Characterization and identification of some medically important bacterial isolates

A Dissertation
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CERTIFICATE

This is to certify that the dissertation entitled "**Characterization and identification of some medically important bacterial isolates**" Submitted by Ms. Suman Tiga, to the department of Life science, National Institute of Technology, Rourkela for the degree of Master of Science in Life science is based on the result obtains in the bonafide project work carried out by her under my Guidance and supervision.

I further certify that to the best of my knowledge Suman Tiga bears a good moral character.

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In the end I must record my special appreciation to GOD who has always been a source of my strength, inspiration & my achievements.

SUMAN TIGA

DECLARATION OF CANDIDATE

I hereby declare that the research work incorporated in this dissertation work entitled **“Characterization and identification of some medically important bacterial isolates”** is an authentic research work carried at Life science Department. National Institute of Technology, Rourkela under the direct guidance and supervision of Dr S Das.

Date: 30.4.2011

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ABSTRACT

The study aimed to isolate and identify the bacterial isolate and determine the antibiotic sensitivity pattern of E *coli, Klebsiella*, Bacillus isolated from human patient. The sensitivity of *E coli Klebsiella pneumoniae , Bacillus cereus* were tested under different antibiotics like chloramphenicol, amoxcilin, vancomycine, tetracycline, penicillin, kanamycine, ciprofloxacin .E coli showed sensitive towards chloramphenicol and resistance to other antibiotics. *Klebsiella pneumoniae* also found to be resistance towards chloramphenicol and tetracycline and sensitive to others. Bacillus was intermediate to ciprofloxacine and amoxicillin and resistance to vancomycine cholaramphenicol and kanamycine.The goals of testing are to detect the possible drugs resistance in common pathogen to assist in treatment of particular infection

INTRODUCTION:-

- A key threat to conservation as well as public health is emerging infectious diseases (Meffe 1999; Daszak et al. 2000). The term antimicrobial agent is used for agents that disrupt the normal physiology of a multicellular animal or plant.

- Pathogens can infect unicellular organisms of the biological kingdoms. A host can be invaded by several substrates and pathways whereby pathogens. A property of pathogenic microorganisms is "Primary invasiveness"

- Infection is also the introduction or entry of a pathogenic microorganism into a susceptible host, whether or not it causes pathological effects or disease, but implies that the organism must enter the body of the host, usually the cells, and be able to reproduce to form new infective units.

- Simply ingesting a pathogen does not imply infection. Pathogenicity is the ability of an organism to invade the host and cause disease.

- This term is applied to groups or species of microorganisms, whereas virulence is used to compare the degree of pathogenicity within the group or species.

- Microbes are living organisms that reproduce, thrive, and spread quickly and efficiently increasing their numbers.

- Microbes include bacteria (*Bacillus cereus*), viruses (colds and influenza, which causes the "flu"), fungi (e.g.,*Candida albicans*, which causes some yeast infections), and parasites (e.g., *Plasmodium falciparum*, which causes malaria).

- The general term given to medicines that kill or slow the growth of microbes is Antimicrobial. The ability of a microbe to grow in the presence of a chemical that would normally kill it or limit its growth is the Antimicrobial drug resistance.

- Bacteria evolution towards resistance to antimicrobial drugs, including multidrug resistance, is unavoidable because it represents a particular aspect of the general evolution of bacteria that is unstoppable. Therefore, the only means of dealing with this situation is to delay the emergence and subsequent dissemination of resistant

bacteria or resistance genes. Mutations in housekeeping structural or regulatory genes can result in increased resistance to antimicrobial drugs in bacteria.

- Scottish scientist Alexander Fleming noticed that a type of mold growing by accident on a laboratory plate was protected from, and even repelled, the bacteria in 1928 while working with *Staphylococcus* bacteria.

- Fleming called penicillin a active substance, was literally an antibiotic - it killed living organisms. Thus began the age of using natural and, later, synthetic drugs to treat people with bacterial infections.

Drug resistance:

Disease-causing microbes evolution has resulted in many antimicrobials losing their effectiveness after more than 50 years of widespread use. As microbes evolve, they adapt to their environment. The microbes evolve new mechanisms to resist the antimicrobials by changing their genetic structure from something that stops them from growing and spreading-such as an antimicrobial. The genetic structure changes ensure that the offspring of the resistant microbes are also resistant. It is to harder to eliminate infections from the body due to antimicrobial resistance. A microbe's ability to survive along antimicrobials, some infectious diseases is now more difficult to treat than a few decades ago.

Causes of antimicrobial drug resistance

Microbes, such as bacteria, viruses, fungi, and parasites, are living organisms that evolve over time. Their primary function is to reproduce, spread-quickly and efficiently. Due to this reason the microbes adapt to their environment that ensure their survival. Something stops their ability to spread, genetic changes can occur that enable the microbe to survive.

Antimicrobial resistance in micro-organisms

The antimicrobial resistance mechanisms are described by using four categories:

1. Based on the structural nature of micro-organism's outer membrane which is considered to be a barrier for drug's entry into the cell Bypass' was described. *Mycobacterium* outer cell wall of makes these bacteria resistant to many antimicrobials (Nikaido, 1994).

2. Enzymatic modification or inactivation of the antimicrobial agent. The β-lactamase enzymes in *E. coli* (Jacoby, 1994), which destroys the β-lactam-ring's of the penicillin; or, amino glycoside-modifying enzymes in *Staphylococcus aureus.*

3. The antimicrobial resistance developed by organism through structural changes of the drug target sites (for example, mutations in the genes*)* lead to a structure of changed drug target sites that inhibit drugs from binding to these target sites (Webber and Paddock, 2001).

4. The self-defense mechanism of a micro-organism is a efflux pump whereby a drug that initially enters the cell through the cytoplasmic membrane is subsequently transported, actively, out of the cell before it can contact the action site and exert its effect (Tenover, 2006). The efflux system requires energy therefore it is consider to be a pump. This mechanism occurs in many natural resistance of various and specific organisms (Bambeke, et al. 2006) for example tetracycline. (Ramon-Garcia, et al. 2006). Or fluroquinolones (Bambeke, et al. 2006).

CLASSIFICATION OF MICROORGANISM:-

Klebsiella

kingdom	:	Bacteria
Phylum	:	Proteobacteria
Class	:	Gammaproteobacteria
Order	:	Enterobacteriales
Family	:	Enterobacteriaceae
Genus	:	Klebsiella
Species	:	K. Pneumonie

E.coli

kingdom	:	Bacteria
Phylum	:	proteobacteria
Class	:	Gammaproteobacter
Order	:	Enterobacteriales
Family	:	Enterobacteriaceae
Genus	:	*Escherichia*
Species	:	E.coli

Bacillus			
	Kingdom	:	Bacteria
	Phylum	:	Firmicutes
	Class	:	Bacilli
	Order	:	Bacillales
	Family	:	Bacillaceae
	Genus	:	*Bacillus*
	Species	:	*B. cereus*

OBJECTIVES:

- Isolation of pure culture of pathogen (*Klebseilla, E.coli, Bacillus*)
- Antibiotic disc diffusion test
- Biochemical characterization
- Determination of Minimum inhibitory concentration (MIC) of antibiotics.

