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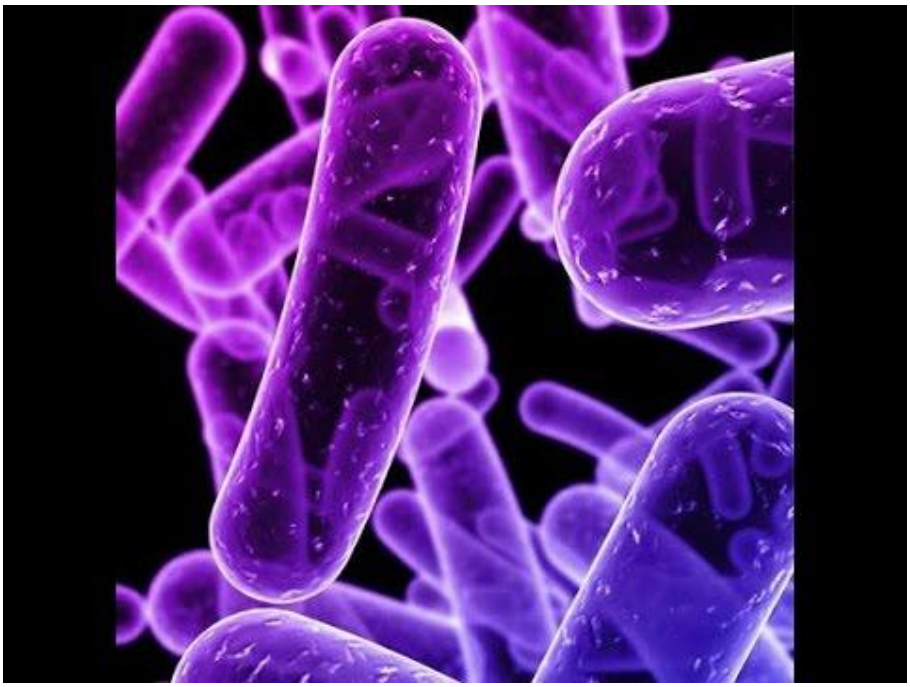
– Lactose fermentor from Clinical Samples



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Dr. Ramakrishna Bagguri, has significantly contributed in the field of Medical Microbiology, Industrial Microbiology and Environmental Microbiology. He did research on Microbial Production of Fructose, Ethanol and also on Multidrug resistance of *Salmonella typhi* and *Staphylococcus aureus* from the Hospital clinical sample.

Dr. Ramakrishna Bagguri has published more than 20 research articles in reputed National and International journals. He has handled three research projects financed by UGC (Major and Minor) Two VGST(SPICE)Student projects are completed under his Guidance. Owing to his research contributions, he has been awarded with "**Research Associate** under state funding agency by Department of Biotechnology, University of Pune, Maharashtra. He received Best paper presentation awards at various National and International conferences

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Dr. Selvam Arjunan has published more than 26 research articles in reputed National and International journals. Currently, he handlingthe Minor research project funded by University Grants Commission (India) (UGC), Department of Higher Education, Ministry of Human Resource Development, Government of India.Considering his contributions in the field of biological sciences, Tamil Nadu Scientific Research Organization (TNSRO) awarded him "Har Gobind Khorana Young Scientist Award - 2017". Owing to his significant contribution, he was awarded as "Distinguish Fellow of Bose Science Society" in the year 2017.

PREFACE

Enterobacteriaceae are the most frequently encountered bacteria's in clinical samples. The members of Enterobacteriaceae may be virtually incriminated in any type of infectious disease and can be recovered from any specimens received in the laboratory. The lactose fermenting Enterobacteriaceae include *E. coli*, *Klebsiella pneumoniae*, *Cirtobacter freundii*, *Cirtobacter koseri* and *Enterobacter* species which evoke hospital acquired infection, including *Pseudomonas aerogenosa, Enterococcus and Staphylococcus aureus*.

The serious problem of hospital acquired infection has become more increase in multiplication of antibiotic resistance bacteria, since the early 1980s. Despite tremendous progress in medical & Pharmaceutical fields, even today hospital acquired infection with *Enterobacteriaceae* has become as troublesome as it is used to in the 2000s one of the reason for this occurrence of multidrug resistance strains of Enterobacriaceae.

The various topics covered in the book are presented in a progression that ranges from basic biology to clinical manifestations and also covers the antibiotic susceptibility of lactose fermenting Enterobacteriaceae from different clinical samples. Early research on Enterobacteriaceae during the last century was aimed at developing a vaccine to prevent its many associated diseases, and in the process, provided much of the basic biology, physiology, and immunology that has been essential for ongoing research. With the advent of genetic and molecular biology approaches, as well as the new tools of genetic engineering, DNA and protein sequencing, a new era of – omics appeared, including genomics, proteomics, and metabolomics. Information from these studies has been applied to the development of vaccines, understanding genetic regulation, and epidemiology.

Epidemiological studies have been important in defining these recent diseases, as well as providing a greater understanding of their transmission, control, and prevention. Moreover, a variety of model systems provide new information about mechanisms of pathogenesis, as well as insights into intracellular invasion and the carrier state. Finally, the development of a

vaccine, as well as new and innovative methods of anti-infective control will be important areas of continued research.

This book represents a concerted effort by a group of scientific researchers, each an expert in their own area of work, who have generously provided their time and energy to present the current status of work in their own field. We hope that this book will serve not only as an important resource and standard reference, but also an aid to stimulate further research that will lead to better methods of disease control and treatment.

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Introduction

CHAPTER – I
INTRODUCTION

The family Enterobacteriaceae is the largest, most heterogeneous collection of medically important Gram negative bacilli. Currently at least 27 genera with 7 enteric groups and more than 110 species have been described. Some of them (e.g., *Escherichia coli, Klebsiella pneumoniae, Proteus vulgries*) are the part of normal enteric microflora and can cause opportunistic infections, *Salmonella, Shigella, Yersinia* pestis are always pathogenic for humans. The enteric bacteria are gram negative rods, not forming spores, have a complex antigenic structure, are facultative anaerobes or aerobes, are motile by peritrichous flagella or non-motile, have simple nutritional requirements, ferment various carbohydrates, reduce nitrates to nitrites, and are oxidase negative. *Escherichia coli* (*E. coli*), a member of normal GI flora, is lactose and mannitol fermenting, gas producing, indole and lysine decarboxylase positive. Enteropathogenic *E. coli* (EPEC) which adheres to mucosal cells of the small bowel (adherence factors are encoded by genes on plasmids) causes watery diarrhea in infants. Well known EPEC strains are identified by O antigen slide agglutination. Also Enterotoxigenic *E. coli* (ETEC) adheres to the epithelial cells of the small bowel. It is a common cause of “travellers diarrhea” and diarrhea in infants when contaminates foods. Some strains produce heat-labile exotoxin (genes carried on plasmids) similar to enterotoxin of *Vibrio cholerae* (activates adenyl cyclase and increase of cAMP). Some ETEC strains produce heat-stable enterotoxin (plasmid encoding). Strains of Enterohemorrhagic *E. coli* (EHEC) produce verotoxin (similar to shigatoxin of *Shigella dysenteriae*) and cause severe diarrhea, hemorrhagic colitis. Clinical syndrome of diarrhea similar to shigellosis is caused by Entero invasive *E. coli* (EIEC), which invades intestinal mucosal cells. Acute or chronic diarrhea can be induced by Entero aggregative *E. coli* (EAEC).

Other than GI clinical syndromes caused by *E. coli* are frequent urinary tract infections (both *E. coli* and hemolytic *E. coli*), sepsis in immuno compromised patients and in newborns, meningitis especially in infants (*E. coli* strains with K1 antigen).

Klebsiella pneumoniae and *Klebsiella oxytoca* cause mainly respiratory and urinary tract infections. *Klebsiella* species are not motile, perform large polysaccharide capsules (mucoid colonies), utilize citrate, and are lysin decarboxylase and urease positive. They are resistant to ampicillin. Similar *Enterobacter aergenes* has smaller capsules, is motile, has similar biochemical activity except urease and is isolated from urinary and respiratory tract infections. It is resistant to cefalotin. Common etiologic agent of nosocomial infections is *Serratia marcescens.* It belongs to the most resistant species with the resistance to penicillin and aminoglycosides. Tests of DNase, lipase and gelatinase production are positive.

The species of *Proteus, Morganella and Providencia* group are motile, swarming is especially characteristic for *Proteus.* All ferment lactose slowly or not at all, they are phenylalanine positive, *Proteus* and *Morganella* urease positive. *Proteus mirabilis*, *Proteus vulgaris, Morganella morgani, Providencia rettgeri* often cause urinary tract infections, contaminate wounds, belong to the agents causing nosocomial infections. Some strains may be resistant to various antibiotics.

Citrobacter, similar to *Salmonella,* slowly ferments lactose. Tests for motility, utilization of citrate are positive, lysine decarboxylase negative. It is found in GI and urinary tract infections.

Gram negative bacilli belonging to the family *Enterobacteriaceae* are the most frequently encountered bacterial isolates recovered from clinical specimens. The members of *Enterobacteriaceae* may be incriminated in virtually

any type of infectious disease and recovered from any specimens received in the laboratory. Immuno compromised patients are highly susceptible environmental strains or following invasive such as catheterization, bronchoscopy, colposcopy or surgical biopsies (Winn *et. al.,* 2006). MacConkey agar is a commonly used primary plating medium in many clinical microbiology laboratories. However, manual and computer literature searches showed only a few past (MacConkey, 1985, Koneman, 1983) and current (MacConkey, 1985, Taylor, 1949, Isenberg, 1985) references dealing with specific interpretation of growth on this agar. Since this medium is so common, and because it can provide timely clues as to the identification of some Gram-negative bacilli, it behooves microbiologists to be efficient in interpreting colonial growth. Initial conjecture as to the possible identification of an organism can then be compared with the final identification and antimicrobial susceptibility results as part of a quality control process. Our main goal was to develop a means for facilitating accurate preliminary grouping of some common lactose fermenting Enterobacteriaceae. *Enterobacteriaceae* may account for 80% of clinical significant isolates of Gram negative bacilli and 50% of clinically significant bacteria in clinical microbiology. They account for nearly 50% of septicemia cases, more than 70% of UTI a significant percentage of intestinal infections (Murray, *et. al.,* 2009) and some important pathogens like *Klebsiella pneumonia* are involved in hospital acquired infections (Shukla *et. al.,* 2004). In some studies on critical illness patients in hospitals *Enterobacteriaceae* accounted for 24.6% of blood streams infections, 48% cases of UTI and 14% of tracheal aspirates (Carvallo and Fillo, 2008). Some members of the Enterobacteriaceae family like *Serratia* and *Citrobacter* are emerging as significant pathogens (Rizvi *et. al.,* 2009). In this study isolation and identification of lactose fermenting bacteria by using different types of gram negative bacilli is morphological, biochemical test used to identify. Mainly lactose fermenting organisms including *E. coli, Klebsiella pneumoniae, Cirtobacter freundii, Cirtobacter koseri* and *Enterobacter* species.

In the present study indicates the lactose fermenting *Enterobacteriaceae* and other many bacteria, which evoke hospital acquired infection, including *E. coli, Pseudomonas aerogenosa* and *Enterococcus, Klebsiella* and *Staphylococcus aureus*. The serious problem of hospital acquired infection has become more increase in multiplication of antibiotic resistance bacteria, since the early 1980s. Despite tremendous progress in Medical and Pharmaceutical fields, even today hospital acquired infection with *Enterobacteriaceae* has become as troublesome as it is used to in the 2000s one of the reason for this occurrence of multidrug resistance strains of *Enterobacteriaceae.*

Escherichia coli, the most prevalent facultative Gram negative bacillus in the human fecal flora, usually inhabit the colon as an innocuous commensal. According to the special pathogenicity theory, special properties enabling *E. coli* to overcome host defenses in a new environment are necessary in order for it to escape the limitations of the colonic mileu and move into new niches devoid of competition from other bacterial species. Virulence (from the Latin word for poisonous) is defined as the ability of organism to cause disease in a particular host. In *E. coli*, virulence results from the cumulative impact of one or several special properties, or virulence factors (VFs), which serve to distinguish potential pathogens from harmless intestinal strains. The practical goal of investigations into the virulence properties of any pathogen is the development of specific anti-VF interventions (such as vaccines) to prevent infection. Urinary tract infection (UTI) is the most common form of extra intestinal *E. coli* infection, and *E. coli* is the most common cause of UTI. At some point during their lives, at least 12% of men and 10 to 20% of women experience an acute symptomatic UTI and an even greater number develops asymptomatic bacteriuria (ABU). More than 100,000 patients are hospitalized annually in the United States because of renal infection, with its attendant risk of Gram-negative sepsis and death. In the past decade there has been a virtual explosion of information regarding the VFs of *E. coli* associated with UTI.

Human epidemiological studies can identify associations between certain bacterial properties and UTI, but direct assessment of the contribution to virulence of these properties requires the use of animal models. Here, selection of an appropriate animal model and of appropriate bacterial strains is crucial. Models involving non physiological manipulations of the urinary tract (renal trauma, ureteral ligation, direct intrarenal injection, etc.) or intravenous injection of bacteria do not faithfully reproduce human UTI (242). Human infection most commonly occurs in patients with anatomically and functionally normal urinary tracts and involves.

Spontaneous ascent of bacteria from the urethra to the bladder and (in a minority of patients) to the kidney and bloodstream. The animal species used must have in common with humans those aspects of the urinary tract that are important in the function of the VFs being studied, e.g., cell surface receptors for adhesins. Comparisons of the virulence of wild-type strains that differ in the property of interest are the simplest way to study that property's contribution to virulence, but as in epidemiological studies in humans, this approach leaves unanswered the question of whether the property itself or associated factors are responsible for observed differences in virulence. Differences can be attributed to the property in question with greater confidence when strains that are genetically identical except for the factor of interest are compared. The more precise the methods used to derive such isogenic strains (e.g., site-directed mutagenesis), the greater the likelihood that the factor of interest is the only variable affecting virulence. Finally, mechanisms of action of possible VFs identified through epidemiological or animal studies are commonly determined *in vitro* at the cellular or sub cellular level. Such information often strengthens the case that a particular property plays a role in virulence and may suggest ways to interfere with its function. Some of the purported VFs of *E. coli* are discrete bacterial structures or products (e.g., fimbriae and hemolysin), in which case the laboratory is helpful in clarifying their functional significance. In contrast, other

VFs are functionally defined properties (e.g., serum resistance), in which case the laboratory is needed to determine the responsible bacterial structures or products.

The *Enterobacteriaceae* is a large family of heterogeneous Gram-negative rods found primarily in the colon of humans and other animals, many as part of normal flora. The family includes many genera e.g. *Esherichia, Klebsiella, Serratia, Proteus, Morganella, Providencia, etc.* Many terms used for these organism; Enterobacteriaceae, enteric bacteria, enteric Gram-negative rods, and they are also called "coliforms". This family is divided into two groups on the basis of fermentation of lactose; lactose fermenters (pink colonies) and non-lactose fermenters (colorless colonies); on MacConkey agar. Features that are common to all members of this family are their anatomic location and the following four metabolic processes, they are facultative anaerobes, Ferment glucose (fermentation of other sugars varies), Oxidase negative, Reduce nitrates to nitrites.

These four reactions can be used to distinguish the *Enterobacteriaceae* from another medically important group of organisms; the non-fermenting Gram-negative rod the most important is *Pseudomonas aeruginosa.*

Chapter-II

Review of Literature

CHAPTER - II
REVIEW OF THE LITERATURE

2.1. Historical Background:

Enterobacteriaceae:

The *Enterobacteriaceae* is a large family of Gram-negative bacteria that includes, along with many harmless symbionts, many of the more familiar pathogens, such as *Salmonella*, *Escherichia coli*, *Yersinia pestis*, *Klebsiella* and *Shigella*. Other disease-causing bacteria in this family include *Proteus*, *Enterobacter*, *Serratia* and *Citrobacter*. This family is the only representative in the order Enterobacteriales of the class Gammaproteobacteria in the phylum Proteobacteria (Garrity, 1984). Phylogenetically, in the Enterobacteriales, several peptidoglycan - less insect endosymbionts form a sister clade to the *Enterobacteriaceae*, but as they are not validly described, this group is not officially a taxon; examples of these species are *Sodalis*, *Buchnera*, *Wigglesworthia*, *Baumannia* and *Blochmannia*, but not formers rickettsias (Williams *et. al.,* 2010). Members of the *Enterobacteriaceae* can be trivially referred to as enterobacteria, as several members live in the intestines of animals. In fact, the etymology of the family is enterobacterium with the suffix to designate a family (aceae) — not after the genus *Enterobacter* (which would be "*Enterobacteraceae*") - and the type genus is *Escherichia*.

2.1.1 Characteristics

Members of the *Enterobacteriaceae* are rod-shaped, and are typically 1-5 μm in length. Like other proteobacteria, enterobacteria have Gram-negative stains and they are facultative anaerobes sugars to produce lactic acid, fermentingid and various other end products. Most also reduce nitrate to nitrite, although exceptions exist (e.g., *Photorhabdus*). Unlike most similar bacteria, enterobacteria generally lack cytochrome oxidase, although there are exceptions

(e.g., *Plesiomonas shigelloides*). Most have many flagella used to move about, but a few genera are non-motile. They are not spore-forming. Catalase reactions vary among *Enterobacteriaceae*.

Many members of this family are a normal part of the gut flora found in the intestines of humans and other animals, while others are found in water or soil, or are parasites on a variety of different animals and plants. *Escherichia coli* is one of the most important model organisms, and its genetics and biochemistry have been closely studied.

Most members of *Enterobacteriaceae* have peritrichous, type I fimbriae involved in the adhesion of the bacterial cells to their hosts. Some enterobacteria produce endotoxins. Endotoxins reside in the cell cytoplasm and are released when the cell dies and the cell wall disintegrates. Some members of the *Enterobacteriaceae* family produce a systemic infection into the blood stream when all the dead bacterial cells release their endotoxins. This is known as endotoxic shock, and can be rapidly fatal.

Identification of Enterobacteriaceae:

To identify different genera of *Enterobacteriaceae*, a microbiologist may run a series of tests in the lab. These include a range of tubes cultures and agar plates cultures such as:

- Phenyl alanine agar for detection of production of deaminase, which converts phenylalanine to phenyl pyruvic acid
- Methyl red or Voges-Proskauer tests depend on the digestion of glucose. The methyl red tests for acid end products. The Voges Proskauer tests for the production of acetylmethylcarbinol.
- Catalase test on nutrient agar tests for the production of catalase enzyme, which splits hydrogen peroxide and releases oxygen gas.

- Oxidase test on nutrient agar tests for the production of the enzyme oxidase, which reacts with an aromatic amine to produce a purple color.
- Nutrient gelatin tests to detect activity of the enzyme gelatinase.

In a clinical setting, three species make up 80 to 95% all isolates identified. These are *Escherichia coli*, *Klebsiella pneumoniae* and *Proteus mirabilis*.

2.1.2 *Escherichia coli:*

"*E. coli*" redirects here. The protozoan parasite, *Entamoeba coli*. *E. coli* in medicine, pathogenic *Escherichia coli*, for a specific strain, *Escherichia coli* (disambiguation). *Escherichia coli* (molecular biology).

Scientific classification

Domain: Bacteria

Kingdom: Eubacteria

Phylum: Proteobacteria

Class: Gammaproteobacteria

Order: Enterobacteriales

Family: Enterobacteriaceae

Genus: *Escherichia*

Species: ***E. coli***

Binomial name

Escherichia coli

(Migula, 1895)

(Castellani and Chalmers, 1919)

Synonyms

Bacillus coli communis (Escherich, 1885)

Escherichia coli commonly abbreviated *E. coli*, is a Gram-negative, rod-shaped bacterium that is commonly found in the lower intestine of warm-blooded organisms (endotherms). Most *E. coli* strains are harmless, but some serotypes can cause serious food poisoning in humans, and are occasionally responsible for product recalls due to food contamination (CDC, 2012, Vogt and Dippold, 2005). The harmless strains are part of the normal flora of the gut, and can benefit their hosts by producing vitamin K_2 (Hudault *et. al.,* 2001) and by preventing the establishment of pathogenic bacteria within the intestine (Eckburg *et. al.,* 2005, Feng *et. al.,* 2002).

E. coli and related bacteria constitute about 0.1% of gut flora (Thompson and Andrea, 2007) and fecal–oral transmission is the major route through which pathogenic strains of the bacterium cause disease. Cells are able to survive outside the body for a limited amount of time, which makes them ideal indicator organisms to test environmental samples for fecal contamination (Ishii and Sadowsky, 2008). There is, however, a growing body of research that has examined environmentally persistent *E. coli* which can survive for extended periods outside of the host (Lecointre *et. al.,* 1998).

The bacterium can also be grown easily and inexpensively in a laboratory setting, and has been intensively investigated for over 60 years. *E. coli* is the most widely studied prokaryotic model organism, and an important species in the fields of biotechnology and microbiology, where it has served as the host organism for the majority of work with recombinant DNA.

2.1.3 History:

The genera *Escherichia* and *Salmonella* diverged around 102 million years ago (credibility interval: 57–176 mya), which coincides with the divergence of their hosts: the former being found in mammals and the latter in birds and reptiles (Haeckel and Ernst, 1867). This was followed by a split of the

escherichian ancestor into five species (*E. albertii*, *E. coli*, *E. fergusonii*, *E. hermannii* and *E. vulneris*.) The last *E. coli* ancestor split between 20 and 30 million years ago (Ecsherich, 1885).

In 1885, Theodor Escherich, a German pediatrician, first discovered this species in the feces of healthy individuals and called it *Bacterium coli* commune due to the fact it is found in the colon and early classifications of Prokaryotes placed these in a handful of genera based on their shape and motility (at that time Ernst Haeckel's classification of Bacteria in the kingdom Monera was in place (Breed and Conn, 1936, Migula,1995). *Bacterium coli* was the type species of the now invalid genus *Bacterium* when it was revealed that the former type species ("*Bacterium triloculare*") was missing (Castellani and Chalmers, 1919). Following a revision of *Bacteria* it was reclassified as *Bacillus coli* by Migula in 1895 (Garrity, 2005) and later reclassified in the newly created genus *Escherichia*, named after its original discoverer.

The genus belongs in a group of bacteria informally known as "coliforms", and is a member of the *Enterobacteriaceae* family ("the enterics") of the Gammaproteobacteria (Samen Von *et. al.,* 2011).

In May 2011, one *E. coli* strain, *Escherichia coli* O104:H4, has been the subject of a bacterial outbreak that began in Germany. Certain strains of *E. coli* are a major cause of food borne illness. The outbreak started when several people in Germany were infected with enterohemorrhagic *E. coli* (EHEC) bacteria, leading to hemolytic-uremic syndrome (HUS), a medical emergency that requires urgent treatment. The outbreak did not only concern Germany, but 11 other countries, including regions in North America (Britannica Online Encyclopedia, 2011). On 30 June 2011 the German *Bundesinstitut für Risikobewertung (BfR)* (*Federal Institute for Risk Assessment*, a federal, fully legal entity under public law of the Federal Republic of Germany, an institute

within the German Federal Ministry of Food, Agriculture and Consumer Protection) announced that seeds of fenugreek from Egypt were likely the cause of the EHEC outbreak (Kubitschek, 1990).

2.1.4 Biology and biochemistry:

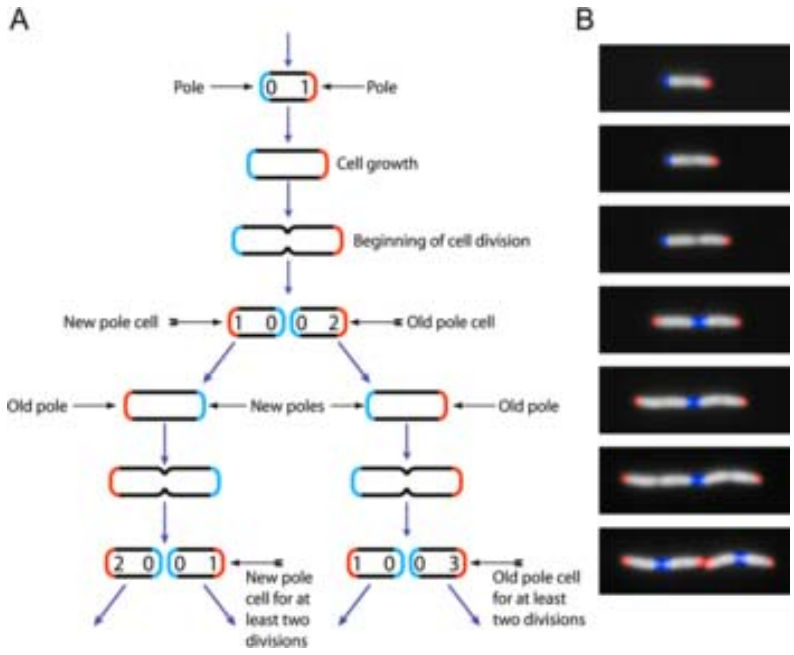

Fig. 1.1: Model of successive binary fission in *E. coli*

E. coli is Gram-negative, facultative anaerobic and non-sporulating. Cells are typically rod-shaped, and are about 2.0 microns (μm) long and 0.5 μm in diameter, with a cell volume of 0.6–0.7 $(\mu m)^3$ (Ingledew and Poole, 1984 and Darnton *et. al.,* 2007). It can live on a wide variety of substrates. *E. coli* uses mixed-acid fermentation in anaerobic conditions, producing lactate, succinate, ethanol, acetate and carbon dioxide. Since many pathways in mixed-acid fermentation produce hydrogen gas, these pathways require the levels of hydrogen to be low, as is the case when *E. coli* lives together with hydrogen-consuming organisms, such as methanogens or sulphate-reducing bacteria (Ingledew and Poole, 1984).

Optimal growth of *E. coli* occurs at 37°C (98.6°F) but some laboratory strains can multiply at temperatures of up to 49°C (120.2°F). Growth can be driven by aerobic or anaerobic respiration, using a large variety of redox pairs, including the oxidation of pyruvic acid, formic acid, hydrogen and amino acids, and the reduction of substrates such as oxygen, nitrate, fumarate, dimethyl sulfoxide and trimethylamine N-oxide (Brussow *et. al.,* 2004).

Strains that possess flagella are motile. The flagella have a peritrichous arrangement (Krieg and Holt, 1984).

E. coli and related bacteria possess the ability to transfer DNA via bacterial conjugation, transduction or transformation, which allows genetic material to spread horizontally through an existing population. This process led to the spread of the gene encoding shiga toxin from *Shigella* to *E. coli* O157:H7, carried by a bacteriophage (Lukjancenko *et. al.,* 2010).

2.1.5 Diversity:

Escherichia coli encompass an enormous population of bacteria that exhibit a very high degree of both genetic and phenotypic diversity. Genome sequencing of a large number of isolates of *E. coli* and related bacteria shows that a taxonomic reclassification would be desirable. However, this has not been done, largely due to its medical importance (Lan and Reeves, 2002) and *E. coli* remains one of the most diverse bacterial species: only 20% of the genome is common to all strains (Orskov *et. al.,* 1977).

In fact, from the evolutionary point of view, the members of genus *Shigella* (*S. dysenteriae*, *S. flexneri*, *S. boydii*, *S. sonnei*) should be classified as *E. coli* strains, a phenomenon termed taxa in disguise (Lawrence and Ochman, 1998). Similarly, other strains of *E. coli* (e.g., the K-12 strain commonly used in

recombinant DNA work) are sufficiently different that they would merit reclassification.

A strain is a sub-group within the species that has unique characteristics that distinguish it from other strains. These differences are often detectable only at the molecular level; however, they may result in changes to the physiology or lifecycle of the bacterium. For example, a strain may gain pathogenic capacity, the ability to use a unique carbon source, the ability to take upon a particular ecological niche or the ability to resist antimicrobial agents. Different strains of *E. coli* are often host-specific, making it possible to determine the source of fecal contamination in environmental samples (Ishii and Sadowsky, 2008, Battistuzzi *et. al.,* 2004). For example, knowing which *E. coli* strains are present in a water sample allows researchers to make assumptions about whether the contamination originated from a human, another mammal or a bird.

Serotype

A common subdivision system of *E. coli*, but not based on evolutionary relatedness, is by serotype, which is based on major surface antigens (O antigen: part of lipopolysaccharide layer; H: flagellin; K antigen: capsule), e.g., O157:H7) (Nataro and Kaper, 1998) (NB: K-12, the common laboratory strain is not a serotype).

2.1.6 Genome plasticity

Like all life forms, new strains of *E. coli* evolve through the natural biological processes of mutation, gene duplication and horizontal gene transfer, in particular 18% of the genome of the laboratory strain MG1655 was horizontally acquired since the divergence from *Salmonella* (Euzeby, 1997). In microbiology, all strains of *E. coli* derive from *E. coli* K-12 or *E. coli* B strains. Some strains develop traits that can be harmful to a host animal. These virulent strains

typically cause a bout of diarrhea that is unpleasant in healthy adults and is often lethal to children in the developing world (Migula, 1895). More virulent strains, such as O157:H7 cause serious illness or death in the elderly, the very young or the immunocompromised.

Neotype strain

E. coli is the type species of the genus (*Escherichia*) and in turn *Escherichia* is the type genus of the family *Enterobacteriaceae*, where it should be noted that the family name does not stem from the genus *Enterobacter* + "i" (sic.) + "aceae", but from "enterobacterium" + "aceae" (enterobacterium being not a genus, but an alternative trivial name to enteric bacterium) (Samen von *et. al.,* 2011, Brzuszkiewicz *et. al.,* 2011, Blattner *et. al.,* 1997).

The original strain described by *Escherichia* is believed to be lost, consequently a new type strain (neotype) was chosen as a representative: the neotype strain is ATCC 11775, also known as NCTC 9001, (Zhaxybayeve and Doolittle, 2011), which is pathogenic to chickens and has an O1:K1:H7 serotype (Arifuzzaman *et. al.,* 2006). However, in most studies either O157:H7 or K-12 MG1655 or K-12 W3110 are used as a representative *E. coli*.

2.1.7 Phylogeny of *Escherichia coli* strains

Escherichia coli is a species. A large number of strains belonging to this species have been isolated and characterized. In addition to serotype (vide supra), they can be classified according to their phylogeny, i.e., the inferred evolutionary history, as shown below where the species is divided into six groups (Orskov *et. al.,* 1997; Hu *et. al.,* 2009).

The link between phylogenetic distance ("relatedness") and pathology is small, e.g., the O157:H7 serotype strains, which form a clade ("an exclusive

group") — group E below — are all enterohaerogic strains (EHEC), but not all EHEC strains are closely related. In fact, four different species of *Shigella* are nested among *E. coli* strains (vide supra), while *Escherichia albertii* and *Escherichia fergusonii* are outside of this group. All commonly used research strains of *E. coli* belong to group A and are derived mainly from Clifton's K-12 strain and to a lesser degree from d'Herelle's *Bacillus coli* strain (B strain).

2.1.8 Genomics

The first complete DNA sequence of an *E. coli* genome (laboratory strain K-12 derivative MG1655) was published in 1997. It was found to be a circular DNA molecule 4.6 million base pairs in length, containing 4288 annotated protein-coding genes (organized into 2584 operons), seven ribosomal RNA (rRNA) operons, and 86 transfer RNA (tRNA) genes. Despite having been the subject of intensive genetic analysis for approximately 40 years, a large number of these genes were previously unknown. The coding density was found to be very high, with a mean distance between genes of only 118 base pairs. The genome was observed to contain a significant number of transposable genetic elements, repeat elements, cryptic prophages, and bacteriophage remnants (Todar, 2007).

Today, over 60 complete genomic sequences of *Escherichia* and *Shigella* species are available. Comparison of these sequences shows a remarkable amount of diversity; only about 20% of each genome represents sequences present in every one of the isolates, while approximately 80% of each genome can vary among isolates. Each individual genome contains between 4,000 and 5,500 genes, but the total number of different genes among all of the sequenced *E. coli* strains (the pan-genome) exceeds 16,000. This very large variety of component genes has been interpreted to mean that two-thirds of the *E. coli*

pangenome originated in other species and arrived through the process of horizontal gene transfer (Evans *et. al.,* 2007).

Proteomics

Full sets of *E. coli* proteins and their interactions have also been isolated and studied. A 2006 study purified 4,339 proteins from cultures of strain K-12 and found interacting part nets for 2,667 proteins, many of which had unknown functions at the time. A 2009 study found 5,993 interactions between proteins of the same *E. coli* strain (Kamada *et. al.,* 2005).

Role as normal microbiota

E. coli normally colonizes an infant's gastrointestinal tract within 40 hours of birth, arriving with food or water or with the individuals handling the child. In the bowel, it adheres to the mucus of the large intestine. It is the primary facultative anaerobe of the human gastrointestinal tract (Lee, 1996). Facultative anaerobes are organisms that can grow in either the presence or absence of oxygen. As long as these bacteria do not acquire genetic elements encoding for virulence factors, they remain benign commensals (Russo, 2000).

Therapeutic use of nonpathogenic *E. coli*

Nonpathogenic *Escherichia coli* strain Nissle, 1917 also known as Mutaflor is used as a probiotic agent in medicine, mainly for the treatment of various gastroenterological diseases (Cornelis, 2000) including inflammatory bowel disease (Toflianit,1994).

2.1.9 Role in disease

Virulent strains of *E. coli* can cause gastroenteritis, urinary tract infections, and neonatal meningitis. In rarer cases, virulent strains are also responsible for

hemolytic-uremic syndrome, peritonitis, mastitis, septicemia and Gram-negative pneumonia (Lee,1996).

UPEC (uropathogenic *E. coli*) is one of the main causes of urinary tract infections. It is part of the normal flora in the gut and can be introduced many ways. In particular for females, the direction of wiping after defecation (wiping back to front) can lead to fecal contamination of the urogenital orifices. Anal sex can also introduce this bacteria into the male urethra, and in switching from anal to vaginal intercourse the male can also introduce UPEC to the female urogenital system. For more info see databases at the end of the article or UPEC pathogenicity.

Role in biotechnology

Because of its long history of laboratory culture and ease of manipulation, *E. coli* also plays an important role in modern biological engineering and industrial microbiology (Bessette *et. al.,*1999).The work of Stanley Norman Cohen and Herbert Boyer in *E. coli*, using plasmids and restriction enzymes to create recombinant DNA, became a foundation of biotechnology (Ihssen *et. al.,* 2010).

E. coli is a very versatile host for the production of heterologous proteins, Wacker *et. al.,* 2002), and various protein expression systems have been developed which allow the production of recombinant proteins in *E. coli*. Researchers can introduce genes into the microbes using plasmids which permit high level expression of protein, and such protein may be mass produced in industrial fermentation processes. One of the first useful applications of recombinant DNA technology was the manipulation of *E. coli* to produce human insulin (Huang *et. al.,* 2012).

Many proteins previously thought difficult or impossible to be expressed in *E. coli* in folded form have also been successfully expressed in *E. coli*. For example, proteins with multiple disulphide bonds may be produced in the periplasmic space or in the cytoplasm of mutants rendered sufficiently oxidizing to allow disulphide-bonds to form, (Fux *et. al.,* 2005), while proteins requiring post-translational modification such as glycosylation for stability or function have been expressed using the N-linked glycosylation system of *Campylobacter jejuni* engineered into *E. coli* (Vidal *et. al.,* 1998, Lederberg *et. al.,* 1946,Cold spring harbour, 2006). Modified *E. coli* cells have been used in vaccine development, bioremediation, and production of immobilized enzymes (Wacker *et. al.,* 2002).

Model organism

E. coli is frequently used as a model organism in microbiology studies. Cultivated strains (e.g., *E. coli* K12) are well-adapted to the laboratory environment, and unlike wild type strains, have lost their ability to thrive in the intestine. Many lab strains lose their ability to form biofilms (Benzer, 1961). These features protect wild type strains from antibodies and other chemical attacks, but require a large expenditure of energy and material resources.

In 1946, Joshua Lederberg and Edward Tatum first described the phenomenon known as bacterial conjugation using *E. coli* as a model bacterium (Keymer *et. al.,* 2006) and it remains the primary model to study conjugation. *E. coli* was an integral part of the first experiments to understand phage genetics, (Deccan Chronicle, 2009) and early researchers, such as Seymour Benzer, used *E. coli* and phage T4 to understand the topography of gene structure. Prior to Benzer's research, it was not known whether the gene was a linear structure, or if it had a branching pattern.

E. coli was one of the first organisms to have its genome sequenced; the complete genome of *E. coli* K12 was published by *Science* in 1997 (Todar, 2009).

