrader's isolation and then slight information on biochemical mechanisms or enzymes is accessible.

Pollutants might undergo biodegradation reactions like cleavage, oxidation, de-chlorination, reduction by different enzymes. Since biodegradation capability is based on enzymes, which are promiscuous and have evolved to detoxifying enzymes, the shorter the duplication time of organism and the more sufficient the organism, the easier to obtain biodegraders. Therefore, bacteria with replication time around or less than minutes are much admirable to respond to natural and artificial pollutant-induced evolutionary pressure; this response is involved in the selection of biotransformation enzymes able to degrade them. These promiscuous enzymes are present in the organisms; even before the exertion of the evolutionary pressure, the induced genetic recombination or mutation in the organisms could lead to enzymes with better biodegradation ability [6].

The carbamates are mainly used in agriculture, as insecticides, fungicides, herbicides, nematocides, or sprout inhibitors; also, it is used as a potential in public health vector control. In addition, these carbamates are used as biocides for industrial or other applications and in household products. As a result, these chemicals are part of the large group of synthetic pesticides that have been developed, produced, and used on a large scale in the society and environment. They are derivatives of carbamic acid and like organophosphates; the mechanism of action of these chemicals is that inhibiting the vital enzyme acetyl cholinesterase is reversible as compared to organophosphates, which is irreversible. Exposure to cholinesterase inhibiting agents is considered as a major health problem for the farm workers throughout the world.

Three classes of carbamate pesticides are generally known. The carbamate herbicides have the general structure  $R^1NHC(O)OR_2$ , in which  $R_1$  and  $R_2$  are aromatic and/or aliphatic moieties. The carbamate ester derivatives, used as insecticides (and nematocides), are usually stable and have a low vapor pressure and low water solubility. Fungicide carbamate contains a benzimidazole group. Carbamates are metabolized by microorganisms, plants, and animals or broken down in water and soil. Soil microorganisms have the capacity of metabolizing (hydrolyzing) carbamates and can easily acclimate themselves to metabolize the different types of carbamates. However, at the high dose levels of carbamates, their metabolites cause changes and can significantly affect the microflora, which may be of importance in soil productivity. Some of the common names of the carbamate insecticide are aldoxycarb,



allyxycarb, aminocarb, bufencarb, butacarb, carbanolate, carbaryl, carbofuran, aldicarb, methomyl, oxamyl, thiofanox, thiodicarb, etc.

## 2.1. Methomyl

Methomyl is widely used throughout the world since it is effective as “contact insecticide” as well as “systemic insecticide.” The IUPAC name of methomyl is S-methyl N-(methylcarbamoyloxy) thioacetimidate. Methomyl belongs to a class of compounds known as oxime carbamates, and it is widely used for the control of insects and nematode pests by inhibiting the enzyme acetylcholinesterase, which hydrolyzes the neurotransmitter acetylcholine [8]. Methomyl has been classified as a pesticide of category-I toxicity [9]. Methomyl is a metabolite of thiodicarb, and acetimidate is a suspected oncogen, which is metabolite in animal tissues [10]. Methomyl is endocrine disruptor and also potent genotoxic, capable of inducing structural and numerical chromosomal aberration in mammalian cells [11]. The World Health Organization (WHO), Environment Protection Agency (EPA), and European Chemical Classification (ECC) classify methomyl as a very toxic and a most hazardous pesticide. Methomyl is highly soluble in water (57.9 g/liter, at 25°C [8]), and since the sorption affinity of soils for this pollutant is rather low, it can easily cause contamination of both ground and surface water resources [12].

The study of methomyl-induced alteration in mice hepatic-oxidative status and methomyl-induced gonadal dysfunction, biochemical contents, and enzyme activities in mice suggests that chronic exposure to methomyl insecticide has deleterious effect on mouse liver and also showed effect on reproductive system in mice. Therefore, the application of such insecticide for designed program should be limited or special care should be taken to minimize its hazards [13, 14].

Many pesticide degradation genes present in soil bacteria have been shown to reside on plasmids, a common location for other degradation genes [15, 16]. Some plasmids are known as catabolic plasmids because they bear genes encoding for enzymes capable of degradation and such plasmids have been of great attraction. The organisms containing the catabolic plasmids have the ability to degrade certain compounds. Many catabolic plasmids have been found in species of *Actinobacter*, *Flavobacterium*, *Pseudomonas*, *Alcaligenes*, *Klebsiella*, *Moraxella*, and *Arthrobacter* [17].

The innovation of microorganisms capable of tolerating or growing in high concentrations of pesticides provides a potentially interesting possibility for treating hazardous wastes [18]. Some investigations resulting in the identification of microbial isolates, which are apparently responsible for the accelerated degradation of individual pesticides, is necessary [19]. Many xenobiotic degradation genes present in soil bacteria have been shown to reside on plasmid, a common location for other degradation genes [20]. The study of plasmid curing suggests that the degrading ability for methomyl is encoded in the plasmid for the genus *Pseudomonas aeruginosa* [21]. Soil microbial populations, particularly members of *Pseudomonas*, *Bacillus*, and *Escherichia coli* have evolved the considerable nutritional versatility and are capable of the degradation of a range of complex, naturally occurring aromatic and aliphatic compounds. An

interesting feature of these degradative plasmids is that they have been isolated almost exclusively from the species of the genus *Pseudomonas*, *Bacillus*, and *E. coli* [22].

It is suggested that the detoxification metabolism occurs when a microorganism uses the pesticide as a carbon and energy source and the process is assisted by resistant microorganisms [23]. The plasmid-coded biodegradation of methomyl may be due to the broad host range plasmids and selection pressures on spontaneous mutants due to the presence of xenobiotics, vertical gene transfer, or horizontal gene transfer, including transposons and broad host range plasmids and selection pressures on spontaneous mutants due to the presence of xenobiotics or due to strains that harbor a single plasmid with a role in pesticide biodegradation [24].