REVIEW LITERATURE

3.1. *Klebsiella*

Klebsiella is a non-motile, Gram-negative, oxidase-negative, rod-shaped bacteria with a prominent polysaccharide-based capsule. It is named after the German microbiologist Edwin Klebs (1834–1913).It is a type of Gram-negative bacteria that causes different types of healthcare-associated infections, including pneumonia, bloodstream infections, wound or surgical site infections, and meningitis. It is found that *Klebsiella* developed antimicrobial resistance, most recently to the class of antibiotics known as carbapenems. *Klebsiella* bacteria are found in the human intestines (where they do not cause disease). They are also found in human stool (feces). *Klebsiella* infections occur among sick patients who are receiving treatment for other conditions. Patients who require devices like ventilators (breathing machines) or intravenous (vein) catheters, and patients who are taking long courses of certain antibiotics are most at risk for *Klebsiella* infections. Healthy people usually do not get *Klebsiella* infection.

3.1.1. **Infection Cause**

Klebsiella bacteria can be spread through person-to-person contact or, less commonly, by contamination of the environment. The bacteria are not spread through the air. Patients in healthcare settings can also be effected Klebsiella when they are on ventilators (breathing machines), or have intravenous (vein) catheters or wounds (caused by injury or surgery). These medical tools and conditions allow *Klebsiella* to enter into the body and cause infection.

3.1.2.. **Disease**

Klebsiella can cause serious infections that may often be fatal. Drug-resistant pneumonia, chronic nasal infections, bronchitis and urinary tract infections, even gangrene are some of the infections these common bacteria can cause in susceptible people.In the Pneumonia, urinary tract infections, septicemia, ankylosing spondylitis, urinary tract infections and soft tissue infection actions can spread rapidly and are most often acquired in the hospital while

being treated for other illnesses or surgical procedures. The main sources of Klebsiella infections are due to the hands of hospital staff and the gastrointestinal tract of patients.

- pneumonia (lung infection)
- bloodstream infection
- wound infection, and
- meningitis
- *Klebsiella pneumonia,* are rare and severe disease with dark brown or red currant–jelly sputum, lung abscess formation and empyema, is most common among diabetics and alcoholics.

3.1.2.1. **Urinary** infections are the common conditions, especially in, the elderly pregnant women and babies. The symptoms are minor and can often be confused with other conditions. However, if left untreated, urinary tract infections causes serious medical conditions, including infections that leave irreparable damage to the kidneys. Klebsiella, resistant to several kinds of antibiotics, is often the cause of complicated urinary infection.

3.1.2.2. **Diarrhea** may be found in immunocompromised individuals. Rhinoscleroma, a the chronic inflammatory disease of the nasopharynx and respiratory system is rarely fatal but can cause deformities of the nose.

3.1.3. **Clinical Feature**

The most clinically important species of the genus is *klebsiella pneumonia*. This large,non-motile bacterium the most clinically important species of this genus is *Klebsiella* pneumoniae. This large, non-produces large sticky colony when plated on nutrient media. *Klebsiella's* pathogenicity can be caused due to its production of a heat-stable enterotoxin. *K. pneumoniae* infections are common in the hospitals where they cause and urinary tract infections pneumonia in catheterized patients. In fact, *K. pneumoniae* is second only to *E. coli* as a urinary tract pathogen. It may be due to probable due to the bacterium's antibiotic resistance properties. *Klebsiella* may develop resistance plasmids (R-plasmids) which cause resistance to such antibiotics as ampicillin and carbenicillin. *Klebsiella pneumonia* affects people

experiencing diseases, such as alcoholism, diabetes and chronic lung disease. *Klebsiella pneumonia* causes rapid-onset illness that often causes areas of destruction in the lung. Infected persons generally get, chills, flu-like symptoms high fever and a cough productive of a lot of mucous. The mucous (or sputum) that is coughed up is often thick and blood tinged and has been referred to as "currant jelly" sputum due to its appearance. *Klebsiella pneumonia* causes mostly lung destruction and pockets of pus in the lung (known as abscesses*). Klebsiella* generally has two types of antigens on the surface of the cell, lipopolysaccharide (O antigen) and capsular polysaccharide (K antigen), both contributing to pathogenicity. Host defense against bacterial invasion by phagocytosis by polymorphonuclear granulocytes and the bactericidal result of serum,mainly mediated by complement proteins. Preclinical studies plays an role for neutrophil myeloperoxidase and lipopolysaccharide-binding protein in host defense against *K pneumoniae* infection. Neutrophil myeloperoxidase mediate oxidative inactivation of elastase, an enzyme involved in the pathogenesis of different tissue destroying infections. The bacteria defeat natural host immunity by various means, as they hold a polysaccharide capsule, which is the major determinant of their pathogenicity. The capsule layer provides protection from phagocytosis by polymorphonuclear granulocytes and avoids bacterial death from bactericidal serum factors. The numerous adhesins produced by the bacteria is responsible for the microorganism to hold on to host cells, which is crucial to the infectious process. *Klebsiellae* are everywhere in nature, while in humans they can inhabit the skin, pharynx or gastrointestinal tract and also sterile wounds and urine. *K pneumoniae* and *K oxytoca* are members of the genus that cause most human infections and they can found in the environment and in mammalian mucosal surfaces. *Klebsiella* infections is the gastrointestinal tract of patients and the hands of hospital staff *Klebsiella* infection takes place in the lungs, causing devastating changes and generally affects old or middle aged men with weakening diseases, such as diabetes or alcoholism. Klebsiella is also responsible for nosocomial infections in such sites as urinary tract, , lower respiratory tract biliary tract, or surgical wound sites. Apparatus, urinary catheters, pollution of respiratory support equipment, and the use of antibiotics are also increasing the probability of nosocomial infection with various *Klebsiella* species. *ozena an Rhinoscleroma d* are two other rare infections set off by Klebsiella species. The extensive use of antibiotics in hospitalized patients resulted in an amplified carriage of *klebsiella* and the appearance of multidrug-resistant strains. *Klebsiella pneumonia* is having an increased mortality rate of

roughly 50% including in the case of antimicrobial therapy. Even more, the mortality rate is close to 100% for people with alcoholism and bacteremia.

3.1.4. **Pathogenesis**

The most common Klebsiella infection is pneumonia and it which causes 50 percent mortality rate with it, even with antimicrobial treatment. *Klebsiella pneumonia*'s Symptoms are, fever, flu-like symptoms, chills and a cough along with thick mucus mixed with blood. *Klebsiella* attack the lung tissue that causes pus to surround the lung causing scar tissue to form. This is very serious and may require surgery to fix. *Klebsiella* even infects the urinary tract and can enter the body through wounds. Contact with the fecal matter is an significant source of infection. Patients with invasive devices such as feeding tubes, central venous catheters indwelling catheters and who are generally in poor health are at particular risk for contracting a *Klebsiella* infection. Those with alcoholism, lung disease and diabetes are also effected . A *Klebsiella* infection may need two powerful antibiotics as the bacteria are resistant to penicillin and many others, due to excessive use by hospitals of broad-spectrum antibiotics.

3.1.5. Treatment

Klebsiella infections which are not drug-resistant can be treated with antibiotics. A microbiology laboratory must tests to determine which antibiotics will treat the infection. *Klebsiella* is mainly a nosocomial infection, which are generally hospital-acquired infections. If an infection appears after 48 hours or more after hospital admission or within 30 days of discharge, it is considered to be nosocomial.

3.1.6. Drug Resistance

Klebsiella infections are often treated with third-generation cephalosporins, carbapenems, fluoroquinolones or aminoglycosides. It is needed to determine the appropriate antibiotic, as some strains may become resistant to multiple antibiotics.