The long-term evolution experiments using *E. coli*, begun by Richard Lenski in 1988, have allowed direct observation of major evolutionary shifts in the laboratory. In this experiment, one population of *E. coli* unexpectedly evolved the ability to aerobically metabolize citrate, which is extremely rare in *E. coli*. As the inability to grow aerobically is normally used as a diagnostic criterion with which to differentiate *E. coli* from other, closely related bacteria, such as *Salmonella*, this innovation may mark a speciation event observed in the lab.

By evaluating the possible combination of nanotechnologies with landscape ecology, complex habitat landscapes can be generated with details at the nanoscale. On such synthetic ecosystems, evolutionary experiments with *E. coli* have been performed to study the spatial biophysics of adaptation in an island biogeography on-chip.

Studies are also being performed into programming *E. coli* to potentially solve complicated mathematics problems, such as the Hamiltonian path problem.

2.2 *Klebsiella*

Klebsiella well known to most clinicians as a cause of community-acquired bacterial pneumonia, occurring particularly in chronic alcoholics (Carpenter, 1990) and showing characteristic radiographic abnormalities (Felson *et. al.,* 1949) due to a severe pyogenic infection which has a high fatality rate if untreated.

The vast majority of *Klebsiella* infections, however, are associated with hospitalization. As opportunistic pathogens, *Klebsiella* spp. primarily attack immunocompromised individuals who are hospitalized and suffer from severe underlying diseases such as diabetes mellitus or chronic pulmonary obstruction. Nosocomial *Klebsiella* infections are caused mainly by *Klebsiella pneumoniae*, the medically most important species of the genus. To a much lesser degree, *K. oxytoca* has been isolated from human clinical specimens. It is estimated that

Klebsiella spp. cause 8% of all nosocomial bacterial infections in the United States and in Europe. No great geographical variations in frequency have been noted. In the United States, *Klebsiella* accounts for 3 to 7% of all nosocomial bacterial infections, placing them among the eight most important infectious pathogens in hospitals (Horan *et. al.,* 1988, Schaberg *et. al.,* 1991), and data collected from the United Kingdom (Bergogne-Berezi, 1995) and from Germany (Ullmann, 1983) are remarkably similar to those reported by the Centers for Disease Control and Prevention.

Table-1 lists the most frequent nosocomial infections caused by *Klebsiella*. The urinary tract is the most common site of infection. *Klebsiella* accounts for 6 to 17% of all nosocomial urinary tract infections (UTI) and shows an even higher incidence in specific groups of patients at risk, e.g., patients with neuropathic bladders or with diabetes mellitus (Bennet *et. al.,* 1985, Lye *et. al.,* 1992). As a cause of nosocomial Gram-negative bacteremia, *Klebsiella* is second only to *Escherichia coli* (Bryan *et. al.,* 1986, Duggan *et. al.,* 1985, Pittet *et. al.,* 1993, Yinnon, 1996).

Table – 2.1

Hospital-acquired bacterial infections caused by *Klebsiella* spp.

Infection	% of infections caused by *Klebsiella*	Rank[a]
UTI	6–17	5–7
Pneumonia	7–14	2–4
Septicemia	4–15	3–8
Wound infections	2–4	6–11
Nosocomial infections in intensive care unit patients	4–17	4–9
Neonatal septicemia	3–20	2–8

[a] Ranking of *Klebsiella* compared to all other bacterial pathogens.

In pediatric wards, nosocomial *Klebsiella* infections are especially troublesome - particularly in premature infants and intensive care units. *Klebsiella* species are often the pathogens involved in neonatal sepsis (Table 1), in both early-manifestation and late-manifestation infections (Gotoff, 1992).

Due to the extensive spread of antibiotic-resistant strains, especially of extended-spectrum β-lactamase (ESβL) - producing strains, there has been renewed interest in *Klebsiella* infections.

2.2.1 Epidemiology

Klebsiella spp. are ubiquitous in nature. *Klebsiellae* probably have two common habitats, one being the environment, where they are found in surface water, sewage, and soil and on plants (Bagley *et. al.,* 1978, Brown and Seidler, 1973, Edberg *et. al.,* 1986, Masten *et. al.,* 1974, Seidler *et. al.,* 1975), and the other being the mucosal surfaces of mammals such as humans, horses, or swine, which they colonize. In this respect, the genus *Klebsiella* is like *Enterobacter* and *Citrobacter* but unlike *Shigella* spp. or *E. coli*, which are common in humans but not in the environment.

In humans, *K. pneumoniae* is present as a saprophyte in the nasopharynx and in the intestinal tract. Carrier rates differ considerably from study to study. The detection rate in stool samples ranges from 5 to 38%, while rates in the nasopharynx range from 1 to 6% (Davis and Masten, 1974, Rose and Schreier, 1968, Rosenthal, 1975, Thom, 1970). Because Gram-negative bacteria do not find good growth conditions on the human skin (Rosenbury, 1962), *Klebsiella* spp. are rarely found there and are regarded simply as transient members of the flora (Kloos and Massel White, 1975).

These carrier rates change drastically in the hospital environment, where colonization rates increase in direct proportion to the length of stay. Even hospital personnel have elevated rates of *Klebsiella* carriage (Caswell and Philips, 1977, Cooke *et. al.,* 1979). Reported carrier rates in hospitalized patients are 77% in the stool, 19% in the pharynx, and 42% on the hands of patients (Cooke *et. al.,* 1979, Davis and Masten, 1974, Johnson *et. al.,* 1969, Pollack *et. al.,* 1972, Rose and Schreier, 1968, Seden *et. al.,* 1971, Smith *et. al.,* 1995). The high rate of nosocomial *Klebsiella* colonization appears to be associated with the use of antibiotics rather than with factors connected with delivery of care in the hospital (Pollack *et. al.,* 1972, Rose and Schreier, 1968). Previous antibiotic therapy is significantly associated with acquisition of *Klebsiella* by the patient. In one study, 2 weeks after admission to the hospital, a two- to fourfold increase in the colonization rates with *Klebsiella* was observed; this increase occurred primarily in patients receiving antibiotics, especially in persons receiving broad-spectrum or multiple antibiotics. In the hospital setting, the local antibiotic policy is a major determinant of the colonization pattern. The significance of increased colonization was illustrated by the observation that the attack rate of *Klebsiella* nosocomial infection in patients carrying hospital-acquired intestinal *Klebsiella* was four times as high as for non-carriers (Seden *et. al.,* 1971). Furthermore, widespread use of antimicrobial therapy has often been held responsible for the occurrence of multiply resistant *Klebsiella* strains in hospitals (Tessin *et. al.,* 1990). Because these undesired effects may be reversed by strict control of antibiotic use, demands for strategies to avoid the overuse of antibiotics in prophylaxis and empirical therapy are increasingly being expressed.

Apart from medical equipment (contaminated due to faulty hygienic procedures) and blood products (Goetz *et. al.,* 1995, Jumaa and Chattopadyay, 1992, Hart, 1993), the principal reservoirs for transmission of *Klebsiella* in the hospital setting are the gastrointestinal tract of patients and the hands of hospital personnel (Montgomerie *et. al.,* 1979). The ability of this organism to spread

rapidly (Kuhn *et. al.,* 1993) often leads to nosocomial outbreaks, especially in neonatal units (Griffiths, 1987). Of the 145 epidemic nosocomial infections reported in the literature published in English between 1983 and 1991, 13 were caused by *Klebsiella* (Doebbeling, 1993). According to the statistics of the Centers for Disease Control and Prevention, *Klebsiella* spp. account for 8% of endemic hospital infections and 3% of epidemic outbreaks (Stamm *et. al.,* 1981).

Especially feared are epidemic hospital infections caused by multiresistant strains. In the 1970s, these strains were chiefly aminoglycoside-resistant *Klebsiella* strains (Curie *et. al.,* 1978, Martin *et. al.,* 1971, Noriega *et. al.,* 1975). Since 1982, strains that produce ESβLs, which render them resistant to extended-spectrum cephalosporins, have evolved (Coovadia *et. al.,* 1992, De Champs *et. al.,* 1991, French *et. al.,* 1996, Gur *et. al.,* 1989, Johnsin *et. al.,* 1969, Medeiros, 1993, Meyer *et. al.,* 1993, Reish *et. al.,* 1993). The hallmark of these strains, resistance to ceftazidime, is observed in both *K. pneumoniae* and *K. oxytoca* isolates (Smith and Chambers, 1995). In Europe, the β-lactamases of ceftazidime-resistant *Klebsiella* strains are commonly of the SHV-5 type, whereas TEM-10 and TEM-12 are more prevalent in the United States (Legakis *et. al.,* 1995, Medeiros, 1993, Pagani *et. al.,* 1994, Schiappa *et. al.,* 1996, Urban and Rahal, 1997, Venezia *et. al.,* 1995). The incidence of ESβL-producing *Klebsiella* isolates in the United States has been reported to be 5% of the *K. pneumoniae* strains tested in the National Nosocomial Infection Study System (Jacoby, 1996). In Europe, the frequency of such strains seems to be even higher. A percentage of 14% to 16% ESβL producers among clinical *Klebsiella* isolates has been reported for France and England (Sirot, 1995). In particular regions or hospitals, the incidence can reach 25% to 40%. However, the percentage of ceftazidime-resistant strains may be much higher, because the conventional disc diffusion criteria used in the routine laboratory underestimate the incidence of these isolates (Jacoby, 1996).

ESβLs are usually plasmid mediated. Since, these plasmids are easily transmitted among different members of the *Enterobacteriaceae*, accumulation of resistance genes results in strains that contain multiresistant plasmids. For this reason, ESβL-producing isolates are resistant to a variety of classes of antibiotics. Moreover, the emergence of these multiply resistant *Klebsiella* strains is unfortunately accompanied by a relatively high stability of the plasmids encoding ESβLs. Even years after discontinuation of ceftazidime and other extended-spectrum cephalosporins, continued colonization of patients by ESβL-producing *Klebsiella* strains has been observed (Hibbertrogers *et. al.,* 1995). Risk factors for acquisition of these strains seem to be the length of stay in hospital and the performance of invasive procedures (Lucet *et. al.,* 1996).

Since ESβL production frequently is accompanied by multiresistance to antibiotics, therapeutic options become limited. So far, however, ESβL-producing *Klebsiella* strains have been susceptible to carbapenems such as imipenem or meropenem. Both antibiotics are the drugs of choice in the treatment of infections due to ESβL-producing organisms. In this respect, a recent observation is very disturbing. For the first time, ESβL-producing *K. pneumoniae* strains which showed an additional resistance to imipenem have been isolated (Bradford, 1997). These strains possessed a transmissible plasmid-mediated AmpC-type β-lactamase. This development should be monitored closely, since the emergence of imipenem-resistant ESβL-producing *Klebsiella* strains will have a serious impact on remaining therapeutic options.

In the last several years, the question has arisen whether it is necessary to determine if each isolated *Klebsiella* strain is an ESβL producer. The answer depends on the epidemiologic situation of a country or a hospital, but it should definitely be positive if a high percentage of ceftazidime-resistant strains are to be expected. To date, two diagnostic tests have been most commonly used for

the detection of such isolates. In the double-disc synergy test, a disc of clavulanic acid and a disc of an extended-spectrum cephalosporin such as ceftazidime are placed close together on an agar surface inoculated with the test organism (Jarlier *et. al.,* 1988). Enhancement of the zone of inhibition around the cephalosporin disc towards the clavulanate-containing disc indicates the presence of an ESβL-producing strain. A commercially available product is the ESβL screening E test strip (AB Biodisk, Solna, Sweden). This method is based on the evaluation of the difference between the antimicrobial activity of ceftazidime alone compared to that of ceftazidime plus clavulanic acid (Katsanis *et. al.,* 1994).

It should be kept in mind that a number of measures have been recommended to prevent the nosocomial spread of *Klebsiella*. Strict adherence to basic epidemiological standards for the management of urinary catheters, intravenous tracheostomies, and wounds, maintenance and care of equipment, and good hand-washing practices all help to prevent the spread of nosocomial *Klebsiella* infections. Detailed information on this subject is given in an excellent review by Montgomerie (Montgomerie, 1979). Another measure to control *Klebsiella* infections is the regulation of antibiotic use in the hospital to prevent misuse and overuse of antibiotics. Furthermore, nosocomial infection surveillance is necessary to collect data that are used in the prevention and control of nosocomial *Klebsiella* infection rates.

2.2.2 Taxonomy of the Genus *Klebsiella*

The taxonomy of *Klebsiella* is characterized by a nomenclature reflecting its colorful taxonomic history. Originally, the medical importance of the genus *Klebsiella* (family *Enterobacteriaceae*) led to its being subdivided into three species corresponding to the diseases they caused: *K. pneumoniae*, *K. ozaenae*, and *K. rhinoscleromatis*. As the taxonomy became increasingly refined due to the

development of new methods such as numerical taxonomy, the species classification in this genus was continually revised. In time, three main classifications emerged, those of Cowan, Bascomb, and Ørskov (Table 2).

TABLE 2

Species classification of the genus *Klebsiella* by different taxonomic systems

Classification by:		
Cowan	**Bascomb**	**Ørskov**
K. aerogenes K. edwardsii	*K. aerogenes/oxytoca/ edwardsii*	*K. pneumoniae* subsp. *pneumoniae*
subsp. *edwardsii*	*K. pneumoniae*	subsp. *ozaenae*
subsp. *atlantae*	sensu stricto	subsp. *rhinoscleromatis*
K. pneumoniae	sensu lato	*K. oxytoca*
K. ozaenae	*K. ozaenae*	*K. terrigena*
K. rhinoscleromatis	*K. rhinoscleromatis*	*K. planticola* (syn. *K. trevisanii)*
	K. "unnamed group"	
	Enterobacter aerogenes	*K. ornithinolytica*

In the early 1980s, *Klebsiella* isolates from the environment, which had previously been classified as "*Klebsiella*-like organisms" (groups J, K, L, and M), were increasingly being classified into provisional taxa (Gavini *et. al.,* 1997). These groups gave rise to four new species: *K. terrigena* (Izard *et. al.,* 1981), *K. ornithinolytica* (Sakazaki *et. al.,* 1989), *K. planticola* and *K. trevisanii* (Ferragut *et. al.,* 1983). In 1986, the last two species were combined into one species, *K. planticola*, because of their extensive DNA sequence homology (Gavini *et. al.,* 1986). While originally considered to be without clinical significance and restricted to aquatic, botanical, and soil environments, *K. terrigena* and *K. planticola* have recently been reported as occurring in human clinical

specimens (Podschun *et. al.,* 1986). According to these findings, particularly *K. planticola* has been isolated from human infections with a surprisingly high frequency of 3.5 to 18.5% among clinical isolates of *Klebsiella* species. More than half of these isolates were recovered from respiratory tract secretions; wound and urine isolates were the next most common (Podschun *et. al.,* 1986). However, since most of the isolates were obtained from polymicrobial specimens, it is difficult to estimate the significance of these strains as causative agents of disease. Nevertheless, 6 of the 94 isolates were recovered from monomicrobial specimens and could be assigned to corresponding infections. Thus, at present it seems possible that in addition to *K. pneumoniae* and *K. oxytoca*, a third *Klebsiella* species exists that is able to cause human infections.

The adoption of a consistent nomenclature has been further complicated by the fact that Great Britain and the former Commonwealth countries adhere to the classification of Cowan while the USA prefers Ørskov's classification. Consequently, the same bacterium may be called *K. pneumoniae* in one country and *K. aerogenes* in another. Most European countries follow the American example and recognize the worldwide predominant classification of Ørskov.

2.2.3 Differentiation of *Klebsiella* Species

Klebsiella species are usually identified and differentiated according to their biochemical reactions. The genus is defined as containing gram-negative, nonmotile, usually encapsulated rod-shaped bacteria of the family *Enterobacteriaceae*, which produce lysine decarboxylase but not ornithine decarboxylase and are generally positive in the Voges-Proskauer test (Edwards and Ewing, 1986). Within the genus *Klebsiella*, the individual species can be differentiated on the basis of the features listed in Table - 3.3. Whereas most *Klebsiella* species can be identified by standard microbiological laboratory tests,

the species *K. terrigena* and *K. planticola* require special, non-conventional reactions (such as utilization of *m*-hydroxybenzoate or hydroxy-l-proline, pectate degradation, acid from melezitose, or growth at 10°C).

TABLE 3
Biochemical reactions of *Klebsiella* species[a]

Characteristic	*Klebsiella pneumoniae*			*K. oxytoca*	*ter*
	subsp. *pneumoniae*	subsp. *ozaenae*	subsp. *rhinoscleromatis*		
Indole	–	–	–	+	
Ornithine decarboxylase	–	–	–	–	
Lysine decarboxylase	+	v	–	+	
Pectate degradation	–	–	–	+	
Gas from lactose at 44.5°C	+	–	–	–	
Growth at 10°C	–	–	–	+	
Acid from:					
Melezitose	–	–	–	v	

2.2.4 Typing of *Klebsiella* Isolates

From an epidemiological point of view, it is often necessary to determine the clonality of the strains. This is particularly important in endemic and epidemic nosocomial outbreaks of *Klebsiella* infections to improve the management of such outbreaks. A variety of methods have been used with various degrees of success in *Klebsiella* typing and are discussed below.

Biotyping

Biotyping based on an extended panel of biochemical and culture tests is certainly the most practicable method of typing for smaller laboratories that are epidemiologically not optimally equipped. Biotyping can be carried out by using macrotube tests alone (Haverkorn and Michel, 1979, Rennie and Duncan, 1974) or by combining a commercially available miniaturized system such as the API 20E system with additional macrotube tests (Podschum *et. al.,* 1994). However, because of the large number of reactions to be tested and the often long cultivation times—up to 90 days for demonstration of gelatinase (Stenzel *et. al.,* 1972) - biotyping of *Klebsiella* spp. is not very suitable as an epidemiological tool.

Serotyping

Serotyping is currently the most widely used technique for typing *Klebsiella* spp. It is based mainly on a division according to the capsule antigens (Orskov and Orskov, 1984). Klebsiellae usually have well-developed polysaccharide capsules, which give their colonies their characteristic mucoid appearance. Of 82 capsule antigens described, 77 types form the basis for an internationally recognized capsule antigen scheme (Orskov, 1977). Although 12 different O-antigen types of *Klebsiella* have also been described, they are difficult to classify because their determination is hampered by the heat-stable capsules (Orskov, 1954, Orskov and Orskov, 1984). Capsule typing, by contrast, shows good reproducibility and is capable of differentiating most clinical isolates (Ayling-Smith and Pitt, 1990). The drawback of this method is the large number of serological cross-reactions that occur among the 77 capsule types. Thus, individual sera have to be absorbed with the cross-reacting K-antigens. Moreover, the typing procedure is cumbersome because of the time needed to perform the test and is susceptible to subjective interpretations because of weak

reactions that are not always easy to interpret. Since anti-capsule antisera are not commercially available, this technique is practiced mostly in specialized laboratories. However, in contrast to capsule typing, neither biochemical typing, bacteriocin typing, nor phage typing alone is sufficiently discriminative and reproducible for epidemiological purposes except under certain conditions (Orskov and Orskov, 1984). The combined use of biotyping and capsule typing enables the differentiation of a large number of bioserotypes (Rennie and Duncan, 1974).

Phage Typing

Phage typing of *Klebsiella* was first developed in the 1960s (Przondo-Hessek, 1996, Slopek, 1978). Although the phage reaction is easily read and the reproducibility of the method is acceptable, this technique shows a relatively poor typing rate of 19 to 67% (Rubin, 1985, Slopek, 1978). Since, it is not an alternative to capsule typing, this procedure has never become widespread and is useful mainly as a secondary method in combination with serologic testing (Christensen and Korner, 1972, Coovadia *et. al.,* 1979, Johanson *et. al.,* 1969). It should be stressed, however, that it is possible to develop capsule- and O-antigen-specific phage typing if appropriate efforts are made, as a number of reports have demonstrated (MccallumK *et. al.,* 1989, Pieroni *et. al.,* 1994, Tomas, 1970).

Bacteriocin Typing

Although capsule typing is the preferred method for *Klebsiella*, it has been advised to include an additional feature independent of the capsule type to enable more precise epidemiological analysis. Many authors recommend typing *Klebsiella* via., bacteriocins (Bauernfeind, 1984, Buffenmayer *et. al.,* 1976, Edmondson and Cooke, 1997, Hall, 1971). Bacteriocins are bactericidal substances, usually proteins, produced by bacteria to inhibit the growth of other

bacteria, usually members of the same species. An isolate can be characterized either by its ability to inhibit specific indicator strains or by its sensitivity to bacteriocins synthesized by a set of producer strains. Since the synthesis of bacteriocins is not frequent enough in *Klebsiella*, the latter technique has become the method of choice for bacteriocin typing of organisms belonging to this genus. However, the two principal early methods-the growth-in-broth method and the cross-streak method-both show considerable disadvantages. Because of the instability of bacteriocin preparations, the reproducibility of the growth-in-broth method is low. In addition, the conventional cross-streak method results in low typability of strains (Edmondson and Cooke, 1979, Hall, 1971). The limitations of both of these methods have been surmounted by a modification of the "scrape-and-point" procedure (Bauernfeind, 1984), which avoids the use of potentially unstable pre-produced and stored bacteriocins. Instead, the bacteriocins are synthesized on an agar medium immediately before the strains to be typed are inoculated by a multipoint inoculator. This method has proven superior for bacteriocin typing of clinical and environmental *Klebsiella* strains (Bauernfeind *et. al.,* 1981, Podschum and Ullmann, 1993, 1994) as well as of nosocomial outbreaks of *Klebsiella* (Bauernfeind *et. al.,* 1993).

2.2.5 Molecular Typing Methods

Molecular typing methods, as applied to the genus *Klebsiella*, are still in their infancy. Preliminary descriptions have been presented on plasmid profiles (Bauernfeid *et. al.,* 1993, Bingen *et. al.,* 1993 Coovadia, 1992, Hartstein *et. al.,* 1993) ribotypes (Arlet *et. al.,* 1994, Bingen *et. al.,* 1993), multilocus enzyme analyses (Combe *et. al.,* 1994) and applications of pulsed-field gel electrophoresis (Gouby *et. al.,* 1994, Porat *et. al.,* 1987). The procedures vary from laboratory to laboratory and lack standardization, making it difficult to compare them.

2.2.6 Pathogenicity Factors of *Klebsiella*

The terms “pathogenicity factor” and “virulence factor” are used synonymously by some authors (Schaberg *et. al.,* 1991), while others lay emphasis on a clear-cut distinction between them. In this review, the term “pathogenicity” defines the ability of a bacterium to cause disease while “virulence” is the measurement or degree of pathogenicity of any bacterial species.

Nosocomial *Klebsiella* infections most commonly involve the urinary and respiratory tracts. Since these two body sites differ considerably with respect to the host defense mechanisms, it should be expected that the pattern of virulence factors found in UTI-causing strains of *Klebsiella* will differ from that observed in strains isolated from pulmonary sources of patients with pneumonia.

The search for the pathogenic mechanisms of *Klebsiella* infections has identified a number of bacterial factors that contribute to the pathogenesis of these bacteria. Both *in vitro* and *in vivo* models have been established to investigate the interaction of bacterial cells and the host. The use of animal models has been a critical element in the study of *Klebsiella* pathogenicity by providing vital information that cannot be obtained from *in vitro* studies. In particular, animal models have been established to study *Klebsiella* virulence factors in UTIs; mice and rats seem to be appropriate animal types. Lower UTIs have been investigated in the diuresis mouse or rat model of cystitis by intravesicular injection of organisms (Fader and Davis, 1980, Maayan *et. al.,* 1985). To study *Klebsiella*-mediated upper UTI, a rat model of experimental retrograde pyelitis has been established. Frequently, both models include scanning electron microscopy of the surface of the bladder or renal pelvis.

The current research into the pathogenicity of *Klebsiella* focuses on the group of five factors shown in Fig.

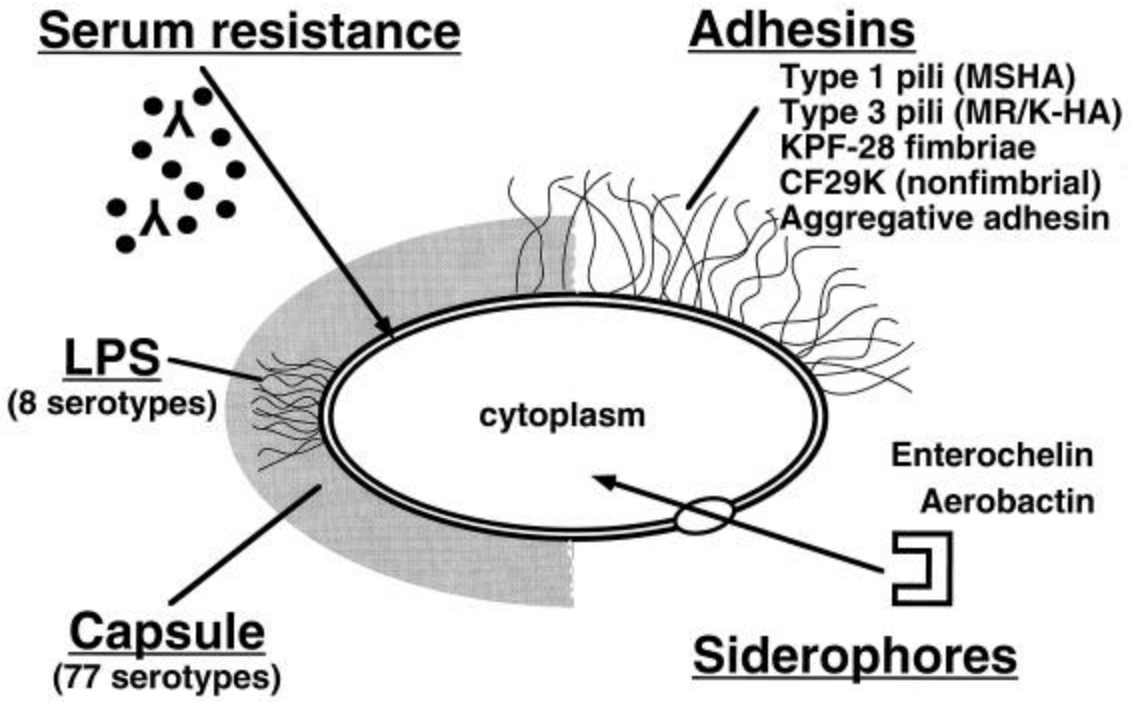

Fig. 2.2: Schematic representation of *Klebsiella* pathogenicity factors

Capsular Antigens

As mentioned above, *Klebsiellae* usually develop prominent capsules composed of complex acidic polysaccharides. The capsular repeating subunits, consisting of four to six sugars and, very often, uronic acids (as negatively charged components), can be classified into 77 serological types (Orskov and Orskov, 1984). Capsules are essential to the virulence of *Klebsiella* (Cryz *et. al.,* 1984, Domenico *et. al.,* 1982, Ehrenwort and Baer, 1956, Highsmith *et. al.,* 1985). The capsular material forms thick bundles of fibrillous structures covering the bacterial surface in massive layers (Fig. 2) (Amako *et. al.,* 1988). This protects the bacterium from phagocytosis by polymorphonuclear granulocytes, on the one hand (Podschum *et. al.,* 1992, Podschum and Uiimann, 1992, Simoons-smit *et. al.,* 1985, 1986), and prevents killing of the bacteria by

bactericidal serum factors, on the other (Williams *et. al.,* 1983). The molecular mechanism presumably consists of inhibiting the activation or uptake of complement components, especially C3b (Williams *et. al.,* 1989). Apart from their antiphagocytic function, *Klebsiella* capsule polysaccharides have been reported to inhibit the differentiation and functional capacity of macrophages *in vitro* (Yokochi *et. al.,* 1977, Yokochi *et. al.,* 1979). Moreover, injection of large doses of *Klebsiella* capsular polysaccharide (CPS) may even produce immunological paralysis, as has been demonstrated in mice that showed a dose-dependent decrease in the production of antibodies to the specific capsular antigen.

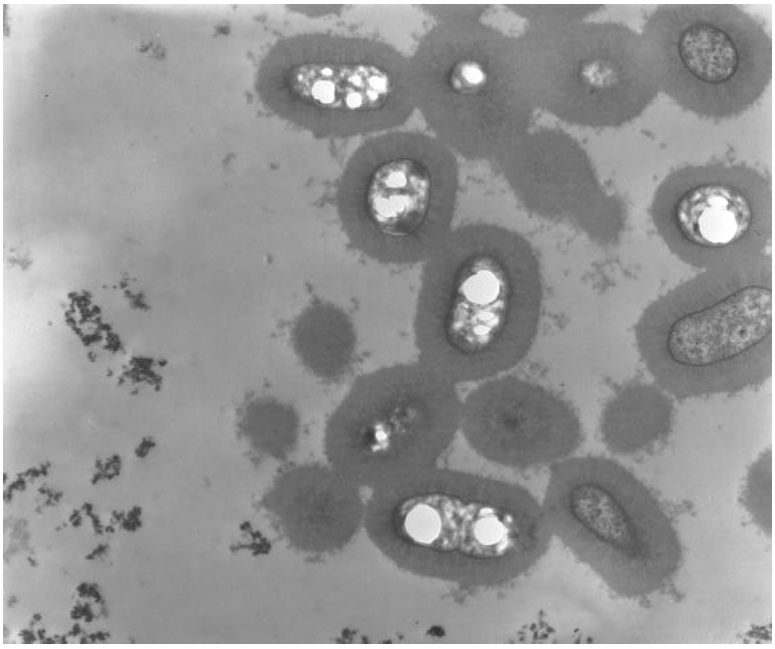

Fig. 2.3: Transmission electron micrograph of *K. pneumoniae* cells surrounded by thick layers of fibrillous capsular material. Courtesy of I. Ofek, Tel Aviv University, Israel.

While *Klebsiella* CPS were generally considered to mediate virulence properties, this consideration has recently been abandoned because of the great differences in virulence observed among different capsular types: strains expressing the capsule antigens K1 and K2 were found to be especially virulent in a mouse peritonitis model, whereas isolates of other serotypes showed little or no virulence (Kauffmann, 1949, Mizuta *et. al.,* 1983). In experimentally induced

skin lesions in mice, *Klebsiella* strains of serotypes K1, K2, K4, and K5 were more virulent than were those expressing other capsule types (Simoons-smit *et. al.,* 1986). At present, strains expressing capsule types K1 and K2 are considered especially likely to be virulent, although only a few of the 77 different K antigens have been systematically studied in this regard.

The degree of virulence conferred by a particular K antigen might be connected to the mannose content of the CPS. Capsular types with low virulence, such as the K7 or K21 antigen (Ofek *et. al.,* 1995 and Podschum and Ullmann, 1992), contain repetitive sequences of mannose-α-2/3-mannose or l-rhamnose-α-2/3-l-rhamnose. These sequences are recognized by a surface lectin of macrophages, which mediates opsonin-independent (i.e., complement- and antibody-independent) phagocytosis, known as lectinophagocytosis. Lectinophagocytosis has been defined as nonopsonic phagocytosis that is based on recognition between surface lectins on one cell and surface carbohydrates on the opposing cell (Ofek *et. al.,* 1995). Lectinophagocytosis may be mediated either by bacterial surface lectins such as fimbriae or by phagocyte lectins that act as receptors. Macrophages with the mannose-α-2/3-mannose-specific lectin or mannose receptor recognize, ingest, and subsequently kill *Klebsiella* serotypes containing the CPS repeating sequences Manα2/3Man or l-Rhaα2/3l-Rha. In contrast, strains that lack these repeating sequences are not recognized by macrophages and hence phagocytosis does not take place. This model is consistent with the marked virulence of K2, which completely lacks mannose-α-2/3-mannose structures (Kabha *et. al.,* 1995, Ofek *et. al.,* 1991). Thus, *Klebsiella* strains bearing capsule types devoid of these mannose or rhamnose sequences should be more closely associated with infectious diseases.

Previous attempts to establish a correlation between individual *Klebsiella* serotypes and the site of infection or clinical symptoms have produced a profusion of contradictory results. Each study reports different capsular types as

predominant (Casewell and Talsnia, 1979, Cryz *et. al.,* 1984, Podschul *et. al.,* 1986, Rennie *et. al.,* 1974, Riser and Noone, 1981, Simmons-smit *et. al.,* 1985, Ullmann, 1983). Geographical differences in serotypes may have contributed to this confusion. Most reports do agree, however, that the K2 serotype is among the most common capsule types isolated from patients with UTI, pneumonia, or bacteremia. It can be assumed, therefore, that K2 is the predominant serotype of human clinical isolates worldwide whereas K2 strains are very rarely encountered in the environment (Brown and Seidler, 1973, Edmondson and Cooke, 1979, Matsen *et. al.,* 1974). Thus, the observed predominance of the K2 serotype in *Klebsiella* infections is quite consistent with the concept of lectinophagocytosis. Since the host innate immune mechanisms interact with structures commonly found on microorganisms, particular *Klebsiella* serotypes, such as the K2 type, which do not bear such structures, become selected.

The significance of capsular mannose-α-2/3-mannose sequences to the clearance of *Klebsiella* in the host is further illustrated by recent findings of Ofek's group (Kabha *et. al.,* 1997). They observed that surfactant protein A (SP-A), the main protein component of lung surfactant, enhances the phagocytosis by alveolar macrophages of *Klebsiella* K21a strains (which bear a mannose-α-2/3-mannose containing capsule) but not of K2 isolates. Since the reaction was inhibited by mannan, the authors suggested that bacterial binding is mediated by the macrophage mannose receptor.

Pili (Fimbriae)

As a critical first step in the infectious process, microorganisms must come as close as possible to host mucosal surfaces and maintain this proximity by attaching to the host cell (adherence). The adhesive properties in the *Enterobacteriaceae* are generally mediated by different types of pili. Pili (otherwise known as fimbriae) are non-flagellar, filamentous projections on

the bacterial surface. These structures are up to 10 µm long and have a diameter of 1 to 11 nm; they consist of polymeric globular protein subunits (pilin) with a molecular mass of 15 to 26 kDa (Jones *et. al.,* 1983).

Pili are demonstrated mainly on the basis of their ability to agglutinate erythrocytes of different animal species. Depending on whether the reaction is inhibited by d-mannose, these adhesins are designated as mannose-sensitive or mannose-resistant hemagglutinins (MSHA and MRHA), respectively (Ottow, 1975). Of the different types of pili described in enterobacteria, there are two predominant types in *Klebsiella* spp. (Old and Adgbola, 1985, Podschum *et. al.,* 1987, Przonda-Hessek *et. al.,* 1983).

Type 1 (common) pili:

Type 1 pili are the best investigated of the bacterial adhesins. They are MSHA which agglutinate guinea pig erythrocytes. The adhesion protein in this pilus type is located on the fimbrial shaft and is capable of binding to mannose-containing trisaccharides of the host glycoproteins (Babu *et. al.,* 1986, Firon *et. al.,* 1984). The sugar structures presumably consist of short oligomannose chains bound via., N-glycosidic linkages to the glycoproteins (Sharon and Ofek, 1986). The relevance of these pili to bacterial virulence is thought to arise mainly from binding of the bacteria to mucus or to epithelial cells of the urogenital, respiratory, and intestinal tracts (Venegas *et. al.,* 1995). Their role in the pathogenesis of UTI was clarified mostly in studies on *E. coli* but has also been described for *K. pneumoniae* in animal models (Fadar and Davis, 1980, French *et. al.,* 1996). Although associated primarily with the pathogenesis of lower UTI (Iwahi *et. al.,* 1983), type 1 pili may also be involved in the pathogenesis of pyelonephritis (Fader and Davis, 1982, Matsumoto *et. al.,* 1987). In this setting, these structures have been shown to bind effectively to proximal tubulus cells (Virkola, 1988). Type 1 fimbriae are also capable of binding to soluble, mannosyl-

containing glycoproteins in urine, such as the Tamm-Horsfall protein (Reinhardt, 1990), or in saliva (Babu *et. al.,* 1986). These findings provide an explanation for the fact that type 1 pili mediate bacterial colonization of the urinogenital and respiratory tracts (Clegg and Gerlach, 1987). Adherence of bacteria to cells of the respiratory tract (Ayar *et. al.,* 1982) leads to impairment of colonization resistance in the upper airways, with a subsequent proliferation of facultative pathogenic bacteria. This impairment may result in the development of pneumonia, especially in patients undergoing long-term mechanical ventilation (Williams *et. al.,* 1989).