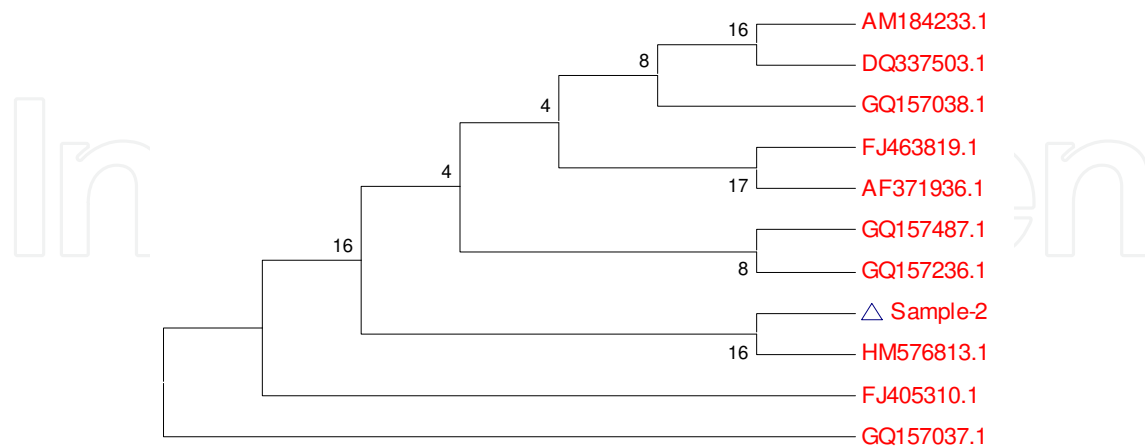
The proteomic profiling of *E. coli* in response to carbamate pesticide–methomyl research work was conducted by Kulkarni and Kaliwal [25]. The study suggests that the proteomic profiling is a sensitive tool for environmental stress diagnosis and that the stress proteins could be used as biomarkers for environmental pollution identification. Thus, biological decontamination methods are preferable to conventional approaches because, in general, microorganisms degrade numerous environmental pollutants without producing toxic intermediates [26, 27].

## 2.2. Imidacloprid

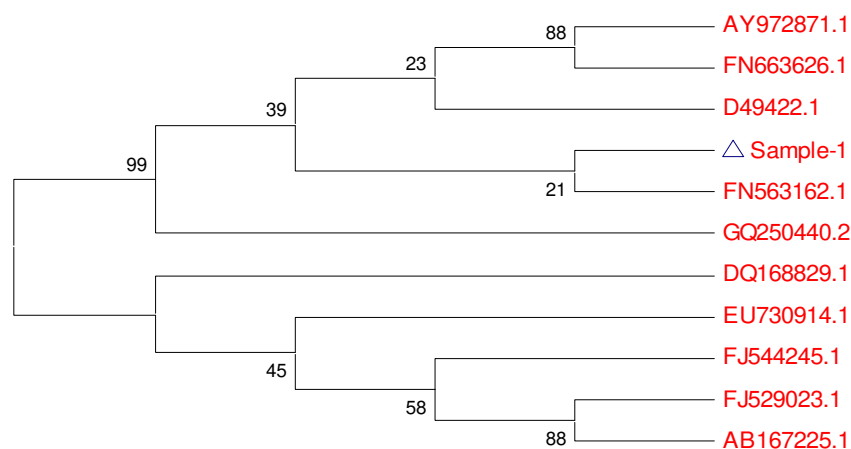
Imidacloprid is the first synthetic neonicotinoid insecticide used against sucking pests, such as rice hoppers, aphids, thrips, and whiteflies. Imidacloprid has been used widely for foliar and seed treatment, soil drench, and stem application [28]. Today imidacloprid is used in over 120 countries to treat more than 140 different crops [29]. It is most commonly used on cotton, rice, cereal, maize, sunflowers, potatoes, and vegetables. The active chemical in imidacloprid works by interfering with the transmission of stimuli in the insect's nervous system. Imidacloprid is a neurotoxic insecticide, which belongs to the class of the neonicotinoid pesticides. Imidacloprid is registered to control insect pests on agricultural and nursery crops, structural pests, and parasites on companion animals. Imidacloprid is an agonist of the nicotinic acetylcholine receptor (nAChR) at the neuronal and neuromuscular junctions in insects and vertebrates. It is structurally and functionally related to nicotine. The toxicity of imidacloprid is largely due to interference of the neurotransmission in the nicotinic cholinergic nervous system. Extended activation of the nAChR by imidacloprid causes desensitization and blocking of the receptor and leads to incoordination, tremors, decreased activity, reduced body temperature, and death.

The ability of some microorganisms to grow in the presence of pesticides may result in the compensation of an adverse effect by the increased activity of remaining part of soil community. Bacteria's are known to become resistant to toxic compound with production of specific degrading enzymes [30]. Three imidacloprid tolerant strains were isolated and identified based on morphology, biochemical characters and 16s rDNA identification as *E. coli* (Fig. 1), *Brevundimonas* sp. MJ 15 (Fig. 2), and *Bacillus weihenstephanensis* (Fig. 3), and they were evaluated for their toxicity toward imidacloprid using standard methods to determine their biochemical contents, growth, and enzyme parameters on exposure to the toxicant and concluded that imidacloprid-induced toxicity and stress in bacterial soil isolates. The knowledge about intoxication effects and conservation of toxicity mechanisms in organisms will

enable to choose appropriate model organisms for relevant monitoring of specific environmental toxicants [31].