3.1.6.1. Beta-lactamase inhibitors

Some bacteria release an enzyme called beta-lactamase, which renders penicillin ineffective. A beta-lactamase prevents the enzyme from forming and allows the penicillin to perform its function. Timenti Unasyn,n and Zosyn are all penicillins with beta-lactamase inhibitors, according to Drugs.com. Intravenously medicine is given to patients with Klebsiella pneumoniae. Side effects include, headaches, stomach upset diarrhea and possibly vomiting although these medications are usually tolerated well. It should not be taken by the patient who are allergic to penicillin. Enzymatic inactivation and modification of the antimicrobial. For example, the secretion of the β-lactamase enzymes in *E. coli* (Jacoby, 1994), which breaks the β-lactam-ring's chemical structure of the penicillins

3.2. *Bacillus cereus*

3.2.1. Characterization

Characteristically, *Bacillus* cultures are the Gram-positive when it is young, but may become Gram-negative as they age. *Bacillus* species are sporulating aerobic, , rod-shaped bacteria which are ubiquitous in nature. Gram-stained cells, 1 µm long, arranged singly or in short chains. *Bacillus cereus* is spore-forming bacterium that can be frequently isolated from soil and some food. *B. cereus* spores are mostly resistant to heat and chemical treatments than vegetative pathogens such as *Salmonella*, *Campylobacter*, *E. coli*, and *Listeria monocytogenes* (Johnson, K.M. 1984) If *B. cereus* can cause two different types of food-borne illness in humans – vomiting very shortly after eating contaminated food or diarrhoea after a longer incubation. *Bacillus cereus* is a cause of food poisoning that is mainly associated with the consumption of rice-based dishes. The organism produces an diarrheal syndrome induced by an emetic toxin and enterotoxin, respectively. Other toxins are produced during growth, including, proteases, phospholipases and hemolysins, one of which, cereolysin, is a thiol-activated hemolysin (Gilbert et al, 1979). These toxins may contribute to the pathogenicity of B. cereus is non gastrointestinal disease (Blakistone et al, 1999). *B. cereus* are often isolated from clinical material other than feces or vomitus was commonly dismissed as a contaminant, but increasingly it is being recognized as a species with pathogenic potential. It causes serious non gastrointestinal infection, particularly in drug

addicts, the immunosuppressed, neonates, and postsurgical patients, especially when prosthetic implants such as ventricular shunts are inserted. Ocular infections are the severe infection, including endophthalmitis, panophthalmitis, and keratitis, usually with the characteristic formation of corneal ring abscesses (Aas et al, 1992) all though prompt surgical and antimicrobial agent treatment, enucleation of the eye and blindness are common sequelae. meningitis, osteomyelitis, endocarditis, Septicemia, and surgical and traumatic wound infections are other manifestations of severe disease (Shinagawa et al, 1993). B. cereus produces beta-lactamases, and so is resistant to beta-lactam antibiotics; it is usually susceptible to treatment with clindamycin, vancomycin, gentamicin, chloramphenicol, and erythromycin. Simultaneous therapy via multiple routes may be required.

3.2.2. **Infection**

Bacillus cereus causes a toxin-mediated food poisoning. *Bacillus cereus* is an gram-positive aerobic and facultative anaerobic, spore-forming, bacillus.A preformed heat stable toxin causes the emetic syndrome. The diarrhoea syndrome is caused by in vivo production of heat-labile enterotoxins.

3.2.3. Clinical Features

There are two clinical syndromes caused by food poisoning:

The diarrheal form of illness:

1. *Bacillus cereus* is having a longer incubation (6 to 24 hours) period similar to that of *Clostridium perfringens* watery diarrhoea, moderate to severe abdominal cramps and vomiting in about one fourth of the patients. Duration of illness is for 20-36 hours, with a median of 24 hours. Associated with meat dishes.

2. **The emetic form of illness**:

Bacillus cereus is having a very short incubation period (1 to 6 hours), similar to that of staphylococcal food poisoning. Vomiting and abdominal cramps. Diarrhoea is present in only about a third of affected individuals. Duration of illness is for 8 to 10 hours, with a median of 9 hours. Of illness. Associated with rice dishes.

3.2.4. **Treatment**

B.cereus food poisoning in person require only supportive treatment. Oral rehydration, intravenous fluid and electrolyte replacement for patients with severe dehydration is

indicated. Antibiotics are not indicated. Patients who are affected by the invasive disease require antibiotic therapy and prompt removal of any potentially infected foreign bodies. *Bacillus cereus* is usually susceptible in vitro to vancomycin, clindamycin. (Kramer et al, 2001)

3.2.5. **Pathogenesis**

B. cereus is responsible for a minority of food borne illnesses (2–5%), causing severe nausea, vomiting and diarrhoea (Kotiranta et al, 2000). Due to survival of the bacterial endospores *Bacillus* food borne illnesses will occur, when food is improperly cooked? (Turnbull PCB 1996). Cooking temperatures less than or equal to 100 °C (212 °F) allows some *B. cereus* spores to survive. Germination and growth generally occurs between 10–50 °C (50–122 °F), though some strains are psychotropic (Davis et al, 2008). Production of enterotoxins results in Bacterial growth, one of which is highly resistant to heat and to pH between 2 and 11; ingestion leads to two types of illness, diarrheal and emetic (vomiting) syndrome.(Ehling-Schulz et al, 2004). The diarrhoeatic syndromes observed in patients are thought to stem from the three toxins Hemolysin BL Hbl, Nonhemolytic Enterotoxin Nhe and Cytotoxin K Cytk(Guinebretière et al,2002). On the chromosome of the bacteria the genes *nhe*/*hbl*/*cytK* are located. Transcription of these genes is controlled by PlcR. These genes occur as well in the taxonomically related *B. thuringensis* and *B. anthracis*. The Hbl and Nhe are the pore-forming toxins that are closely related to ClyA of *E. coli*.

3.3. E Coli

The total coliform and fecal colifom are referred to as indicator organism because a quantization of their presence is used to indicate the potential presence of 1 5 pathogens in foods. The fecal coliform group includes rod-shaped, Gram-negative, non-spore forming organisms that live in the gastrointestinal tract of humans and other warm blooded animals, ferment lactose at 44.5 ^{o}C within 48 hour. A member of the fecal coliform group is *Escherichia coli* (Oram, 2005). The name *Escherichia* comes from the name of the paediatrician Escherich. Who in 1895 first isolated and characterized this bacterium as “bacterium coli commune” (‘enteric bacteria’) (Todar, 2005). Its involvement in several cases

of food poisoning has suggested that *E. coli* should be used as an indicator for sanitary quality. *E. coli* is an inhabitant of the intestine tract of humans and animals as well as in the environment, water and food. The presence of *E. coli* in food or water is an indication of uncleanliness and careless handing. It also implies that enteric pathogens may be present (Fratamico and Smith, 2006). The limits of temperature for growth of *E. coli* are 7-46°C and the optimum growth temperature is approximately 37°C but *E. coli* can survive for weeks at -20°C to 4°C. *E. coli* generally grows within the pH range of 4.4–9.0 (Bell and Kyriakides, 1998).