In the appraisal of the pathogenic role of type 1 pili, however, the phenomenon of “phase variation” has to be taken into account. As mentioned above, this type of adhesin mediates bacterial colonization of the host mucosal surfaces via., a rather nonspecific binding. In pathogenic microorganisms, colonization of the mucous membrane is followed by invasion of the underlying tissue, with all of the subsequent events of infectious pathogenesis. Once in the host tissue, however, the type 1 pili are no longer of use to the bacteria, since they trigger an opsonin-independent leukocyte activity known as lectinophagocytosis (OfekI and Sharon, 1988). The repulsion forces separating bacterium and leukocyte are weakened by the hydrophilic character of these pili (Ohmann *et. al.,* 1985), thus enabling the adhesins to bind to specific mannose-containing receptors on the leukocyte surface (Rodriguezortega *et. al.,* 1987). Adhesin-binding triggers stimulation of the leukocyte, which ultimately leads to phagocytosis and intracellular killing of the bacterium (Lock *et. al.,* 1990). The bacterium counters this form of host defense by switching off the expression of type 1 pili in tissue (Maayan *et. al.,* 1885). Thus, while type 1 pili are important for host colonization, their contribution to subsequent steps of pathogenesis is less clear.

Type 3 pili:

Unlike other fimbriae, type 3 pili agglutinate only erythrocytes that have been treated with tannin. Although its name, mannose-resistant, *Klebsiella*-like hemagglutination (MR/K-HA), implies that this fimbrial type is synthesized only by *Klebsiella*, later studies demonstrated that type 3 pili occur in many enteric genera (Clegg and Gerlach, 1987). Moreover, type 3 pili apparently are not identical in all genera of enterobacteria, since serological studies showed considerable antigenic diversity. Originally described as the adhesion organelles of *Klebsiella* inhabiting plant roots (Korhonen *et. al.,* 1983), these pili were later found to be capable of binding to various human cells. Strains of *K. pneumoniae* expressing type 3 pili adhere to endothelial cells, epithelia of the respiratory tract, and uroepithelial cells (Hornick *et. al.,* 1992). In the kidneys, these pili mediate bacterial adhesion to tubular basement membranes, Bowman's capsules, and renal vessels (Tarkkanen *et. al.,* 1997). Binding to tannic acid-treated erythrocytes is inhibited by spermidine, a polyamine that is also secreted in urine (Gerlach *et. al.,* 1989). Since spermidine is exposed on the cell surface of damaged erythrocytes, it has been suggested that MR/K hemagglutination is mediated by spermidine. This might explain why type 3 pili bind to tannic acid- or heat-treated erythrocytes but not to untreated erythrocytes.

The role of this fimbrial type in the pathogenetic process is largely unknown. So far, the only evidence of a correlation between the type 3 MrkD hemagglutinin and disease has been the observation of expression of type 3 pili in *Providencia stuartii* in catheter-associated bacteriuria. This species is not a common cause of UTI in short-term-catheterized or non-catheterized persons but has a much higher prevalence in the urine of patients with long-term indwelling catheters. In the above-mentioned study, it was demonstrated that the higher prevalence of *P. stuartii* in catheter-associated bacteriuria was due to its ability to adhere and persist to the catheter in the catheterized urinary tract by expression

of the MR/K hemagglutinin. Unfortunately, so far, no experimental animal model has been established investigate the role of these pili in infection. The structure of the corresponding host receptors is unknown.

Three new types of *Klebsiella* adhesins have been recently reported. The R-plasmid-encoded CF29K adhesin of *K. pneumoniae* has been demonstrated to mediate adherence to the human intestinal cell lines Intestine-407 and $CaCO^{-2}$ (Darfeuille-Michaud *et. al.,* 1992). This adhesin type seems to be identical to the CS31-A adhesive protein of human diarrheal *E. coli* strains (Di Martino *et. al.,* 1995) and belongs to the K88 adhesin family. The available data suggest that CF29K probably is a product of the transfer of CS31A genetic determinants from *E. coli* to *K. pneumoniae* strains in the human intestine. A particular adherence pattern characterized by aggregative adhesion to intestinal cell lines is mediated by another new *Klebsiella* adhesin that seems to be composed of capsule-like extracellular material (Favre-Bonte *et. al.,* 1995). While the two adhesins mentioned above are non-fimbrial, a third putative colonization factor of the human gut is a new fimbria that has been termed KPF-28 (Di Martino *et. al.,* 1996). Interestingly, this fimbrial type has been found in the majority of *K. pneumoniae* strains producing CAZ-5/SHV-4 type ESβL.

To date, however, little is known about the frequency and distribution of these newly described adhesins, their geographical variations, their expression by different species of *Klebsiella*, their site of isolation from the host, or their significance in pathogenicity.

Serum Resistance and Lipopolysaccharide

The first line of defense by the host against invading microorganisms includes, in addition to phagocytosis by polymorphonuclear granulocytes, the bactericidal effect of serum. The serum bactericidal activity is mediated primarily by complement proteins. After their cascade-like activation, these proteins

accumulate as membrane attack complex on the surface of the microorganism. This complex consists of the terminal complement proteins C5b–C9, which produce a trans-membranous pore in the outer membrane of Gram-negative bacteria, leading to an influx of Na^+ and subsequent osmotic lysis of the bacteria. The complement cascade can be activated by two different mechanisms: the classic complement pathway, which typically requires specific antibodies to be activated, and the alternative complement pathway, which can be activated even in the absence of antibodies. The alternative pathway is also regarded as an early defense system of innate immunity, which enables the host to react to invading microorganisms even before specific antibodies are formed (Jioner, 1988). Both complement pathways lead, via the activation of C3, to the formation of the opsonin C3b, which ultimately results in formation of the terminal C5b–C9 complex and thus plays a key role in this defense system.

In response to this host defense, pathogenic microorganisms have developed strategies to counter the serum bactericidal effect. Most commensal Gram-negative bacteria are sensitive to the bactericidal effect of human serum, whereas pathogenic strains often exhibit serum resistance properties (Olling, 1977). Thus, clinical isolates of enterobacteria often show resistance to serum, and the feature “serum resistance” has been correlated with the onset of infection (Oling, 1977, Roantree *et. al.,* 1960) and severity of symptoms (Bjorksten and Kaijser, 1978, Gower *et. al.,* 1972). Since, the main role of the serum bactericidal system is thought to prevent microorganisms from invading and persisting in the blood, even differences in the degree of bacterial serum susceptibility may determine whether a strain is able to infect as well as the length of time it takes the organisms to establish the infection.

To date, the exact mechanism underlying bacterial serum resistance is unknown. Aside from various proteins of the outer membrane, such as the TraT lipoprotein or porins, primarily CPS and O antigens (lipopolysaccharides

[LPS]) have been implicated (Ciurana *et. al.,* 1987, Opal *et. al.,* 1982, Porat *et. al.,* 1987). For *Klebsiella*, two hypotheses have been propounded (Merino *et. al.,* 1992). First, capsule polysaccharides may cover and mask the underlying LPS and exhibit a surface structure that does not activate complement. On the other hand, the O side chains of the LPS may reach through the capsule layer and be exposed to the exterior milieu in certain *Klebsiella* capsule types. Since LPS is generally able to activate complement, C3b is subsequently deposited onto the LPS molecules. However, since it is fixed preferentially to the longest O-polysaccharide side chains, C3b is far away from the bacterial cell membrane. Thus, the formation of the lytic membrane attack complex (C5b–C9) is prevented, and subsequent membrane damage and cell death do not take place.

In addition to this steric hindrance of the lytic complement action by LPS, the quantity of deposited C3b also determines the degree of serum resistance. While serum-sensitive strains activate both the classical and the alternative complement pathways, the smooth LPS of serum-resistant strains activates only the alternative pathway. The activation of both complement pathways by serum-sensitive strains leads to higher levels of deposited C3b, resulting in greater damage and bacterial killing.

It should be borne in mind, however, that all previous studies in this field were done with strains expressing the O1 serotype. Even though O1 is the most common O antigen found among clinical *Klebsiella* isolates, a number of different O serotypes, many of them neutral polysaccharides, are known. Originally there were 12 chemically different O types, but the results of structural investigations later reduced the number of *Klebsiella* O1 antigens to 8. To date, it is unclear whether serum resistance is mediated solely by the O1 antigen or whether this feature is generally conferred by *Klebsiella* LPS.

Nevertheless, even within a given O serotype, serum resistance does not seem to be a stable characteristic; environmental factors affect the composition and effect of LPS. Recently, the influence of different osmolarity conditions on LPS was demonstrated in *Aeromonas hydrophila* serotype O:34; cells grown at high osmolarity showed smooth LPS, whereas growth at low osmolarity resulted in rough LPS. Correspondingly, cells cultivated at high osmolarity were resistant to normal human serum while bacteria grown at low osmolarity proved to be serum sensitive. Thus, the same bacterial strain may be serum resistant at host body sites with a high-osmolarity milieu, such as the urinary tract, and serum sensitive at low-osmolarity body locations like the respiratory tract.

Siderophores

The growth of bacteria in host tissue is limited not only by the host defense mechanisms but also by its supply of available iron. Iron is an essential factor in bacterial growth, functioning mainly as a redox catalyst in proteins participating in oxygen and electron transport processes (Griffiths, 1987). The supply of free iron available to bacteria in the host milieu is extremely low, since this element is bound intracellularly to proteins such as hemoglobin, ferritin, hemosiderin, and myoglobin and extracellularly to high-affinity iron-binding proteins such as lactoferrin and transferrin. The level of free, bioavailable iron (10^{-18} M) is several thousand fold too low for normal bacterial growth (Bullen *et. al.,* 1978). The marked effect of the iron supply in the host body on the pathogenesis of infections has been demonstrated for *Klebsiella*. After parenteral administration of iron in a guinea pig model, the susceptibility to *K. pneumoniae* infections increased dramatically (Khimji and Miles, 1978).

Many bacteria attempt to secure their supply of iron in the host by secreting high-affinity, low-molecular-weight iron chelators, called siderophores, that are capable of competitively taking up iron bound to host proteins (Griffiths,

1987). Under iron-deficient conditions, e.g., in the host milieu, enterobacteria synthesize a variety of siderophores, which belong to two different chemical groups, one consisting of the phenolate-type siderophores and one consisting of the hydroxamate-type siderophores.

The more common group consists of the phenolate-type siderophores. Their best-known representative, enterobactin (also known as enterochelin), is a cyclic trimer of 2,3-dihydroxy-benzoyl-serine. This siderophore appears to comprise the main iron uptake system of enterobacteria and is synthesized by almost all clinical isolates of *E. coli* and *Salmonella* spp. (Griffiths *et. al.,* 1987). Studies on the contribution of enterobactin to virulence have produced conflicting results. For example, Yancey *et. al.,* reported that *Salmonella typhimurium* mutants unable to produce this siderophore were less virulent in mice, while Benjamin et al. 1985 found no association between virulence and the ability to synthesize enterobactin. To date, the role of enterobactin in virulence remains uncertain.

Among the hydroxamate-type siderophores, the ferrichromes (which are synthesized only by fungi), the ferrioxamines, and aerobactin are the most important. In contrast to enterobactin, the contribution of aerobactin to bacterial virulence has been clearly demonstrated (De Lorenzo *et. al.,* 1988). While the thermodynamic stability constant of ferric enterobactin indicates a much higher affinity for Fe (III) than that of ferric aerobactin (K_s 10^{52} and 10^{23}, respectively), aerobactin still seems to be far more effective than enterobactin because of a number of physical advantages such as greater stability and better solubility (Wooldridge and Williams, 1993). Moreover, while enterobactin becomes hydrolyzed by an esterase after delivery of iron, aerobactin can be recycled after each turn of iron transport.

The observations of Martinez *et. al.,* 1987 indicate that enterobacterial genera can be divided into two groups according to their incidence of aerobactin synthesis. The group with a low rate of aerobactin-producing strains (<20%) comprises genera such as *Serratia*, *Proteus*, and *Salmonella*. The second group, which includes the genus *Escherichia*, shows a high incidence of aerobactin synthesis (>40%).

In the genus *Klebsiella*, the production of both enterobactin and aerobactin has been demonstrated. However, while enterobactin is synthesized by almost all strains (Podschum *et. al.,* 1992, Rassbrodt and Rabsch, 1988, Williams *et. al.,* 1987), aerobactin-positive *Klebsiella* isolates, irrespective of the species or source of isolation, have been observed rarely (Marrinez *et. al.,* 1987, Podschun *et. al.,* 1993). Enterobactin-positive *Klebsiella* isolates in animal models were no more virulent than enterobactin-negative strains (Miles and Khimij, 1975). In contrast, an association between aerobactin synthesis and the virulence of *Klebsiella* strains was unequivocally demonstrated by Nassif and Sansonetti, 1986. In this study, the aerobactin gene was cloned from the plasmids of some *K. pneumoniae* strains of serotypes K1 and K2 and transferred to a non-virulent (siderophore-negative) strain. The transformant then exhibited markedly enhanced virulence in a mouse peritonitis model.

Because of the common ability of strains to produce enterobactin, it has been speculated for a long time what additional advantage a bacterium, which already synthesizes enterobactin, might derive from aerobactin. The great advantage of enterobactin over aerobactin is its high iron affinity, the highest ever recorded for a ferric iron chelator. At pH 7.4, the formation constant for ferric enterobactin is 10^{52}, which is magnitudes higher than that of transferrin (Griffiths, 1987). In contrast to enterobactin, aerobactin is not an effective competitor for transferrin-bound iron because of its much lower affinity for ferric iron. Indeed, investigations have demonstrated that enterobactin sequesters iron

predominantly from transferrin, while the iron source of aerobactin is host cells. Thus, production of these two siderophores may give access to both sources of iron, resulting in enhanced growth in the host.

Data on the incidence of aerobactin-producing *Klebsiella* indicates that this siderophore does not play a central role in the pathogenicity of the genus *Klebsiella*. It should be pointed out, however, that clinical *K. pneumoniae* isolates, which do not synthesize aerobactin themselves, are entirely capable of using exogenously introduced aerobactin as their sole source of iron (Williams *et. al.,* 1989). By synthesizing only the intrinsically expressed aerobactin receptor, such strains could derive an advantage over other aerobactin-synthesizing bacteria in mixed infections. The aerobactin-mediated iron uptake system would thus be an indirect contributor to the pathogenicity of the genus *Klebsiella*.

As in many enterobacteria, and as has been especially well studied in *E. coli*, other factors have also been demonstrated in *Klebsiella* spp. Although the production of cytotoxins (Higaki *et. al.,* 1990, Koo and Stein, 1986, Minami *et. al.,* 1992, Straus, 1987), enterotoxins (Gaurino *et. al.,* 1989, Klipstein and Engert, 1975, 1977, 1976, 1983, Minami *et. al.,* 1994) and hemolysin (Barberis *et. al.,* 1986) has been sporadically described, these features probably play a rather minor role in *Klebsiella*.

2.2.7 Vaccination:

As stated above, most *Klebsiella* infections are acquired during hospital stays and account for 5 to 7.5% of all nosocomial infections. The morbidity and mortality of severe systemic infections, such as bacteremia and pneumonia, remain high despite the use of appropriate antibiotic therapy. Fatality rates of 20 to 50% in *Klebsiella* bacteremia and of more than 50% in *Klebsiella pneumonia* have been reported (Bryan *et. al.,* 1983, Cryz, 1983, De la Torre *et. al.,* 1985). Moreover, *Klebsiella* infections in pediatric wards have become a

major concern. In neonatal intensive care units, *Klebsiella* is one of the three or four most common pathogens (Hart, 1993). This is apparently related to the observation that premature neonates, especially those in neonatal intensive care units are more likely than other neonates to develop an intestinal flora in which *Klebsiella* spp. are highly prevalent. These findings, taken together with the emergence of ESβL-producing multiresistant strains, indicate the need for a means of immunological control of *Klebsiella* infections. Such measures may include immunoprophylaxis (active vaccination of patients who are at risk) as well as immunotherapy (passive immunization by hyperimmune sera).

Among the different cell constituents, two surface components are mainly being discussed as candidates for an anti-*Klebsiella* vaccine: LPS and CPS.

Lipopolysaccharides

Due to their endotoxic properties, LPS are considered important in the pathology of septicemia. Until recently, *Klebsiella* LPS O antigens were generally considered to be masked by the capsule polysaccharides and thus not to be exposed on surface, leaving them inappropriate as vaccine candidates. Recent studies, however, demonstrated surface exposure of O-antigens in strains expressing particular capsular serotypes (Tomas *et. al.,* 1988). The small number of different *Klebsiella* O-types is a great advantage with respect to their applicability as vaccines. In contrast to the K antigens, only eight O types are known, O1 being the most commonly found O type in clinical isolates. A multivalent LPS vaccine composed of these eight O antigens or at least the inclusion of the common O1 antigen in a broad spectrum capsular polysaccharide vaccine might be a promising approach. Recently, the administration of monoclonal antibodies to the *Klebsiella* O1 antigen has been reported to be protective in a mouse model of lethal endotoxemia (Thom, 1970). Moreover, the inclusion of O antigens in a multivalent *Klebsiella* vaccine

formulation might be of additional benefit due to strong adjuvant action, as has been demonstrated for the *Klebsiella* O3 lipopolysaccharide (Yokochi *et. al.,* 1995). A great drawback of active immunization with LPS-containing vaccines, however, is adverse toxic reactions, which must be expected because of the endotoxin content. Thus, each *Klebsiella* vaccine composed of O antigens has to be rendered safe by sufficient detoxification of the LPS.

Capsular Polysaccharides

CPS have been the obvious vaccine candidates for several reasons. Capsules are produced by almost all *Klebsiella* strains; they represent the outermost layer of surface structures in contact with the host milieu, and they have been proven to be highly immunogenic and nontoxic. A serious disadvantage of a *Klebsiella* CPS vaccine is the great number of K antigens (77 different antigens). However, in a study of the incidence of the capsule types among bacteremic *Klebsiella* isolates, Cryz *et. al.,* 1986 observed that only 25 serotypes made up 70% of all bacteremic strains. Based on their seroepidemiological findings, they formulated a 24-valent *Klebsiella* CPS vaccine that subsequently was proven to be safe and immunogenic (Cryz *et. al.,* 1985). To date, this vaccine seems to be the most promising approach for preventing sepsis caused by *Klebsiella* and has already passed phase I human trials (Edelman *et. al.,* 1994). The most recent study of the 24-valent *Klebsiella* CPS vaccine demonstrated an excellent antibody response after active immunization in patients with acute blunt or penetrating trauma (Campbell *et. al.,* 1996).

Indeed, investigations have demonstrated that enterobactin sequesters iron predominantly from transferrin, while the iron source of aerobactin is host cells (Brock *et. al.,* 1991). Thus, production of these two siderophores may give access to both sources of iron, resulting in enhanced growth in the host.

Concluding Remarks:

Klebsiella are opportunistic pathogens and can give rise to severe diseases such as septicemia, pneumonia, UTI, and soft tissue infection. Typically, *Klebsiella* infections are nosocomial. The hospitalized, immunocompromised patient with underlying diseases is the main target of these bacteria. Thus, *Klebsiella* infections may serve as a paradigm of hospital-acquired infections. Their incidence of 5 to 7% of all hospital-acquired infections ranks them among the most important nosocomial pathogens.

In this context, some new trends have been observed in the past several years.

(i) An increasing number of endemic and epidemic outbreaks in pediatric wards has been reported. Especially common are *Klebsiella* infections causing septicemia and meningitis in newborns in neonatal intensive care units. Since more and more of these outbreaks have been caused by multidrug-resistant strains, *Klebsiella* neonatal infections are becoming a major concern of the pediatrician. Especially peculiar has been the repeated frequent isolation of multidrug-resistant *Klebsiella* isolates expressing serotype K55. It remains to be seen whether this observation reflects the spread of a particular “neonatal” *Klebsiella* clone.

(ii) Hospital outbreaks of multidrug-resistant *Klebsiella* spp. are often caused by a new type of strain, the ESβL producers. The incidence of ESβL-producing strains among clinical *Klebsiella* isolates has been steadily increasing over the past several years. Frequencies of up to 40% have been reported in certain regions. Currently, the available data suggest a further increase in the incidence of ESβL-producing isolates. As a result, the therapeutic options are becoming limited, so that in the near future there will be an urgent need for hospital

infection control measures that counter the spread of ESβL-producing bacteria.

(iii) Until recently, *K. pneumoniae* and *K. oxytoca* have been considered to be the only pathogenic *Klebsiella* species. However, the newer species *K. terrigena* and *K. planticola*, formerly regarded as "environmental" *Klebsiella* species, have been demonstrated to occur in human clinical specimens. *K. planticola*, in particular, has been isolated with astonishing frequency from human infectious processes. So far, it is unclear what kind of pathogenicity factors *K. planticola* might possess or whether this species expresses the same factors that have been described for *K. pneumoniae*. *K. planticola* can, however, be expected to be of clinical significance, and the question remains whether the identification of this species by standard laboratory procedures should be recommended.

Nosocomial *Klebsiella* infections continue to be a heavy burden on the economy and on the life expectancy of patients in developed countries. Hospital infection prevention and control programs led in the past to considerable improvements in the management and control of these infections. However, there is a general agreement that further progress in prevention of hospital-acquired infections will require new approaches to infection control. As a future challenge for the advancement of preventive measures against nosocomial *Klebsiella* infections, the usefulness of new concepts needs to be evaluated. A number of different approaches are conceivable.

One possible measure is the vaccination of persons at risk. Regardless of whether active or passive immunization is performed (the latter preferentially by a cocktail of monoclonal anti-*Klebsiella* antibodies), the question has to be raised about whom to vaccinate. While little controversy is to be expected for vaccinating immunocompromised hospitalized patients to prevent fatal *Klebsiella*

pneumonia and septicemia, the justification of immunological measures in other patient groups is being debated. A good example in this respect is *Klebsiella* UTI in elderly individuals. Most cases of bacterial pyelonephritis are not caused by *Klebsiella* but by *E. coli* strains. However, although *Klebsiella* species are not a predominant cause of UTI, they can cause significant renal scarring even after a single episode of infection. Moreover, infections with these uropathogens are more likely to lead to death than are infections with most *E. coli* strains. The question whether a *Klebsiella* vaccine should be recommended for persons older than 60 years has to be clarified by cost-benefit analyses.

Another point of interest is the possible eradication of *Klebsiella* in patients during their hospital stay. One of the new approaches is the use of cranberry juice. This juice shows a pronounced anti-adhesive effect on enterobacteria and therefore might prevent colonization of hospitalized patients or even eradicate these bacteria in colonized persons. Cranberry juice might be of benefit both because of its high content of fructose as an inhibitor of type 1 pili and because of the presence of a high-molecular-weight constituent that blocks mannose-resistant adhesion. It has been postulated that the juice acts on the gastrointestinal organisms to eliminate the source of infection rather than on the bladder because recurrent but not acute UTIs were prevented. Daily consumption of cranberry juice cocktail has been demonstrated to cause bacteriuric elderly persons to become and remain abacteriuric. The use of cranberry juice cocktail in the hospital setting might be a paradigm for cheap and simple preventive intervention without the use of antibiotics or vaccines.

Fascinating future aspects are also raised by new findings on so far unknown host defense mechanisms. As mentioned above, lung SP-A increases phagocytosis of *Klebsiella* by acting as an opsonin and by activating alveolar macrophages. Another surfactant protein (SP-D) has been reported to interact with bacterial LPS. It has been suggested that SP-D plays an important role in

the defense of the lungs against gram-negative bacteria. Investigations in the near future will show whether surfactant proteins may be useful in new therapeutic approaches and whether they are a meaningful addition to the management of nosocomial *Klebsiella* infections.

2.3 Citrobacter

The *Citrobacter* species, including *Citrobacter freundii*, are aerobic gram-negative bacilli. *Citrobacter freundii* are long rod-shaped bacteria typically 1-5 μm in length (Wang *et. al.,* 2000). Most *C. freundii* cells are surrounded by many flagella used to move about, but a few are non-motile. Its habitat includes the environment (soil, water, sewage), food, and the intestinal tracts of animals and humans (Wang *et. al.,* 2000). It belongs to the family of *Enterobacteriaceae*.

As an opportunistic pathogen, *C. freundii* is responsible for a number of significant opportunistic infections. It is known to be the cause of a variety of nosocomial infections of the respiratory tract, urinary tract, blood and several other normally sterile sites in patients (Whalen *et. al.,* 2007). *C. freundii* represents approximately 29% of all opportunistic infections. Therefore, one of the chief reasons many different strains and plasmids of the *C. freundii* genome are being sequenced is in order to find antibiotics that can fight these opportunistic infections.

Surprisingly, this infectious microbe in humans plays a positive role in the environment. *C. freundii* is responsible for reducing nitrate to nitrite in the environment (Puchenkova, 1996). This crucial conversion is an important stage in the nitrogen cycle. And recycling nitrogen is very essential because the earth's atmosphere is about 85% nitrogen (Puchenkova, 1996). Therefore, due to its important contribution to the environment is another motivation for sequencing the genome of *C. freundii*.

The *Citrobacter* genus was discovered in 1932 by Werkman and Gillen. Cultures of *C. freundii* were isolated and identified in the same year from soil extracts (Wang *et. al.,* 2000).

2.3.1 Genome structure

No information about the complete genome of *C. freundii* is available online, although some individual strains and plasmids of the microbe have been sequenced. The most prominent one is the plasmid pCTX-M#3 because it is the largest plasmid and encodes a large amount of proteins. Its sequence was completed on January 6, 2005. It is a circular DNA plasmid and it is 89,468 nucleotide base pairs long. The length of the plasmid is 0.089468 (Mbp). It is composed of 51.0% GC content, and encodes 105 proteins.

Another important feature of the *C. freundii* genome is that it is the only microbe in the *Enterobacteriaceae* family that contains a plasmid which encodes L-methionine γ-lyase (MGL). The nucleotide sequence of the plasmid contains a 3000 bp long EcoRI insert (Llaya *et. al.,* 2005). The fragment also contains two open reading frames. The first frame consists of 1,194 nucleotides, and the second, 1,296 nucleotides. The first frame, known as the megL gene, encodes a protein of 398 amino acid residues that has sequence homology with MGLs from different sources. The second frame encodes a protein with sequence homology with proteins belonging to the family of permeases (Llaya *et. al.,* 2005).

The *C. freundii* OS60 AmpC β-lactamase gene has also been sequenced and it is composed of 1197 nucleotides. It encodes a 380 amino acid long precursor and contains a 19 residue signal peptide in the 5' end (Lindberg *et. al.,* 1985). This gene encodes a mature protein that has a molecular mass of 39781 Daltons. The amino acid positions in these precursor are surprisingly identical to residues in the *E. coli* K12 chromosomal AmpC β-lactamases (Lindberg *et. al.,* 1986).

Another important strain in the genome of *C. freunii* is GN346, which is a clinical isolate recovered in 1965. This strain produces the enzyme cephalosporinase, which has the ability to hydrolyzes and inactivate the antibiotics cephalosporins and cephamycins (Tsukamoto *et. al.,* 1990). The structural and promoter regions of the cephalosporinase gene are 1408 nucleotides long. The amino acid sequence of the mature enzyme, is composed of 361 amino acids with a molecular mass of 39,878 Da (Tsukamoto *et. al.,* 1990).

2.3.2 Cell structure and metabolism

The cell structure of *C. freundii* is long and rod-shaped usually 1-5 μm in length. The outside of the cell contains many flagella used for motality (Wang *et. al.,* 2000). Since *C. freundii* is Gram-negative bacteria, it contains two membranes (inner and outer). The periplasmic space lies in between the two membranes. The outer membrane does not contain an energy source; but it does contain many porins embedded within that help the organism acquire important ions (Wang *et. al.,* 2000). Unlike Gram-positive bacteria, *C. freundii* cells do not contain a thick cell wall made up of peptidoglycan.

For metabolism, *C. freundii* has an amazing ability to grow on glycerol as the sole carbon and energy source. In this process, glycerol is fermented by a dismutation process. This process requires two pathways (Keevil *et. al.,* 1997). In the first pathway, glycerol is dehydrogenated by a NAD1-linked glycerol dehydrogenase to dihydroxyacetone. The dihydroxyacetone is then phosphorylated and funneled to glycolysis by dihydroxyacetone kinase (Keevil *et. al.,* 1997). In the second pathway, glycerol is dehydrated by the coenzyme B12-dependent glycerol dehydratase to form 3-hydroxypropionaldehyde. This product is reduced to the major fermentation product 1,3-propanediol by the NADH-linked 1,3-propanediol dehydrogenase, which regenerates NAD1.

The dha regulon encodes the four essential enzymes of these two pathways. Amazingly, the expression of the dha regulon is only induced when glycerol is present.

Cells of *C. freundii* are also able to metabolize lactose or citrate as a carbon source.

2.3.3 Ecology

Citrobacter freundii are commonly found in the environment, mainly in soil, water, and sewages. They are an indicator of potential contamination of water. They are also found on different organs of diseased animals, including mammals, birds, reptiles, and amphibians (Wang *et. al.,* 2000). They are not known to interact with other organism.

In the environment, *C. freundii* can convert nitrate or the ammonium ion (which is a nitrogen atom combined with four hydrogen atoms) to nitrite; this reaction occurs in the environment as well as within the digestive tract of humans and other animals (Puchenkova, 1996). After it converts nitrate to nitrite in the environment, the nitrite is converted to nitrogen, and this final step completes the nitrogen cycle in the earth's atmosphere, which is made up of 85% nitrogen (Puchenkova, 1996). This organism's ecological role not only includes its important role in the nitrogen cycle, because it can also accumulate uranium (which is the basic material for nuclear technology) by building phosphate complexes.

Citrobacter freundii has also been investigated for biodegradation of tannic acid used in tannerys (Puchenkova, 1996).

2.3.4 Pathology

As an opportunistic pathogen, *Citrobacter freundii* is often the cause of significant opportunistic infections, meaning that it does not generally cause disease in healthy human hosts. They only affect patients with a weak immune system, signifying that they need an "opportunity" to infect the person (Whalen *et. al.,* 2007). Therefore, in patients with a suppressed immune system, *Citrobacter* species are known to cause a wide variety of nosocomial infections of the respiratory tract, urinary tract, and the blood (Whalen *et. al.,* 2007). Hepatic, biliary and pancreatic diseases are also common diseases that are caused by *C. freundii*. The biliary tract is the most common site of infection by the *C. freundii* bacilli (Marco Sanchez *et. al.,* 1985).

One fatal disease that *C. freundii* has been associated with is neonatal meningitis. Neonatal meningitis is the inflammation of the meninges (the system of membranes which surround the CNS) due to bacterial invasion (Julie *et. al.,* 1985). The mortality rate of *Citrobacter meningitis* is unacceptably high, with death rates of patients ranging from 25 to 50 %. Moreover, serious neurological problems still persist in 75% of survivors. In this disease, *Citrobacter freundii* is able to penetrate the blood-brain barrier that consists of the choroid plexus epithelium and the brain capillary endothelium (JulieL *et. al.,* 1985).

Tests performed by Badger *et. al.,* in the article "*Citrobacter freundii* Invades and Replicates in Human Brain Microvascular Endothelial Cells" suggest that bacterial proliferation of *C. freundii* takes place at the intracellular level, which had been contrary to the general scientific thought. The findings indicate that *C. freundii* traverses vacuoles, replicates and is released into the basolateral side of the human brain microvascular endothelial cells (HBMEC) in order to cross the blood-brain barrier. Further analysis may potentially allow for

therapeutic strategies to treat infections. There is still no therapeutic treatment available (Badger *et. al.,* 1999).

Certain diseases studied in trout and cyprinids are also caused by *C. freundii. C. freundii* causes abnormal inflammatory changes in the intestine of trout and inflammatory and necrotic changes in the internal organs of cyprinids. The illness was discovered by means of artificial infection with a pure culture of *C. freundii*. This discovery established *C. freundii* as a cause of fish disease (Drelichman *et. al.,* 1985).

In a case study by the Journal of Medical Microbiology, a patient developed peritonitis and tunnel infection due to *Citrobacter freundii* which is uncommon. The patient was on continuous ambulatory peritoneal dialysis. Usually the causing agents are Gram-positive micro-organisms, particularly *Staphylococcus aureus* and *Staphyloccus edpidermis*. Also there are no known reports of tunnel infection due to *C. freundii*. Initial antibiotic therapy did not work and the infection continued to persist until the catheter was removed. This is clinically significant because *Citrobacter freundii* show different antibiotic susceptibility which is why initial therapy was not successful. The patient did not respond to treatment until the catheter was removed showing *Citrobacter freundii* are opportunistic pathogens that affect hospitalized and immunocompromised patients (Dervisoglu *et. al.,* 2008).

2.3.5 Current Research

A small scale research concerning certain strains of *C. freundii* was done recently at the University of Tennessee, Knoxville. The importance of certain tetracycline and streptomycin resistance genes and class 1 integrons in *C. freundii* isolated from dairy farm soil and nondairy soils were evaluated. One strain of *C. freundii* extracted from dairy farm soils carried class 1 integrons with different inserted gene cassettes. Results of this small study suggested that

the presence of multiple resistance genes and class 1 integrons in *C. freundii* in dairy farm soil may act as a reservoir of antimicrobial resistance genes and could play a role in the dissemination of these antimicrobial resistance genes to other commensal and indigenous microbial communities in soil. However, additional longer-term studies conducted in more locations are needed to support this hypothesis (Srinivasan *et. al.,* 2007).

A second research concerning *C. freundii* was done in order to devise a polymerase chain reaction (PCR) method that simultaneously uses three pairs of specific primers to detect genes of certain microbes (including *C. freundii*). The method included designing three primer pairs which were: SPVC-1 and SPVC-2, INVA-1 and INVA-2; and VIAB-1 and VIAB-2. PCR was performed using these three primers to identify 14 clinically important bacterial organisms. The following strains were quickly identified using the PCR: (1) *C. freundii*; (2) *S. typhi*; and *S. paratyphi* C; (3) *S. dublin* (virulence antigen-positive); and (4) *Salmonella serovars* that harbor an spv-type virulence plasmid. Although this PCR method is new, with the advance of technology in the future this method can allow the identification of *C. freundii* in mammals immediately so that appropriate antibiotic treatment can be initiated without delay (Sanchez-cespedes and Vila, 2007).

A third study concerning *C. freundii* was done at the University of Barcelona, Spain. The mechanisms of resistance to fluoroquinolones in two *Citrobacter freundii* strains were studied. Both strains were isolated from the same patient. This study allowed partial characterization of the *acrA* and *acrB* genes of this microorganism. Expression of genes in both strains was analysed using DNA micro-arrays for *Escherichia coli*. Nucleotide similarity between the partially sequenced *acrA* and *acrB* genes of *C. freundii* and *E. coli* was 80.7% and 85%, respectively. The *acrA* and *acrB* genes of *C. freundii* are similar to those in *E. coli* and their over expression may play an important role in

modulating the final minimum inhibitory concentration of fluoroquinolones (Cigerci *et. al.,* 2007).

A fourth study concerning *C. freundii* was done in Taiwan. A team of researchers isolated a diabetic patient that developed necrotizing fasciitis which was caused by *C. freundii* from an injury incited by a marine animal. Necrotizing fasciitis is an infection within the deeper layer of the skin and subcutaneous tissues. When treating the patient they took a sample from the fluid in the wound and found *C. freundii*. After three days of starting the antibiotic treatments with cefotaxmine and cefepmine, there was accumulation of subcutaneous abscesses. After 6, 10, 14, and 21 days of tending to the patient, some antibiotics were giving the patient some relief, but no long term recovery was reached. Cefotoaxime, cefepime, ciprofloxamin are among the antibiotics that were no match for *C. freundii*. The patient fully recovered after 42 days of ertapenem treatment. The researchers had isolated two colonies of *C. freundii* within 5 days of each other. As the isolates were grown they became immensely resistant to cefotaxime and cefepime. The reason why entrapenem worked against *C. freundii* is because is active against *AmpC* producing *Enterobacteriaceae*.

2.3.6 Antibiotic resistance

Citrobacter species are a common cause of nosocomial infections associated with patients that are undergoing prolonged hospital treatments. *C. freundii* has recently been reported to express resistance to broad-spectrum antibiotics including piperacillin, piperacillin, tazobactam, vancomycin and cephalosporins. Isolation of ceftriaxone-resistant *Citrobacter freundii* (CRCF) has been associated with the over prescribed broad spectrum antibiotics. The emerging new CRCF strains could suggest induction or depression of resistance genes as well as elimination of competing organisms. CRCF has been mostly isolated from patients with significant comorbidities including AIDS,

peripheral vascular disease, and cerebrovascular disease. The usage of fluoroquinolone has also been reported to have no affect against the isolation of CRCF (Kim *et. al.,* 2003).