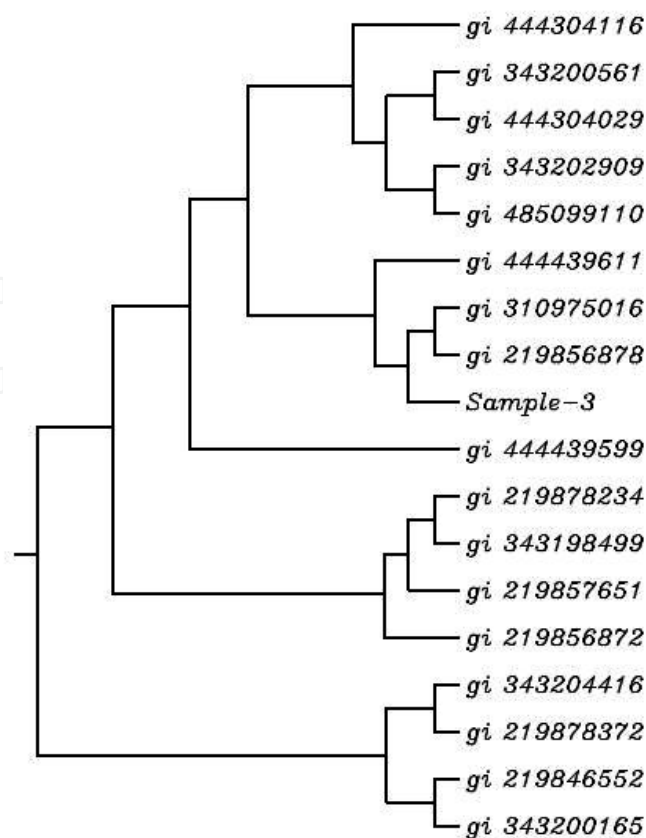
**Figure 1.** Phylogenetic tree of *E. coli*.



**Figure 2.** Phylogenetic tree of *Brevundimonas* sp. MJ 15.

Genes responsible for pesticide degradation in microorganism have been found to be located on plasmids, transposons, and chromosomes. Some microbial strains possess genetic determinants that confer resistance. In bacteria, these determinants are often found on plasmids, which have facilitated their study at the molecular level [32]. The involvements of plasmids in degradation of organic xenobiotics were first reported 20 years ago, and the list is increasing continuously till today [33]. Such type of plasmids have mostly found in xenobiotic degrading bacteria, after selective isolation of the strain by many methods. Also, direct exogenous plasmid isolation has yielded several novel catabolic plasmids more recently. The involvement of plasmids in degradation of pesticides is reported in several studies. Plasmid encode the genes were found responsible for degradation of anthracene, fenitrothion, carbofuran, dimethoate, phenanthrene, and dichlorophenoxyacetic acid by *Pseudomonas* sp., *Burkholderia*





**Figure 3.** Phylogenetic tree of *Bacillus weihenstephanensis*.

sp., *Sphingomonas* sp., *Bacillus licheniformis*, *Flavobacterium* sp., and *Alcaligenes paradoxus*, respectively [34, 35].

**3. Antibiotic resistance in bacteria**

Antibiotic use promotes the development of antibiotic-resistant bacteria. Every time a person takes antibiotics, sensitive bacteria are killed, but resistant bacteria may be left to grow and multiply. Bacteria can be described as being susceptible to, tolerant of, or resistant to specific antibiotics. When an antibiotic attacks a group of bacteria, those cells that are susceptible will die.

The primary cause of increase in drug-resistant bacteria is mainly because of repeated and improper uses of antibiotics. The keen use of antibiotics is the key to controlling the spread of resistance bacteria. For example, antibiotics should be used for the treatment of bacterial infections, but many times, they are given for the viral infections such as common cold, most sore throats, and the flu, for which they have not prepared. Extensive and widespread use of antibiotics promotes the spread of antibiotic resistance. Antibiotic resistance occurs when bacteria changes in some way that decreases or eliminates the effectiveness of drugs or other

agents designed to cure or prevent infections. As a result, the bacteria survive and continue to multiply causing more harm to the host. This can be performed by bacteria by several mechanisms. Some bacteria develop the ability to neutralize the antibiotic before it can do harm, others can rapidly impel the antibiotic out, and still others can change the antibiotic attack site so it cannot affect the normal function of the bacteria.

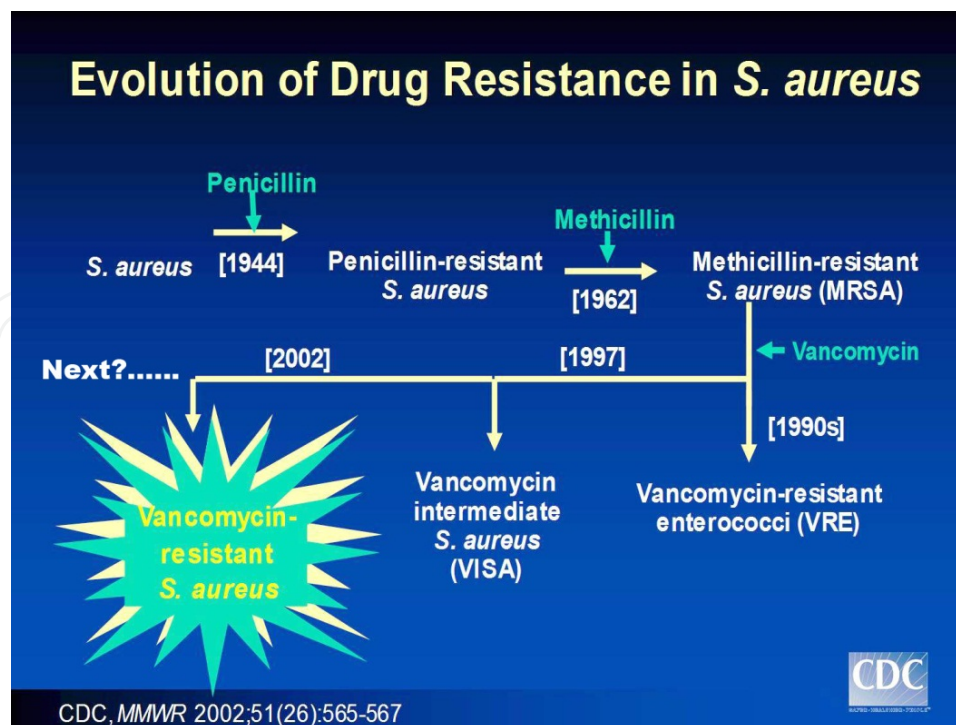
An increase in the frequency of antibiotic resistance in bacteria since the 1950s has been observed for all major classes of antibiotics used to treat a wide variety of diseases. There may be many reasons such as resistance is the result of bacteria evolving new genes in response to the presence of antibiotics, or the antibiotic-resistant bacteria selected for in the environment by possessing antibiotic resistance genes and also several factors involved in antibiotic resistance will show that resistance is a designed feature of preexisting genes enabling bacteria to compete with the antibiotic producers in their environment.