E. coli grows very quickly and the generation time in the intestine is thought to be about 12 hours. Under optimum conditions the generation time is 20 minutes. Some strains of *E. coli* bacteria produce an enzyme called extended-spectrum β-lactamase (ESBL) which helps *E. coli* resist to many types of antimicrobials. Among all the strains of *E. coli some* strains, some are useful for producing sources of 'B' and 'K' vitamins for the host and some are harmless to hosts but some strains can cause illness such as urinary tract infections, meningitis, peritonitis, mastitis, septicemia and Gram-negative pneumonia. *E. coli* has three kinds of antigens including 'H' or flagellar antigens, 'O' or somatic antigens which are the lipopolysaccharide complexes and 'K' or the capsular antigens which are mainly acidic polysaccharide (Todar, 2005). *E. coli* strains can cause illness such as enteroinvasive *E. coli* (EIEC), entero-toxigenic *E. coli* (ETEC), entero-pathogenic *E. coli* (EPEC) and entero-hemorrhagic *E. coli* (EHEC) (Fratamico and Smith, 2006). Escherichia coli (E. coli) are a group of genus of Gramnegative, anaerobic, rod-shaped bacteria, which inhabits the intestine of all healthy humans and most warm blooded animals. There are many strains of E. coli bacteria and most of them are harmless and serves a useful function in the body by suppressing the growth of harmful bacterial species and by synthesizing appreciable amounts of vitamins. A very few strains *E. coli* are having the capability of causing human illness by several different mechanisms. This is the case of Escherichia coli O157 which is the most frequent and virulent. This serotype belongs to the enter hemorrhagic E. coli (EHEC) group. EHEC bacteria contain one or more virulent attributes as: The ability to produce shiga-like toxin(s) also called verotoxin(s) Adherence factors which enable the organism to attach to and to colonize intestinal mucosal cells Enterohemolysin Chromosomal eae gene which encodes the production of intimin (an adherence marker). E. coli O157 was first identified as a human pathogen capable of causing foodborne illness in 1982. However, the public was generally unaware of the existence of O157 until a decade later when a large outbreak occurred. Therefore, the focus of most research has been on the serotype O157:H7.. Excluding O157, the most commonly implicated sero-groups include O26, O103, O111 and O145.

3.3.1. Disease Diagnosis:

Microscopy studies in stool samples will show Gram negative rods, with no particular cell wall. Stool is inoculated on the either MacConkey agar or EMB agar (or both). Deep red colonies are produced on the surface of MacConkey agar, as the organism is lactose-positive,

and fermentation of this sugar will cause the medium's pH to drop, leading to darkening of the medium. This is diagnostic of *E. coli*. Mammalian cells in tissue culture are used for the tests for toxin production, which are rapidly killed by shiga toxin. , This method is very slow and expensive ,although sensitive and very specific. (Johnson et al, 2006).

Gastrointestinal infection

Transmission of pathogenic *E. coli* often occurs via fecal-oral transmission. (Gehlbach et al, 1973) Common routes of transmission include: unhygienic food preparation, farm contamination due to manure fertilization, (Russell, 2006). Irrigation of crops with contaminated grey water or raw sewage (Heaton, 2008). Feral pigs on cropland,(Thomas, et al 2007-08) or direct consumption of sewage-contaminated water.(Chalmers et al, 2000. Food products associated with outbreaks of *E. coli* include raw ground beef, raw seed sprouts or spinach. Cooking the food properly, preventing cross-contamination, instituting health care policies so food industry employees seek treatment when they are ill, pasteurization of juice or dairy products and proper hand washing requirements will cause the disruption faecal-oral cycle of transmission.

Neonatal meningitis

A serotype produced by *Escherichia coli* that contains a capsular antigen called K1. The newborn's colonisation in the intestines with these stems, that are present in the mother's vagina, lead to bacteraemia, which leads to meningitis. The IgM antibodies absence of the from the mother, plus the fact that the body recognises as self the K1 antigen, as it resembles the cerebral glycopeptides, this leads to a severe meningitis in the neonate.

3.3.2. Symptoms

Bloody diarrhea is the main symptom of an E.coli infection. You may also havestomach cramps and nausea and vomiting. Some people do not notice any symptoms. Children are more likely than adults to have symptoms. Usually symptoms start 3 or 4 days after you come in contact with the *E. coli*. Most people get better in about a week. They often don't see a doctor and don't know that *E. coli* caused their problems.

3.3.3. Clinical feature

HUS is the reason of acute kidney failure in children. The infection may be caused by a very small dose which can be as few as 50-100 bacteria. The disease caused by E. coli O157:H7 is

hemorrhagic colitis and is characterized by severe cramping (abdominal pain) and diarrhoea (watery and/or bloody). Other symptoms may include vomiting and low-grade fever. The illness is usually self-limited and lasts an average of 8 days. Sometimes the infection causes non-bloody diarrhoea or no symptoms at all. Serious complications occur in 0 to 15% of cases and are experienced more frequently by the children less than five years of age and the elderly. These complications are haemolytic uremic syndrome -in which the red blood cells are destroyed and the kidney fail- and thrombotic thrombocytopenic purpura (TTP) which is characterized by HUS plus fever and neurologic symptoms. TTP has a mortality rate as high as 50%.

3.3.4. Treatment

Without antibiotics most people can recover within 5 to 10 days. The diarrhoea occurs for a few days but it is important to drink plenty of fluids to prevent dehydration (fluid loss). The treatment with some antibiotics may precipitate kidney complications but there is no proper evidence that antibiotics improve the course of disease. Blood transfusions and kidney dialysis are often required. The death rate for haemolytic uremic syndrome is only 3%-5%.

3.4. ANTIMICROBIAL DRUG RESISTANCE

The arsenal of available drugs against protozoal pathogens has been severely limiting the emergence of drug resistance. The toxicity of drugs have been evolved (Box) by parasites in numerous ways to overcome. Mutations in the drug target so that the binding of the drug or inhibit the target are due to the Drug resistance. Where a single point mutation can confer resistance ,drug resistance can develop quickly in situations.

3.4.1. MECHANISM OF RESISTANCE

1. Antimicrobial agent’s altered permeability
2. Inactivation of the antimicrobial agent
3. Altered target site

4. Replacement of a sensitive pathway

3.4.2.. **Mode of action of antimicrobials**

An antimicrobial (either bactericidal or bacteriostatic) has an effect on micro-organisms antimicrobially when it interferes well with their site of activity. Antimicrobial mechanisms of action are (Abedon, 2003):

- Interference with cell wall synthesis:
- Protein synthesis inhibition
- Interference with nucleic acid synthesis:
- Bacterial membrane structure disruption

3.5. Different antibiotics and their mode of action

3.5.1. Tetracyclines

Tetracyclines have the antimicrobial activity. These may include: Aureomycin, Terramycin, and Panmycin. There are 4 fused 6-membered rings, as shown in the figure below, that form the basic structure from which the various tetracyclines are made. Different derivatives are different at one or more of four sites on the rigid, planar ring structure. *Streptomyces spp*., gives classical tetracyclines but the newer derivatives are semi-synthetic as is generallytruefor newer members of other drug groups. (Fig 1).

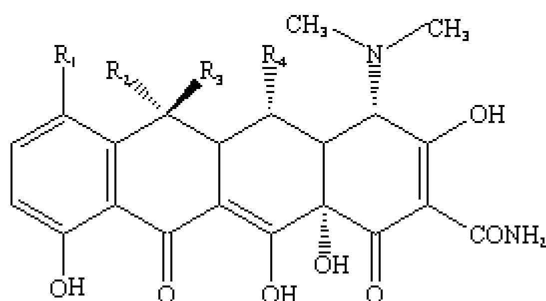

Fig-1: Tetracycline core structure.