Citrobacter freundii is also known to contain in its chromosome a gene coding for cephalosporinase. This enzyme hydrolyses −CO−NH− bond in the lactam ring of cephalosporins and cephamycis thus rendering the bacteria resistant to this type of antibiotics. However, when exposed to new third generation cephems and cerbapenems, clinically isolated *C. freundii* showed sensitivity to those substances. A small outbreak of *C. freundii* resistant to third generation cephems has been observed in the surgical ward of Nagoya University Hospital in patients that underwent surgical procedures. The *C. freundii* was isolated from patient's bile, wound gauze, feces, pus, and ascites. It was suggested that these new strains of *C. freundii* contained a plasmid encoding *AmpC* celphalosporinase but upon failure to transfer the cephems resistance from *C. freundii* to *E. coli* it was concluded that the enzyme must be encoded in the chromosome of *C. freundii*. Since *C. freundii* is associated with nosocomial infections caution to this new strains is recommended (Nada *et. al.,* 2004).

2.3.7 Treatment:

Antimicrobial therapy is indicated in virtually all *Enterobacter* infections.

With few exceptions, the major classes of antibiotics used to manage infections with these bacteria include the beta-lactams, carbapenems, the fluoroquinolones, the aminoglycosides, and TMP-SMZ. Because most *Enterobacter* species are either very resistant to many agents or can develop resistance during antimicrobial therapy, the choice of appropriate antimicrobial agents is complicated. Consultation with experts in infectious diseases and microbiology is usually indicated. In 2006, Paterson published a good review of

resistance among various Enterobacteriaceae (Nada *et. al.,* 2004). Ritchie et. al. (2009) published a good discussion regarding antibiotic choices for infection encountered in the ICU (Zhang *et. al.,* 2011).

Newer options include tigecycline. Although not indicated specifically for *Enterobacter pneumonia* or bloodstream infections, tigecycline has excellent *in vitro* activity against these Gram-negative bacilli (Jacoby, 2009, HellerI *et. al.,* 2012, Deshpande *et. al.,* 2010). In one laboratory study of multidrug-resistant Gram-negative bacilli, tigecycline maintained a low MIC against all of the organisms. Older options might include intravenous administration of polymyxin B or colistin, drugs that are rarely used, even in large medical centers, and for which standard susceptibility criteria are not available. A study of 89 carbapenem non-susceptible *Enterobacteriaceae* isolates from China showed that polymyxin B was much more active than tigecycline (Bush *et. al.,* 1995).

Beta-lactams

With rare exceptions, *E. cloacae, E. aerogenes,* and most other *Enterobacter* species are resistant to the narrow-spectrum penicillins that traditionally have good activity against other *Enterobacteriaceae* such as *E. coli* (e.g., ampicillin, amoxicillin) and to first-generation and second-generation cephalosporins (e.g., cefazolin, cefuroxime). They also are usually resistant to cephamycins such as cefoxitin. Initial resistance to third-generation cephalosporins (e.g., ceftriaxone, cefotaxime, ceftazidime) and extended-spectrum penicillins (e.g., ticarcillin, azlocillin, piperacillin) varies but can develop during treatment. The activity of the fourth-generation cephalosporins (e.g., cefepime) is fair, and the activity of the carbapenems (e.g., imipenem, meropenem, ertapenem, doripenem) is excellent. However, resistance has been reported, even to these agents.

The bacteria designated by the acronym SERMOR-PROVENF (SER = *Serratia,* MOR = *Morganella,* PROV = *Providencia,* EN = *Enterobacter,* F = *freundii* for *Citrobacter freundii*) have similar, although not identical, chromosomal beta-lactamase genes that are inducible. With *Enterobacter,* the expression of the gene *AmpC* is repressed, but derepression can be induced by beta-lactams. Of these inducible bacteria, mutants with constitutive hyper-production of beta-lactamases can emerge at a rate between 10^5 and 10^8. These mutants are highly resistant to most beta-lactam antibiotics and are considered stably derepressed.

AmpC beta-lactamases are cephalosporinases from the functional group 1 and molecular class C in the Bush-Jacoby-Medeiros classification of beta-lactamases. They are not inhibited by beta-lactamase inhibitors (e.g., clavulanic acid, tazobactam, sulbactam). Ampicillin and amoxicillin, first- and second-generation cephalosporins, and cephamycins are strong *AmpC* beta-lactamase inducers. They are also rapidly inactivated by these beta-lactamases; thus, resistance is readily documented *in vitro* but may emerge rapidly *in vivo*. Jacoby (2009) recently published a good discussion about the emerging importance of *AmpC* beta-lactamases (Woodford *et. al.,* 2007).

Third-generation cephalosporins and extended-spectrum penicillins, although labile to *AmpC* beta-lactamases, are weak inducers. Resistance is expressed *in vitro* only with bacteria that are in a state of stable derepression (mutant hyper-producers of beta-lactamases). However, the physician must understand that organisms considered susceptible with *in vitro* testing can become resistant during treatment by the following sequence of events: (1) induction of *AmpC* beta-lactamases, (2) mutation among induced strains, (3) hyperproduction of *AmpC* beta-lactamases by mutants (stable derepression), and (4) selection of the resistant mutants (the wild type sensitive organisms being killed by the antibiotic).

For unknown reasons, extended-spectrum penicillins are less selective than third-generation cephalosporins. The in-therapy resistance phenomenon is less common with carboxy, ureido (e.g., piperacillin), or acylaminopenicillins. This phenomenon has been well documented as a cause of treatment failure with pneumonia and bacteremia; however, the phenomenon is rare with UTIs.

The fourth-generation cephalosporins are relatively stable to the action of *AmpC* beta-lactamases; consequently, they retain moderate activity against the mutant strains of *Enterobacter,* hyperproducing *AmpC* beta-lactamases.

Carbapenems are strong *AmpC* beta-lactamase inducers, but they remain very stable to the action of these beta-lactamases. As a consequence, no resistance to carbapenems, either *in vitro* or *in vivo*, can be attributed to *AmpC* beta-lactamases. However, *Enterobacter* species can develop resistance to carbapenems via., other mechanisms. The New Delhi metallo-beta-lactamase (NDM-1) has affected *Enterobacter* species around the world (Souli *et. al.,* 2008, Pintabo *et. al.,* 2008, Gupta *et. al.,* 2009).

The production of extended-spectrum beta-lactamases (ESβLs) has been documented in *Enterobacter*. Usually, these ESβLs are *TEM1* -derived or *SHV1* -derived enzymes, and they have been reported since 1983 in *Klebsiella pneumoniae, Klebsiella oxytoca,* and *E. coli*. Bush et al classify these ESβLs in group 2be and in molecular class A in their beta-lactamase classification. The location of these enzymes on plasmids favors their transfer between bacteria of the same and of different genera. Many other Gram-negative bacilli may also possess such resistant plasmids.

Among *Enterobacter* species, reports indicate that *E. aerogenes* has been the most common carrier of ESβL. Unlike the *AmpC* beta-lactamases, these enzymes are encoded by plasmid DNA and do not possess a molecular

mechanism of induction or stable derepression. They are inactivated by the beta-lactamase inhibitors and remain susceptible to cefoxitin (testing cefoxitin is then a useful tool to help differentiate *AmpC* beta-lactamases from ESβLs).

Bacteria-producing ESβLs should be considered resistant to all generations of cephalosporins, all penicillins, and to the monobactams such as aztreonam, even if the in vitro susceptibilities are in the sensitive range according to the CLSI breakpoints. In the past, the CLSI has cautioned physicians regarding the absence of a good correlation with susceptibility when its breakpoints are applied to ESβL-producing bacteria.

In 1999, the CLSI published guidelines for presumptive identification and for confirmation of ESβL production by *Klebsiella* and *E. coli,* guidelines that are often applied to other *Enterobacteriaceae*. From the above, one can conclude that, when a bacterium of the genus *Enterobacter* produces ESβL(s) (more than 1 ESβL can be produced by the same bacteria), it does so in addition to the *AmpC* beta-lactamases that are always present, either in states of inducibility or in states of stable derepression. With stable derepressed mutants, ESβL is almost impossible to detect unless molecular methods such as polymerase chain reaction (PCR) or isoelectric focusing (IEF) electrophoresis are used. For inducible strains, no recommendations have been issued by the CLSI for the detection of ESβL (i.e., if PCR and IEF electrophoresis are not readily available).

Carbapenems are the most reliable beta-lactam drugs for the treatment of severe *Enterobacter* infections, and fourth-generation cephalosporins are a distant second choice. The association of an extended-spectrum penicillin with a beta-lactamase inhibitor remains a controversial issue for therapy of ESβL-producing organisms.

Resistance to carbapenems is rare but has been reported and is considered an emerging clinical threat posed by *Enterobacter* species, as well as by other Enterobacteriaceae (Souli *et. al.,* 2008, Pintabo *et. al.,* 2008). The beta-lactamases first implicated in imipenem resistance were NMC-A and IMI-1, both molecular class A and functional group 2f carbapenemases, which are inhibited by clavulanic acid and then able to hydrolyze all the beta-lactams not associated with a beta-lactamase inhibitor.

Hyperproduction (stable derepression) of *AmpC* beta-lactamases associated with some decrease in permeability to the carbapenems may also cause resistance to these agents. *In vitro* low-level ertapenem resistance was not associated with resistance to imipenem or meropenem, but high-level ertapenem resistance predicted resistance to the other carbapenems (Gales *et. al.,* 2006) Metallo-beta-lactamases cause resistance across the carbapenem class, are transmissible, and have been associated with clinical outbreaks in hospitals worldwide. In one reported outbreak of 17 cases of infection (2 due to *Enterobacter* species), molecular studies demonstrated presence of a gene belonging to bla (VIM-1) cluster (Walkty *et. al.,* 2009) KPC-type carbapenemases have emerged in New York City. The new NDM-1 carbapenemase has already rapidly spread to many countries (Keevil and Hough, 1997).

Aminoglycoside resistance is relatively common and varies widely among centers. As with other members of *Enterobacteriaceae*, this resistance results from the production of different aminoglycoside-inactivating enzymes. Resistance to fluoroquinolones is relatively rare but may be high in some parts of the world.

Resistance to TMP-SMZ is more common. Colistin and polymyxin B: These drugs are being used more frequently to treat serious infection caused by multidrug-resistant organisms, sometimes as monotherapy or in combination with

other antibiotics. Clinical experience, including documentation of success rates and attributable mortality is broadening (Hawser *et. al.,* 2009, Bassetti *et. al.,* 2007). Heteroresistance to colistin was demonstrated in a few *Enterobacter* isolates collected from ICU patients and was best identified using broth microdilution, agar dilution, or E-test methods (Yang *et. al.,* 2010). Polymyxin B was not as active against *Enterobacter* species as it was against other *Enterobacteriaceae* but did demonstrate an MIC50 of less than or equal to 1, with 83% of *Enterobacter* isolates considered susceptible (Abbott and Janda, 1997). One recent *in vitro* study documented a colistin MIC90 of 2 mcg/mL or less in more than 90% of *Enterobacter* isolates from Canada (Alhambra *et. al.,* 2004).

Chapter-III

Materials and Methods

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MATERIALS AND METHODS

MATERIALS

Media:

1) Peptone Broth:

Peptone	-	5 grams
Distilled water	-	1000 ml
pH	-	7.2

2) Brain heart infusion media (pH 7.4)

Infusion from calf brain	-	200 g
Infusion from beef heart	-	250 g
Peptone	-	10 g
Disodium phosphate	-	2.5 g
Sodium chloride	-	5.0 g
Dextrose	-	2.0 g
Agar	-	1.0 g
Distilled water	-	1000 ml

3) Nutrient Broth (pH 7.2)

Peptone	-	5 gms
Beef Extract	-	3 gms
Sodium Chloride	-	3 gms
Distilled water	-	1000 ml

4**) Nutrient agar Media (pH 7.2)**

Peptone	-	5 gms
Beef Extract	-	3 gms
Sodium Chloride	-	3 gms
Distilled water	-	1000 ml
Agar	-	16 g

5) Mac Conkey's Agar Media (pH 7.1)

Peptone	- 20 g
Sodium chloride	- 5 g
Bile salts	- 1.5 g
Lactose	- 10 g
Neutral red solution (1% aqueous)	- 10 ml
Crystal violet	- 0.001 g
Distilled water	- 1000 ml
Agar	- 16 g

6) Blood Agar Media (pH 7.3)

Infusion from beef heart	- 500 g
Tryptone	- 10 g
Sodium chloride	- 5 g
Distilled water	- 1000 ml
Agar	- 15 g

Dissolve all the ingredients and autoclave at 121^0C for 15 min. cool to 45-50^0C and add 50 ml of sterile defibrinated sheep blood, aseptically. Rotate to mix thoroughly, avoiding accumulation of air bubbles and pour immediately into sterile tubes or plates i.e., before solidification.

7) Citrate Agar Media (pH6.8)

Magnesium sulphate	- 0.2 g
Sodium chloride	- 5 g
Ammonium dihydrogen phosphate	- 1 g
Dipotassium hydrogen phosphate	- 1 g
Sodium citrate (citric acid)	- 2 g
Bromothymol blue	- 0.08 g
Distilled water	- 1000 ml
Agar	- 20 g

8) Eosin Methylene Blue Agar Media (EMB) (pH 7.2):

Peptone	- 10 g
Lactose	- 5 g
Sucrose	- 5 g
Disodium hydrogen phosphate	- 2 g
Eosin Y	- 0.4 g
Methylene blue	- 0.065 g
Agar	- 15 g
Distilled water	- 1000 ml

9) MR-VP Broth (pH 6.9):

Peptone	- 7 g
Potassium phosphate	- 5 g
Dextrose	- 5 g
Distilled water	- 1000 ml

10) Muller's Hinton's Agar Meida (pH 7.4)

Beef infusion	- 300 g
Casamino acids	- 17.5 g
Starch	- 1.5 g
Agar	- 17 g
Distilled water	- 1000 ml

METHODS:

3.1 Selection of individuals:

For the isolation of lactose fermenting *Enterobacteriaceae* from the clinical and different laboratory samples and before that selection of individuals is very important because we have selected different age groups and from that different sex groups also we categorized. It is very important aspect to study and also distinguish the one individual to other individuals.

3.2) Collection of clinical samples:

Isolation and identification of *Enterobacteriaceae* species from different clinical laboratory amples. Collection of clinical samples: Collect the different clinical samples like Urine, pus, sputum, blood, faecal matter, etc from different clinical laboratories and hospitals twice or trice a week and transport he samples to lad with transport media and kept for incubation. Isolation of lactose fermenting Enterobacteriaceae: Collected samples are incubated and after incubation inoculated in to the separate culture media plates with different media. kept for incubation then observe the colonies on the media some get lactose fermenting colonies and they are very small and pink in colour and non lactose fermenting colonies are colourless. Further studies are carried out by using other morphological, staining and Biochemical methods are used for identification.

3.3) Enrichment of samples:

In this study collected samples are enriched by using initially samples are added to some broth media for proper growth as pre innoculum and kept for incubation period after incubation all the cultures are transferred to the different media for the isolation of lactose fermenting Enterobacteriaceae.

3.4) Isolation of Lactose fermenting Enterobacteriaceae:

All the collected and enriched samples are using for the isolation of lactose fermenting Enterobacteriaceae by different culture media like Nutrient agar, MacConkey agar, EMB agar etc and after proper growth occurs on the media, further used for morphological, cultural and biochemical characteristics.

3.5) Cultural characteristics:

MacConkey's Agar media:

This media is selective media for the growth of the lactose fermenting organisms in some times contaminating organisms also grows so, further identification is essential.

Screening of lactose fermenting organisms: Further screened lactose fermenting organisms by using different biochemical studies like sugar fermentation, IMVIC tests.

Lactose fermenters are divided into two groups on the basis of indole test:

i) Indole positive group of lactose fermenters

ii) Indole negative group of lactose fermenters

i) Indole positive group includes: *E. coli*, *Citrobacter* species (other than *Citrobacter freundii*) *Klebsiella oxytoca*. These further differentiate on the basis of their ability to grow on Simmons citrate medium, Urease and indole test.

ii) Indoel Negative group includes: *Citrobacter freundii, klebsiella pneumonia* and *Enterobacter* species, enterobacter are differentiate from other by H_2S production in iron agar. *Klebsiella* and *Enterobacter* are differentiated by growth in Simmons citrate medium and urease test.

3.6) Biochemical characteristics for the isolation of lactose fermenting enterobacteriacece:

i) Indole Production test:

Tryptophan, an essential amino acid, is oxidized by some bacteria by the enzyme tryptophan resulting in the formation of indole, pyruvic acid and ammonia. The indole test is performed by inoculating a bacterium into tryptone broth, the indole produced during the reaction in detected by adding Kovac's reagent (dimethylaminobenzaldehyde) which produces a cherry-red reagent layer.

ii) MR-VP test (Methyl red and Yoges-Proskauer test)

The methyl red (MR) and the Yoges-Proskauer (V-P) tests are used to differentiate two major types of facultatively anaerobic entric bacteria that produce large amounts of acid and those that produce the neutral product acetoin as end product. Both these are performed simultaneously because they are physiologically related and are performed on the same medium MR-VP broth. Opposite results are usually obtained for the methyl red and Yoges–Proskauer tests, i.e., MR+, VP- or MR- , VP+.

MRVP tests are of value in the separation of *Escherichia coli* and *Enterobacter aerogens* (both coliform bacteria) which appear virtually identical except for certain physiological differences that are used as indicators of the sanitary quality of water, food, food productions and eating establishments.

iii) Citrate Utilization Test: Citrate test is to differentiate among enrtic bacteria on the basis of their ability to utilize ferment citrate as the sole carbon source. The utilization of citrate depends on the presence of an enzyme citrase produced by the organisms that breaks down the citrate to oxaloacetic acid and acetic acid. These products are later converted to pyruvic acid and carbon dioxide.

The citrate test is performed by inoculating the microorganisms into an organic synthetic medium, Simmon's citrate agar, where sodium citrate is the only source of carbon and energy. Bromothymol blue is used as an indicator. When the citric acid is metabolized, the CO_2 generated combines with sodium and water to form sodium carbonate an alkaline product, which changes the colour of the indicator from green to blue and this constitutes a positive test.

iv) Urease test:

Urea is a major organic waste product of protein digestion in most vertebrates and is excreted in the urine. Some microorganisms have the ability to produce the enzyme urease. The urease is a hydrolytic enzyme which attacks the carbon and nitrogen bond amide compounds (e.g., urea). With the liberation

of ammonia it is a useful diagnostic test for identifying bacteria, especially to distinguish members of the Enterobacteriaceae.

This test is performed by growing the test organisms on urea broth or agar medium containing the pH indicator phenol red during incubation, microorganisms possessing urease will produce ammonia that raises the pH of the medium/broth. As the pH becomes higher, the phenol red changes from a yellow colour to a red or deep pink colour.

v) Hydrogen sulphide production:

Hydrogen sulphide (H_2S) commonly called 'rotten egg' gas because of the copious amounts liberated, when eggs decompose, by certain bacteria, such as *Proteus vulgaris*, *E. coli* through reduction of sulphur containing amino acids (e.g., cystein, cysteine and methionine) or through the reduction of inorganic sulphur compounds such as thiosulfates, sulfates or sulfites blackening of the culture medium will be seen in *Enterobacteriaceae* species which are positive test for H_2S production.

3.7) Antimicrobial susceptibility pattern:

Totally all isolates are subjected to test antimicrobial susceptibility test. Our objective is to study resistance to the commonly used conventional antibiotics in this area. Penicillin, ampicillin, amoxicillin, gentamycin, tetracycline, erythromycin, methicillin and vancomycin the disc diffusion method for *in vitro* antibiotics susceptibility test Kirby – Bauer was used in the study.

Chapter-IV

Results and Discussion

CHAPTER - VI
RESULTS AND DISCUSSION

4.1 Isolation and Characterization of Lactose Fermenting *Enterobacteriaceae* and Incidence of Intestinal Gastroenteritis in Gulbarga Region:

The present investigation was carried out during the period from November 2010 – November 2012. During this period, the Gulbarga region has witnessed several intestinal gastroenteritis outbreaks. Efforts were made to collect the samples from the patients affected during these outbreaks. In addition, samples from patients suffering from diarrhea and other intestinal infections and admitted to hospitals also formed the basis for this study. Overall a total 1500 samples (850 males and 650 females) from patients affected individuals formed the source of the samples. The age and sex wise distribution of these subjects is indicated in the Table – 4.1 and Figure - 1.

The sex wise incidence rate of lactose fermenting Enterobacteriaceae indicated a significantly higher incidence in small children and adults are more sufferer than the middle age and more lactose fermenting Enterobacteriaceae strains isolated from the adult females than males.

The incidence of lactose fermenting Enterobacteriaceae organisms ranged from a minimum of 10.3% in the age group of below 5 years to a maximum of 10.5% in the age group of 44-55 years. The age groups of young 5-15 years and middle aged 35-45 years recorded an incidence of Gram negative Enterobacteriaceae higher than the average incidence.

The age wise distribution of isolation rate indicated a slightly different trend in males and females. In case of males its ranges from a minimum of 80% in the age group of 25-30 years to a maximum of 11.5% in the age group of 45-50 onwards. However in both males and females the age groups of 5-10 and 31-45 years and in females the age group 25-35 years recorded. Lactose fermenting Enterobacteriaceae incidences higher than their respective averages in addition to the age group, this is recorded the highest incidence in each sex.

Table – 4.1: Age and sex distribution of patients suffering from *Enterobacteriaceae* infections

Sl. No	Age	Males	Females	Total
1	Neonates (0-28 days)	02	05	07
2	Pediatrics (1-10)	25	40	65
3	Teenage (11-20)	60	80	140
4	Adults (21-30)	75	98	173
5	Middle age (31-40)	40	20	60
6	Late middle (41-50)	20	25	45
7	Old age (50 onwards)	25	35	60

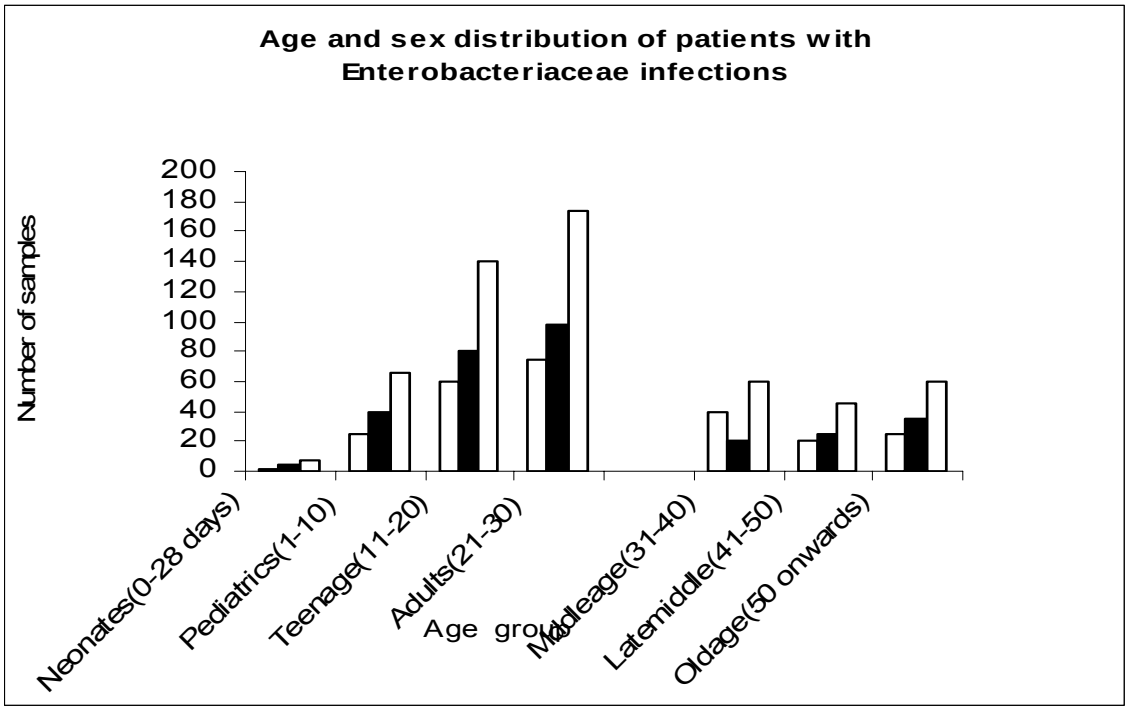


Fig. – 4.1: Age and sex distribution of patients suffering from *Enterobacteriaceae* infections

Overall observations showed higher incidence of lactose fermenting Enterobacteriaceae among females (20%) than males (16.5%) with the highest incidence, the age wise distribution of lactose fermenting Enterobacteriaceae incidences indicated that the age groups late middle 41-55 and 55 onwards year both in males and females in more vulnerable for Urinary tract infections, followed by the age group 15-20 teenage years. significantly the lowest incidence of lactose fermenting Enterobacteriaceae Urinary tract infection in both males and females is recorded in the young children's below the age group of 1-10 years, closely followed by the incidence in older people above 55 years of age. This observation is in the country to the reports that the young children's and old aged people are more susceptible for gastroenteritis, Urinary tract infections, diarrhea and enterotoxins infections. This observation suggests that majority of the Urinary tract infection, diarrhea infections recorded in these vulnerable age groups (more than 5 and above 55) are caused by ethiological agents other than *E. coli, Klebsiella*. This inference is strengthened when the observations recorded in a parallel study conducted during the same period and using almost the same samples on the incidence of toxigenic *Escherichia coli* (STEC) are taken into consideration. In this investigation, STEC are (Shigo toxic producing *Escherichia coli*) has been recorded as one of the major ethological agent of gastroenteritis in this region, out of the nearly 600 patients screened in only one occasion both *V. cholera* and STEC has been isolated together from the same sample.

In addition, samples were collected from other sources such as human urine samples, pus samples, stool samples, watery stools, blood samples, sewage samples and serum samples etc. (Table – 4.2 and Figure 4.2).

Table – 4.2: Incidence of lactose fermenting Enterobacteriaceae in different samples

Sl. No.	Samples		No. of isolates	% of isolation
1	Faecal matter	Sample L.F.E.	400 35	09
2	Urine sample	Sample L.F.E.	150 20	10
3	Blood Sample	Sample L.F.E.	200 25	12.5
4	Water sample	Sample L.F.E.	100 12	10.0
5	Serum sample	Sample L.F.E.	200 23	11.12
6	Sewage sample	Sample L.F.E.	100 12	12.0
7	Pus Intestinal	Sample L.F.E.	50 05	10.0

L.F.E –Lactose fermenting Enterobacteriaceae

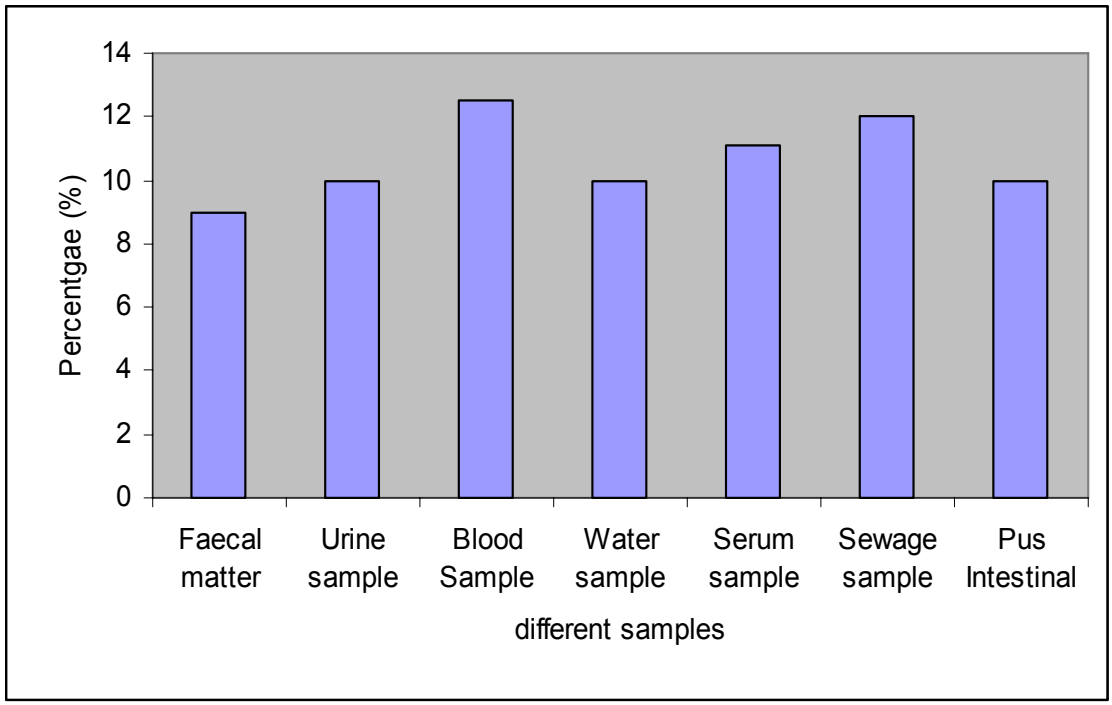


Fig. – 4.2: Sample wise incidence of Lactose fermenting Enterobacteriaceae

During the period of this investigation regular outbreaks of lactose fermenting Enterobacteriaceae infections and are common in all the seasons were reported from different places of clinical laboratories stools, urine, blood, sputum, sewage, pus sample, serum sample were collected from the clinical laboratories of the patient samples.

The different samples and sample wise incidence of lactose fermenting Enterobacteriaceae and these samples from different diagnostic laboratories in this region and maximum number organisms isolated form blood samples and sewage samples (12.2% and 11.5%) because these bacteria is contacted by the ingestion of contaminated water or food and is associated with poor hygienic and inadequate sanitation as a result these Enterobacteriaceae are endemic mainly in most of the developing countries in the world. These two samples are very good habitats of *Enterobacteriaceae* like *E. coli, Klebsiella*, *Coilforms* etc so, most of the lactose fermenting Enterobacteriaceae strains isolated from this samples. Next to these samples are serum and Intestinal diarrhaeal samples and the samples also containing more number lactose fermenting Enterobacteriaceae especially in this samples nearly (11.2% and 10.1%). And other samples also containing in the most of the developing countries contaminated foods, especially under cooked seafood is the usual vehicle for transmission, while contaminated water is more pronounced in the developed countries (Glass *et. al.,* 1991; Shapira *et. al.,* 1999). Most of the infections through different sources and outbreaks of intestinal, diarrhaeal and gastroentritis infections cause deaths estimated around 1,20,000 annually world wide and large number of cases reported every year of which vast majority occur in children's (WHO, 1995).

Isolation and characterization of lactose fermenting Enterobacteriaceae from different samples indicated that Enterobacteriaceae in this *E. coli* is the predominately present in the sewage samples and closely followed in the

intestinal discharge from the patients suffering from gastroenteritis. *E. coli* and Coliform strains have been frequently isolated from certain extra intestinal infections but are rarely isolated from others. As per the records of the sources of lactose fermenting Enterobacteriaceae are often isolated from blood and Urinary tract infections and rarely from wounds of arms, legs, infected eyes and ears, but they can occasionally occurs.

Lactose fermenting Enterobacteriaceae has been found to be well adapted to the human intestinal tract. As per the report majority of the lactose fermenting Enterobacteriaceae isolates are from human faecal or intestinal samples. Significant incidence of coliforms and *E. coli* have been recorded in water and sewage samples, the food samples and other animal sources are very low incidence of lactose fermenting Enterobacteriaceae. The observation of the present investigation to some extent agree with report in that majority of the lactose fermenting Enterobacteriaceae isolates are from human intestine, sewage and water samples.

The isolation, incidence and characterization of Enterobacteriaceae from these samples are presented in this section.

a) Microscopic Characteristics:

All the isolates were subjected for microscopic observation for motility and gram's staining. The results are presented in Plate 4.1 – 4.6 some of the isolates showing characteristic motility and some of them are non-motile and it is one of the very important characters to distinguish the motile and non-motile lactose fermenting Enterobacteriaceae.

When subjected for motility test also indicated the presence of highly motile bacteria showing darting motility characteristics and some of the Enterobacteriaceae are non–motile and few are very active like a *E. coli* organisms. Gram's staining indicated the presence of Gram negative small rods and some of them are Gram-negative big rod shaped and most of the

Enterobacteriaceae are Gram negative rods, which are non-spore forming and some of them are capsulated.

b) Cultural Characteristics:

All the 1500 different samples collected from patients suffering from gastroentertitis and other infections were processed for the isolation of lactose fermenting Enterobacteriaceae using various cultural media. The cultural characteristics of the lactose fermenting organisms observed on different cultural media are as follows:

On Nutrient broth: Different samples collected aseptically bring to the laboratory and inoculated on nutrient broth for few hours for incubation after incubation then transfer to the solid culture media. Further studies, if growth occurs the broth media turns to turbidity.

On Peptone broth: It is also using as a transport media and if aseptically inoculate the collected samples into this peptone broth and then bring to the laboratory for further transfer or incubation and it is a liquid media and cells are very active in this broth.

On Nutrient Agar: After incubation in the broth the samples are transfer into the Nutrient agar media and colonies were circular, small, 1-3 mm in diameter, pale moist, raised, translucent with colourless colonies appears after 18-24 hours incubation.

On MacConkey's agar: collected samples are inoculated on to the MacConkey agar media colonies were circular, small, lactose fermenting pink coloured, 1-2 mm in diameter, translucent and some Enterobacteriaceae colonies are colourless because they are non-lactose fermenting organisms so, this media is very important to screen the lactose and non lactose fermenting Enterobacteriaceae.