### 3.1. Antibiotic resistance in humans

The development of resistance in bacteria is a major concern for another reason of human health. Historically, infectious diseases have killed billions of people and have the reason for most devastating chapters in the history of humankind. Scientists have been so successful in the earlier century in preventing and curing infectious diseases that only a few years ago, it was thought that modern science had at last enabled us to “close the book on infectious diseases.” Bacteria that have become resistant to several antibiotics, said to be multidrug resistant, are often called super bugs by the media. An important mechanism by which bacteria become resistant is by obtaining one or more specific resistance genes from other bacteria. This type of resistance can be acquired by the transfer of a plasmid, already existing in the bacterium gene pool that carries a gene for an enzyme which either destroys or inactivates the antimicrobial substance.

*Staphylococcus aureus* is a major cause of hospital-acquired infections, causing high morbidity and mortality throughout the world. Vancomycin has been the drug of choice for 30 years for the treatment of methicillin-resistant *S. aureus* (MRSA). Over the last decade, methicillin-resistant *S. aureus* (MRSA) strains have become endemic in hospitals worldwide. In addition, it is now incipient community pathogen in many geographical regions. The emergence of high levels of penicillin resistance followed by development and spread of strains resistant to the semisynthetic penicillins (methicillin, nafcillin, and oxacillin), macrolides, tetracyclins, and amino glycosides has made therapy of staphylococcal disease a global challenge. Now a day's resistance to semisynthetic penicillin's had spread throughout the world, compromising the use of these drugs for empiric therapy for staphylococcal infections in a number of regions. This had led to increased reliance on vancomycin for the treatment of documented MRSA infections. As a consequence, selective pressure was established that eventually lead to the emergence of strains of *S. aureus* and other species of staphylococci with decreased susceptibility to vancomycin and other glycopeptides (Fig. 4).

Vancomycin is the only effective agent against some pathogens, and even in recent years, it has been lost some of its effectiveness. This problem has many causes, including the improper and misuse of antibiotics and the result of transfer of resistance genes from one bacterium to



**Figure 4.** Evolution of drug resistance in *Staphylococcus aureus*.

another. This might be possible because many bacteria have an integral natural resistance to a number of antibiotics, and the genes that provide this resistance can be passed on to other bacteria by a variety of means. Multidrug-resistant *S. aureus* (MDRS) is a much more serious threat, particularly to hospitalized patients in globally, and it represents a challenge for public health, as community-acquired infections seem to be on the increase in adults and children. *S. aureus* colonization has been shown to be a major risk factor for community-acquired and nosocomial infections. In epidemiological study, Unakal and Kaliwal [36] reported the multidrug resistance and vancomycin resistance *S. aureus* isolated from Health Care Centres in India for the first time and detected Van A gene in 4 strains, Van B gene in one strain from the 14 VRSA strains procured from the clinical samples pus, infected blood, and cerebrum spinal fluid.

The first report of the *S. aureus* with reduced susceptibility to vancomycin was from Japan [37]. This report was quickly followed by similar ones from other countries, including the United States [38] and Belgium [39]. The extensive longitudinal study of the current situation of vancomycin resistance has reported the first incidence of VRSA emergence from northern part of India [40]. The first clinical infection with vancomycin-resistant *S. aureus* was reported from Michigan, with second case in Pennsylvania. Further, the second confirmed VRSA from Pennsylvania was reported, which represents the VRSA isolate from patient in the United States. The emergence of decreased vancomycin susceptibility in MRSA strains presents a significant clinical problem with few therapeutic options. The rapid evolution of antibiotic resistance is of considerable concern. Considering high prevalence of MRSA and increased use of vancomycin, the development of vancomycin resistance (VRSA) in clinical strains seems

likely to occur. In 1996, the documented infection caused by *S. aureus* with reduced susceptibility to vancomycin (vancomycin-intermediate *S. aureus* [VISA]) was reported in Japan [37]. Thereafter, about 20 cases of VISA infections have been reported in several countries, including Korea [41].

Vancomycin is a glycopeptide antibiotic, and glycopeptide resistance has emerged in *S. aureus* with the interspecies transfer of resistant gene from nonpathogenic *Enterococcus faecalis* *in vitro* [42]. Moreover, cellular modification due to prolonged use of vancomycin results in an increased extracellular material, which cause thickening of cell wall [43, 44].