Mechanism of Action

Bacterial protein synthesis is inhibited by tetracyclines by blocking the attachment of the transfer RNA-amino acid to the ribosome. They are the inhibitors of the codon-anticodon

interaction. Protein synthesis in the host can be inhibited by Tetracyclines, but are less likely to reach the concentration required because eukaryotic cells do not have a tetracycline uptake mechanism.

3.5.2. **Chloramphenicol**

Chloramphenicol is an antibiotic that possesses activity similar to the tetracycline. It is the only antibiotic prepared synthetically. For the treatment of serious infections it is reserved because it is potentially highly toxic to bone marrow cells. Protein synthesis has been inhibited by attaching to the ribosome and interferes with the formation of peptide bonds between amino acids. Chloramphenicol acts as an anti-metabolite for the essential amino acid phenylalanine at ribosomal binding sites. (Fig 2).

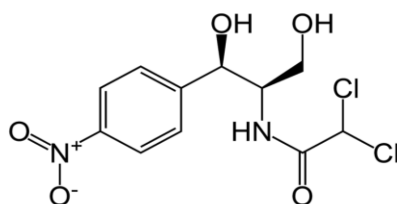

Fig-2: Chloramphenicol core structure.

Mechanism of Chloramphenicol

a) Inhibits protein synthesis by binding to a subunit of the bacterial ribosome
b) blocks cross-linking of the cell wall structure
c) inhibits enzymes needed in the biosynthesis of folic acid
d) inhibits bacterial DNA gyrase
e) inhibits DNA-dependent RNA polymerase(Fig 3)

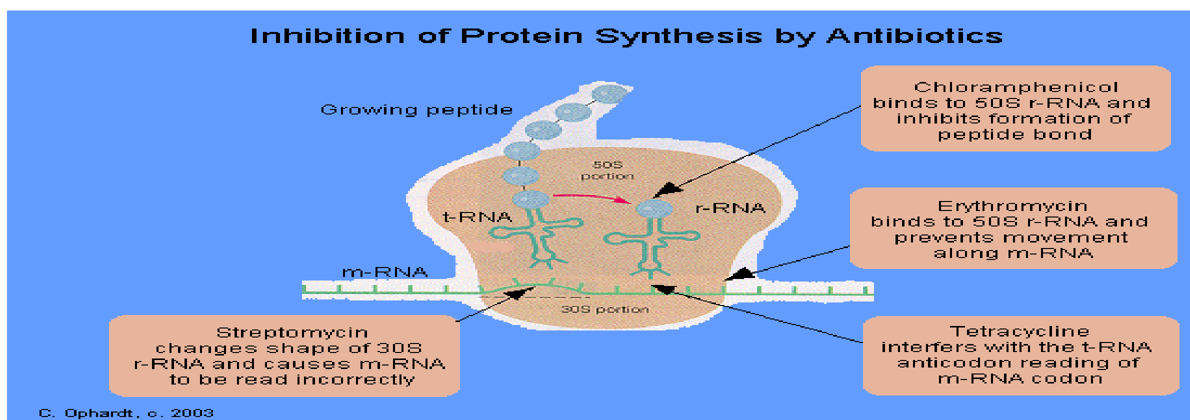

Fig-3: Action of Chloramphenicol.

3.5.3. **Vancomycin**

Vancomycin is an antibiotic that is a glycopeptide used in the prophylaxis and treatment of infections may be caused by Gram-positive bacteria. It is used as a drug of "last resort", and it is used only after treatment with other antibiotics had failed, although the emergence of vancomycin-resistant organisms means that it is increasingly being displaced from this role by linezolid (Zyvox) available PO and IV and daptomycin (Cubicin) IV and quinupristin/dalfopristin .(Fig 4)

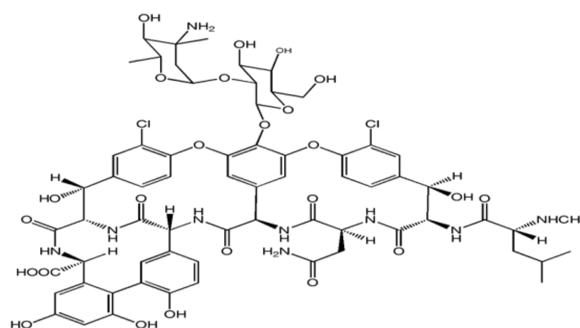

Fig-4: Vancomycin core structure.

Mode of action and mechanism of resistance

1. Vancomycin has been to the bacterial environment while the bacterium is trying to synthesize new cell wall. The cell wall strands have been not yet cross-linked but are synthesized.

2. Vancomycin can recognize and bind on the end of the peptide chains of the two D-ala residues. The D-lactate replaces the last D-ala residue, so vancomycin cannot binding resistant bacteria.

3. In resistant bacteria, cross-links are successfully formed. However, the vancomycin bound to the peptide chains prevents them from interacting properly with the cell wall cross-linking enzyme, in the non-resistant bacteria.

4. In the resistant bacteria, stable cross links are formed (Fig 5).

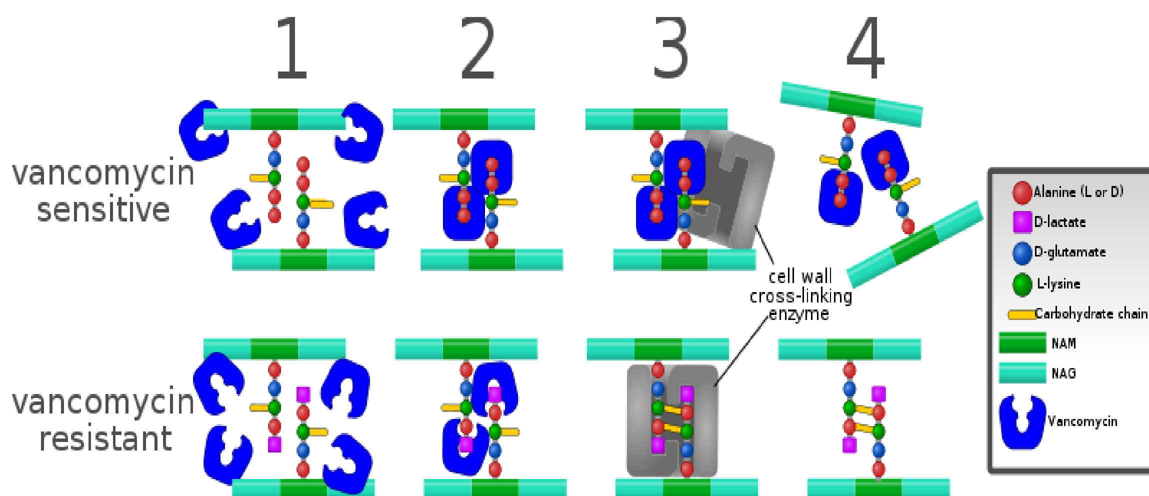

Fig-5: Mode of action and mechanism of vancomycine.