Plate – 4.1: Lactose fermenting Macconkey agar

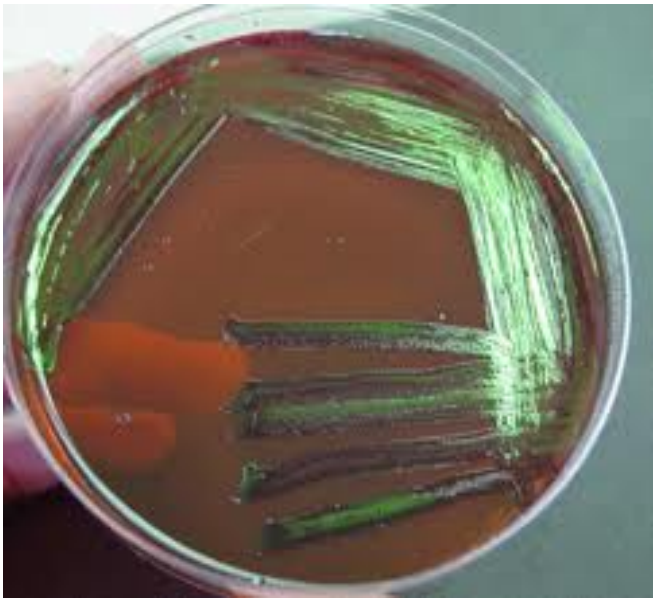


Plate – 4.2: Lactose fermenting organisms on EMB agar

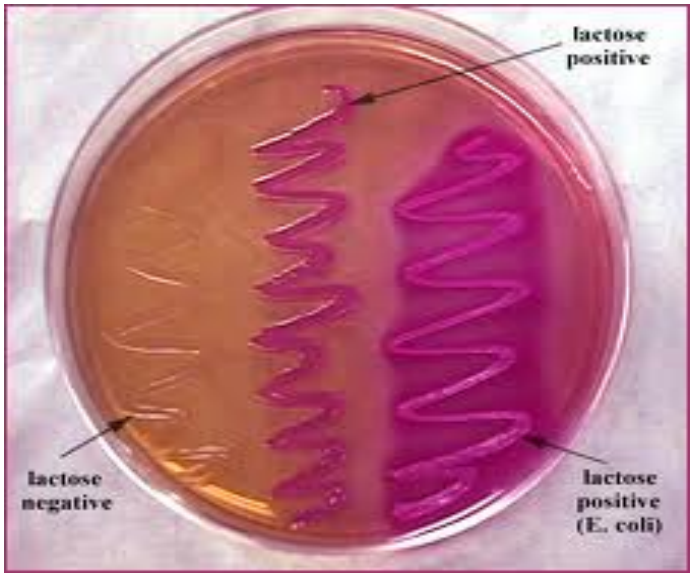


Plate – 4.3: Lactose fermenting positive and lactose fermenting negative Enterobacteriacece

Plate – 4.4: Lactose fermenting strains are pink in colour

Plate – 4.5 a): Lactose fermenting Enterobacteriacece on Blood agar media (*Klebsiella* Spp)

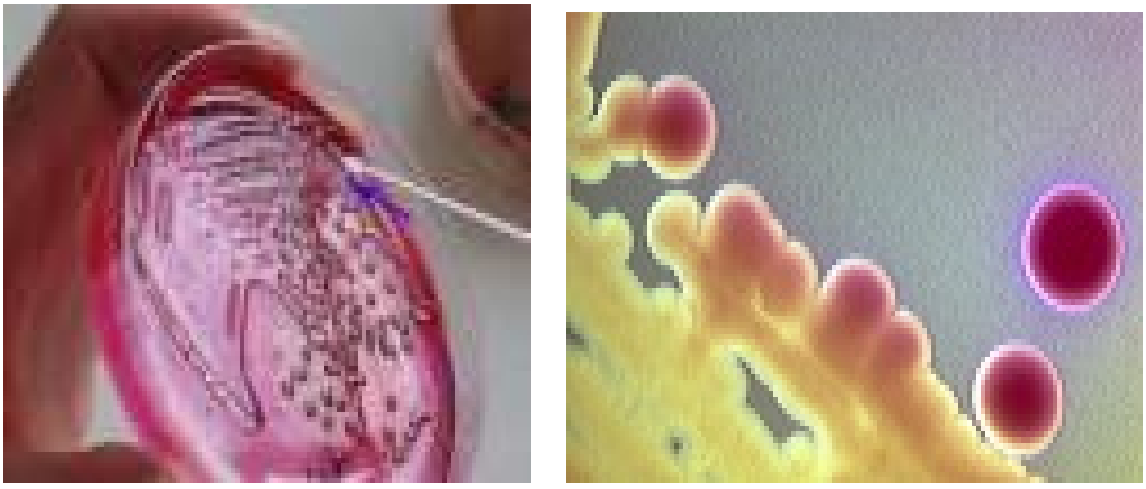
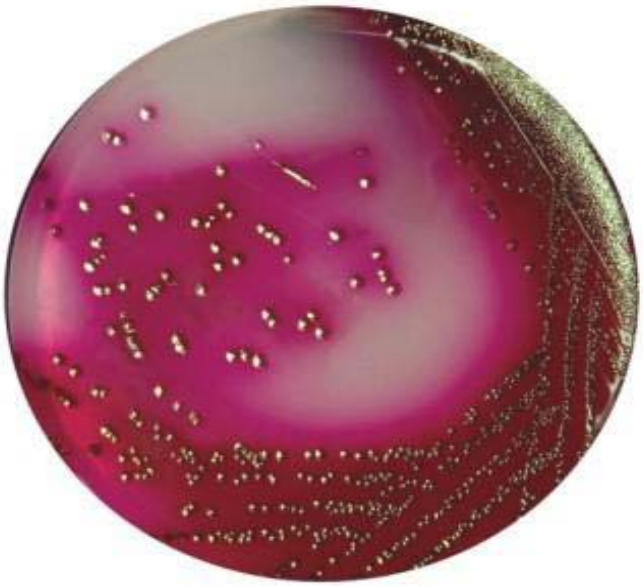
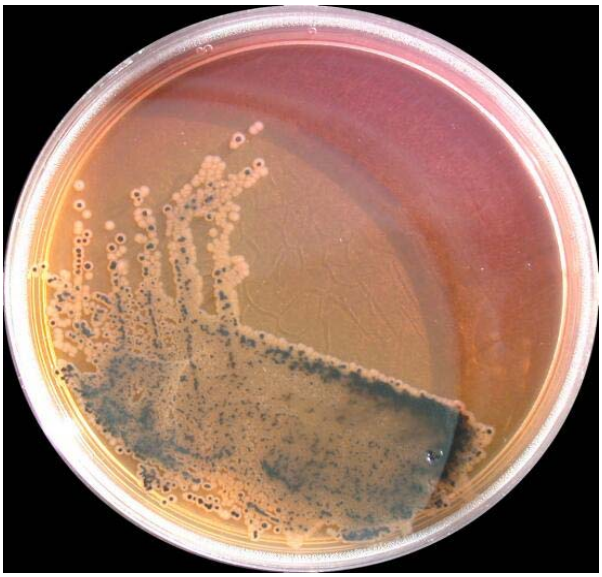


Plate – 4.5 b): *Klebsiella* Spp.



a) *E. coli*



b) *Citrobacter*

Plate – 4.6 (a) & (b): Lactose fermenting Enterobacteriacece on MacConkey's agar media

c) Biochemical characteristics:

IMVIC Tests:

IMVIC tests are the most widely used and preliminary biochemical tests very important tests for the differentiation of Enterobacteriaceae members. IMVIC includes for important tests for all the isolates were tested individually because these tests to differentiate some species like *E. coli, Klebsiella* and other members of the lactose fermenting Enterobacteriaceae (Table – 4.3 and Figure 4.7 to 4.12).

The lactose fermenting are divided into two groups on the basis of indole test- Indole positive lactose fermenters and indole negative lactose fermenters.

E. coli - I + U + C -
K. oxytoca - I + U + C +
C. koseri - I + U + C +

i) Indole positive group includes:

E. coli, Citrobacter sp*. Klebsiella oxytoca*. These can be further differentiation on the basis of their ability to grow on Simmonsons citrate medium (C) Urease (U) and indole (I). Urease +ve strains of *Citrobacter koseri* can be differentiated from *Klebsiella oxytoca* on the basis of a group simple test like motility. *Citrobacter* are motile whereas *K. oxytoca* are non motile if it is in doubt capsular staining can be resorted to or methyl red (MR) test can be used but it will take 48 hrs for confirmation.

Table – 4.3: Biochemical characterization of lactose fermenting *Enterobacteriaceae*

Test	*E. coli*	*Klebsiella*	*C. koseri*	*E. cloacae*	*C. fereundii*	*K. oxy*
Indole	+	-	+	-	+	-
Methyl red	+	-	-	-	+	+
V-P test	-	+	-	+	-	+
Citrate	-	+	+	+	+	+
Lactose	+	+	+	+	+	+
Glucose	+	+	+	+	+	+
Mannitol	+	+	+	+	+	+
H_2S gas	-	-	-	-	+	-
Urease	-	-	+	+	-	+
Nitrate Red	-	-	+	+	-	-

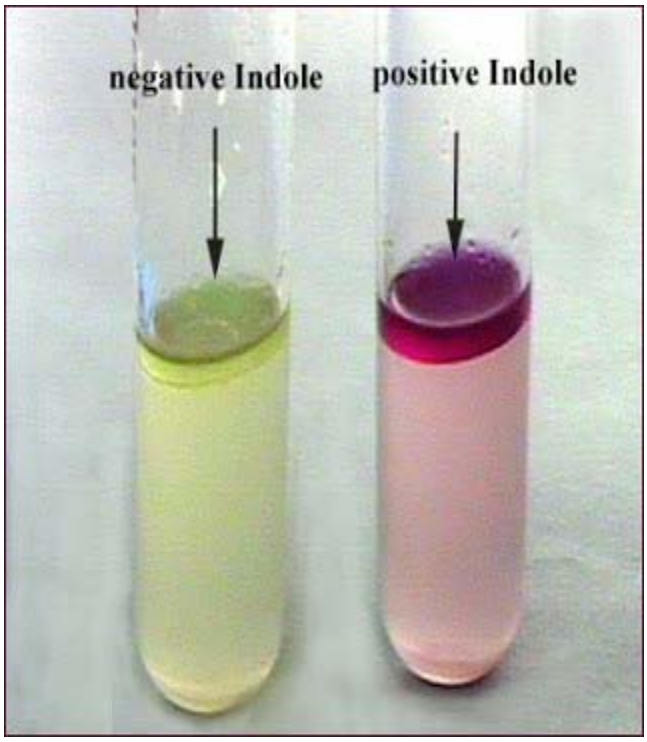


Plate – 4.7: Indole test

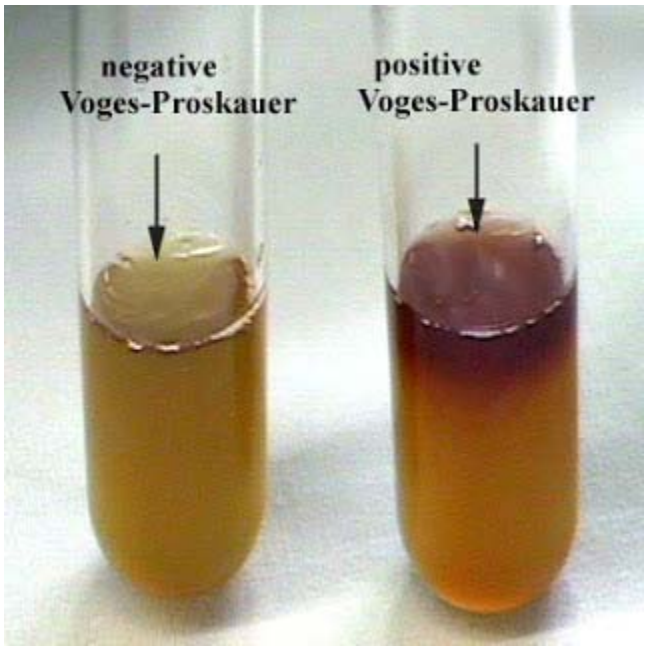


Plate – 4.8: Voges Praskauer test

Plate – 4.9: Citrate Utilization Test

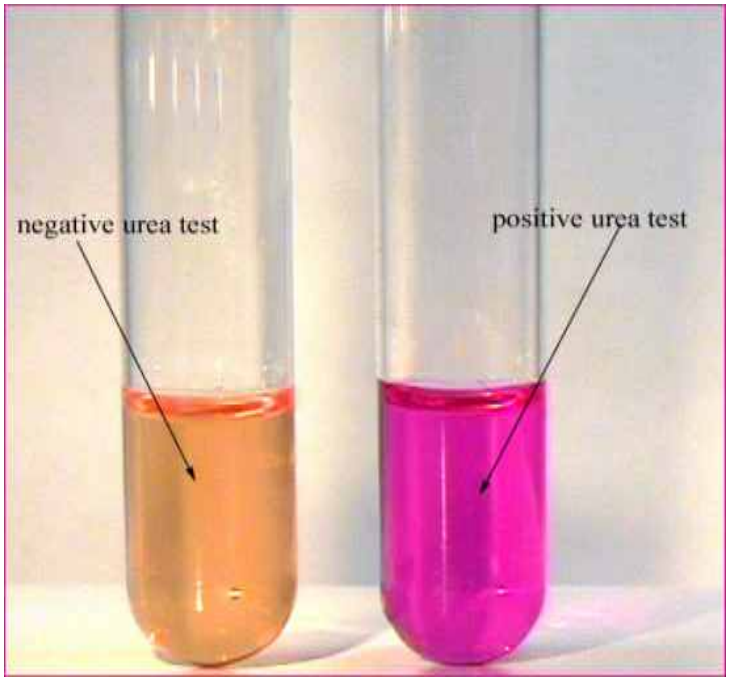


Plate – 4.10: Urease Test

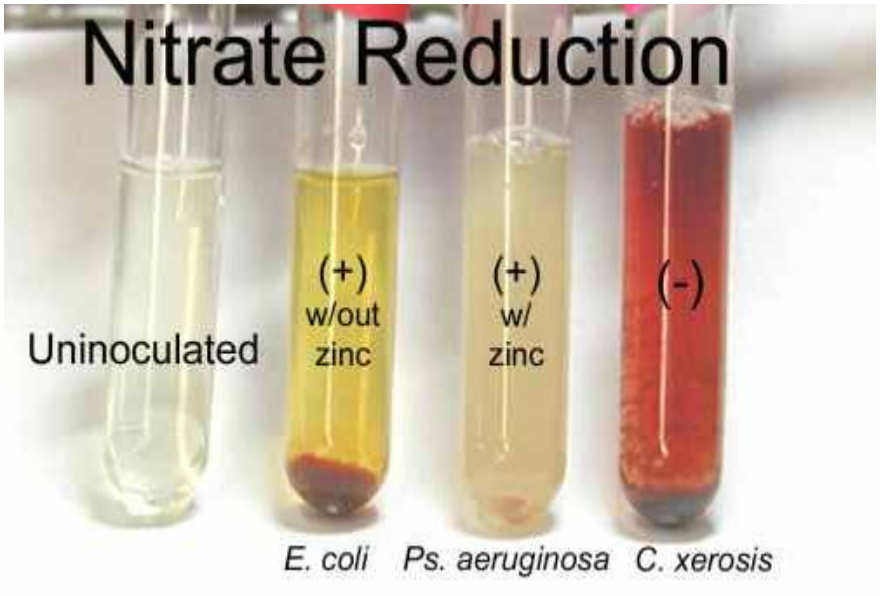


Plate – 4.11: Nitrate Reductions Test

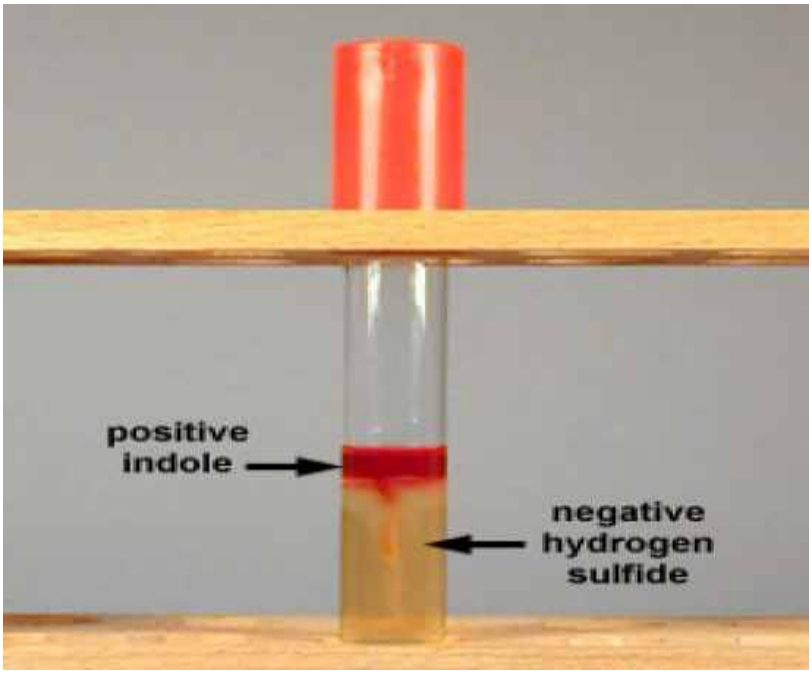


Plate – 4.12: Lactose fermenting negative hydrogen sulphide and indole Positive

ii) Indole negative group includes:

Citrobacter freundii, *Klebsiella pneumonia*, *Enteobacter* species. *Citrobacter freundii* can be differentiated from others by H_2S production in Klinger iron agar (KI) *Klebsiella* and *Enterobacter* are differentiated by growth in Simmons citrate medium and Urease test.

Citrobacter freundii - I + U -/+ C +/-
H_2S

Enterobacter aerogenes A/A in the gas - I – U - C +

Klebsiella pneumonia A/A with gas - I – U + C +

Enterobacter cloacae - I - U + C +

Urease positive strains of *E. cloacae* are differentiated from *Klebsiella* with the help of motility, when necessary urease negative strains of *E. cloacae* can be differentiated from *E. aerogens* on the basis of lysine and ornithine decarboxylase tests. *E. aerogenes* with decarboxylate lysine and *E. cloacae* with decarboxylate orginine.

4.2. Incidence of lactose fermenting Enterobacteriaceae:

a) Location wise incidence of lactose fermenting Enterobacteriaceae:

Samples were collected from different clinical diagnostic and hospital laboratories from different samples are collected major from faecal matter, urine and blood samples. The incidence rate of lactose fermenting Enterobacteriaceae depends on the seasons and maximum is 6.75- 9.0%. The incidence rate was highest (9%) in the samples collected from the rainy seasons because mainly contaminated food and water. The overall average rate of lactose fermenting Enterobacteriaceae incidence was 8.8% - 9%.

In our study we collected more than 1200 samples for this study and more strains are isolated from stool, urine, blood and more strains are contaminated with lactose fermenting Enterobacteriaceae compare with other samples.

b) Incidence of lactose fermenting Enterobacteriaceae in different seasons:

Total samples collected for this study to isolate the lactose fermenting Enterobacteriaceae mainly it depends on the different seasons. Different seasons having variation in the incidence mainly in the rainy season most of the people suffering from this infection especially food and water contamination through faecal matter these lactose fermenting Enterobacteriaceae easily contaminate and cause the different types of infections, so in this season incidence rate is maximum when compare with the winter and summer seasons. Incidence rate in the winter is very less when compare with the summer as well as rainy. In summer also incidence rate is high because the food and water scarcity and food spoilages due to high temperature especially in this region and extremely very high temperature nearly 48^0C (Figure – 4.3).

In the present investigation 65-70 lactose fermenting Enterobacteriaceae isolates have been collected from different gastroenteritis and Intestinal infected patients indicating lactose fermenting Enterobacteriaceae incidence of 10% which is almost the light variation in different seasons as recorded. This suggests that the incidence of lactose fermenting Enterobacteriaceae in this region has some changes during the last two decades. The records maintained the total incidence of lactose fermenting Enterobacteriaceae based on the number of cases reported every year also strength this view. It has also observed that lactose fermenting Enterobacteriaceae infections recure at regular intervals every year, reaching their peak during the period from March to May and July to September.

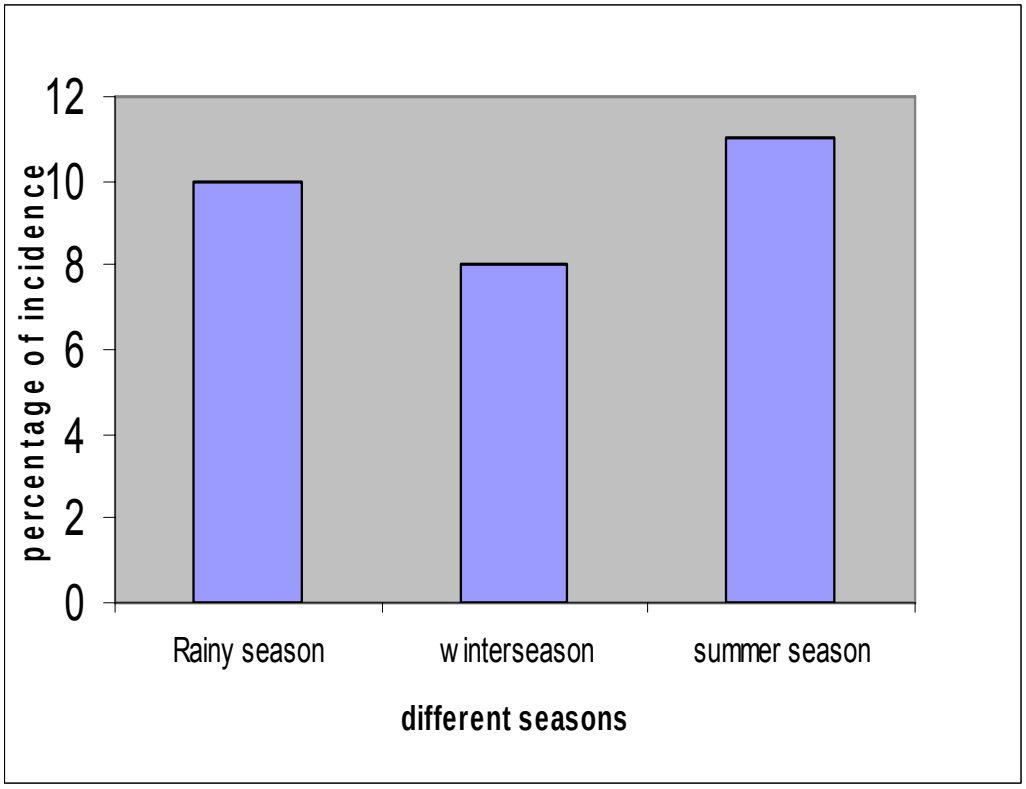


Figure – 4.3: Incidence of lactose fermenting Enterobacteriaceae in different seasons

c) Year wise incidence of lactose fermenting Enterobacteriaceae:

The overall year wise incidence of lactose fermenting Enterobacteriaceae in different samples in the given in Figure – 4.4.

There was not a much significant differences in the year wise incidence of lactose fermenting Enterobacteriaceae during the year 2011 to 2012. The highest incidence (11.5%) was recorded during 2011, where as out of the 250 samples collected as many as 40 were positive for lactose fermenting Enterobacteriaceae. The lowest incidence was observed during the year 2012 and 250 samples out of 25 were positive for lactose fermenting Enterobacteriaceae.

The incidence of lactose fermenting Enterobacteriaceae among the intestinal infections, gastroenteritis and diarrhaeal cases are on the gradual declining trend as per our incidence during 2011& 2012.

Different location and hospital samples & different centres surveyed, where lactose fermenting Enterobacteriaceae is epidemics were frequently reported during the year 2011-2012, lactose fermenting Enterobacteriaceae were found to be associated with 11.5% of these Urinary tract infection, diarrhea and pyogenic infections. Out of the different locations the highest incidence of lactose fermenting Enterobacteriaceae 11.5%, while the lowest 8.5% incidence. In the remaining different samples of lactose fermenting Enterobacteriaceae ranged from about 10% of to 11%.

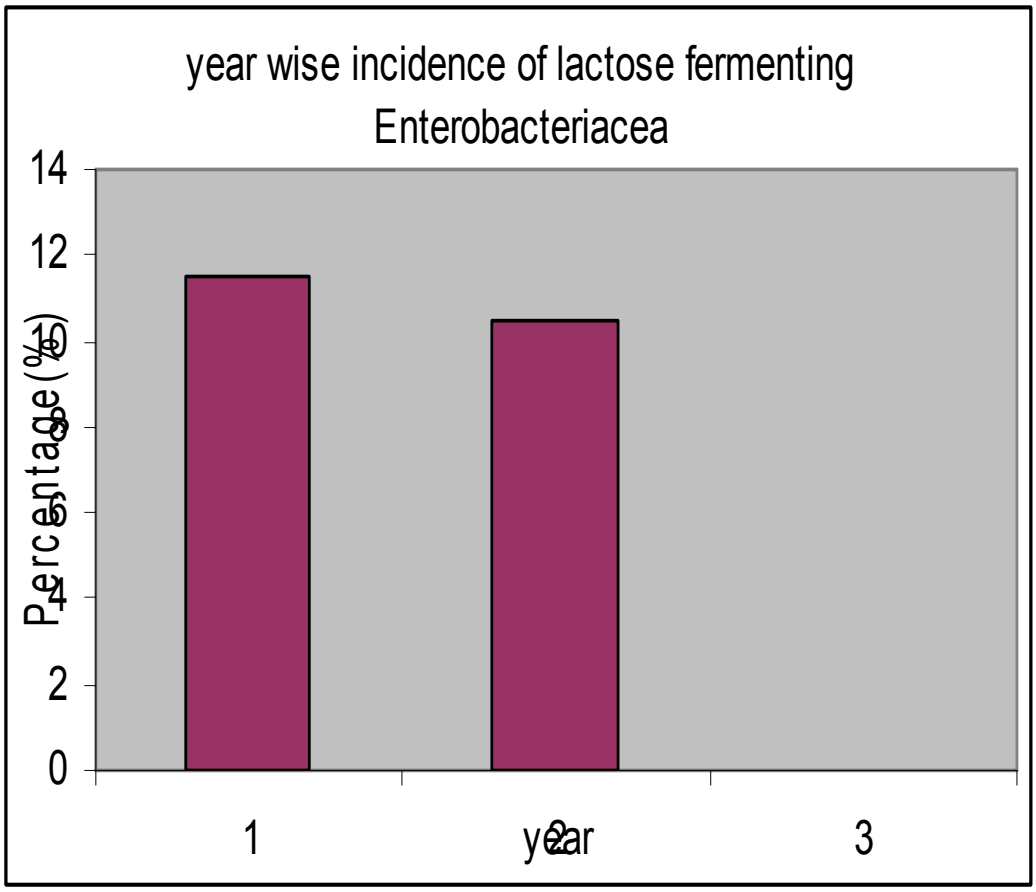


Figure – 4.4: Year wise incidence of lactose fermenting Enterobacteriaceae

4.3 Antibiogram typing of lactose fermenting Enterobacteriaceae:

Antimicrobial drugs have undoubtedly saves the lives of millions of people. However the widespread use of such drugs in hospitals, health centres, the community and agriculture has led to the emergence of resistance among bacteria. Antimicrobials are commonly used in food producing animals for treatment prophylaxis and growth promotion. However, such use can also lead to the development of drug resistance bacteria, which may be transmitted to humans through the food supply. Over the past years, the emergence and spread of antimicrobial resistance has become a major public concern.

Several monitoring programmes have been initiated to generate baseline data about the prevalence of resistance in different bacterial species, including lactose fermenting Enterobacteriaceae (Aaresttup *et. al.,* 2004). The genetic mechanisms that lead to bacterial resistance manifold and their spread in different bacterial population is enabled by highly efficient transfer systems of mobile genetic elements (Schwarz and Chaslus-Danota, 2003). The characterization of resistance mechanisms provides additional information about the epidemiology of resistant clones (Aarestup *et. al.,* 2004).

All the 60-70 isolates from the human sources were screened for antibiotic resistance pattern against the 8-10 different antibiotics (Plate – 4.13). The antibiotics were chosen on the basis of information collected from physician and the most common antibiotics prescribed for the treatment of urinary tract infections, diarrheal infections in this region as well as in general. The antibiotic resistance pattern expressed by the 60-70 lactose fermenting Enterobacteriaceae isolates. All the isolates exhibited as many as 34 different resistance patterns.

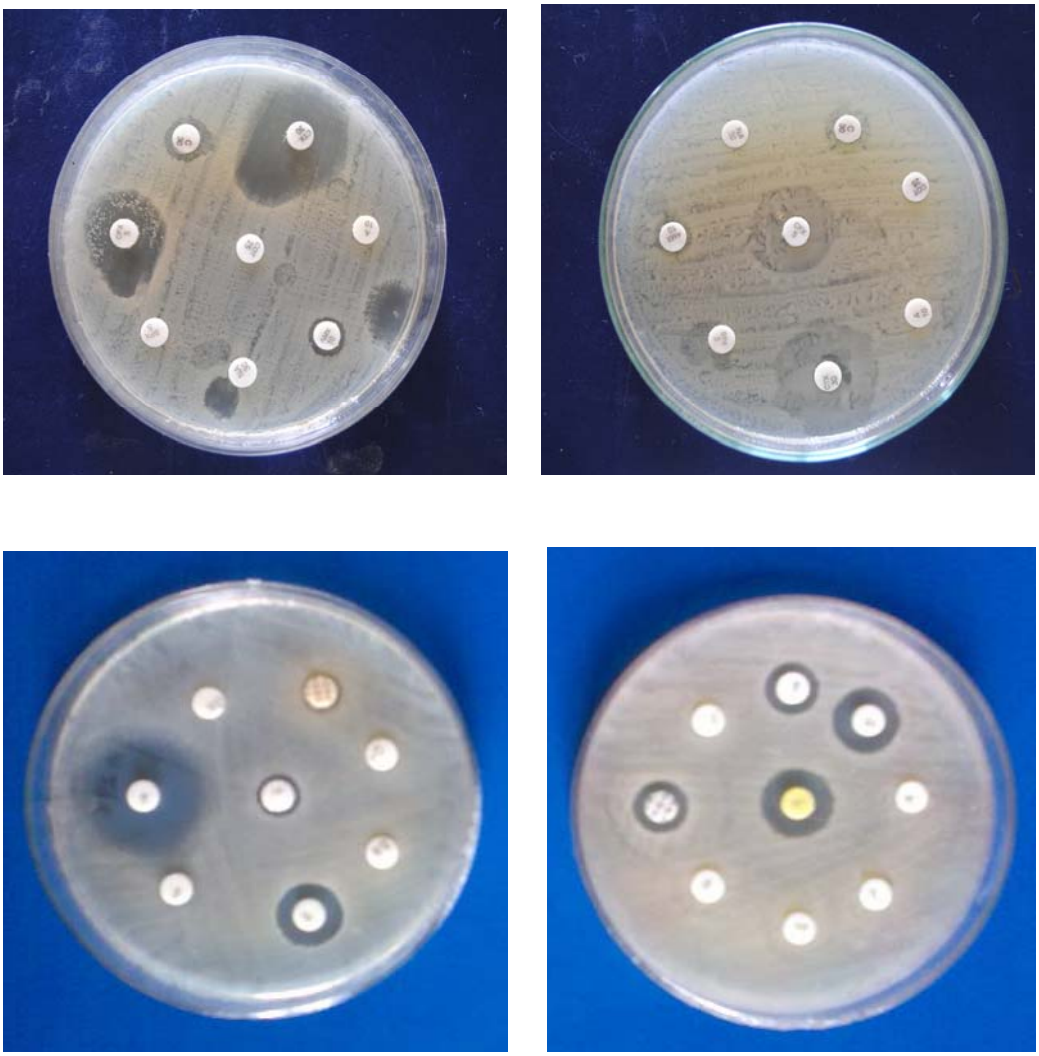


Plate 4.13: Antibiotic susceptibility resistant pattern of lactose fermenting Enterobacteriaceae

In the present investigation all the 65 isolates of lactose fermenting Enterobacteriaceae of human intestinal origin were found to be resistant to multiple antibiotics. As many as 08 different antibiotic resistant patterns were depicted by these 65 isolates. However, one pattern with resistance to ampicillin, nalidixic acid, strepomucin, and ciproflaxcin was most prevalent. All the 65 strains showed resistance to at least two antibiotics. Resistance to five antibiotics was most prevalent, as many as 31.6% of the lactose fermenting Enterobacteriaceae isolates showed resistance to 5 antibiotics.

Multiple antibiotics resistance was most common and all the isolates exhibited multiple antibiotic resistances. The distribution of all lactose fermenting Enterobacteriaceae isolates among the 22 resistance patterns in very diverse, in most cases only one lactose fermenting Enterobacteriaceae isolates exhibited by more than one isolate. The resistance pattern A showing resistance to ampicillin and nalidixic acid was exhibited by 9 isolates the pattern of G showing resistance to ampicillin, nalidixic acid, gentamicin and norflaxacin was exhibited by 5-isolates, while the pattern M showing resistance to ampicillin, nalidixic acid, ciprofloxacin and neomycin was recorded with four isolates (Table – 4.4 and Figure – 4.5). The pattern (F) and G showing resistance to three to seven antibiotics were exhibited by 3-isolates each while the patterns.

The multiple resistance patterns among all the lactose fermenting Enterobacteriaceae resistance to four antibiotic was most prevalent nearly 36% i.e., 20 isolates of the 60 isolates falling in this group, though there was a variation in the four antibiotics in this group, resistance to ampicillin, streptomycin, co-trimoxazole and gentamycin was most prevalent 30-35%. Incidence of resistance was also high for four antibiotics as 20 of the 60 isolates (30%) were resistant to as many as four antibiotics.

Table – 4.4: Multiple antibiotics resistance pattern of lactose fermenting Enterobacteriaceae

Sl. No.	Number of Antibiotics	Incidence of pattern (No. of isolates	Resistance Percentage (%)
1	One	10	12.0
2	Two	12	13
3	Three	15	20.2
4	Four	20	25.5
5	Five	15	20.2
6	Six	10	12.0

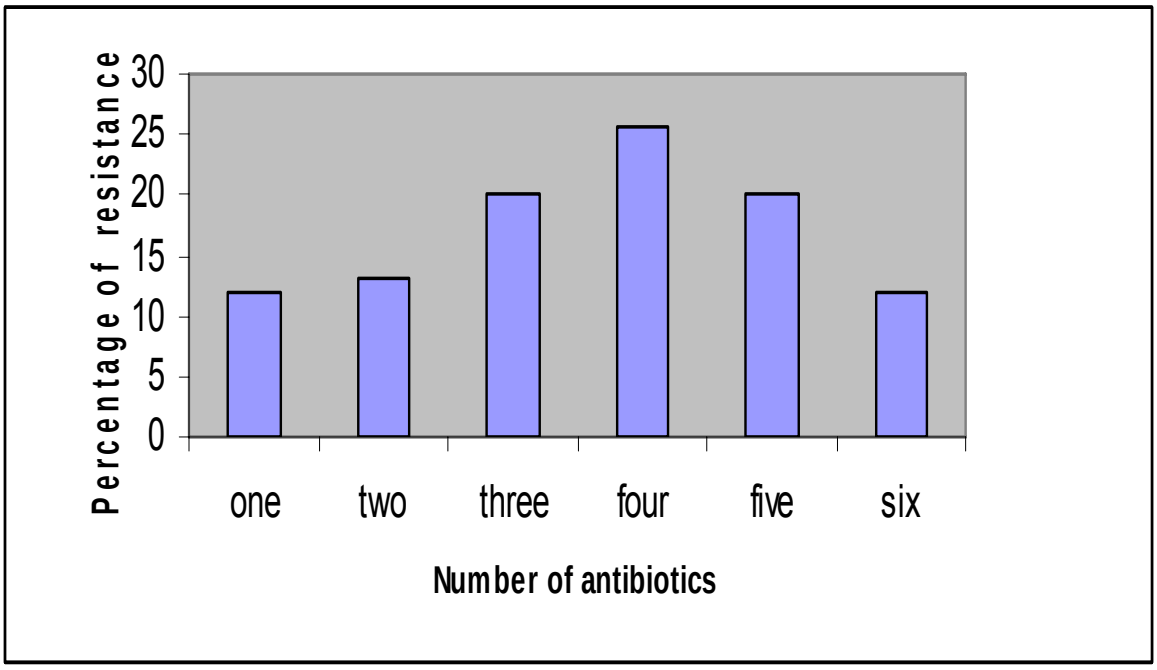


Figure – 4.5: Multiple antibiotic resistance pattern of lactose fermenting Enterobacteriaceae

Resistance the three antibiotics was recorded is 12 (15.5%) isolates where as, resistance to six antibiotics was recorded in 8 isolates (08%) and ten isolates (11.5%). The susceptibility and resistance of lactose fermenting Enterobacteriaceae drugs like streptomycin, azythromicin, ciprofloxacin, nalidixic acid, tetracycline, cefaxime etc are the main antibiotics (Table – 4.5) shows the pattern of the lactose fermenting Enterobacteriaceae.

It is also significant to note that more than 60% of the isolates are resistance to for antibiotics or more. More than 40% are resistance to three or more antibiotics and fortunately resistance to all 8 antibiotics tested was not recorded. tetracycline resistances is observed only when the isolate is resistant to 5-6 antibiotics and Impeneme resistance is very less compare to other antibiotics.

Comparison of the incidence of resistance among lactose fermenting Enterobacteriaceae to individual antibiotic indicated the presence of a high degree of resistance to as many as eight antibiotics, while resistance to Chlorophenicol and tetracycline was very low. The highest (86.08%) percent of resistance was recorded against Ampicillin and closely followed by that against tetracycline (65%) and streptomycin (40%), impeneme (30%), cefaxime (40%), azithromycin (35%) and nalidixic acid (42%) (Table – 4.6 and Figure – 4.6 and 4.7).

Among the individual antibiotics, highest incidence of resistances was observed against ampicillin (80.2%) closely followed by that against streptomycin (62.5%) and ciproflaxcin (56.4%), cefaxime (40%) and moderate resistance was observed against Azithromicin, fortunately, the lactose fermenting Enterobacteriaceae strains isolated from this region exhibited very low resistance against impeneme and cefaxime. These observations indicate that for treatment of Urinary tract infections, diarrhea and related infections in this regions, impenem and cefaxime showed to be drug of choice.