### 3.2. Antibiotic resistance in bovines

Bovine mastitis is a common disease entity of dairy cows, accompanied by physical, chemical, pathological, and bacteriological changes in milk and glandular tissue [45]. It is a harmful disease affecting the dairy industry worldwide and is a matter of great concern for leading milk-producing countries like India because of the losses incurred due to high morbidity, discarded milk, treatment costs, and reduced milk production, thus drawing in more attention toward its treatment and control [46]. Mastitis is produced by a variety of pathogenic microorganisms. The majority of cases in bovines are infectious, and it has been estimated that up to 200 microbial species are potential causative agents [47].

The antibiotic efficacy of tetracycline, cefixime, ofloxacin, amoxicillin, and ampicillin was investigated in *S. aureus*, *E. coli*, and *B. subtilis*-induced mastitis in mice by Chinchali [48], and the results were evidenced by the bacterial count and inflammatory enzyme activities of mammary gland tissue against the induced bacterial pathogens in comparison with control group. In the results, there was an orderly decrease in the bacterial counts of *S. aureus*, *E. coli*, and *B. subtilis*, which showed susceptibility for the antibiotics used in the study and indicated their effectiveness of bactericidal activity and their efficacy level against the induced inflammatory reaction. This study demonstrates the effectiveness of the antibiotic in the treatment of the disease. However, continued use and overuse of the antibiotics without an *in vitro* study will lead to the antibiotic resistance strains.

The usage of antibiotics in the bovine mastitis correlates with the emergence and maintenance of antibiotic-resistant traits within pathogenic strains [49]. These traits are coded by particular genes that may be carried on the bacterial genome or plasmids [50]; hence, these are easily transferred among isolates. The prevalence of antibiotic resistance usually varies between isolates from the different sampled area, environment, and even between isolates from different herds on the same farm or environment [51].

The evolution of antibiotic resistance in *S. aureus* strains is a serious cause of concern in dairy animals [52]. Antibiotic-resistant *S. aureus* isolates pose a severe challenge to both in veterinary and health professions and in dairy cattle producers because they have a serious negative impact on the therapy management. *S. aureus* has been and become the main issue of studies on antibiotic resistance because of its importance for all forms of mastitis in dairy cows [53]. Multiple antibiotic-resistant *S. aureus* strains have been isolated and screened from milk obtained from cattle, beef, and human samples from the various part of the world.

Antimicrobials have been used frequently as a conventional measure to prevention and control diseases in dairy farming. Especially in mastitis control programs, more and more antibiotics were applied even without any clinical symptoms in dairy cattle herds. However, long-term in-feed use of antibiotics on dairy farms has led to the alarming increase of antibiotic-resistant bacteria, which has become a public health issue worldwide, e.g., methicillin-resistant *S. aureus* (MRSA) from raw milk and environmental samples constitutes a great threat to food safety. In order to better understand the potential of dairy cattle as a reservoir for antibiotic-resistant bacteria, it is important to investigate the prevalence of antimicrobial resistance among bacteria isolated from raw milk [54].

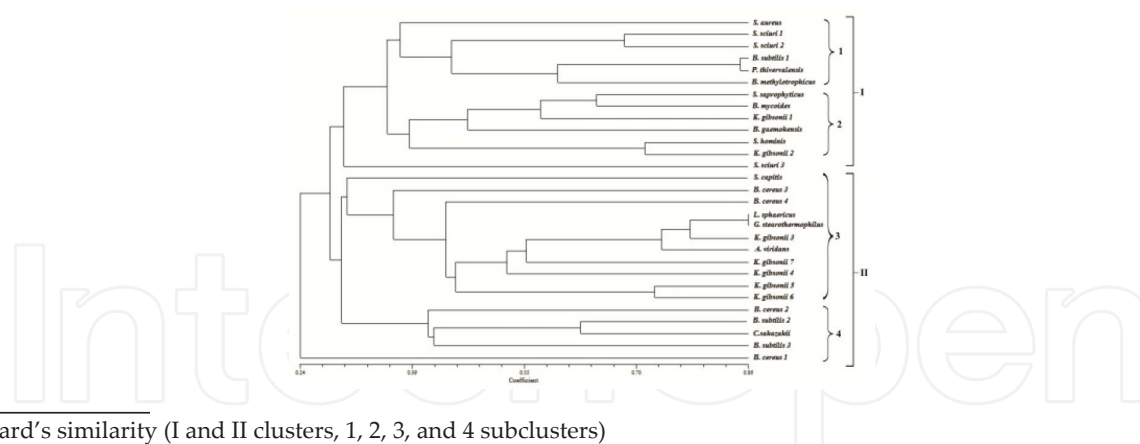
References [55–59] reported many bovine mastitic bacteria and reported the isolated bacteria were resistant to many antibiotics. Sadashiv [60] isolated a total of 878 strains of bacteria from bovine mastitis (Table 1), which showed multidrug resistance to the antibiotics such as amikacin, amoxyclav, ampicillin, methicillin, oxacillin, penicillin G, cefaclor, cefixime, cefpodoxime, ceftriaxone, ciprofloxacin, norfloxacin, ofloxacin, gentamicin, azithromycin, erythromycin, streptomycin, tetracycline, and chloramphenicol. Further, the strains were identified by 16s rDNA method. Due to wide sampling area, the identified strains were subjected to random amplified polymorphic DNA (RAPD) polymorphism analysis and found huge diversity among the strains, demonstrating the migration of the antibiotic resistance strains (Fig. 5). The study concluded that the examination of the antibiotic resistance profiles of the isolates must be done earlier to the use of antibiotics in both to choose appropriate antibiotic for treatment and prevention of the disease.