3.5.4.. **Amoxicillin**

Amoxicillin belongs to a class of penicillins. Other members are as follows : ampicillin (Unasyn), piperacillin (Pipracil), ticarcillin (Ticar) and several others. These antibiotics all have a similar mechanism of action. They do not kill bacteria, but stops bacteria from multiplying by preventing bacteria from forming the walls that surround them. Bacteria cannot survive without a cell wall. Amoxicillin acts against many different bacteria including *N. gonorrhoea* , *H. influenzae, Pneumococci, E. coli, Streptococci,* and certain strains of *Staphylococci*.(Fig 6)

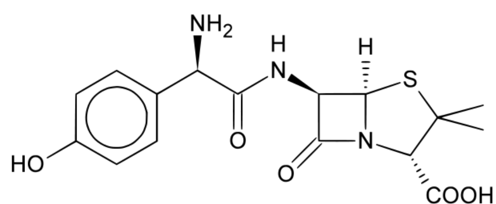

Fig-6: Amoxcillin core structure.

Mechanism of action

Amoxicillin binding occurs at the site Penicillin-binding protein 1A (PBP-1A) located inside the bacterial cell well. Penicillins helps in acylating the penicillin sensitive transpeptidase C-terminal domain by opening the lactam ring. Prevention of the formation of a cross-link of two linear peptidoglycan strands, inhibiting the third and last stage of bacterial cell wall synthesis is due to the inactivation of the enzyme. Bacterial cell wall autolytic enzymes such as autolysins mediates cell lysis by; it is possible that amoxicllin interferes with an autolysin inhibitor (Fig 7)

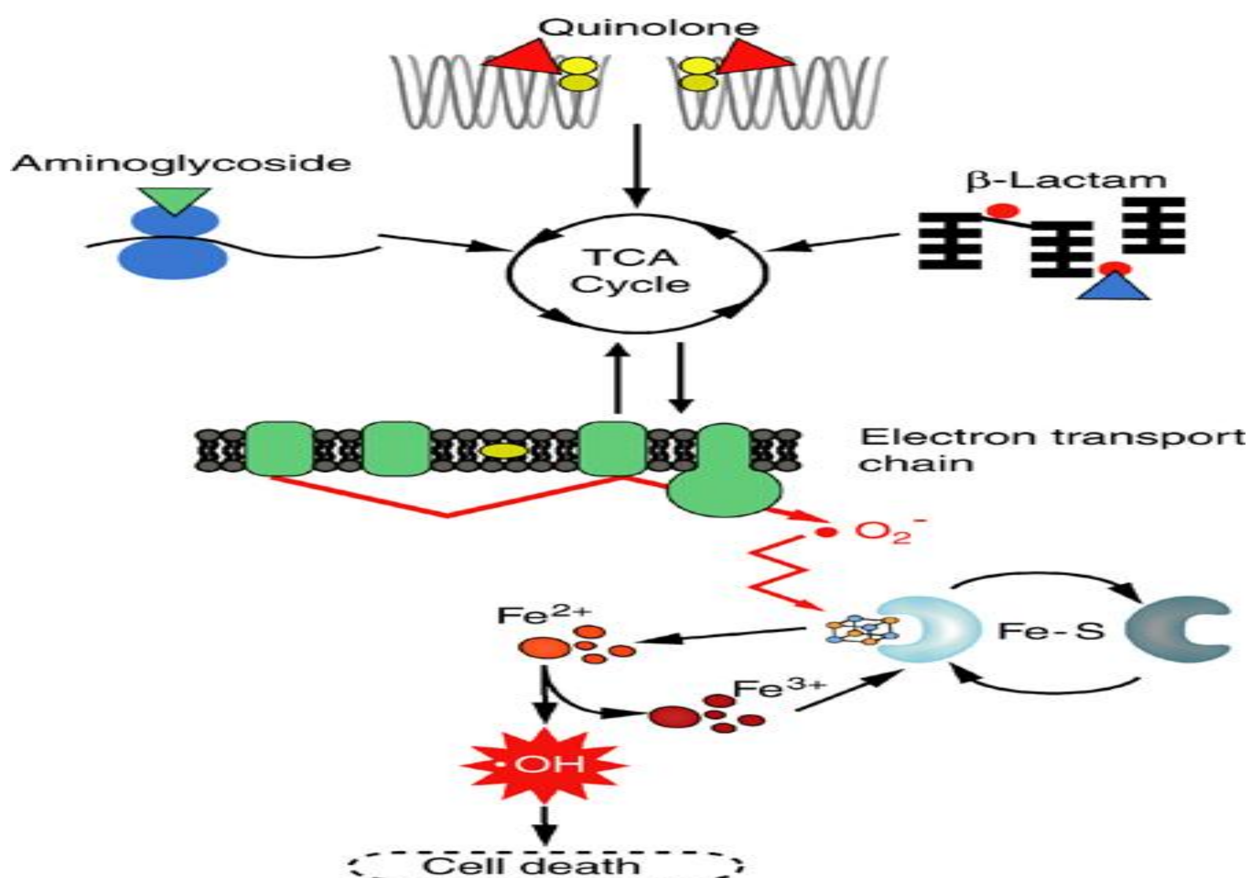

Fig 7: Mechanism of action.

3.5.5. **Ciprofloxacin**

Ciprofloxacin: Rapid bactericidal activity and high potency are its characteristics. It spares protective intestinal streptococci and anaerobes. It is active against many Beta lactum and amino glycoside resistant bacteria. At acidic pH it is less active. It has low frequency of mutational resistance and low propensity to select plasmid resistant mutants. Ciprofloxacin is used for treating certain bacterial infections. It belongs to the class of fluoroquinolones. It is present available in both tablet and suspension (liquid) form for the treatment of various diseases like bronchitis, diarrhoea, sinus, urinary tract infections etc..(Fig 8).

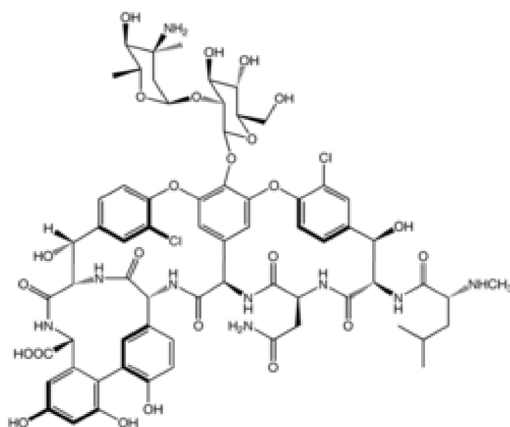

Fig 8: Ciprofloxacin core structure.

Mechanism of Action

Inhibition of topoisomerase enzymes, inhibits relaxation of supercoiled DNA and promotes breakage of double stranded DNA. The drug acts by decreasing the rate of multiplication of bacteria by stopping the reproduction and repair of their genetic material or (DNA)

3.5.6. Penicillin

Penicillium fungi is a microbe that helps in derivation of Penicillin - group of antibiotics . penicillin G,procaine penicillin, benzathine penicillin, and penicillin V are included under this group. Penicillin antibiotics are the first drugs that were effective against many

previously serious diseases such as syphilis and Staphylococcus infections. Penicillins are most widely used today, though many types of bacteria are now resistant. All penicillins comes under the category of beta-lactam antibiotics and are used in the treatment of bacterial infections caused by susceptible, usually Gram-positive, organisms (Fig 9)

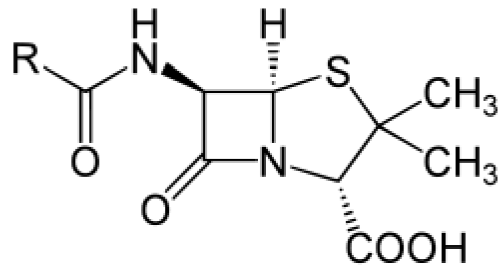

Fig 9: structure of penicillin.