Table – 4.5: Antibiotic susceptibility and resistance pattern among lactose fermenting Enterobacteriaceae

SNO	AMP10	AMX	C30	COT25	CIP5	TE30	NA30	CFX5	IMP10	SPX5
1	R	R	I	S	S	R	S	R	S	I
2	R	R	I	S	S	R	S	R	S	I
3	R	R	I	S	S	R	S	S	S	I
4	S	I	R	S	S	R	S	S	I	I
5	I	R	I	R	R	R	S	S	S	I
6	I	S	I	R	R	R	S	R	I	S
7	R	S	R	R	R	R	S	R	S	S
8	I	I	S	I	R	R	S	R	S	R
9	R	I	S	I	I	R	I	R	S	R
10	R	I	S	I	I	R	I	R	S	R
11	R	I	R	S	I	S	I	I	S	R
12	R	I	R	R	I	S	I	I	S	R
13	R	R	R	R	I	I	I	I	R	R
14	R	R	R	S	R	I	I	I	R	R
15	I	R	R	S	R	I	R	I	R	R
16	I	R	S	I	R	I	R	I	S	S
17	I	R	S	I	R	R	I	I	S	R
18	I	R	I	I	S	R	S	S	R	S
19	R	I	I	R	S	S	S	S	S	S
20	R	I	I	R	S	I	S	I	R	I
21	R	S	I	R	I	I	I	I	S	I
22	S	S	R	R	I	I	I	I	R	S
23	S	I	R	R	I	R	I	R	S	S
24	S	I	R	R	R	R	S	R	I	S
25	R	R	R	S	I	I	S	R	I	I
26	R	R	R	I	R	I	S	R	S	S

SNO	AMP10	AMX	COT25	CIP5	TE30	NA30	CFX5	AZM15	IMP10	SPX5
27	R	R	R	R	I	I	I	I	R	R
28	R	R	R	S	R	I	I	I	R	R
29	I	R	R	S	R	I	R	I	R	R
30	I	R	S	I	R	I	R	I	S	S
31	I	R	S	I	R	R	I	I	S	R
32	I	R	I	I	S	R	S	S	R	S
33	R	I	I	R	S	S	S	S	S	S
34	R	I	I	R	S	I	S	I	R	I
35	R	S	I	R	I	I	I	I	S	I
36	S	S	S	R	I	I	I	I	R	S
37	S	I	S	R	I	R	I	R	S	S
38	S	I	S	R	R	R	S	R	I	S
39	R	R	R	S	I	I	S	R	I	I
40	R	R	R	I	R	I	S	R	S	S
41	R	R	R	R	I	I	I	I	R	R
42	R	R	S	S	R	I	I	I	R	R
43	I	R	R	S	R	I	R	I	R	R
44	I	R	S	I	R	I	R	I	S	S
45	I	R	S	I	R	R	I	I	S	R
46	I	R	I	I	S	R	S	S	R	S
47	R	I	I	R	S	S	S	S	S	S
48	R	I	I	R	S	I	S	I	R	I
49	R	S	I	R	I	I	I	I	S	I
50	S	S	R	R	I	I	I	I	R	S
51	S	I	R	R	I	R	I	R	S	S
52	S	I	R	R	R	R	S	R	I	S
53	R	R	R	S	I	I	S	R	I	I
54	R	R	R	I	R	I	S	R	S	S

SNO	AMP10	AMX	C30	CIP5	TE30	NA30	CFX5	AZM15	IMP10
55	R	R	R	I	I	I	I	I	I
56	R	R	R	R	I	I	I	I	I
57	I	R	R	R	I	R	I	R	I
58	I	R	S	R	I	R	I	R	I
59	I	R	S	R	R	I	I	I	I
60	I	R	I	S	R	S	S	S	S
61	R	I	I	S	S	S	S	S	S
62	R	I	I	S	I	S	I	S	I
63	R	S	I	I	I	I	I	I	I
64	S	S	R	I	I	I	I	I	I
65	S	I	R	I	R	I	R	I	R
66	S	I	R	R	R	S	R	S	R
67	R	R	R	I	I	S	R	S	R
68	R	R	R	R	I	S	R	S	R
69	R	R	R	I	I	I	I	I	I
70	R	R	R	R	I	I	I	I	I
71	I	R	R	R	I	R	I	R	I
72	I	R	S	R	I	R	I	R	I
73	I	R	S	R	R	I	I	I	I
74	I	R	I	S	R	S	S	S	S
75	R	I	I	S	S	S	S	S	S
76	R	I	I	S	I	S	I	S	I
77	R	S	I	I	I	I	I	I	I
78	S	S	R	I	I	I	I	I	I
79	S	I	R	I	R	I	R	I	R
80	S	I	R	R	R	S	R	S	R
81	R	R	R	I	I	S	R	S	R
82	R	R	R	R	I	S	R	S	R

Amp10-Ampicillin, Am-Amoxicillin, C30-Chlorophenicol, CIP-Ciproflocin, TE-Tetracycline, NA30-Nalidixicacid, CFX5-Cefaixime, CTR30-Ceftriaxone, AZM15-Azithromycin, IMP10-Impenem
R-Resistance, I-Intermediate, S-Susceptible

Table – 4.6: Antibiotic susceptibility and resistant pattern of lactose fermenting Enterobacteriaceae

Sl. No.	Name of the antibiotics	Susceptibility (%)	Resistance (%)
1	Streptomycin	60	40
2	Azythromycin	65	35
3	Ciprofloxacin	60	40
4	Nalidixic acid	60	40
5	Tetracycline	50	50
6	Cefaxime	50	50
7	Impenem	70	30
8	Ampicillin	40	60

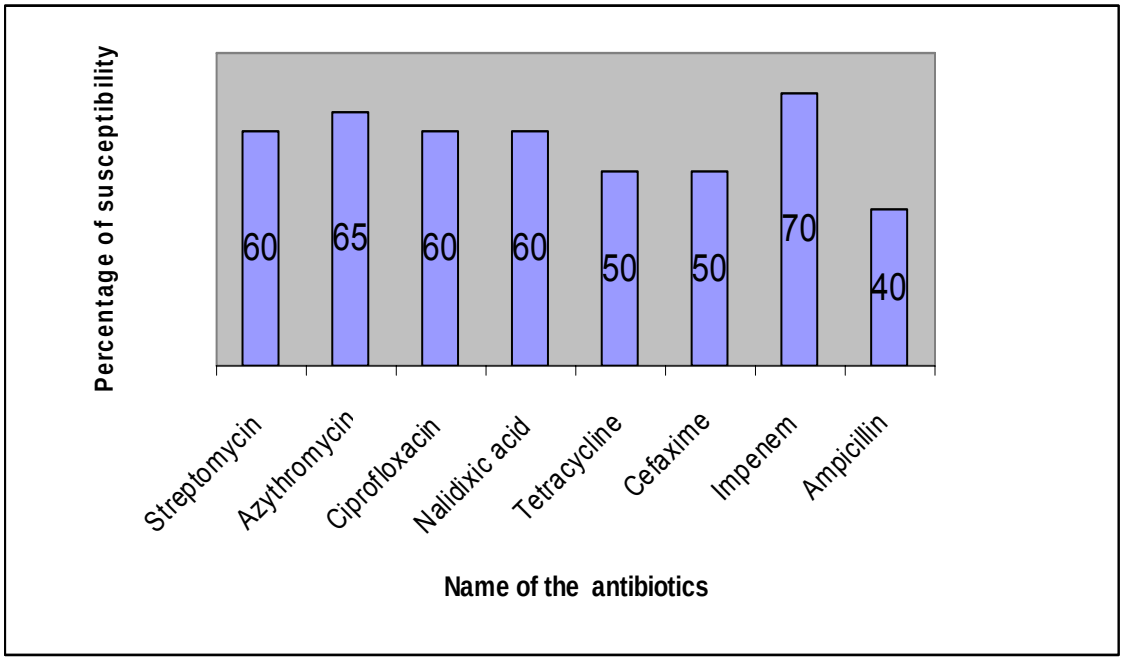


Figure – 4.6: Antibiotics Susceptibility pattern of lactose fermenting Enterobacteriaceae

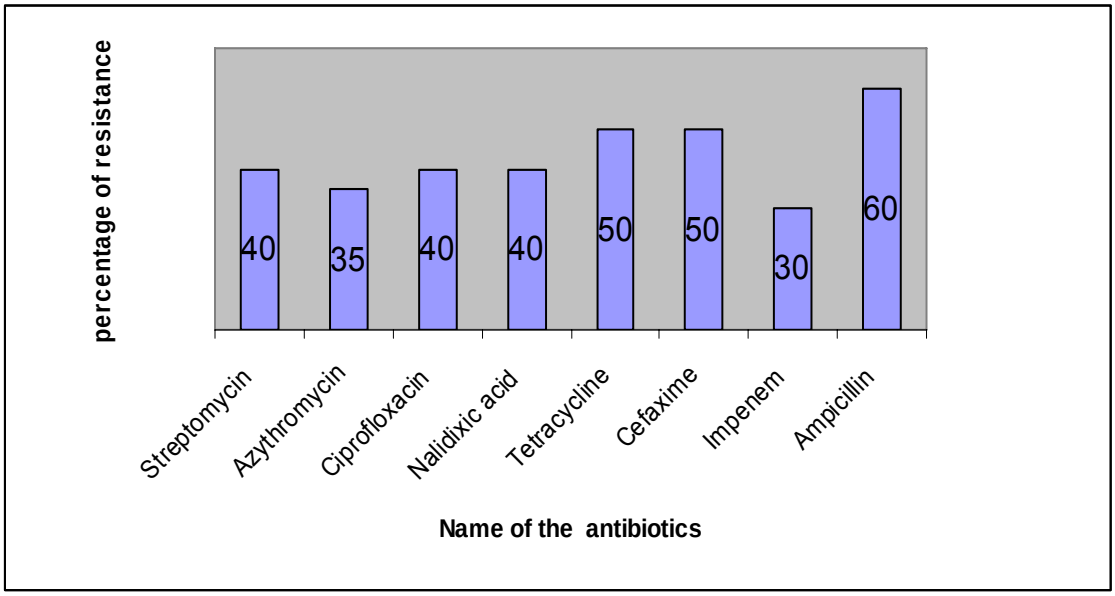


Figure – 4.7: Antibiotic Resistance pattern of lactose fermenting Enterobacteriaceae

The degree of highest resistance to tetracycline, streptomycin and other antibiotics and sensitivity to impenem, cefaxime observed in the present study among the lactose fermenting Enterobacteriaceae isolated from the different locations and epidemics in this region of Gulbarga are in agreement with the above reports from different places in India.

From the table it is clear that co-resistance between certain antibiotics was very high. It was among tetracycline, ciprofloxacin, gentamycin and also between chlorophenicol and nalidix acid. There was co-resistance between streptomycin, tetracycline and ampicillin.

Chapter-V

Summary and Conclusion

CHAPTER – VI
SUMMARY AND CONCLUSION

Enterobacteriaceae especially Gram negative bacilli mainly *E. coli, Klebsiella, Shigella* and *Citrobacter* are very important *Enterobacteriaceae* species. Among theses very common pathogenic organisms are *E. coli, Proteus,* and *Klebsiella* are causes infections in the urinary tract and other species of organisms are highly pathogenic and are not easily contaminate. They are causes severe infections and are very specific type of organisms like *Shigella, E. coli, Citrobacter,* etc.

The present study indicates that the antibiotic susceptibility of lactose fermenting Enterobacteriaceae form the different clinical samples as shown in Figure 2 the most of the strains are resistance, some of them are susceptible. It shows poor sanitation and hygienic maintenance and Figure 1 as compare with the different age groups of patients, most of the isolates from adults or middle age and children's is more sufferer than the other age groups. One more observation we observed in this study males are more sufferer than the females.

The present investigation was undertaken with an aim of studying the epidemiology of lactose fermenting Enterobacteriaceae in human diarrhaeal, epidemics and urinary tract infections in this region and to identify lactose fermenting by different morphological, cultural, and biochemical methods. The observation made in the present study can be summarized as follows.

1. The incidence of gastroenteritis, diarrhaeal infections in this regions including reported gastroenteritis epidemics during period from 2003-05 have recorded. A total of 650 samples from different patients were collected and analysed.
2. The incidence of lactose fermenting Enterobacteriaceae in faecal matter samples of patients suffering from diarrhaeal infection was found to be 12-5%.

3. The different samples from different patients have been assessed and the highest incidence was recorded in faecal matter and lowest in the other body fluids (means other than blood and urine).
4. Sex wise distribution of lactose fermenting Enterobacteriaceae like *E. coli*, *Klebsiella, Coliforms* etc., occurs in all types samples and showed highest incidences in females age group form 40-50 and above i.e., nearly 42.5% and lowest incidence rate in teenage females is 20%. In males the highest incidence adults (21-30) teenagers (11-20 years) and the lowest incidence rate was pediatrics.
5. Age wise incidence of lactose fermenting Enterobacteriaceae was found to be highest in middle age group and 25% and teenage group is 22% was found. The lowest incidence 15.5% was found in the pediatrics below 10 years age group children's.
6. The year wise incidence of lactose fermenting Enterobacteriaceae indicated highest in 2011 (24.5%). Though the incidence of lactose fermenting Enterobacteriaceae recorded in the present investigation indicated a slight declining trend from 2011-12. However, it has remained almost the slightly change as prepared earlier in 2002.
7. The analysis of the incidence of lactose fermenting Enterobacteriaceae in different samples indicated highest in sewage samples (18%). Closely followed by that recorded in the faecal matter of the diarrhael patients (15.5%). The incidence of lactose fermenting Enterobacteriaceae was also significant (9.5%) in water samples.
8. Antibiotic resistance pattern of lactose fermenting Enterobacteriaceae isolated from gastroenteritis and diarrhael patients were determined. A total of 60 isolates showing different pattern were depicted, showing resistance to ampicillin, tetracycline, streptomycin and less resistance shows to azithromycin, ciproflaxicin was observed with as many as 15 isolates.

9. Multiple antibiotic resistances were most common all the isolates were resistance to two or more antibiotics. However, only five isolates were resistance to four antibiotics and none was resistant to more than five antibiotics.
10. Highest incidence of resistance was observed against tetracycline and streptomycin and cifroflaxcin (35.5%) and streptomycin (40%), ciprofloxacin (42%) were recorded.
11. The incidence of urinary tract infections, diarrhael infections and intestinal infections in this region has been observed to be unchanged from 1995 to the present investigation, epidemics being recorded at regular intervals and the region being identified as lactose fermenting Enterobacteriaceae organisms endemic area. Further and continued surveillance of lactose fermenting Enterobacteriaceae as incidences in this region and for applying modern molecular epidemiological approaches for determining the clonal relatedness of the etiological agents, which will help in the effective control and management of lactose fermenting Enterobacterial infections prone regions.

References

REFERENCES

1. Abbott SL, Janda JM. *Enterobacter cancerogenus* ("*Enterobacter taylorae*") infections associated with severe trauma or crush injuries. *Am J Clin Pathol*. Mar 1997; 107(3):359-61.
2. Aguilar A, Merino S, Rubires X, Tomas J M. Influence of osmolarity on lipopolysaccharides and virulence of *Aeromonas hydrophila* serotype O:34 strains grown at 37°C. *Infect Immun.* 1997; 65:1245–1250.
3. Albertí S, Alvarez D, Merino S, Casado M T, Vivanco F, Tomás J M, Benedí V J. Analysis of complement C3 deposition and degradation on *Klebsiella pneumoniae*. *Infect Immun.* 1996; 64:4726–4732.
4. Albertí S, Marqués G, Camprubi S, Merino S, Tomás J M, Vivanco F, Benedí V J. C1q binding and activation of the complement classical pathway by *Klebsiella pneumoniae* outer membrane proteins. *Infect Immun.* 1993; 61:852–860.
5. Albesa I, Barberis L I, Pájaro M C, Eraso A J. Actividad hemolítica de *Klebsiella pneumoniae* sobre glóbulos rojos de conejo. *Rev Latinoam Microbiol.* 1985; 27:83–87.
6. Albesa I, Barberis L I, Pajaro M C, Farnochi M C, Eraso A J. A thiol-activated hemolysin in Gram-negative bacteria. *Can J Microbiol*. 1985; 31:297–300.
7. Albesa I. *Klebsiella pneumoniae* haemolysin adsorption to red blood cells. *J Appl Bacteriol.* 1989; 67:263–266.
8. Alhambra A, Cuadros JA, Cacho J, et al. *In vitro* susceptibility of recent antibiotic-resistant urinary pathogens to ertapenem and 12 other antibiotics. *J Antimicrob Chemother*. Jun 2004; 53(6):1090-4.
9. Amako K, Meno Y, Takade A. Fine structures of the capsules of *Klebsiella pneumoniae* and *Escherichia coli* K1. *J Bacteriol.* 1988; 170:4960–4962.

10. Arifuzzaman M, Maeda M, Itoh A, Nishikata K, Takita C, Saito R, Ara T, Nakahigashi K, Huang HC, Hirai A, Tsuzuki K, Nakamura S, Altaf-Ul-Amin M, Oshima T, Baba T, Yamamoto N, Kawamura T, Ioka-Nakamichi T, Kitagawa M, Tomita M, Kanaya S, Wada C, Mori H (May 2006). "Large-scale identification of protein-protein interaction of *Escherichia coli* K-12"]. *Genome Res.* 16 (5): 686–91.
11. Arlet G, Rouveau M, Casin I, Bouvet P J M, Lagrange P H, Philippon A. Molecular epidemiology of *Klebsiella pneumoniae* strains that produce SHV-4 β-lactamase and which were isolated in 14 French hospitals. *J Clin Microbiol.* 1994; 32:2553–2558.
12. Athamna A, Ofek I, Keisari Y, Markowitz S, S. D G G, Sharon N. Lectinophagocytosis of encapsulated *Klebsiella pneumoniae* mediated by surface lectins of guinea pig alveolar macrophages and human monocyte-derived macrophages. *Infect Immun.* 1991; 59:1673–1682.
13. Avorn J, Monane M, Gruwitz J H, Glynn J, Choodnovskiy I, Lipsitz L A. Reduction of bacteriuria and pyuria after ingestion of cranberry juice. *JAMA*. 1994; 271:751–754.
14. Ayars G H, Altman L C, Fretwell M D. Effect of decreased salivation and pH on the adherence of *Klebsiella* species to human buccal epithelial cells. *Infect Immun.* 1982; 38:179–182.
15. Ayling-Smith B, Pitt T L. State of the art in typing: *Klebsiella* spp. *J Hosp Infect.* 1990; 16:287–295.
16. Babu J P, Abraham S N, Dabbous M K, Beachey E H. Interaction of a 60-Kilodalton d-mannose-containing salivary glycoprotein with type 1 fimbriae of *Escherichia coli*. *Infect Immun*. 1986; 54:104–108.
17. Badger, J., Stins, M., and Sik Kim, K. "*Citrobacter freundii* Invades and Replicates in Human Brain Microvascular Endothelial Cells" *Infection and Immunity*, 1999. Volume 67. P. 4208-4215.

18. Bagley S T, Seidler R J, Talbot H W J, Morrow J E. Isolation of *Klebsiellae* from within living wood. *Appl Environ Microbiol*. 1978; 36:178–185.
19. Bagley S, Seidler R J, Brenner D J. *Klebsiella planticola sp. nov*.: a new species of Enterobacteriaceae found primarily in non-clinical environments. *Curr Microbiol*. 1981; 6:105–109.
20. Balish M J, Jensen J, Uehling D T. Bladder mucin: a scanning electron microscopy study in experimental cystitis. *J Urol.* 1982; 128:1060–1063.
21. Barberis L I, Eraso A J, Pájaro M C, Albesa I. Molecular weight determination and partial characterization of *Klebsiella pneumoniae* hemolysins. *Can J Microbiol.* 1986; 32:884–888.
22. Bascomb S, Lapage S P, Willcox W R, Curtis M A. Numerical classification of the tribe *Klebsiellae*. *J Gen Microbiol.* 1971; 66:279–295.
23. Bassetti M, Righi E, Fasce R, et al. Efficacy of ertapenem in the treatment of early ventilator-associated pneumonia caused by extended-spectrum beta-lactamase-producing organisms in an intensive care unit. *J Antimicrob Chemother*. Aug 2007; 60(2):433-5.
24. Batshon B A, Baer H, Shaffer M F. Immunologic paralysis produced in mice by *Klebsiella pneumoniae* type 2 polysaccharide. *J Immunol*. 1963; 90:121–126.
25. Battistuzzi FU, Feijao A, Hedges SB (November 2004). "A genomic timescale of prokaryote evolution: insights into the origin of methanogenesis, phototrophy, and the colonization of land". *BMC Evol. Biol.* 4: 44.
26. Bauernfeind A, Petermüller C, Schneider R. Bacteriocins as tools in analysis of nosocomial *Klebsiella pneumoniae* infections. *J Clin Microbiol*. 1981; 14:15–19.

27. Bauernfeind A, Rosenthal E, Eberlein E, Holley M, Schweighart S. Spread of *Klebsiella pneumoniae* producing SHV-5 beta-lactamase among hospitalized patients. *Infection*. 1993; 21:18–22.
28. Bauernfeind A. Epidemiological typing of *Klebsiella* by bacteriocins. *Methods Microbiol.* 1984; 16:213–224.
29. Benjamin W H, Turnbough C L, Posey B S, Briles D E. The ability of *Salmonella typhimurium* to produce the siderophore enterobactin is not a virulence factor in mouse typhoid. *Infect Immun.* 1985; 50:392–397.
30. Bennet R, Eriksson M, Melen B, Zetterström R. Changes in the incidence and spectrum of neonatal septicemia during a fifteen-year period. *Acta Paediatr Scand.* 1985; 74:687–690.
31. Bennett C J, Young M N, Darrington H. Differences in urinary tract infection in male and female spinal cord injury patients on intermittent catheterization. *Paraplegia*. 1995; 33:69–72.
32. Bentley R, Meganathan R (1 September 1982). "Biosynthesis of vitamin K (menaquinone) in bacteria". *Microbiol. Rev.* 46 (3): 241–80.
33. Benzer S (March 1961). "On the topography of the Genetic fine structure". *Proc. Natl. Acad. Sci. U.S.A.* 47 (3): 403–15.
34. Bergogne-Berezin E. Nosocomial pathogens: new pathogens, incidence, prevention. *Presse Med.* 1995; 24:89–97.
35. Bessette PH, Aslund F, Beckwith J, Georgiou G (November 1999). [//www.ncbi.nlm.nih.gov/pmc/articles/PMC24128/ "Efficient folding of proteins with multiple disulfide bonds in the Escherichia coli cytoplasm"]. *Proc. Natl. Acad. Sci. U.S.A.* **96** (24):
36. Bingen E H, Denamur E, Elion J. Use of ribotyping in epidemiological surveillance of nosocomial outbreaks. *Clin Microbiol Rev.* 1994; 7:311–327.
37. Bingen E H, Desjardins P, Arlet G, Bourgeois F, Marianikurkdjian P, Lambertzechovsky N Y, Denamur E, Philippon A, Elion J. Molecular epidemiology of plasmid spread among extended broad-spectrum

beta-lactamase-producing *Klebsiella pneumoniae* isolates in a pediatric hospital. *J Clin Microbiol*. 1993; 31:179–184.

38. Björkstén B, Kaijser B. Interaction of human serum and neutrophils with *Escherichia coli* strains: differences between strains isolated from urine of patients with pyelonephritis or asymptomatic bacteriuria. *Infect Immun*. 1978; 22:308–311.
39. Blattner FR, Plunkett G, Bloch CA, Perna NT, Burland V, Riley M, Collado-Vides J, Glasner JD, Rode CK, Mayhew GF, Gregor J, Davis NW, Kirkpatrick HA, Goeden MA, Rose DJ, Mau B, Shao Y (September 1997). "The complete genome sequence of *Escherichia coli* K-12". *Science* 277 (5331): 1453–62.
40. Bradford P A, Urban C, Mariano N, Projan S J, Rahal J J, Bush K. Imipenem resistance in *Klebsiella pneumoniae* is associated with the combination of ACT-1, a plasmid-mediated AmpC β-lactamase, and the loss of an outer membrane protein. *Antimicrob Agents Chemother*. 1997; 41:563–569.
41. Breed RS, Conn HJ (May 1936). [//www.ncbi.nlm.nih.gov/pmc/articles/PMC543738/ "The Status of the Generic Term Bacterium Ehrenberg 1828"]. *J. Bacteriol.* **31** (5): 517–8.
42. Brenner D J, Farmer III J J, Hickman F W, Asbury M A, Steigerwalt A G. Taxonomic and nomenclature changes in Enterobacteriaceae. HEW publication (CDC) 79-8356. Atlanta, Ga: Centers for Disease Control; 1977.
43. Brock J H, Williams P H, Licéaga J, Wooldridge K G. Relative availability of transferrin-bound iron and cell-derived iron to aerobactin-producing and enterobactin-producing strains of *Escherichia coli* and to other microorganisms. *Infect Immun*. 1991; 59:3185–3190.

44. Brown C, Seidler R J. Potential pathogens in the environment: *Klebsiella pneumoniae*, a taxonomic and ecological enigma. Appl Microbiol. 1973; 25:900–904.
45. Brüssow H, Canchaya C, Hardt WD (September 2004). "Phages and the evolution of bacterial pathogens: from genomic rearrangements to lysogenic conversion". *Microbiol. Mol. Biol. Rev.* 68 (3): 560–602.
46. Bryan C S, Hornung C A, Reynolds K L, Brenner E R. Endemic bacteremia in Columbia, South Carolina. *Am J Epidemiol*. 1986; 123:113–127.
47. Bryan C S, Reynolds K L, Brenner E R. Analysis of 1,186 episodes of Gram-negative bacteremia in non-university hospitals: the effects of antimicrobial therapy. *Rev Infect Dis.* 1983; 5:629–638.
48. Brzuszkiewicz E, Thürmer A, Schuldes J, Leimbach A, Liesegang H, Meyer FD, Boelter J, Petersen H, Gottschalk G, Daniel R (December 2011). "Genome sequence analyses of two isolates from the recent *Escherichia coli* outbreak in Germany reveal the emergence of a new pathotype:Entero-Aggregative-Haemorrhagic *Escherichia coli* (EAHEC)". *Arch. Microbiol.* 193 (12): 883–91.
49. Buffenmeyer C L, Rychek R R, Yee R B. Bacteriocin (klebocin) sensitivity typing of *Klebsiella*. *J Clin Microbiol*. 1976; 4:239–244.
50. Bullen J J, Rogers H J, Griffiths E. Role of iron in bacterial infection. *Curr Top Microbiol Immunol.* 1978; 80:1–35.
51. Burwen D R, Banerjee S N, Gaynes R P. Ceftazidime resistance among selected nosocomial gram-negative bacilli in the United States. *J Infect Dis.* 1994; 170:1622–1625.
52. Bush K, Jacoby GA, Medeiros AA. A functional classification scheme for beta-lactamases and its correlation with molecular structure. *Antimicrob Agents Chemother*. Jun 1995; 39(6):1211-33.

53. Campbell W N, Hendrix E, Cryz S, Cross A S. Immunogenicity of a 24-valent *Klebsiella* capsular polysaccharide vaccine and an eight-valent *Pseudomonas* O-polysaccharide conjugate vaccine administered to victims of acute trauma. *Clin Infect Dis.* 1996; 23:179–181.
54. Caplan ES, Hoyt NJ. Identification and treatment of infections in multiply traumatized patients. *Am J Med*. Jul 15 1985; 79(1A):68-76.
55. Carpenter J L. *Klebsiella* pulmonary infections: occurrence at one medical center and review. *Rev Infect Dis.* 1990; 12:672–682.
56. Carvallo RH, Fillo PPG, [2008] Epidemiologically relevant antimicrobial resistance phenotypes in pathogens isolated from critically ill patients in a Brazilian University Hospital, *Brazilian Journal of Microbiology*, 39:623-630.
57. Casewell M W, Phillips I. Epidemiological patterns of *Klebsiella* colonization and infection in an intensive care ward. *J Hyg Camb*. 1978; 80:295–300.
58. Casewell M W, Phillips I. Hands as a route of transmission for *Klebsiella* species. *Br Med J.* 1977; 2:1315–1317.
59. Casewell M, Talsania H G. Predominance of certain *Klebsiella* capsular types in hospitals in the United Kingdom. *J Infect*. 1979; 1:77–79.
60. Castellani A. and Chalmers A.J.: Manual of Tropical Medicine, 3rd ed., Williams Wood and Co., New York, 1919.
61. Centers for Disease Control. National nosocomial infections study report. Annual Summary 1983. Atlanta, Ga: *Centers for Disease Control*; 1985. pp. 9SS–21SS.
62. Centers for Disease Control. National nosocomial infections study report. Annual Summary 1975. *Atlanta, Ga: Centers for Disease Control;* 1977.

63. Centers for Disease Control. National nosocomial infections study report. Annual Summary 1979. *Atlanta, Ga: Centers for Disease Control;* 1982.
64. Centers for Disease Control. Nosocomial infection surveillance, 1983. *CDC Surveillance Summaries.* 1984; 33:955–22SS.
65. Chocat P, Esaki N, Tanizawa K, Nakamura K, Tanaka H, Soda K. "Purification and characterization of selenocysteine beta-lyase from *Citrobacter freundii*." *Journal of Bacteriology*. 1985 Aug; 163(2): 669-76.
66. Chow JW, Satishchandran V, Snyder TA, et al. *In vitro* susceptibilities of aerobic and facultative Gram-negative bacilli isolated from patients with intra-abdominal infections worldwide: the 2002 Study for Monitoring Antimicrobial Resistance Trends (SMART). *Surg Infect (Larchmt)*. *Winter* 2005; 6(4):439-48.
67. Christensen S C, Korner B. An endemic caused by multiresistant *Klebsiella* in an urological unit. *Scand J Urol Nephrol.* 1972; 6:232–238.
68. Chuang, Y., Tseng, S., Teng, L., Ho, Y., and Hsueh, P. "Emergence of cefotaxime resistance in *Citrobactor freundii* causing necrotizing fasciitis and osteomyelitis" *Journal of Infection*, 2006. Volume 53. p. e161-e163.
69. Ciğerci IH, Korcan SE, Konuk M, Oztürk S. "Comparison of ALAD activities of *Citrobacter* and *Pseudomonas* strains and their usage as biomarker for Pb contamination." *Environmental Monitoring and Assessment*. 2007 May 22.
70. Ciurana B, Tomás J M. Role of lipopolysaccharide and complement in susceptibility of *Klebsiella pneumoniae* to non-immune serum. *Infect Immun.* 1987; 55:2741–2746.
71. Clark NM, Patterson J, Lynch JP 3rd. Antimicrobial resistance among Gram-negative organisms in the intensive care unit. *Curr Opin Crit Care*. Oct 2003; 9(5):413-23.

72. Clegg S, Gerlach G F. Enterobacterial fimbriae. *J Bacteriol.* 1987; 169:934–938.
73. Combe M L, Pons J L, Sesboue R, Martin J P. Electrophoretic transfer from polyacrylamide gel to nitrocellulose sheets: a new method to characterize multilocus enzyme genotypes of *Klebsiella* strains. *Appl Environ Microbiol.* 1994; 60:26–30.
74. Cooke E M, Pool R, Brayson J C, Edmondson A S, Munro M E, Shinebaum R. Further studies on the source of *Klebsiella aerogenes* in hospital patients. *J Hyg Camb*. 1979; 83:391–395.
75. Coovadia Y M, Johnson A P, Bhana R H, Hutchinson G R, George R C, Hafferjee I E. Multiresistant *Klebsiella pneumoniae* in a neonatal nursery: the importance of maintenance of infection control policies and procedures in the prevention of outbreaks. *J Hosp Infect.* 1992; 22:197–205.
76. Cornelis P (2000). "Expressing genes in different *Escherichia coli* compartments". *Curr. Opin. Biotechnol.* **11** (5): 450–454.
77. Cosgrove SE, Kaye KS, Eliopoulous GM, et al. Health and economic outcomes of the emergence of third-generation cephalosporin resistance in *Enterobacter* species. *Arch Intern Med*. Jan 28 2002; 162(2):185-90.
78. Cowan S T, Steel K J, Shaw C, Duguid J P. A classification of the *Klebsiella* group. *J Gen Microbiol.* 1960; 23:601–612.
79. Cryz S J, Fürer E, Germanier R. Experimental *Klebsiella pneumoniae* burn wound sepsis: role of capsular polysaccharide. *Infect Immun.* 1984; 43:440–441.
80. Cryz S J, Jr, Fürer E, Germanier R. Safety and immunogenicity of *Klebsiella pneumoniae* K1 capsular polysaccharide vaccine in humans. *J Infect Dis.* 1985; 151:665–671.

81. Cryz S J, Jr, Mortimer P, Cross A S, Fürer E, Germanier R. Safety and immunogenicity of a polyvalent *Klebsiella* capsular polysaccharide vaccine in humans. *Vaccine*. 1986; 4:15–20.
82. Cryz S J, Mortimer P M, Mansfield V, Germanier R. Seroepidemiology of *Klebsiella* bacteremic isolates and implications for vaccine development. *J Clin Microbiol*. 1986; 23:687–690.
83. Cryz S J. Progress in immunization against *Klebsiella* infections. *Eur J Clin Microbiol.* 1983; 2:523–528.
84. Cunha BA, McDermott B, Nausheen S. Single daily high-dose tigecycline therapy of a multidrug-resistant (MDR) *Klebsiella pneumoniae* and *Enterobacter aerogenes* nosocomial urinary tract infection. *J Chemother*. Dec 2007; 19(6):753-4.
85. Cunha BA, Theodoris AC, Yannelli B. *Enterobacter cloacae* graft infection/bacteremia in a hemodialysis patient. *Am J Infect Control*. Apr 2000; 28(2):181-3.
86. Cunha BA. *Antibiotic Essentials*. 9th ed. Sudbury, MA: Jones & Bartlett; 2010.
87. Cunha BA. Enterobacter: Colonization & infection. *Infect Dis Pract*. 1999; 23:41-3.
88. Cunha BA. Once-daily tigecycline therapy of multidrug-resistant and non-multidrug-resistant Gram-negative bacteremias. *J Chemother*. Apr 2007; 19(2):232-3.
89. Cunha BA. Pharmacokinetic considerations regarding tigecycline for multidrug-resistant (MDR) *Klebsiella pneumoniae* or MDR *Acinetobacter baumannii urosepsis*. *J Clin Microbiol*. May 2009; 47(5):1613.
90. Curie K, Speller D C E, Simpson R A, Stephens M, Cooke D I. A hospital epidemic caused by a gentamicin-resistant *Klebsiella aerogenes*. *J Hyg Camb*. 1978; 80:115–123.

91. Darfeuille-Michaud A, Jallat C, Aubel D, Sirot D, Rich C, Sirot J, Joly B. R-plasmid-encoded adhesive factor in *Klebsiella pneumoniae* strains responsible for human nosocomial infections. *Infect Immun.* 1992; 60:44–45.
92. Darnton NC, Turner L, Rojevsky S, Berg HC (March 2007). "On torque and tumbling in swimming *Escherichia coli*". *J. Bacteriol.* 189 (5): 1756–64.
93. Davis T J, Matsen J M. Prevalence and characteristics of *Klebsiella* species: relation to association with a hospital environment. *J Infect Dis.* 1974; 130:402–405.
94. De Champs C, Rouby D, Guelon D, Sirot J, Sirot D, Beytout D, Gourgand J M. A case-control study of an outbreak of infections caused by *Klebsiella pneumoniae* strains producing CTX-1 (TEM-3) beta-lactamase. *J Hosp Infect.* 1991; 18:5–13.
95. De Champs C, Sirot D, Chanal C, et al. A 1998 survey of extended-spectrum beta-lactamases in Enterobacteriaceae in France. The French Study Group. *Antimicrob Agents Chemother*. Nov 2000; 44(11):3177-9.
96. de la Torre M G, Romero-Vivas J, Martinez-Beltran J, Guerrero A, Meseguer M, Bouza E. *Klebsiella bacteremia:* an analysis of 100 episodes. *Rev Infect Dis.* 1985; 7:143–150.
97. de Lorenzo V, Martinez J L. Aerobactin production as a virulence factor: a reevaluation. *Eur J Clin Microbiol Infect Dis.* 1988; 7:621–629.
98. Deal EN, Micek ST, Ritchie DJ, et al. Predictors of in-hospital mortality for bloodstream infections caused by *Enterobacter* species or *Citrobacter freundii*. *Pharmacotherapy*. Feb 2007; 27(2):191-9.

99. Dervisoglu, E., Yegenaga, I., Yumuk, Z. "*Citrobacter freundii* peritonitis and tunnel infection in a patient on continuous ambulatory peritoneal dialysis." *Journal of Medical Microbiology*, 2008. Volume 57. p. 125-127.
100. Deshpande P, Rodrigues C, Shetty A, Kapadia F, Hedge A, Soman R. New Delhi Metallo-beta lactamase (NDM-1) in Enterobacteriaceae: treatment options with carbapenems compromised. *J Assoc Physicians India*. Mar 2010; 58:147-9.
101. Di Martino P, Bertin Y, Girardeau P, Livrelli V, Joly B, Darfeuille-Michaud A. Molecular characterization and adhesive properties of CF29K, an adhesin of *Klebsiella pneumoniae* strains involved in nosocomial infections. *Infect Immun.* 1995; 63:4336–4344.
102. Di Martino P, Livrelli V, Sirot D, Joly B, Darfeuille-Michaud A. A new fimbrial antigen harbored by CAZ-5/SHV-4-producing *Klebsiella pneumoniae* strains involved in nosocomial infections. *Infect Immun*. 1996; 64:2266–2273.
103. Dijk Y, Bik EM, Hochstenbach-Vernooij S, VD Vlist GJ, Savelkoul PH, Kaan JA, et al. Management of an outbreak of *Enterobacter cloacae* in a neonatal unit using simple preventive measures. *J Hosp Infect*. May 2002; 51(1):21-6.
104. DiPersio JR, Dowzicky MJ. Regional variations in multidrug resistance among Enterobacteriaceae in the USA and comparative activity of tigecycline, a new glycylcycline antimicrobial. *Int J Antimicrob Agents*. May 2007; 29(5):518-27.
105. Doebbeling B N. Epidemics: identification and management. In: Wenzel R P, editor. Prevention and control of nosocomial infections. 2nd ed. Baltimore, Md: The Williams & Wilkins Co.; 1993. pp. 177–206.
106. Domenico P, Johanson W G, Straus D C. Lobar pneumonia in rats produced by clinical isolates of *Klebsiella pneumoniae*. *Infect Immun.* 1982; 37:327–335.