Sl. no	Bacteria	Isolates	Percentage (%)
1	<i>Staphylococcus aureus</i>	210	23.91
2	Coagulase-negative <i>Staphylococcus</i>	165	18.79
3	<i>Bacillus</i> spp.	221	25.17
5	<i>Escherichia coli</i>	97	11.04
4	<i>Pseudomonas</i> spp.	72	8.20
7	<i>Aerococcus</i> spp.	34	3.87
6	<i>Cronobacter</i> spp.	23	2.61
8	Others	56	6.37
Total		878	100

**Table 1.** Prevalence of different bacterial isolates from the bovine mastitis milk.

It is possible that mastitogenic bacteria can lose the sensitivity to antibiotics over the time or even acquire sometimes this feature [61]. Important reasons for the failure of treatment of mastitis are the indiscriminate use of antibiotics without *in vitro* sensitivity of causal organisms. It is necessary to monitor mastitis pathogens to assess any changes in their antibiotic resistance patterns. Careful use of antibiotics can avoid the increase and dissemination in antimicrobial resistance arising from the use of antimicrobial drugs in animals.





Jaccard's similarity (I and II clusters, 1, 2, 3, and 4 subclusters)

**Figure 5.** RAPD analysis of the bacterial strains. Dendrogram showing the relationship of the bacterial strains generated by neighbor-joining method using cluster analysis.

Some pharmaceutical companies have expanded their R&D efforts due to in response to the increased number of bacteria that have developed resistance to the one or more number of antibiotics. New drugs are being developed which interfere with the resistant cells and the method of new defence against certain antibiotics. For example, bacteria resistant to penicillin produce the enzyme penicillinase, which breaks up the penicillin before it can perform its work. Presently, a penicillinase inhibitor is available that is taken in tandem with the penicillin, therefore by preventing the penicillinase from destroying the penicillin and by allowing the antibiotic to perform its work [62].

The increased use, and sometimes misuse, of antibiotic drugs has resulted in bacterial resistance to a large and growing number of these drugs. However, much more research into newer and newer antibiotics continues, and measures can and must be taken to reverse the practices that usually promote the development of antibiotic resistance in bacteria.

## 4. Conclusion

Presently, the development of resistance in all microorganisms is one of the major concerns and challenges throughout the world. The development of resistance can be achieved by the microorganisms by many ways. The smart, keen, and controlled use of pesticides, chemicals, and antibiotics will be much helpful in controlling the development of resistance. However, screening newer microbes and using newer recombinant technology on the screened microbes will help us reduce the resistance to xenobiotics compounds.

## Acknowledgements

The authors are grateful to the Department of Biotechnology (DBT), Ministry of Science and Technology, Government of India, New Delhi, for funding the Interdisciplinary Program for

Life Science Project (BT/PR/4555/INF/22/126/2010 dated 30-09-2010), the Bioinformatics Infrastructure Facility Project (BT/BI/25/001/2006 VOL II dt. 05-03-2012), and the P.G. Departments of Microbiology and Biotechnology Karnatak University, Dharwad, for providing the facilities.

## Author details

S.O. Sadashiv<sup>1</sup> and Basappa B. Kaliwal<sup>2\*</sup>

\*Address all correspondence to: b\_kaliwal@yahoo.com

1 Department of Microbiology, Davangere University, Davangere, Karnataka, India

2 Department of Biotechnology, Vice-Chancellor, Davangere University, Davangere, Karnataka, India

## References

- [1] Davies J, Davies D. Origins and evolution of antibiotic resistance. *Microbiol. Mol. Biol. Rev.*, 2010; 417–433. doi:10.1128/MMBR.00016-10.
- [2] Naphade SR, Durve AA, Bhot M, Varghese J, Chandra N. Isolation, characterization and identification of pesticide tolerating bacteria from garden soil. *Euro. J. Exp. Biol.*, 2012; 2(5): 1943–1951.
- [3] Green T, Toghill A, Lee R, Waechter F, Weber E, Peffer R, Noakes J. *Toxicol Sci.*, 2005; 86: 36–47.
- [4] Gilden RC, Huffling K, Sattler B. Pesticides and health risks. *J. Obstet. Gynecol. Neonatal Nurs.*, 2010; 39(1): 103–110.
- [5] Singh DK. Biodegradation and bioremediation of pesticide in soil: concept, method and recent developments. *Indian J. Microbiol.* 2008; 48: 35–40.
- [6] Velázquez-Fernández JB, Martínez-Rizo AB, Ramírez-Sandoval M, Domínguez-Ojeda D. Biodegradation and bioremediation of organic pesticides, pesticides—recent trends in pesticide residue assay. Dr. R.P. Soundararajan (Ed.), InTech, (2012). ISBN: 978-953-51-0681-4, DOI: 10.5772/48631.
- [7] PBS. Pesticide Resistance. 2007. Retrieved September 15, 2007.
- [8] Tomlin CDS. *The Pesticide Manual*. BCPC Publications. 13th ed. 2003: 1344; ISBN 978-1-90139613-3.