Mode of action

Cross-links formation of the bacterial cell wall by a penicillin binding protein (PBP, an enzyme) and subsequent suicide inhibition by penicillin.

1. The bacterial cell wall consists repeating units of N-acetylglucosamine (NAG) and N-acetylmuramic acid (NAM) subunits. The subunits of NAM have short peptide chains attached to them.

2. The PBP forms the cross-link and binds the peptide side chains.

3. The PBP the cross-link has been formed and dissociates from the wall once.

4. Penicillin enters the active site of the PBP is added to the system.

5. The beta-lactam ring springs open and penicillin covalently binds to the PBP, permanently blocking its active site (Fig 10).

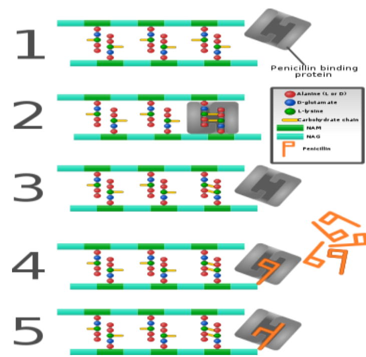

Fig 10: Mode of action.

3.5.7. Kanamycin

Kanamycin is an amino glycoside antibiotic. Sensitive bacteria can be killed by its action by stopping the production of essential proteins needed by the bacteria to survive (Fig 11)

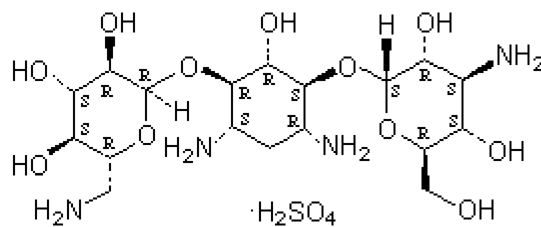

Fig 11: Kanamycin core structure.

Mode of Action

Bacteriocidal.It is capable of diffusing through the porin channels in the outer membrane of gram-negative bacteria. Three ribosomal proteins will be interacting with it that enables inhibiting protein synthesis and increasing translation errors.

Mechanism of Resistance

Bacterial aminophosphotransferases (APHs) inactivates Kanamycin .The APHs can inactivate the actions of kanamycin by transfer of the γ-phosphate of ATP to the hydroxyl group in the 3' position of the pseudosaccharide. The *Kan*^{R} gene codes for kanamycin resistance.

MATERIAL AND METHODOLOGY

4.1 .COLLECTION OF SAMPLE:

Various pathogenic strains were collected from ispat general hospital, Rourkela.

4.2 ISOLATION OF PURE CULTURE:

Pure cultures of the pathogens were isolated by using nutrient agar plate. That pure culture was used for biochemical analysis, antibiotic test, MIC test.

4.3. BIOCHEMICAL ANALYSIS:

Biochemical identification kit was used for the identification o the species of the pathogenic strain. The kit used are a) enterobactericeaae identification kit (part I, part II), b) carbohydrate utilization test kit (part A, part B, part C). Biochemical Identification test kit is a standardized colorimetric identification system utilizing conventional biochemical tests and carbohydrate utilization tests. Based on the pH change and substrate utilization the tests are done. On incubation metabolic changes occur organisms which are indicated by a colour change in the media that is either interpreted visually or after addition of a reagent.

4.4. Antimicrobial susceptibility test:

Kirby-Bauer disk diffusion method was used to test the susceptibility of the pathogen isolates to different antimicrobial agents: ampicillin (30 µg), tetracycline (30 µg), chloramphenicol (30 µg), vancomycin (30 µg), kanamycin (30 µg), ciprofloxacin (30 µg), The inocula were prepared by growing the *various pathogen* on separate agar plates and colonies from the plate were transferred with inoculating loop into 3 ml of normal saline in a test tube. The density of these suspensions was adjusted to 0.5 McFarland standards. The surface of Muller-Hinton agar plate was evenly inoculated with the organisms using a sterile swab. The swab was dipped into the suspension and pressed against the side of the test tube to remove excess fluid. Using the wet swab inoculation was done in the Muller-Hinton agar by evenly streaking across the surface. By means of Disc Dispenser the antibiotic discs were applied to the surface of the inoculated agar and the plates were incubated overnight at 37 ^{0}C. The zone of growth-inhibition’s diameter was observed and measured and compared to the chart provided by National Committee for Clinical Laboratory Standards (NCCLS).

4.5. McFarland standard

McFarland standards include the suspensions of either barium sulfate or latex particles that helps for visual comparison of bacterial density. Now-a-days in the market , commercially prepared standards are available for purchase from companies such as Remel or BD BBL. There is a small card containing parallel black lines- a Wickerham card.

4.5.1. A 0.5 McFarland standard preparation

1. Add a 0.5-ml aliquot of a 0.048 mol/liter BaCl2 (1.175% wt/vol BaCl2 • 2H20) to 99.5 ml of 0.18 mol/liter H2SO4 (1% vol/vol) with constant stirring to maintain a suspension.
2. Correct density verification of the turbidity standard by measuring absorbance using a spectrophotometer with a 1-cm light path and matched cuvette.The absorbance at 625 nm should be 0.08 to 0.13 for the 0.5 McFarland standards.
3. Barium sulfate suspension is transferred in the 4- to 6-ml aliquots into screw-cap tubes of the same size as those used in standardizing the bacterial inoculums.
4. The tubes are sealed tightly and stored in the dark at room temperature.

4.5.2. Use of the McFarland standard in the Kirby-Bauer procedure.

1. Vigorous agitation of the barium sulfate standard on a mechanical vortex mixer and inspect for a uniformly turbid appearance. Replace the standard if large particles appear. Standard is composed of latex particles, mix by inverting gently, not on a vortex mixer.
2. Bacterial colonies was added to the saline in the “preparation of the inoculum” step of the procedure, should compare the resulting suspension to the McFarland standard. By holding both the standard and the inoculum tubes side by side comparison has been done and the appearance of the lines through both suspensions the process is done. Do not hold the tubes flush against the card. If the bacterial suspension appears lighter than the 0.5 McFarland standards, more organisms should be added to the tube from the culture plate. If the suspension appears denser than the 0.5 McFarland standards, additional saline should be added to the inoculums tube in order to dilute the suspension to the appropriate density.

4.6. **Preparation of Mueller-Hinton plate**

1. Allow a MH agar plate undisturbed until it comes to the room temperature. It is most important to allow the plates to remain in the plastic sleeve while they warm to minimize condensation.

2. Set the plate inverted, if the surface of the agar has visible liquid present, and place a jar on its lid to allow the excess liquid to drain from the agar surface and evaporate. Plates may be placed in a 35°C incubator or in a laminar flow hood at room temperature until dry (usually 10 to 30 minutes).

3. Label each of the MH agar plate for each organism to be tested, appropriately.

4.7. **Preparation of inoculum**

1. Use sterile inoculating loop or needle and touch four or five isolated colonies of the organism to be tested.
2. Suspend the organism in 2 ml of sterile saline.
3. Vortex the saline tube to create a smooth suspension.

4. By adjusting the turbidity of this suspension to a 0.5 McFarland standard by adding more organism if the suspension is too light or diluting with sterile saline if the suspension is too heavy.
5. Use this suspension within 15 minutes of preparation.