107. Donati L, Scamazzo F, Gervasoni M, et al. Infection and antibiotic therapy in 4000 burned patients treated in Milan, Italy, between 1976 and 1988. *Burns*. Aug 1993; 19(4):345-8.

108. Drelichman V, Band JD. “Bacteremias due to *Citrobacter diversus* and *Citrobacter freundii.* Incidence, risk factors, and clinical outcome.” *Archives of Internal Medicine*. 1985 Oct; 145(10): 1808-10.

109. Drudy D, Mullane NR, Quinn T, et al. *Enterobacter sakazakii*: an emerging pathogen in powdered infant formula. *Clin Infect Dis*. Apr 1 2006; 42(7):996-1002.

110. Duggan J M, Oldfield G S, Ghosh H K. Septicaemia as a hospital hazard. *J Hosp Infect.* 1985; 6:406–412.

111. Durand ML, Calderwood SB, Weber DJ, et al. Acute bacterial meningitis in adults. A review of 493 episodes. *N Engl J Med*. Jan 7 1993; 328(1):21-8.

112. Eckburg PB, Bik EM, Bernstein CN, Purdom E, Dethlefsen L, Sargent M, Gill SR, Nelson KE, Relman DA (June 2005). "Diversity of the human intestinal microbial flora"]. *Science* **308** (5728): 1635–8.

113. Edberg S C, Piscitelli V, Cartter M. Phenotypic characteristics of coliform and noncoliform bacteria from a public water supply compared with regional and national clinical species. *Appl Environ Microbiol.* 1986; 52:474–478.

114. Edelman R, Taylor D N, Wasserman S S, Mcclain J B, Cross A S, Sadoff J C, Que J U, Cryz S J. Phase 1 trial of a 24-valent *Klebsiella* capsular polysaccharide vaccine and an eight-valent Pseudomonas O-polysaccharide conjugate vaccine administered simultaneously. *Vaccine*. 1994; 12:1288–1294.

115. Edmondson A S, Cooke E M, Wilcock A P D, Shinebaum R. A comparison of the properties of *Klebsiella* strains isolated from different sources. *J Med Microbiol.* 1980; 13:541–550.

116. Edmondson A S, Cooke E M. The development and assessment of a bacteriocin typing method for *Klebsiella*. *J Hyg Camb.* 1979; 82:207–233.
117. Edwards P R, Ewing W H. Identification of *Enterobacteriaceae*. 4th ed. Minneapolis, Minn: Burgess Publishing Co.; 1986.
118. Ehrenwort L, Baer H. The pathogenicity of *Klebsiella pneumoniae* for mice: the relationship to the quantity and rate of production of type-specific capsular polysaccharide. *J Bacteriol.* 1956; 72:713–717.
119. Escherich T (1885). "Die Darmbakterien des Neugeborenen und Säuglinge". *Fortschr. Med.* **3**: 515–522.
120. Evans Jr., Doyle J.; Dolores G. Evans. "*Escherichia coli*". *Medical Microbiology, 4th edition*. The University of Texas Medical Branch at Galveston. Archived from the original on 2007-11-02.
121. Fader R C, Davis C P. Effect of piliation on *Klebsiella pneumoniae* infection in rat bladders. *Infect Immun.* 1980; 30:554–561.
122. Fader R C, Davis C P. *Klebsiella pneumoniae*-induced experimental pyelitis: the effect of piliation on infectivity. *J Urol.* 1982; 128:197–201.
123. Farmer J J, III, Davis B R, Hickman-Brenner F W, McWhorter A, Huntley-Carter G P, Asbury M A, Riddle C, Wathen-Grady H G, Elias C, Fanning G R, Steigerwalt A G, O'Hara C M, Morris G K, Smith P B, Brenner D J. Biochemical identification of new species and biogroups of *Enterobacteriaceae* isolated from clinical specimens. *J Clin Microbiol.* 1985; 21:46–76.
124. Favre-Bonte S, Darfeuille-Michaud A, Forestier C. Aggregative adherence of *Klebsiella pneumoniae* to human Intestine-407 cells. *Infect Immun.* 1995; 63:1318–1328.
125. Felson B, Rosenberg L S, Hamburger M J. Roentgen findings in acute Friedländer's pneumonia. *Radiology*. 1949; 53:559–565.

126. Feng P, Weagant S, Grant, M (2002-09-01). "Enumeration of *Escherichia coli* and the Coliform Bacteria". *Bacteriological Analytical Manual (8th ed.)*. FDA/Center for Food Safety & Applied Nutrition. Retrieved 2007-01-25.
127. Ferragut C, Izard D, Gavini F, Kersters K, De Ley J, Leclerc H. *Klebsiella trevisanii*: a new species from water and soil. *Int J Syst Bacteriol*. 1983; 33:133–142.
128. Firon N, Ofek I, Sharon N. Carbohydrate-binding sites of the mannose-specific fimbrial lectins of enterobacteria. *Infect Immun.* 1984; 43:1088–1090.
129. Foster DR, Rhoney DH. *Enterobacter meningitis*: organism susceptibilities, antimicrobial therapy and related outcomes. *Surg Neurol*. Jun 2005; 63(6):533-7.
130. Fotadar U, Zaveloff P, Terracio L (2005). "Growth of *Escherichia coli* at elevated temperatures". *J. Basic Microbiol.* 45 (5): 403–4.
131. Freedman R M, Ingram D L, Gross I, Ehrenkranz R A, Warshaw J B, Baltimore R S. A half century of neonatal sepsis at Yale. *Am J Dis Child.* 1981; 135:140–144.
132. French G L, Shannon K P, Simmons N. Hospital outbreak of *Klebsiella pneumoniae* resistant to broad-spectrum cephalosporins and β-lactam–β-lactamase inhibitor combinations by hyperproduction of SHV-5 β-lactamase. J Clin Microbiol. 1996; 34:358–363.
133. Fritsche TR, Stilwell MG, Jones RN. Antimicrobial activity of doripenem (S-4661): a global surveillance report (2003). *Clin Microbiol Infect*. Dec 2005; 11(12):974-84.
134. Fritsche TR, Strabala PA, Sader HS, et al. Activity of tigecycline tested against a global collection of Enterobacteriaceae, including tetracycline-resistant isolates. *Diagn Microbiol Infect Dis*. Jul 2005; 52(3):209-13.

135. Fux CA, Shirtliff M, Stoodley P, Costerton JW (2005). "Can laboratory reference strains mirror "real-world" pathogenesis?". *Trends Microbiol.* 13 (2): 58–63.
136. Gales AC, Jones RN, Sader HS. Global assessment of the antimicrobial activity of polymyxin B against 54 731 clinical isolates of Gram-negative bacilli: report from the SENTRY antimicrobial surveillance programme (2001-2004). *Clin Microbiol Infect*. Apr 2006; 12(4):315-21.
137. Gallagher PG, Ball WS. Cerebral infarctions due to CNS infection with *Enterobacter sakazakii*. *Pediatr Radiol*. 1991; 21(2):135-6.
138. Gallagher PG. *Enterobacter bacteremia* in pediatric patients. *Rev Infect Dis*. Sep-Oct 1990; 12(5):808-12.
139. Gavini F, Izard D, Grimont P A D, Beji A, Ageron E, Leclerc H. Priority of *Klebsiella planticola* Bagley, Seidler, and Brenner 1982 over *Klebsiella trevisanii* Ferragut, Izard, Gavini, Kersters, DeLey, and Leclerc 1983. *Int J Syst Bacteriol.* 1986; 36:486–488.
140. Gavini F, Leclerc H, Lefèbvre B, Ferragut C, Izard D. Etude taxonomique d'entérobactéries appartenant ou apparentées au genre *Klebsiella*. *Ann Microbiol (Inst Pasteur)* 1977; 128B:45–49.
141. George M. Garrity, ed. (July 26, 2005) [1984 (Williams & Wilkins)]. The Gammaproteobacteria. Bergey's Manual of Systematic Bacteriology. 2B (2nd ed.). *New York: Springer.* pp. 1108.
142. George M. Garrity, ed. (July 26, 2005) [1984 (Williams & Wilkins)]. *The Gammaproteobacteria*. Bergey's Manual of Systematic Bacteriology. 2B (2nd ed.). New York: Springer. pp. 1108.
143. Gerlach G F, Allen B L, Clegg S. Type 3 fimbriae among enterobacteria and the ability of spermidine to inhibit MR/K hemagglutination. *Infect Immun.* 1989; 57:219–224.

144. Goetz A M, Rihs J D, Chow J W, Singh N, Muder R R. An outbreak of infusion-related *Klebsiella pneumoniae* bacteremia in a liver transplantation unit. *Clin Infect Dis.* 1995; 21:1501–1503.

145. Gotoff S P. Sepsis in the newborn. In: Krugman S, Katz S L, Gershon A A, Wilfert C M, editors. Infectious diseases of children. 9th ed. St. Louis, Mo: Mosby—Year Book; 1992. pp. 402–418.

146. Gouby A, Neuwirth C, Bourg G, Bouziges N, Carlesnurit M J, Despaux E, Ramuz M. Epidemiological study by pulsed-field gel electrophoresis of an outbreak of extended-spectrum beta-lactamase-producing *Klebsiella pneumoniae* in a geriatric hospital. *J Clin Microbiol.* 1994; 32:301–305.

147. Gower P E, Taylor P W, Koutsaimanis K G, Roberts A P. Serum bactericidal activity in patients with upper and lower urinary tract infections. *Clin Sci.* 1972; 43:13–22.

148. Griffiths E, Chart H, Stevenson P. High-affinity iron uptake systems and bacterial virulence. In: Roth J A, editor. Virulence mechanisms of bacterial pathogens. Washington, D.C: American Society for Microbiology; 1988. pp. 121–137.

149. Griffiths E. The iron-uptake systems of pathogenic bacteria. In: Bullen J J, Griffiths E, editors. Iron and infection. New York, N.Y: John Wiley & Sons, Inc.; 1987. pp. 69–137.

150. Grozdanov L, Raasch C, Schulze J, Sonnenborn U, Gottschalk G, Hacker J, Dobrindt U (August 2004). "Analysis of the genome structure of the nonpathogenic probiotic *Escherichia coli* strain Nissle 1917"]. *J. Bacteriol.* 186**.**

151. Guarino A, Guandalini S, Alessio M, Gentile F, Tarallo L, Capano G, Migliavacca M, Rubino A. Characteristics and mechanism of action of a heat-stable enterotoxin produced by *Klebsiella pneumoniae* from infants with secretory diarrhea. *Pediatr Res.* 1989; 25:514–518.

152. Gupta S, Govil D, Kakar PN, Prakash O, Arora D, Das S. Colistin and polymyxin B: A re-emergence. *Indian J Crit Care Med*. Apr-Jun 2009; 13(2):49-53.
153. Gür D, Pitt T L, Hall L M C, Erdal Akalin H, Livermore D M. Diversity of klebsiellae with extended-spectrum β-lactamases at a Turkish university hospital. *J Hosp Infect.* 1992; 22:163–178.
154. Haeckel, Ernst (1867). *Generelle Morphologie der Organismen*. Reimer, Berlin. ISBN 1-144-00186-2.
155. Hall F A. Bacteriocine typing of *Klebsiella spp. J Clin Pathol.* 1971; 24:712–716.
156. Halstead DC, Abid J, Dowzicky MJ. Antimicrobial susceptibility among *Acinetobacter calcoaceticus-baumannii* complex and *Enterobacteriaceae* collected as part of the Tigecycline Evaluation and Surveillance Trial. *J Infect*. Jul 2007; 55(1):49-57.
157. Hanna H, Afif C, Alakech B, Boktour M, et al. Central venous catheter-related bacteremia due to Gram-negative bacilli: significance of catheter removal in preventing relapse. *Infect Control Hosp Epidemiol*. Aug 2004; 25(8):646-9.
158. Hart C A. *Klebsiellae* and neonates. *J Hosp Infect.* 1993; 23:83–86.
159. Hartstein A I, Morthland V H, Rourke J W, Sykes R, Rashad A L. Plasmid DNA analysis, biotyping, and antimicrobic susceptibility as subtyping tests for *Klebsiella pneumoniae* and *Klebsiella oxytoca*. *Diagn Microbiol Infect Dis.* 1993; 16:35–41.
160. Haverkorn M L, Michel M F. Nosocomial *Klebsiellas* I. Colonization of hospitalized patients. *J Hyg Camb.* 1979; 82:177–193.
161. Hawser SP, Bouchillon SK, Hoban DJ, Badal RE. *In vitro* susceptibilities of aerobic and facultative anaerobic Gram-negative bacilli from patients with intra-abdominal infections worldwide from 2005-2007: results from the SMART study. *Int J Antimicrob Agents*. Dec 2009; 34(6):585-8.

162. Heller I, Grif K, Orth D. Emergence of VIM-1-carbapenemase-producing *Enterobacter cloacae* in Tyrol, Austria. *J Med Microbiol*. Apr 2012; 61:567-71.
163. Hibbertrogers L C F, Heritage J, Gascoynebinzi D M, Hawkey P M, Todd N, Lewis I J, Bailey C. Molecular epidemiology of ceftazidime resistant Enterobacteriaceae from patients on a paediatric oncology ward. *J Antimicrob Chemother.* 1995; 36:65–82.
164. Higaki M, Chida T, Takano H, Nakaya R. Cytotoxic component(s) of *Klebsiella oxytoca* on HEp-2 cells. *Microbiol Immunol.* 1990; 34:147–151.
165. Highsmith A K, Jarvis W R. *Klebsiella pneumoniae*: selected virulence factors that contribute to pathogenicity. *Infect Control.* 1985; 6:75–77.
166. Hillel S Levinson, Inga Mahler. “Phosphatase activity and lead resistance in *Citrobacter freundii* and *Staphylococcus aureus*.” *FEMS Microbiology Letters.* 1998 Apr; 161(1): 135-138.
167. Hoffmann H, Sturenburg E, Heesemann J, et al. Prevalence of extended-spectrum beta-lactamases in isolates of the *Enterobacter cloacae* complex from German hospitals. *Clin Microbiol Infect*. Apr 2006; 12(4):322-30.
168. Horan T, Culver D, Jarvis W, Emori G, Banerjee S, Martone W, Thornsberry C. Pathogens causing nosocomial infections. *Antimicrobic Newsl.* 1988; 5:65–67.
169. Hornick D B, Allen B L, Horn M A, Clegg S. Adherence to respiratory epithelia by recombinant *Escherichia coli* expressing *Klebsiella pneumoniae* type 3 fimbrial gene products. *Infect Immun.* 1992; 60:1577–1588.
170. Hu P, Janga SC, Babu M, Díaz-Mejía JJ, Butland G, Yang W, Pogoutse O, Guo X, Phanse S, Wong P, Chandran S, Christopoulos C, Nazarians-Armavil A, Nasseri NK, Musso G, Ali M, Nazemof N, Eroukova V, Golshani A, Paccanaro A, Greenblatt JF, Moreno-Hagelsieb G, Emili A (April 2009).

171. Huang CJ, Lin H, Yang X. (2012). "Industrial production of recombinant therapeutics in *Escherichia coli* and its recent advancements". *J Ind Microbiol Biotechnol* **39** (3): 383–99.
172. Hudault S, Guignot J, Servin AL (July 2001). [//www.ncbi.nlm.nih.gov/pmc/articles/PMC1728375/ "*Escherichia coli* strains colonizing the gastrointestinal tract protect germ-free mice against *Salmonella typhimurium* infection"]. *Gut* **49** (1): 47–55. doi:10.1136/gut.49.1.47.
173. Ihssen J, Kowarik M, Dilettoso S, Tanner C, Wacker M, Thöny-Meyer L. (2010). [//www.ncbi.nlm.nih.gov/pmc/articles/PMC2927510/ "Production of glycoprotein vaccines in *Escherichia coli*"]. *Microbial Cell Factories* **9** (61): 494–7.
174. Ilya V. Manukhov, Daria V. Mamaeva, Sergei M. Rastorguev, Nicolai G. Faleev, Elena A. Morozova, Tatyana V. Demidkina, and Gennadii B. Zavilgelsky. “A Gene Encoding L-Methionine γ-Lyase Is Present in Enterobacteriaceae Family Genomes: Identification and Characterization of *Citrobacter freundii* L-Methionine γ-Lyase.” *The Journal of Bacteriology.* 2005 June; 187(11): 3889-3893.
175. Ingledew WJ, Poole RK (1984). "The respiratory chains of *Escherichia coli*"]. *Microbiol. Rev.* 48 (3): 222–71.
176. International Bulletin of Bacteriological Nomenclature and Taxonomy 8:73–74 (1958).
177. Isenberg, H.D., J.A. Washington, III, A. Balows and A.C. Sonnenwirth, *in* H. Lennette, A. Balows, W.J. Hausler, Jr. and H.J. Shadomy (Eds.), [1985] Manual of Clinical Microbiology*,* 4th ed., American Society for Microbiology, Washington, DC, pp. 73-98.
178. Ishii S, Sadowsky MJ (2008). "*Escherichia coli* in the Environment: Implications for Water Quality and Human Health". *Microbes Environ.* 23 (2): 101–8.

179. Iversen C, Lehner A, Mullane N, Marugg J, Fanning S, Stephan R, et al. Identification of "*Cronobacter*" spp. (*Enterobacter sakazakii*). *J Clin Microbiol*. Nov 2007; 45(11):3814-6.

180. Iwahi T, Abe Y, Nakao M, Imada A, Tsuchiya K. Role of type 1 fimbriae in the pathogenesis of ascending urinary tract infection induced by *Escherichia coli*. *Infect Immun.* 1983; 39:1307–1315.

181. Izard D, Ferragut C, Gavini F, Kersters K, De Ley J, Leclerc H. *Klebsiella terrigena*, a new species from soil and water. *Int J Syst Bacteriol.* 1981; 31:116–127.

182. Jacoby G A, Han P. Detection of extended-spectrum β-lactamases in clinical isolates of *Klebsiella pneumoniae* and *Escherichia coli*. *J lin Microbiol.* 1996; 34:908–911.

183. Jacoby G A. Antimicrobial-resistant pathogens in the 1990s. *Annu Rev Med*. 1996; 47:169–179.

184. Jacoby GA. AmpC beta-lactamases. *Clin Microbiol Rev*. Jan 2009; 22(1):161-82, Table of Contents.

185. Jain K, Radsak K, Mannheim W. Differentiation of the oxytocum group from *Klebsiella* by deoxyribonucleic acid-deoxyribonucleic acid hybridization. *Int J Syst Bacteriol.* 1974; 24:402–407.

186. Jarlier V, Nicolas M H, Fournier G, Philippon A. Extended broad-spectrum β-lactamases conferring transferable resistance to newer β-lactam agents in *Enterobacteriaceae*: hospital prevalence and susceptibility patterns. *Rev Infect Dis.* 1988; 10:867–878.

187. Jiang X, Ni Y, Jiang Y, et al. Outbreak of infection caused by *Enterobacter cloacae* producing the novel VEB-3 beta-lactamase in China. *J Clin Microbiol*. Feb 2005; 43(2):826-31.

188. Johanson W G, Pierce A K, Sanford J P. Changing pharyngeal bacterial flora of hospitalized patients. Emergence of Gram-negative bacilli. *N Engl J Med*. 1969; 281:1137–1140.

189. Johnson A P, Weinbren M J, Ayling-Smith B, Du Bois S K, Amyes S G B, George R C. Outbreak of infection in two UK hospitals caused by a strain of *Klebsiella pneumoniae* resistant to cefotaxime and ceftazidime. *J Hosp Infect*. 1992; 20:97–103.

190. Joiner K A. Complement evasion by bacteria and parasites. *Annu Rev Microbiol.* 1988; 42:201–230.

191. Jones G W, Isaacson R E. Proteinaceous bacterial adhesins and their receptors. *Crit Rev Microbiol.* 1983; 10:229–260.

192. Julie L. Badger, Monique F. Stins, and Kwang Sik Kim. "*Citrobacter freundii* Invades and Replicates in Human Brain Microvascular Endothelial Cells." *Hinyokika kiyo. Acta Urologica Japonica.* 1985 Jul; 31(7): 1159-70.

193. Jumaa P, Chattopadhyay B. Pseudobacteraemia with multiply-resistant *Klebsiella pneumoniae* resulting from contamination from the blood gas machine on a neonatal unit. *J Hosp Infect.* 1992; 22:251–255.

194. Kabha K, Nissimov L, Athamna A, Keisari Y, Parolis H, Parolis L A S, Grue R M, Schlepper-Shafer J, Ezekowitz A R B, Ohman D E, Ofek I. Relationships among capsular structure, phagocytosis, and mouse virulence in *Klebsiella pneumoniae*. *Infect Immun*. 1995; 63:847–852.

195. Kabha K, Schmegner J, Keisari Y, Parolis H, Schlepper-Schaefer J, Ofek I. SP-A enhances phagocytosis of *Klebsiella* by interaction with capsular polysaccharides and alveolar macrophages. *Am J Physiol.* 1997; 272:L344–L352.

196. Kamada N, Inoue N, Hisamatsu T, Okamoto S, Matsuoka K, Sato T, Chinen H, Hong KS, Yamada T, Suzuki Y, Suzuki T, Watanabe N, Tsuchimoto K, Hibi T (May 2005). "Nonpathogenic Escherichia coli strain Nissle1917 prevents murine acute and chronic colitis". *Inflamm. Bowel Dis.* 11 (5): 455–63.

197. Kang CI, Kim SH, Park WB, et al. Bloodstream infections caused by *Enterobacter* species: predictors of 30-day mortality rate and impact of broad-spectrum cephalosporin resistance on outcome. *Clin Infect Dis*. Sep 15 2004; 39(6):812-8.

198. Katsanis G P, Spargo J, Ferraro M J, Sutton L, Jacoby G A. Detection of *Klebsiella pneumoniae* and *Escherichia coli* strains producing extended-spectrum β-lactamases. *J Clin Microbiol*. 1994; 32:691–696.

199. Kauffmann F. On the serology of the *Klebsiella* group. *Acta Pathol Scand*. 1949; 26:381–406.

200. Kaye KS, Cosgrove S, Harris A, et al. Risk factors for emergence of resistance to broad-spectrum cephalosporins among *Enterobacter* spp. *Antimicrob Agents Chemother*. Sep 2001; 45(9):2628-30.

201. Keevil CW, Hough JS, Cole JA. “Prototrophic growth of *Citrobacter freundii* and the biochemical basis for its apparent growth requirements in aerated media.” *Journal of General Microbiology*. 1997 Jan; 98(1): 273-6.

202. Keymer JE, Galajda P, Muldoon C, Park S, Austin RH (November 2006). "Bacterial metapopulations in nanofabricated landscapes". *Proc. Natl. Acad. Sci. U.S.A.* 103 (46): 17290–5.

203. Khimji P L, Miles A A. Microbial iron-chelators and their action on *Klebsiella* infections in the skin of guinea-pigs. *Br J Exp Pathol.* 1978; 59:137–147.

204. Kim PW, Harris AD, Roghmann MC, Morris JG Jr, Strinivasan A, Perencevich EN. "Epidemiological risk factors for isolation of ceftriaxone-resistant versus -susceptible *Citrobacter freundii* in hospitalized patients" *Antimicrob Agents Chemother*, 2003 Sep; 47(9): p. 2882-2887.

205. Kirby-Bauer, J.C.Sherris & M.Turcle [1996] Antibiotic sensitivity testing by a standard single disk method. *American Journal of clinical Pathology*, 45:493 -496.

206. Klipstein F A, Engert R F, Houghten R A. Immunological properties of purified *Klebsiella pneumoniae* heat-stable enterotoxin. *Infect Immun*. 1983; 42:838–841.

207. Klipstein F A, Engert R F. Enterotoxigenic intestinal bacteria in tropical sprue. III. Preliminary characterization of *Klebsiella pneumoniae* enterotoxin. *J Infect Dis.* 1975; 132:200–203.

208. Klipstein F A, Engert R F. Immunological interrelationships between cholera toxin and the heat-labile and heat-stable enterotoxins of coliform bacteria. *Infect Immun.* 1977; 18:110–117.

209. Klipstein F A, Engert R F. Purification and properties of *Klebsiella pneumoniae* heat-stable enterotoxin. *Infect Immun.* 1976; 13:373–381.

210. Kloos W E, Musselwhite M S. Distribution and persistence of *Staphylococcus* and *Micrococcus* species and other aerobic bacteria on human skin. *Appl Microbiol.* 1975; 30:381–395.

211. Koneman, E.W., S.D. Allen, V.R. Dowell, Jr. and H.M. Sommers (Eds.), [1983] *Color* Atlas and Textbook of Diagnostic Microbiology, 2nd ed., J.B. Lippincott Co., Philadelphia, PA, pp. 57-124.

212. Koo F C W, Stein M D. Detection of an extracellular toxin produced by burn isolates of *Klebsiella pneumoniae*. *Curr Microbiol.* 1986; 13:23–27.

213. Korhonen T K, Tarkka E, Ranta H, Haahtela K. Type 3 fimbriae of *Klebsiella sp*.: molecular characterization and role in bacterial adhesion to plant roots. *J Bacteriol.* 1983; 155:860–865.

214. Krieg, N. R.; Holt, J. G., eds. (1984). *Bergey's Manual of Systematic Bacteriology*. 1 (First ed.). Baltimore: The Williams & Wilkins Co. pp. 408–420. ISBN 0-683-04108-8.

215. Kubitschek HE (1 January 1990). "Cell volume increase in *Escherichia coli* after shifts to richer media". *J. Bacteriol.* 172 (1): 94–101.
216. Kühn I, Ayling-Smith B, Tullus K, Burman L G. The use of colonization rate and epidemic index as tools to illustrate the epidemiology of faecal *Enterobacteriaceae* strains in Swedish neonatal wards. *J Hosp Infect.* 1993; 23:287–297.
217. Lan R, Reeves PR (September 2002). "*Escherichia coli* in disguise: molecular origins of Shigella". *Microbes Infect.* 4 (11): 1125–32.
218. Larson EL, Cimiotti JP, Haas J, et al. Gram-negative bacilli associated with catheter-associated and non-catheter-associated bloodstream infections and hand carriage by healthcare workers in neonatal intensive care units. *Pediatr Crit Care Med*. Jul 2005; 6(4):457-61.
219. Lawrence JG, Ochman H (August 1998). "Molecular archaeology of the *Escherichia coli* genome"]. *Proc. Natl. Acad. Sci. U.S.A.* 95 (16): 9413–7.
220. Lecointre G, Rachdi L, Darlu P, Denamur E (December 1998). "*Escherichia coli* molecular phylogeny using the incongruence length difference test". *Mol. Biol. Evol.* 15 (12): 1685–95.
221. Lederberg, Joshua; E.L. Tatum (October 19 1946). "Gene recombination in *E. coli"* (PDF). *Nature* 158 (4016): 558. Bibcode 1946 *Natur*.158. 558L.
222. Lee SY (1996). "High cell-density culture of *Escherichia coli*". *Trends Biotechnol.* 14 (3): 98–105.
223. Legakis N J, Maniatis A, Tzouvelekis L S. Prevalent mechanisms of resistance among common enterobacterial isolates in Greek hospitals. *Microb Drug Resist.* 1995; 1:331–333.
224. Lindberg, Frederik; Westman, Lennart; Normark, Staffan. “Regulatory Components in *Citrobacter freundii* ampC β-lactamase Induction.” *Proceedings of the National Academy of Sciences of the United States of America.* 1985 July; 82(14): 4620-4624.

225. Liu CP, Wang NY, Lee CM, et al. Nosocomial and community-acquired Enterobacter cloacae bloodstream infection: risk factors for and prevalence of SHV-12 in multiresistant isolates in a medical centre. *J Hosp Infect*. Sep 2004; 58(1):63-77.

226. Livermore DM, Oakton KJ, Carter MW, et al. Activity of ertapenem (MK-0826) versus *Enterobacteriaceae* with potent beta-lactamases. *Antimicrob Agents Chemother*. Oct 2001; 45(10):2831-7.

227. Livermore DM. beta-Lactamases in laboratory and clinical resistance. *Clin Microbiol Rev*. Oct 1995; 8(4):557-84.

228. Lock R, Dahlgren C, Lindén M, Stendahl O, Svensbergh A, Öhman L. Neutrophil killing of two type 1 fimbria-bearing *Escherichia coli* strains: dependence on respiratory burst activation. *Infect Immun*. 1990; 58:37–42.

229. Lo-Ten-Foe JR, de Smet AM, Diederen BM, et al. Comparative evaluation of the VITEK 2, disk diffusion, etest, broth microdilution, and agar dilution susceptibility testing methods for colistin in clinical isolates, including heteroresistant *Enterobacter cloacae* and *Acinetobacter baumannii* strains. *Antimicrob Agents Chemother*. Oct 2007; 51(10):3726-30.

230. Lowman W, Sriruttan C, Nana T, et al. NDM-1 has arrived: first report of a carbapenem resistance mechanism in South Africa. *S Afr Med J*. Nov 25 2011; 101(12):873-5.

231. Lucet J C, Chevret S, Decre D, Vanjak D, Macrez A, Bedos J P, Wolff M, Regnier B. Outbreak of multiply resistant Enterobacteriaceae in an intensive care unit: Epidemiology and risk factors for acquisition. *Clin Infect Dis.* 1996; 22:430–436.

232. Lukjancenko O, Wassenaar TM, Ussery DW (November 2010). [//www.ncbi.nlm.nih.gov/pmc/articles/PMC2974192/ "Comparison of 61 sequenced *Escherichia coli* genomes"]. *Microb. Ecol.* **60** (4): 708–20.

233. Luzzaro F, Docquier JD, Colinon C, et al. Emergence in *Klebsiella pneumoniae* and *Enterobacter cloacae* clinical isolates of the VIM-4 metallo-beta-lactamase encoded by a conjugative plasmid. *Antimicrob Agents Chemother*. Feb 2004; 48(2):648-50.

234. Lye W C, Chan R K T, Lee E J C, Kumarasinghe G. Urinary tract infections in patients with diabetes mellitus. *J Infect.* 1992; 24:169–174.

235. Maayan M C, Ofek I, Medalia O, Aronson M. Population shift in mannose-specific fimbriated phase of *Klebsiella pneumoniae* during experimental urinary tract infection in mice. *Infect Immun.* 1985; 49:785–789.

236. MacFaddin, Jean F. Biochemical Tests for Identification of Medical Bacteria. Williams & Wilkins, 1980, p 441.

237. Madigan MT, Martinko JM (2006). *Brock Biology of microorganisms* (11th ed.). Pearson. ISBN 0-13-196893-9.

238. Maki D G. Epidemic nosocomial bacteremias. In: Wenzel R P, editor. CRC Handbook of hospital acquired infections. Boca Raton, Fla: CRC Press, Inc.; 1981. pp. 371–512.

239. Maki DG, Weise CE, Sarafin HW. A semiquantitative culture method for identifying intravenous-catheter-related infection. *N Engl J Med*. Jun 9 1977; 296(23):1305-9.

240. Mandine E, Salles M F, Zalisz R, Guenounou M, Smets P. Murine monoclonal antibodies to *Klebsiella pneumoniae* protect against lethal endotoxemia and experimental infection with capsulated *K. pneumoniae*. *Infect Immun.* 1990; 58:2828–2833.

241. Mangan D F, Snyder I S. Mannose-sensitive stimulation of human leucocyte chemiluminescence by *Escherichia coli*. *Infect Immun.* 1979; 26:1014–1019.

242. Marco Sánchez F, Turabian Fernández JL, Durán Pérez-Navarro A. “Fatal *Citrobacter freundii* Bronchopneumonia Acquired in the Community in an Uncompromised Patient.” *Revista Clínica Española*. 1985 Apr; 176(6): 320.

243. Markowitz SM, Smith SM, Williams DS. Retrospective analysis of plasmid patterns in a study of burn unit outbreaks of infection due to *Enterobacter cloacae*. *J Infect Dis*. Jul 1983; 148(1):18-23.

244. Martin, C. M., N. S. Ikari, J. Zimmerman, and J. A. Waitz. 1971. A virulent nosocomial *Klebsiella* with a transferable R factor for gentamicin: emergence and suppression. *J. Infect. Dis.* 124 (Suppl.): S24–S29.

245. Martinez J L, Cercenado E, Baquero F, Pérez-Díaz J C, Delgado-Iribarren A. Incidence of aerobactin production in Gram-negative hospital isolates. *FEMS Microbiol Lett*. 1987; 43:351–353.

246. Matsen J M, Spindler J A, Blosser R O. Characterization of *Klebsiella* isolates from natural receiving waters and comparison with human isolates. *Appl Microbiol.* 1974; 28:672–678. [PMC free article]

247. Matsumoto T, Mizunoe Y, Sakamoto N, Tanaka M, Kumazawa J. Increased renal scarring by bacteria with mannose-sensitive pili. *Urol Res.* 1990; 18:299–303.

248. Mayhall C G. Surgical infections including burns. In: Wenzel R P, editor. Prevention and control of nosocomial infections. 2nd ed. Baltimore, Md: The Williams & Wilkins Co.; 1987. pp. 614–664.

249. McCallum K L, Schoenhals G, Laakso D, Clarke B, Whitfield C. A high-molecular-weight fraction of smooth lipopolysaccharide in *Klebsiella* serotype O1:K20 contains a unique O-antigen epitope and determines resistance to nonspecific serum killing. *Infect Immun.* 1989; 57:3816–3822.

250. Medeiros A A. Nosocomial outbreaks of multiresistant bacteria—extended-spectrum beta-lactamases have arrived in North America. *Ann Intern Med.* 1993; 119:428–430.

251. Merino S, Camprubí S, Albertí S, Benedí V J, Tomás J M. Mechanisms of *Klebsiella pneumoniae* resistance to complement-mediated killing. *Infect Immun.* 1992; 60:2529–2535.

252. Meyer K S, Urban C, Eagan J A, Berger B J, Rahal J J. Nosocomial outbreak of *Klebsiella* infection resistant to late-generation cephalosporins. *Ann Intern Med.* 1993; 119:353–358.
253. Migula (W.): Bacteriaceae (Stabchenbacterien). In: A. ENGLER and K. PRANTL (eds): Die Naturlichen Pfanzenfamilien, W. Engelmann, Leipzig, Teil I, Abteilung Ia, 1895, pp. 20–30.
254. Miles A A, Khimji P L. Enterobacterial chelators of iron: their occurrence, detection, and relation to pathogenicity. *J Med Microbiol.* 1975; 8:477–490.
255. Minami J, Katayama S I, Matsushita O, Sakamoto H, Okabe A. Enterotoxic activity of *Klebsiella oxytoca* cytotoxin in rabbit intestinal loops. *Infect Immun.* 1994; 62:172–177.
256. Minami J, Okabe A, Shiode J, Hayashi H. Production of a unique cytotoxin by *Klebsiella oxytoca*. *Microb Pathog.* 1989; 7:203–211.
257. Minami J, Saito S, Yoshida T, Uemura T, Okabe A. Biological activities and chemical composition of a cytotoxin of *Klebsiella oxytoca*. *J Gen Microbiol*. 1992; 138:1921–1927.
258. Mizuta K, Ohta M, Mori M, Hasegawa T, Nakashima I, Kato N. Virulence for mice of *Klebsiella* strains belonging to the O1 group: relationship to their capsule (K) types. *Infect Immun.* 1983; 40:56–61.
259. Mobley H L T, Chippendale G R, Tenney J H, Mayrer A R, Crisp L J, Penner J L, Warren J W. MR/K hemagglutination of *Providencia stuartii* correlates with adherence to catheters and with persistence in catheter-associated bacteriuria. *J Infect Dis.* 1988; 157:264–271.
260. Monnet D, Freney J, Brun Y, Boeufgras J M, Fleurette J. Difficulties in identifying *Klebsiella* strains of clinical origin. *Zentbl Bakteriol.* 1991; 274:456–464.
261. Monnet D, Freney J. Method for differentiating *Klebsiella planticola* and *Klebsiella terrigena* from other *Klebsiella* species. *J Clin Microbiol.* 1994; 32:1121–1122.

262. Montenegro M A, Bitter-Suermann D, Timmis J K, Agüero M E, Cabello F C, Sanyal S C, Timmis K N. TraT gene sequences, serum resistance and pathogenicity-related factors in clinical isolates of *Escherichia coli* and other Gram-negative bacteria. *J Gen Microbiol.* 1985; 131:1511–1521.