- [9] Baron RL, Hayes WJ, Laws ER (Eds.). San Diego, Calif, Carbamate insecticides. In Handbook of Pesticide Toxicology. Vol 3. Academic Press, New York. 1991; 1125–1190.
- [10] EPA. Drinking Water Regulations and Health Advisories, USEPA, 1996; 822-B-96-002, Washington, DC.
- [11] Andersen HR, Vinggaard AM, Rasmussen TH, Gjermansen IM, Bonefeld-Jorgensen EC. Toxicol. Appl. Pharmacol., 2002; 179: 1–12.
- [12] Strathmann TJ, Stone AT. Reduction of the carbamate pesticides oxamyl and methomyl by dissolved FeII and CuI. Environ. Sci. Technol., 2001; 35(12): 2461–2469.
- [13] Manawadi S, Kaliwal BB. Methomyl induced alteration in mice hepatic-oxidative status. Int. J. Biotechnol. Appl., 2010 2(2): 11–19.
- [14] Manawadi SI, Kaliwal BB. Methomyl induced gonadal dysfunction, biochemical contents and enzyme activities in male albino mice. Int. J. Biotechnol. Appl., 2010; 2(2): 20–32
- [15] Chung MJ, Ka JO. Isolation and characterization of 2,4-dichlorophenoxyacetic acid-degrading bacteria from paddy soils. J. Microbiol., 1998; 36(4): 256–261.
- [16] Laemmli CM, Leveau JHJ, Zehnder AJB, van der meer JR. Characterization of a second tfd gene cluster for chlorophenol and chlorocatechol metabolism on plasmid pJP4 in *Ralstonia eutropha* JMP134 (pJP4). J. Bacteriol., 2000; 182(15): 4165–4172.
- [17] Sayler GS, Hooper SW, Layton AC, King JMH. Catabolic plasmids of environmental and ecological significance. Microb. Ecol., 1990; 19(1): 1–20.
- [18] Manisha DM, Shyamapada M, Nishith KP. Plasmid-mediated dimethoate degradation by *Bacillus licheniformis* isolated from a fresh water fish *Labeo rohita*. J. Biomed. Biotechnol., 2005; 3: 280–286.
- [19] Racke KD, Coats JR, Comparative degradation of organophosphorus insecticides in soil: specificity of enhanced microbial degradation. J Agric. Food Chem. 1988; 36: 193–199.
- [20] Altalhi AD, Plasmid-mediated detoxification of mycotoxin zearalenone in *Pseudomonas* Sp. ZEA-1. Am. J. Biotechnol. Biochem., 2007; 3: 150–158.
- [21] Kulkarni AG, Kaliwal BB. Bioremediation of methomyl by soil isolate—*Pseudomonas aeruginosa*. J. Bioremed. Biodeg. 2015; 6: 281. doi:10.4172/2155-6199.1000281
- [22] Don RH, Pemberton JM. Properties of six pesticide degradation plasmids isolated from *Alcaligenes paradoxus* and *Alcaligenes eutrophus*. J Bacteriol., 1981; 145: 681–686.
- [23] Matsumura F. Degradation of pesticides in the environment by microorganisms and sunlight. In Matsumura, F and Krishna Murti CR (eds), Biodegradation of pesticides. New York, Academic Press, 1988; 3: 67–87.

- [24] Bhagobaty RK, Malik A. Utilization of chlorpyrifos as a sole source of carbon by bacteria isolated from wastewater irrigated agricultural soils in an industrial area of Western Uttar Pradesh, India. *Res. J. Microbiol.*, 2008; 3: 293–307.
- [25] Kulkarni AG, Kaliwal BB. Proteomic Profiling of *Escherichia coli* in Response to Carbamate Pesticide—Methomyl. *Insecticides—Basic and Other Applications*. Intech Publication. ISBN 979-953-307, 2012; 200–216.
- [26] Pieper DH, Reineke W. Engineering bacteria for bioremediation. *Curr. Opin. Biotechnol.*, 2000; 11(3): 262–270.
- [27] Furukawa K. ‘Super bugs’ for Bioremediation. *Trends Biotechnol.*, 2003; 21(5): 187–190.
- [28] Nauen R, Hungenberg H, Tollo B, Tietjen K, Elbert A. Antifeedant effect, biological efficacy and high affinity binding of imidacloprid to acetylcholine receptors in *Myzus Persicae* and *Myzusnicotianae*. *Pest Manag. Sci.*, 1998; 53: 133–140.
- [29] Krohn J, Hellpointner E. Environmental fate of imidacloprid. *Pflanzenschutz-Nachr Bayer*. 2002; 55: 3–26
- [30] Kulkarni AG, Kaliwal BB. Methomyl induced effects on free and immobilized *Escherichia coli*. *Int. J. Biotechnol. Res.* 2009; 2(2), 97–101.
- [31] Shetti AA, Kaliwal BB. Imidacloprid induced intoxication in soil isolate *Brevundimonas* sp. Mj 15. *Int. J. Life Sci. Pharma Res.*, 2012; 2(3): 105–117.
- [32] Bahig AE, Aly EA, Khaleed AA, Amel KA. Isolation, characterization and application of bacterial population from agricultural soil at Sohag province, Egypt. *Malays. J. Microbiol.*, 2008; 4(2), 42–50.
- [33] Top EM, Moenne-Loccoz Y, Pembroke T, Thomas CM. Phenotypic traits conferred by plasmids. In: *The horizontal gene pool bacterial plasmids and gene spread* (Thomas, C.M., Ed.). Harwood Academic Publishers. 2000; 249–285.
- [34] DebMandal M, Shyamapada M, Nishith K. Potential metabolites of dimethoate produced by bacterial degradation. *World J. Microbiol. Biotechnol.*, 2008; 24; 69–72.
- [35] Kumar G, Singla R, Kumar R. Plasmid associated anthracene degradation by *Pseudomonas* sp. isolated from filling Station site. *Appl. Environ. Microbiol.*, 2010; 8(4); 89.
- [36] Unakal CG, Kaliwal BB. Phenotypic characterization and risk factors of nosocomial *Staphylococcus aureus* from health care centers. *Adv. Microbiol.*, 2012; 2: 122–128.
- [37] Hiramatsu K, Aritaka N, Hanaki H, Kawasaki S, Hosoda Y, Hori S, Fukuchiand Y, Kobayashi I. Dissemination in Japanese hospitals of strains of *Staphylococcus aureus* heterogeneously resistant to vancomycin. *Lancet*, 1997; 350: 1670–1673.