263. Montgomerie J Z, John J F, Atkins L M, Gilmore D S, Ashley M A. Increased frequency of large R-plasmids in *Klebsiella pneumoniae* colonizing patients with spinal cord injury. *Diagn Microbiol Infect Dis.* 1993; 16:25–29.

264. Montgomerie J Z. Epidemiology of *Klebsiella* and hospital-associated infections. *Rev Infect Dis.* 1979; 1:736–753.

265. Mori M, Ohta M, Agata N, Kido N, Arakawa Y, Ito H, Komatsu T, Kato N. Identification of species and capsular types of *Klebsiella* clinical isolates, with special reference to *Klebsiella planticola*. *Microbiol Immunol.* 1989; 33:887–895.

266. Murakami K, Yoshida T. “Covalent binding of moxalactam to cephalosporinase of *Citrobacter freundii*.” *Antimicrobial Agents and Chemotherapy.* 1985 May; 27(5): 727-32.

267. Murray PR, et. al, [2009] Extended spectrum Amp C and metallo-beta-lactamases in *Serratia & Citorbacer* Spp. In a disc Approximation assay. *Journal of Infection in developing Countries*. 3(4) 285-294.

268. Mushtaq S, Ge Y, Livermore DM. Comparative activities of doripenem versus isolates, mutants, and transconjugants of *Enterobacteriaceae* and *Acinetobacter* spp. with characterized beta-lactamases. *Antimicrob Agents Chemother*. Apr 2004; 48(4):1313-9.

269. Nada T, Baba H, Kawamura K, Ohkura T, Torii K, Ohta M. "A small outbreak of third generation cephem-resistant *Citrobacter freundii* infection on a surgical ward" *Jpn J Infect Dis.* 2004 Aug;57(4): p.181-182.

270. Nassif X, Sansonetti P J. Correlation of the virulence of *Klebsiella pneumoniae* K1 and K2 with the presence of a plasmid encoding aerobactin. *Infect Immun.* 1986; 54:603–608.

271. Nataro JP, Kaper JB (January 1998). "Diarrheagenic *Escherichia coli*". *Clin. Microbiol. Rev.* 11 (1): 142–201.

272. Noriega, E. R., R. E. Leibowitz, A. S. Richmond, E. Rubinstein, S. Schaefler, M. S. Simberkoff, and J. J. Rahal. 1975. Nosocomial infection caused by gentamicin-resistant, streptomycin-sensitive *Klebsiella*. *J. Infect. Dis.* 131(Suppl):S45–S50.

273. Nouvellon M, Pons J L, Sirot D, Combe M L, Lemeland J F. Clonal outbreaks of extended-spectrum β-lactamase-producing strains of *Klebsiella pneumoniae* demonstrated by antibiotic susceptibility testing, β-lactamase typing, and multilocus enzyme electrophoresis. *J Clin Microbiol*. 1994; 32:2625–2627.

274. Ofek I, Beachey E H. Mannose binding and epithelial cell adherence of *Escherichia coli*. *Infect Immun*. 1978; 22:247–254.

275. Ofek I, Doyle R J. Bacterial adhesion to cells and tissues. London, United Kingdom: Chapman & Hall, Ltd.; 1994.

276. Ofek I, Goldhar J, Keisari Y. Nonopsonic phagocytosis of microorganisms. *Annu Rev Microbiol*. 1995; 49:239–276.

277. Ofek I, Goldhar J, Zafriri D, Lis H, Adar R, Sharon N. Anti-*Escherichia coli* adhesin activity of cranberry and blueberry juices. *N Engl J Med.* 1991; 324:1599.

278. Ofek I, Kabha K, Athamna A, Frankel G, Wozniak D J, Hasty D L, Ohman D E. Genetic exchange of determinants for capsular polysaccharide biosynthesis between *Klebsiella pneumoniae* strains expressing serotypes K2 and K21a. *Infect Immun.* 1993; 61:4208–4216.

279. Ofek I, Sharon N. Lectinophagocytosis: a molecular mechanism of recognition between cell surface sugars and lectins in the phagocytosis of bacteria. *Infect Immun.* 1988; 56:539–547.

280. Ohlsson A, Bailey T, Takieddine F. Changing etiology and outcome of neonatal septicemia in Riyadh, Saudi Arabia. *Acta Paediatr Scand.* 1986; 75:540–544.
281. Öhman L, Magnusson K-E, Stendahl O. Mannose-specific and hydrophobic interaction between *Escherichia coli* and polymorphonuclear leucocytes—influence of bacterial culture period. *Acta Pathol Microbiol Immunol Scand Sect B*. 1985; 93:125–131.
282. Old D C, Adegbola R A. Antigenic relationships among type-3 fimbriae of Enterobacteriaceae revealed by immunoelectronmicroscopy. *J Med Microbiol.* 1985; 20:113–121.
283. Old D C, Tavendale A, Senior B W. A comparative study of the type-3 fimbriae of *Klebsiella* species. *J Med Microbiol.* 1985; 20:203–214.
284. Olling, S. 1977. Sensitivity of Gram-negative bacilli to the serum bactericidal activity: a marker of the host parasite relationship in acute and persisting infections. *Scand. J. Infect. Dis.* 10(Suppl.):1–40.
285. Opal S, Cross A, Gemski P. K antigen and serum sensitivity of rough *Escherichia coli*. *Infect Immun.* 1982; 37:956–960.
286. Ørskov I, Fife-Asbury M A. New *Klebsiella* capsular antigen, K82, and the deletion of five of those previously assigned. *Int J Syst Bacteriol.* 1977; 27:386–387.
287. Orskov I, Orskov F, Jann B, Jann K (September 1977). "Serology, chemistry, and genetics of O and K antigens of *Escherichia coli*"]. *Bacteriol Rev* 41 (3): 667–710.
288. Ørskov I, Ørskov F. Serotyping of *Klebsiella*. *Methods Microbiol.* 1984; 14:143–164.
289. Ørskov I. Genus *Klebsiella*. In: Krieg N R, Holt J G, editors. Bergey's manual of systematic bacteriology. Vol. 1. Baltimore, Md: The Williams & Wilkins Co.; 1984. pp. 461–465.

290. Ørskov I. O antigens in the *Klebsiella* group. *Acta Pathol Microbiol Scand.* 1954; 34:145–156.
291. Ottow J C G. Ecology, physiology, and genetics of fimbriae and pili. *Annu Rev Microbiol.* 1975; 29:79–108.
292. Pagani L, Ronza P, Giacobone E, Romero E. Extended spectrum beta-lactamases from *Klebsiella pneumoniae* strains isolated at an Italian hospital. *Eur J Epidemiol.* 1994; 10:533–540.
293. Palmer DL, Kuritsky JN, Lapham SC, et al. *Enterobacter mediastinitis* following cardiac surgery. *Infect.*
294. Palmer DL, Kuritsky JN, Lapham SC, et al. *Enterobacter mediastinitis* following cardiac surgery. *Infect Control*. Mar 1985; 6(3):115-9.
295. Paterson DL, Rossi F, Baquero F, et al. *In vitro* susceptibilities of aerobic and facultative Gram-negative bacilli isolated from patients with intra-abdominal infections worldwide: the 2003 Study for Monitoring Antimicrobial Resistance Trends (SMART). *J Antimicrob Chemother*. Jun 2005; 55(6):965-73.
296. Paterson DL. Resistance in Gram-negative bacteria: Enterobacteriaceae. *Am J Med*. Jun 2006; 119(6 Suppl 1):S20-8; discussion S62-70.
297. Pathengay A, Trehan HS, Mathai A, et al. *Enterobacter endophthalmitis*: clinicomicrobiologic profile and outcomes. *Retina*. Mar 2012; 32(3):558-62.
298. Pieroni P, Rennie R P, Ziola B, Deneer H G. The use of bacteriophages to differentiate serologically cross-reactive isolates of *Klebsiella pneumoniae*. *J Med Microbiol*. 1994; 41:423–429.
299. Pintado V, San Miguel LG, Grill F, et al. Intravenous colistin sulphomethate sodium for therapy of infections due to multidrug-resistant Gram-negative bacteria. *J Infect*. Mar 2008; 56(3):185-90.
300. Pitout JD, Nordmann P, Laupland KB, et al. Emergence of *Enterobacteriaceae* producing extended-spectrum beta-lactamases (ESBLs) in the community. *J Antimicrob Chemother*. Jul 2005; 56(1):52-9.

301. Pitout JD, Thomson KS, Hanson ND, et al. Plasmid-mediated resistance to expanded-spectrum cephalosporins among *Enterobacter aerogenes* strains. *Antimicrob Agents Chemother*. Mar 1998; 42(3):596-600.
302. Pittet D, Li N, Wenzel R P. Association of secondary and polymicrobial nosocomial bloodstream infections with higher mortality. *Eur J Clin Microbiol Infect Dis.* 1993; 12:813–819.
303. Podschun R, Fischer A, Ullmann U. Siderophore production of *Klebsiella species* isolated from different sources. *Zentbl Bakteriol*. 1992; 276:481–486.
304. Podschun R, Heineken P, Sonntag H G. Hemagglutinins and adherence properties to HeLa and Intestine 407 cells of *Klebsiella pneumoniae* and *Klebsiella oxytoca* isolates. *Zentbl Bakteriol Mikrobiol Hyg Ser A.* 1987; 263:585–593.
305. Podschun R, Heineken P, Ullmann U, Sonntag H G. Comparative investigations of *Klebsiella* species of clinical origin: plasmid patterns, biochemical reactions, antibiotic resistances and serotypes. *Zentbl Bakteriol Mikrobiol Hyg Ser A.* 1986; 262:335–345.
306. Podschun R, Penner I, Ullmann U. Interaction of *Klebsiella* capsule type 7 with human polymorphonuclear leucocytes. *Microb Pathog.* 1992; 13:371–379.
307. Podschun R, Ullmann U. Bacteriocin typing of environmental *Klebsiella* isolates. Zentbl Hyg Umweltmed. 1993; 195:22–26.
308. Podschun R, Ullmann U. Bacteriocin typing of *Klebsiella* spp. isolated from different sources. *Zentbl Hyg Umweltmed.* 1996; 198:258–264.
309. Podschun R, Ullmann U. Incidence of *Klebsiella planticola* among clinical *Klebsiella* isolates. *Med Microbiol Lett.* 1994; 3:90–95.
310. Podschun R, Ullmann U. Isolation of *Klebsiella terrigena* from clinical specimens. *Eur J Clin Microbiol Infect Dis.* 1992; 11:349–352.

311. Podschun R, Ullmann U. *Klebsiella* capsular type K7 in relation to toxicity, susceptibility to phagocytosis and resistance to serum. *J Med Microbiol.* 1992; 36:250–254.

312. Podschun R. Phenotypic properties of *Klebsiella pneumoniae* and *K. oxytoca* isolated from different sources. *Zentbl Hyg Umweltmed*. 1990; 189:527–535.

313. Poh C L, Yap S C, Yeo M. Pulsed-field gel electrophoresis for differentiation of hospital isolates of *Klebsiella pneumoniae*. *J Hosp Infect.* 1993; 24:123–128.

314. Pollack M, Niemann R E, Reinhardt J A, Charache P, Jett M P, Hardy P H., Jr Factors influencing colonization and antibiotic-resistance patterns of Gram-negative bacteria in hospital patients. *Lancet*. 1972; ii: 668–671.

315. Porat R, Johns M A, McCabe W R. Selective pressures and lipopolysaccharide subunits as determinants of resistance of clinical isolates of Gram-negative bacilli to human serum. *Infect Immun.* 1987; 55:320–328.

316. Przondo-Hessek A, Pulverer G. Hemagglutinins of *Klebsiella pneumoniae* and *Klebsiella oxytoca*. *Zentbl Bakteriol Mikrobiol Hyg Ser A.* 1983; 255:472–478.

317. Przondo-Hessek A. Bacteriophages of *Klebsiella* bacilli. Isolation properties and use in typing. *Arch Immunol Ther Exp*. 1966; 14:413–435.

318. Puchenkova SG. “Enterobacteria in areas of water along the Crimean Coast.” *Mikrobiolohichnyĭ zhurnal.* 1996 Mar-Apr; 58(2): 3-7.

319. Ramm L E, Whitlow M B, Mayer M M. Size distribution and stability of the trans-membrane channels formed by complement complex C5b-9. *Mol Immunol.* 1983; 20:155–160.

320. Ransjö U, Good Z, Jalakas K, Kühn I, Siggelkow I, berg B, Anjou E. An outbreak of *Klebsiella oxytoca* septicemias associated with the use of invasive blood pressure monitoring equipment. *Acta Anaesthesiol Scand.* 1992; 36:289–291.

321. Ratnam I, Franklin C, Spelman DW. *In vitro* activities of 'new' and 'conventional' antibiotics against multi-drug resistant Gram negative bacteria from patients in the intensive care unit. *Pathology*. Dec 2007; 39(6):586-8.

322. Reid G, Howard J, Gan BS (September 2001). "Can bacterial interference prevent infection?". *Trends Microbiol.* **9** (9): 424–428.

323. Reinert RR, Low DE, Rossi F, et al. Antimicrobial susceptibility among organisms from the Asia/Pacific Rim, Europe and Latin and North America collected as part of TEST and the *in vitro* activity of tigecycline. *J Antimicrob Chemother*. Nov 2007; 60(5):1018-29.

324. Reinhardt H H, Obedeanu N, Sobel J D. Quantitation of Tamm-Horsfall protein binding to uropathogenic *Escherichia coli* and lectins. *J Infect Dis.* 1990; 162:1335–1340.

325. Reish O, Ashkenazi S, Naor N, Samra Z, Merlob P. An outbreak of multiresistant *Klebsiella* in a neonatal intensive care unit. *J Hosp Infect.* 1993; 25:287–291.

326. Reissbrodt R, Rabsch W. Further differentiation of Enterobacteriaceae by means of siderophore-pattern analysis. *Zentbl Bakteriol Mikrobiol Hyg Ser A.* 1988; 268:306–317.

327. Rennie R P, Duncan I B R. Combined biochemical and serological typing of clinical isolates of *Klebsiella*. *Appl Microbiol.* 1974; 28:534–539.

328. Riser E, Noone P. Klebsiella capsular type versus site of isolation. *J Clin Pathol.* 1981; 34:552–555.

329. Ristuccia PA, Cunha BA. Enterobacter. *Infect Control*. Mar 1985; 6(3):124-8.

330. Ritchie DJ, Alexander BT, Finnegan PM. New antimicrobial agents for use in the intensive care unit. *Infect Dis Clin North Am*. Sep 2009; 23(3):665-81.

331. Rizvi M. et al., [2009] Extended spectrum AmpC and Metallo-beta Lactamases in *Serratia & Citrobacter* Spp. In a disc approximation assay. *Journal of infection in developing Countries*, 3(9): 285-294.
332. Roantree R J, Rantz L A. A study of the relationship of the normal bactericidal activity of human serum to bacterial infection. *J Clin Invest.* 1960; 39:72–81.
333. Rodriguez-Ortega M, Ofek I, Sharon N. Membrane glycoproteins of human polymorphonuclear leucocytes that act as receptors for mannose-specific *Escherichia coli*. *Infect Immun*. 1987; 55:968–973.
334. Rose H D, Schreier J. The effect of hospitalization and antibiotic therapy on the gram-negative fecal flora. *Am J Med Sci*. 1968; 255:228–236.
335. Rosebury T. Microorganisms indigenous to man. New York, N.Y: McGraw Hill Book Co.; 1962.
336. Rosenthal S, Tager I B. Prevalence of Gram-negative rods in the normal pharyngeal flora. *Ann Intern Med*. 1975; 83:355–357.
337. Rossi F, Baquero F, Hsueh PR, et al. *In vitro* susceptibilities of aerobic and facultatively anaerobic Gram-negative bacilli isolated from patients with intra-abdominal infections worldwide: 2004 results from SMART (Study for Monitoring Antimicrobial Resistance Trends). *J Antimicrob Chemother*. Jul 2006; 58(1):205-10.
338. Rubin S J. *Klebsiella* marker systems. *Infect Control*. 1985; 6:59–63.
339. Rupp ME, Fey PD. Extended spectrum beta-lactamase (ESBL)-producing Enterobacteriaceae: considerations for diagnosis, prevention and drug treatment. *Drugs*. 2003; 63(4):353-65.
340. Russo E (January 2003). "The birth of biotechnology". *Nature* 421 (6921): 456–457.
341. Sader HS, Jones RN, Dowzicky MJ, et al. Antimicrobial activity of tigecycline tested against nosocomial bacterial pathogens from patients hospitalized in the intensive care unit. *Diagn Microbiol Infect Dis*. Jul 2005; 52(3):203-8.

342. Sader HS, Jones RN, Stilwell MG, et al. Tigecycline activity tested against 26,474 bloodstream infection isolates: a collection from 6 continents. *Diagn Microbiol Infect Dis*. Jul 2005; 52(3):181-6.

343. Sakazaki R, Tamura K, Kosako Y, Yoshizaki E. *Klebsiella ornithinolytica* sp. nov., formerly known as ornithine-positive *Klebsiella oxytoca*. *Curr Microbiol.* 1989; 18:201–206.

344. Samen von Bockshornklee mit hoher Wahrscheinlichkeit für EHEC O104:H4 Ausbruch verantwortlich *in English: Fenugreek seeds with high probability for EHEC O104: H4 responsible outbreak*" (in German) (PDF). Bundesinstitut für Risikobewertung (BfR) *in English: Federal Institute for Risk Assessment*. 30 June 2011.

345. Sánchez-Céspedes J, Vila J. "Partial characterization of the acrAB locus in two *Citrobacter freundii* clinical isolates." *International Journal of Antimicrobial Agents.* 2007 Sep; 30(3): 259-63.

346. Sanders CC, Sanders WE Jr. beta-Lactam resistance in Gram-negative bacteria: global trends and clinical impact. *Clin Infect Dis*. Nov 1992; 15(5):824-39.

347. Sanders WE Jr, Sanders CC. *Enterobacter* spp.: pathogens poised to flourish at the turn of the century. *Clin Microbiol Rev*. Apr 1997; 10(2):220-41.

348. Schaberg D R, Culver D H, Gaynes R P. Major trends in the microbial etiology of nosocomial infection. *Am J Med.* 1991; 91:72S–75S.

349. Schaechter M, Medoff G, Eisenstein B I. Mechanisms of microbial disease. 2nd ed. Baltimore, Md: The Williams & Wilkins Co.; 1993.

350. Schiappa D A, Hayden M K, Matushek M G, Hashemi F N, Sullivan J, Smith K Y, Miyashiro D, Quinn J P, Weinstein R A, Trenholme G M. Ceftazidime-resistant *Klebsiella pneumoniae* and *Escherichia coli* bloodstream infection: a case-control and molecular epidemiologic investigation. *J Infect Dis.* 1996; 174:529–536.

351. Seidler R J, Knittel M D, Brown C. Potential pathogens in the environment: cultural reactions and nucleic acid studies on *Klebsiella pneumoniae* from clinical and environmental sources. *Appl Microbiol.* 1975; 29:819–825.

352. Selden R, Lee S, Wang W L, Bennett J V, Eickhoff T C. Nosocomial *Klebsiella* infections: intestinal colonization as a reservoir. *Ann Intern Med.* 1971; 74:657–664.

353. Sharon N, Ofek I. Mannose specific bacterial surface lectins. In: Mirelman D, editor. Microbial lectins and agglutinins. New York, N.Y: John Wiley & Sons, Inc.; 1986. pp. 55–81.

354. Shukla I. et al., [2004] prevalence of extended spectrum Beta-lactamase producing *Klebsiella pneumonia* in a tertiary care hospital. *Indian Journal of Medical Microbiology,* 22(2):87-91.

355. Siegel JD, Rhinehart E, Jackson M, Chiarello L and the HICPAC. Guideline for Isolation Precautions: Preventing Transmission of Infectious Agents in Healthcare Settings 2007.

356. Siegel JD, Rhinehart E, Jackson M, Chiarello L, HICPAC. Management of Multidrug-Resistant Organisms In Healthcare Settings, 2006. Centers for Disease Control, 2008.

357. Simoons-Smit A M, Verweij-van Vught A M J J, Kanis I Y R, MacLaren D M. Biochemical and serological investigations on clinical isolates of *Klebsiella*. *J Hyg Camb.* 1985; 95:265–276.

358. Simoons-Smit A M, Verweij-van Vught A M J J, Kanis I Y R, MacLaren D M. Chemiluminescence of human leucocytes stimulated by clinical isolates of *Klebsiella*. *J Med Microbiol.* 1985; 19:333–338.

359. Simoons-Smit A M, Verweij-van Vught A M J J, MacLaren D M. The role of K antigens as virulence factors in *Klebsiella*. *J Med Microbiol.* 1986; 21:133–137.

360. Simoons-Smit A M, Verweij-van Vught A M J J, MacLaren D M. Virulence of *Klebsiella* strains in experimentally induced skin lesions in the mouse. *J Med Microbiol.* 1984; 17:67–77.

361. Sirot D. Extended-spectrum plasmid-mediated beta-lactamases. *J Antimicrob Chemother*. 1995; 36:19–34.

362. Slopek S, Przondo-Hessek A, Milch H, Deak S. A working scheme for bacteriophage typing of *Klebsiella* bacilli. *Arch Immunol Ther Exp.* 1967; 15:589–599.

363. Slopek S. Phage typing of *Klebsiella*. *Methods Microbiol*. 1978; 11:193–222.

364. Smith J M B, Chambers S T. *Klebsiella oxytoca* revealing decreased susceptibility to extended spectrum beta-lactams. *J Antimicrob Chemother.* 1995; 36:265–267.

365. Smith R F, Dayton S L, Chipps D D, Blasi D. Intestinal carriage of *Klebsiella* and *Pseudomonas* in burned children and their comparative role in nosocomial infection. Health Lab Sci. 1973; 10:173–179.

366. Sonnenwirth, A.C. *in* A.C. Sonnenwirth and L.Jarett (Eds.), [1980] Gradwohl's Clinical Laboratory Methods and Diagnosis, 8th ed., C.V. Mosby Co., St. Louis, MO, Vol. 2, pp. 1554-1628.

367. Souli M, Kontopidou FV, Papadomichelakis E, et al. Clinical experience of serious infections caused by Enterobacteriaceae producing VIM-1 metallo-beta-lactamase in a Greek University Hospital. *Clin Infect Dis*. Mar 15 2008; 46(6):847-54.

368. Spencer R C. Predominant pathogens found in the European Prevalence of Infection in Intensive Care Study. *Eur J Clin Microbiol Infect Dis.* 1996; 15:281–285.

369. Srinivasan V, Nam HM, Sawant AA, Headrick SI, Nguyen LT, Oliver SP. “Distribution of Tetracycline and Streptomycin Resistance Genes and Class 1 Integrons in Enterobacteriaceae Isolated from Dairy and Nondairy Farm Soil.” *Microbial Ecology*. 2007 Aug 15.

370. Stamm W E, Weinstein R A, Dixon R E. Comparison of endemic and epidemic nosocomial infections. In: Dixon R E, editor. Nosocomial infections. Atlanta, Ga: Yorke Medical Books; 1981. pp. 9–13.
371. Stenzel W, Bürger H, Mannheim W. Zur Systematik und Differentialdiagnostik der Klebsiella-Gruppe mit besonderer Berücksichtigung der sogenannten Oxytocum-Typen. Zentbl Bakteriol Mikrobiol Hyg Ser A. 1972; 219:193–203.
372. Straus D C. Production of an extracellular toxic complex by various strains of *Klebsiella pneumoniae*. *Infect Immun.* 1987; 55:44–48.
373. Suerbaum S, Friedrich S, Leying H, Opferkuch W. Expression of capsular polysaccharide determines serum resistance in *Escherichia coli* K 92. Zentbl Bakteriol. 1994; 281:146–157.
374. Tarkkanen A M, Virkola R, Clegg S, Korhonen T K. Binding of the type 3 fimbriae of *Klebsiella pneumoniae* to human endothelial and urinary bladder cells. *Infect Immun.* 1997; 65:1546–1549.
375. Tarkkanen A-M, Allen B, Westerlund B, Holthöfer H, Kuusela P, Risteli L, Clegg S, Korhonen T K. Type V collagen as target for type-3 fimbriae, enterobacterial adherence organelles. *Mol Microbiol*. 1990; 4:1353–1361.
376. Taylor P W, Kroll H-P. Effect of lethal doses of complement on the functional integrity of target enterobacteria. *Curr Top Microbiol Immunol*. 1985; 121:135–158.
377. Taylor P W. Bactericidal and bacteriolytic activity of serum against Gram-negative bacteria. *Microbiol Rev*. 1983; 47:46–83.
378. Taylor, E.W. *in* J.C. Clough, J.F. Beale and E.V. Suckling, [1949] The Examination of Waters and Water Supplies*,* 6th ed., The Blakiston Co., Philadelphia, PA,.

379. Tessin I, Trollfors B, Thiringer K. Incidence and etiology of neonatal septicaemia and meningitis in Western Sweden 1975–1986. *Acta Paediatr Scand.* 1990; 79:1023–1030.

380. The Phage Course – Origins". Cold Spring Harbor Laboratory. 2006. http://www.cshl.edu/History/phagecourse.html. Retrieved 2007-12-03.

381. Thompson, Andrea (2007-06-04). "E. coli Thrives in Beach Sands". Live Science. http://www.livescience.com/health/070604_beach_ecoli.html. Retrieved 2007-12-03.

382. Todar, K. "Pathogenic *E. coli*". *Online Textbook of Bacteriology*. University of Wisconsin–Madison Department of Bacteriology. http://www.textbookofbacteriology.net/e.coli.html. Retrieved 2007-11-30.

383. Tof, Ilanit (1994). "Recombinant DNA Technology in the Synthesis of Human Insulin". Little Tree Pty. Ltd. http://www.littletree.com.au/dna.htm. Retrieved 2007-11-30.

384. Tomás J M, Benedí V J, Ciurana B, Jofre J. Role of capsule and O antigen in resistance of *Klebsiella pneumoniae* to serum bactericidal activity. *Infect Immun.* 1986; 54:85–89.

385. Tomás J M, Camprubi S, Williams P. Surface exposure of the O-antigen in *Klebsiella pneumoniae* O1:K1 serotype strains. *Microb Pathog.* 1988; 5:141–147.

386. Tresoldi AT, Padoveze MC, Trabasso P, et al. *Enterobacter cloacae* sepsis outbreak in a newborn unit caused by contaminated total parenteral nutrition solution. *Am J Infect Control*. Jun 2000; 28(3):258-61.

387. Tsukamoto K, Tachibana K, Yamazaki N, Ishii Y, Ujiie K, Nishida N, Sawai T. “Role of lysine-67 in the active site of class C beta-lactamase from *Citrobacter freundii* GN346.” *European Journal of Biochemistry /FEBS.* 1990 Feb 22; 188(1): 15-22.

388. Tullus K, Berglund B, Fryklund B, Kühn I, Burman L G. Epidemiology of fecal strains of the family *Enterobacteriaceae* in 22 neonatal wards and influence of antibiotic policy. *J Clin Microbiol.* 1988; 26:1166–1170.

389. Tullus K, Olsson-Liljequist B, Lundström G, Burman L G. Antibiotic susceptibility of 629 bacterial blood and CSF isolates from Swedish infants and the therapeutic implications. *Acta Paediatr Scand*. 1991; 80:205–212.

390. Tunkel AR, Fisch MJ, Schlein A, et al. *Enterobacter endocarditis*. *Scand J Infect Dis*. 1992; 24(2):233-40.

391. Ullmann U. Pattern of microbial isolates in hospitalized patients. *Zentbl Bakteriol Mikrobiol Hyg Ser B.* 1986; 183:103–113.

392. Ullmann U. The distribution of *Klebsiella pneumoniae* serotypes from different sources and their sensitivity to cephalosporins. *Infection*. 1983; 11:S28–S31.

393. Urban C, Rahal J J. *Klebsiella* and extended spectrum β-lactamases. *Int J Antimicrob Agents.* 1997; 8:37–43.

394. Venegas M F, Navas E L, Gaffney R A, Duncan J L, Anderson B E, Schaeffer A J. Binding of type 1-piliated *Escherichia coli* to vaginal mucus. *Infect Immun.* 1995; 63:416–422.

395. Venezia R A, Scarano F J, Preston K E, Steele L M, Root T P, Limberger R, Archinal W, Kacica M A. Molecular epidemiology of an SHV-5 extended-spectrum beta-lactamase in Enterobacteriaceae isolated from infants in a neonatal intensive care unit. *Clin Infect Dis*. 1995; 21:915–923.

396. Vermeulen C, Cross A, Byrne W R, Zollinger W. Quantitative relationship between capsular content and killing of K1-encapsulated *Escherichia coli*. *Infect Immun*. 1988; 56:2723–2730.

397. Vesikari T, Isolauri E, Tuppurainen N, Renlund M, Koivisto M, Janas M, Ikonen R S, Kero P, Heinonen K, Nyman R, Kunnas M. Neonatal septicaemia in Finland 1981–85. *Acta Paediatr Scand.* 1989; 78:44–50.

398. Vidal O, Longin R, Prigent-Combaret C, Dorel C, Hooreman M, Lejeune P (1998). "Isolation of an *Escherichia coli* K-12 mutant strain able to form biofilms on inert surfaces: involvement of a new ompR allele that increases curli expression"]. *J. Bacteriol.* 180 (9): 2442–9.

399. Virkola R, Westerlund B, Holthöfer H, Parkkinen J, Kekomäki M, Korhonen T K. Binding characteristics of *Escherichia coli* adhesins in human urinary bladder. *Infect Immun*. 1988; 56:2615–2622.

400. Vogt RL, Dippold L (2005). "*Escherichia coli O157:H7* outbreak associated with consumption of ground beef, June–July 2002"]. *Public Health Rep* 120 (2): 174–8.

401. Vosti K L, Randall E. Sensitivity of serologically classified strains of *Escherichia coli* of human origin to the serum bactericidal system. *Am J Med Sci.* 1970; 259:114–119.

402. Wacker M, Linton D, Hitchen PG, Nita-Lazar M, Haslam SM, North SJ, Panico M, Morris HR, Dell A, Wren BW, Aebi M (2002). "N-linked glycosylation in *Campylobacter jejuni* and its functional transfer into *E. coli*". *Science* 298 (5599): 1790–1793.

403. Walia S, Madhavan T, Reuman P, Tewari R, Duckworth D. Plasmid profiles and klebocin types in epidemiologic studies of infections by *Klebsiella pneumoniae*. *Eur J Clin Microbiol Infect Dis*. 1988; 7:279–284.

404. Walkty A, DeCorby M, Nichol K, Karlowsky JA, Hoban DJ, Zhanel GG. *In vitro* activity of colistin (polymyxin E) against 3,480 isolates of Gram-negative bacilli obtained from patients in Canadian hospitals in the CANWARD study, 2007-2008. *Antimicrob Agents Chemother*. Nov 2009; 53(11):4924-6.

405. Wang JT, Chang SC, Chen YC, Luh KT. “Comparison of antimicrobial susceptibility of *Citrobacter freundii* isolates in two different time periods.” *The Journal of Microbiology, Immunology and Infection*. 2000 Dec; 33(4): 258-62.

406. Watson JT, Jones RC, Siston AM, et al. Outbreak of catheter-associated *Klebsiella oxytoca* and *Enterobacter cloacae* bloodstream infections in an oncology chemotherapy center. *Arch Intern Med*. Dec 12-26 2005; 165(22):2639-43.

407. Wendt C, Lin D, von Baum H. Risk factors for colonization with third-generation cephalosporin-resistant enterobacteriaceae. *Infection*. Oct 2005; 33(5-6):327-32.

408. Whalen JG, Mully TW, Enlgish JC 3rd. “Spontaneous *Citrobacter freundii* infection in an immunocompetent patient.” *Archives of dermatology*. 2007 Jan; 143(1): 124-5.

409. Williams P, Chart H, Griffiths E, Stevenson P. Expression of high affinity iron uptake systems by clinical isolates of *Klebsiella*. *FEMS Microbiol Lett.* 1987; 44:407–412.

410. Williams P, Lambert P A, Brown M R W, Jones R J. The role of the O and K antigens in determining the resistance of *Klebsiella aerogenes* to serum killing and phagocytosis. *J Gen Microbiol*. 1983; 129:2181–2191.

411. Williams P, Smith M A, Stevenson P, Griffiths E, Tomas J M T. Novel aerobactin receptor in *Klebsiella pneumoniae. J Gen Microbiol*. 1989; 135:3173–3181.

412. Williams P, Tomas J M. The pathogenicity of *Klebsiella pneumoniae*. *Rev Med Microbiol.* 1990; 1:196–204.

413. Williams, K. P.; Gillespie, J. J.; Sobral, B. W. S.; Nordberg, E. K.; Snyder, E. E.; Shallom, J. M.; Dickerman, A. W. (2010). [//www.ncbi.nlm.nih.gov/pmc/articles/PMC2863478/ "Phylogeny of Gammaproteobacteria"]. *Journal of Bacteriology* 192 (9): 2305–2314. doi: "Dorlands Medical Dictionary :Enterobacteriaceae".

414. Winn W.Jr.Allen et al., [2006] *Enterobacteriaceae* Koneman’s Atlas and text book of Diagnostic Microbiology Lippincott Williams and Wilkins, USA, 212-289.

415. Wisplinghoff H, Bischoff T, Tallent SM, et al. Nosocomial bloodstream infections in US hospitals: analysis of 24,179 cases from a prospective nationwide surveillance study. *Clin Infect Dis*. Aug 1 2004; 39(3):309-17.
416. Woodford N, Dallow JW, Hill RL, et al. Ertapenem resistance among *Klebsiella* and *Enterobacter* submitted in the UK to a reference laboratory. *Int J Antimicrob Agents*. Apr 2007; 29(4):456-9.
417. Wooldridge K G, Williams P H. Iron uptake mechanisms of pathogenic bacteria. *FEMS Microbiol Rev.* 1993; 12:325–348.
418. Würker M, Beuth J, Ko H L, Przondo-Modarska A, Pulverer G. Type of fimbriation determines adherence of *Klebsiella* bacteria to human epithelial cells. *Zentbl Bakteriol.* 1990; 274:239–245.
419. Yan QQ, Condell O, Power K, Butler F, Tall BD, Fanning S. Cronobacter species (formerly known as *Enterobacter sakazakii*) in powdered infant formula: a review of our current understanding of the biology of this bacterium. *J Appl Microbiol*. Mar 16 2012.
420. Yancey R J, Breeding S A L, Lankford C E. Enterochelin (enterobactin): virulence factor for *Salmonella typhimurium*. *Infect Immun.* 1979; 24:174–180.
421. Yang FC, Yan JJ, Hung KH, Wu JJ. Characterization of ertapenem-resistant *Enterobacter cloacae* in a Taiwanese university hospital. *J Clin Microbiol*. Feb 2012; 50(2):223-6.
422. Ye Y, Li JB, Ye DQ, et al. *Enterobacter bacteremia*: Clinical features, risk factors for multiresistance and mortality in a Chinese University Hospital. *Infection*. Oct 2006; 34(5):252-7.
423. Yinnon A M, Butnaru A, Raveh D, Jerassy Z, Rudensky B. Klebsiella bacteremia: community versus nosocomial infection. *Monthly J Assoc Physicians*. 1996; 89:933–941.

424. Yokochi T, Inoue Y, Kato Y, Sugiyama T, Jiang G Z, Kawai M, Fukada M, Takahashi K. Strong adjuvant action of Klebsiella O3 lipopolysaccharide and its inhibition of systemic anaphylaxis. *FEMS Immunol Med Microbiol.* 1995; 10:181–184.

425. Yokochi T, Nakashima I, Kato N. Effect of capsular polysaccharide of *Klebsiella pneumoniae* on the differentiation and functional capacity of macrophages cultured *in vitro*. *Microbiol Immunol*. 1977; 21:601–610.

426. Yokochi T, Nakashima I, Kato N. Further studies on generation of macrophages in *in vitro* cultures of mouse spleen cells and its inhibition by the capsular polysaccharide of *Klebsiella pneumoniae*. *Microbiol Immunol.* 1979; 23:487–499.

427. Zhang R, Cai JC, Zhou HW, Nasu M, Chen GX. Genotypic characterization and *in vitro* activities of tigecycline and polymyxin B for members of the Enterobacteriaceae with decreased susceptibility to carbapenems. *J Med Microbiol*. Dec 2011; 60:1813-9.

428. Zhaxybayeva O, Doolittle WF (April 2011). "Lateral gene transfer". *Curr. Biol.* 21 (7): R242–6.

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