- [38] Smith TL, Pearson ML, Wilcox KR, Cruz C, Lancaster MV, Robinson-Dunin B, Tenover EC, Zervos ML, Band ID, White E, Larvis WR. Emergence of vancomycin resistance in *Staphylococcus aureus*. N. Engl. J. Med., 1999; 340: 493–501.
- [39] Denis O, Nonhoff C, Byl B, Knoop C, Bobin-Dubreux S, Struelens MJ. Emergence of vancomycin-intermediate *Staphylococcus aureus* in a Belgian hospital: microbiological and clinical features. J. Antimicrob. Chemother., 2002; 50, 383–391.
- [40] Hare KT, Malay RS. Emergence of vancomycin resistant *Staphylococcus aureus* (VRSA) from a tertiary care hospital from northern part of India. BMC Infect. Dis., 2006; 6: 156.
- [41] Kim MN, Hwang SH, Pyo YJ, Munand HM, Pai CH. Clonal spread of *Staphylococcus aureus* heterogeneously resistant to vancomycin in a university hospital in Korea. J. Clin. Microbiol., 2002; 40; 1376–1380.
- [42] Noble WC, Virani Z, Cree RC. Co-transfer of vancomycin and other resistance genes from *Enterococcus faecalis* NCTC 12201 to *Staphylococcus aureus*. FEMS Microbiol. Lett., 1992; 72: 195–198.
- [43] Sieradzki K, Roberts RB, Haberland SW, Tomasz A. The development of vancomycin resistance in a patient with methicillin-resistant *Staphylococcus aureus* infection. N. Engl. J. Med., 1999; 340: 517–523.
- [44] Cui L, Ma X, Sato K, Okuma K, Tenover FC, Mamizuka EM, Gemmell CG, Kim MN, Ploy MC, Solh NE, Ferrazand V, Hiramatsu K. Cell wall thickening is a common feature of vancomycin resistance in *Staphylococcus aureus*. J Clin. Microbiol., January 2003; 41(1): 5–14.
- [45] Samad MA. Animal Husbandry and Veterinary Science, Volume II, LEP Pub No. 11, Bangladesh, 2008.
- [46] Mohanty NN, Das P, Pany SS, Sarangi LN, Ranabijuli S, Panda HK. Veterinary World, 2013; 6: 739–743.
- [47] Blowey R, Edmondson P. Mastitis Control in Dairy Herds: An Illustrated and Practical Guide. Ipswich, UK: Farming Press Books, 1995.
- [48] Chinchali JF. Studies on experimental bacterial mastitis in swiss albino mice [thesis]. Karnatak University, Dharwad, India, 2014
- [49] Shitandi A, Sternesjö A. Prevalence of multidrug resistant *Staphylococcus aureus* in milk from large and small-scale producers in Kenya. J. Dairy Sci., 2004; 87: 4145–4149.
- [50] Rychlik I, Gregorova D, Hradecka H. Distribution and function of plasmids in *Salmonella enterica*. Vet. Microbiol., 2006; 112(1): 1–10.



- [51] Waage S, Bjorland J, Caugant DA. Spread of *Staphylococcus aureus* resistant to penicillin and tetracycline within and between dairy herds. *Epidemiol. Infect.*, 2002; 129: 193–202.
- [52] Wang Y, Wu CM, Lu LM, Ren GWN, Cao XY, Shen JZ. Macrolide. Incosamide resistant phenotypes and genotypes of *Staphylococcus aureus* isolated from bovine clinical mastitis. *Vet. Microbiol.*, 2008; 130:118–125
- [53] Malinowski E, Klossowska A, Kaczmarowski M, Lassa H, Kuzma K. Antimicrobial susceptibility of staphylococci isolated from affected with mastitis cows. *Bull. Vet. Inst. Pulawy*, 2002; 46: 289–294.
- [54] Holmes MA, Zadoks RN. Methicillin resistant *S. aureus* in human and bovine mastitis. *J. Mammary Gland. Biol. Neoplasia*, 2011; 16(4):373–382.
- [55] Kurjogi MM, Kaliwal BB. Prevalence and antimicrobial susceptibility of bacteria isolated from bovine mastitis. *Adv. Appl. Sci. Res.*, 2011; 2(6): 229–235.
- [56] Sadashiv SO, Kaliwal BB, Kurjogi MM, Sanakal RD. Prevalence and antimicrobial susceptibility of coagulase-negative staphylococci isolated from bovine mastitis. *Veterinary World*, 2011; 4(4): 158–161.
- [57] Sadashiv SO, Kaliwal BB. Antibiotic resistance of *Staphylococcus aureus* and coagulase-negative staphylococci (CNS) isolated from bovine mastitis in the region of north Karnataka, India. *World J. Pharm. Res.*, 2014; 3(01): 571–586.
- [58] Sadashiv SO, Kaliwal BB. Isolation, characterization and antibiotic resistance of *Bacillus* spp. from bovine mastitis in the region of north Karnataka India. *Int. J. Curr. Microbiol. Appl. Sci.*, 2014; 3(4): 360–373.
- [59] Sadashiv SO, Kaliwal BB. Screening and antimicrobial resistance of *Escherichia coli* isolated from bovine mastitis in the region of north Karnataka, India. *Indo Am. J. Pharm. Res.*, 2015; 5(04): 1309–1316.
- [60] Sadashiv SO. Studies on isolation, molecular characterization of bacteria and their effects on milk in Bovine Mastitis [thesis]. Karnatak University, Dharwad. India; 2014
- [61] Malinowski E, Lassa H, Smulski S, Kłossowska A, and Michał Kaczmarowski, Antimicrobial susceptibility of bacteria isolated from cows with mastitis in 2006–2007. *Bull. Vet. Inst. Pulawy.*, 2008; 52: 565–572
- [62] Norris S. Antibiotic Resistance. Library of Parliament, 2008.