4.8. **Inoculation of the MH plate**

1. Dip a sterile swab into the inoculums tube.
2. Rotate the swab against the side of the tube (above the fluid level) using firm pressure, to remove excess fluid. The swab should not be dripping wet.
3. Inoculate the dried surface of a MH agar plate by streaking the swab three times over the entire agar surface; rotate the plate approximately 60 degrees each time to ensure an even

distribution of the inoculums .

4. Rim the plate with the swab to pick up any excess liquid .
5. Discard the swab into an appropriate container.
6. Leaving the lid slightly ajar, allow the plate to sit at room temperature at least 3 to 5 minutes, but no more than 15 minutes, for the surface of the agar plate to dry before proceeding to the next step.

4.9. **Placement of the antibiotic disks**

1. Antimicrobial-impregnated disks should be placed on the surface of the agar, using either forceps to dispense each antimicrobial disk one at a time, or a multi disk dispenser to dispense multiple disks at one time.

a. To use a multi disk dispenser, place the inoculated MH agar plate on a flat surface and remove the lid .

b. The dispenser's placed over the agar plate and the plunger was firmly pressed once to dispense the disks onto the surface of the plate.

c. Dispenser off the plate has been lift and using forceps sterilized by cleaning them with an alcohol pad or flaming them with isopropyl alcohol, touch each disk on the plate to ensure complete contact with the agar surface. This should be done before replacing the petri dish lid as static electricity may cause the disks to relocate themselves on the agar surface or adhere to the lid.

d. Do not move a disk once it has contacted the agar surface even if the disk is not in the proper location, because some of the drug begins to diffuse immediately upon contact with the agar.

e. To add disks one at a time to the agar plate using forceps, place the MH plate on the template provided in this procedure . Sterilize the forceps by cleaning them with a sterile alcohol pad and allowing them to air dry or immersing the forceps in alcohol then igniting.

f. Using the forceps carefully remove one disk from the cartridge .

g. Partially remove the lid of the petri dish. Place the disk on the plate over one of the dark spots on the template and gently press the disk with the forceps to ensure complete contact with the agar surface. Replace the lid to minimize exposure of the agar surface to room air.

h. Continue to place one disk at a time onto the agar surface until all disks have been

placed as directed in steps f. and g. above.

4.10. Incubation of the plates

Once all disks are in place, replace the lid, invert the plates, and place them in a 35°C air incubator for 16 to 18 hours.

4.11. Measuring zone sizes

1. Following incubation, measure the zone sizes to the nearest millimeter using a ruler or caliper; include the diameter of the disk in the measurement.
2. When measuring zone diameters, always round up to the next millimetre.
3. All measurements are made with the unaided eye while viewing the back of the Petri dish. Hold the plate a few inches above a black, non-reflecting surface illuminated with reflected light.
4. View the plate using a direct, vertical line of sight to avoid any parallax that may result in misreading.
5. Record the zone size on the recording sheet.
6. If the placement of the disk or the size of the zone does not allow you to read the diameter of the zone, measure from the center of the disk to a point on the circumference of the zone where a distinct edge is present (the radius) and multiply the measurement by 2 to determine the diameter.
7. Growth up to the edge of the disk can be reported as a zone of 0 mm.
8. Organisms such as Proteus mirabilis, which swarm, must be measured differently than no swarming organisms. Ignore the thin veil of swarming and measure the outer margin in an otherwise obvious zone of inhibition.
9. Distinct, discrete colonies within an obvious zone of inhibition should not be considered swarming. These colonies are either mutant organisms that are more resistant to the drug being tested, or the culture was not pure and they are a different organism. If the phenomenon repeats itself by repeat testing ,the organism must be considered resistant to that drug.

4.12. Minimum Inhibitory Concentration Test (MIC Test):

- A single microorganism's pure culture is grown in Mueller-Hinton broth, or other broth as appropriate.

- Standardized culture was prepared by using standard microbiological techniques to have a concentration of very near 1 million cells per millilitre. The more reproducible the test results are produced by using he more standard the microbial culture.
- Diluted of the antimicrobial agent has been done a number of times, 1:1, through a sterile diluent (usually Mueller-Hinton broth).
- After the antimicrobial agent has been diluted, a volume of the standardized inoculums equal to the volume of the diluted antimicrobial agent is added to each dilution vessel, bringing the microbial concentration to approximately 500,000 cells per millilitre.
- Incubate the inoculated, serially diluted antimicrobial agent at a proper temperature for the test organism for a pre-set period, usually 18 hours. The more reproducible the test results were obtained by using the more standard the incubation period.
- After incubation, the series of dilution vessels is observed for microbial growth, usually indicated by turbidity and/or a pellet of microorganisms in the bottom of the vessel. The last tube in the dilution series that does not demonstrate growth corresponds with the minimum inhibitory concentration (MIC) of the antimicrobial agent.

4.13. PREPARATION OF SERIALLY DILUTED ANTIMICROBIAL AGENT:

The antimicrobial agents are diluted to a number of times with ratio 1:1. The concentration of antibiotic in the solution is in microgram/mL.
The quantity of antimicrobial agent to be taken for the preparation of a known concentration of antibiotic solution was carried out by using the following formula.

$$\text{WEIGHT (mg)} = \frac{\text{VOLUME (mL)} * \text{Conc. } (\mu g/mL)}{\text{POTENCY } (\mu g/mg)}$$

RESULTS

5.1. Result Analysis:

The characterization and identification of strain was done by using Hi Enterobacteriaceae for the identification of Enterobacteriaceae. Biochemical Identification test kit is a standardized colorimetric identification system utilizing conventional biochemical tests and carbohydrate utilization tests. The tests are based on the principle of pH change and substrate utilization.and the antimicrobial susceptibility profile was evaluated by the Kirby-Bauer technique. The minimum inhibitory concentrations (MICs) were determined by dilution technique. . On incubation organisms undergo metabolic changes which are indicated by a colour change in the media that is either interpreted visually or after addition of a reagent (Figs 12-14)

Fig 12- *E. coli* identification

Fig 13- *Bacillus* identification

Fig 14- *Klebsiella* identification

Table 1: Enterobacteriaceae Identification.

Strip *1*	Result Entry Datasheet			
SL.NO	Test	*E. coli*	*Bacillus*	Klebsiella
1	ONPG	+	-	+
2	Lysine utilization	+	+	+
3	Ornithine utilization	-	+	+
4	Urease	-	-	-
5	Phenylalanine deamination	-	-	-
6	Nitrate reduction	+	+	+
7	H_2S production	-	-	-
8	Citrate utilization	-	+	+
9	Voges proskauer’s	-	-	-
10	Methyl red	-	-	-
11	Indole	-	-	+
12	Malonate utilization	-	-	-

Strip 2	Result Entry datasheet			
Sl.No	Test	*E. coli*	*Bacillus*	*Klebsiella*
13	Esculin hydrolysis	-	+	-
14	Arabinose	+	-	+
15	Xylose	+	-	-
16	Adonitol	-	-	-
17	Rhamnose	+	-	-
18	Cellobiose	-	+	-
19	Melibiose	+	-	+
20	Saccharose	+	-	-
21	Raffinose	+	-	-
22	Trehalose	+	+	-
23	Glucose	+	+	+
24	Lactose	+	-	+
25	Oxidase	-	+	-

Table 2 Carbohydrate utilization test

SL.NO	TEST	E. coli	Bacillus	Klebsiella
PART A				
1	Lactose	+	-	-
2	Xylose	+	-	-
3	Maltose	+	+	-
4	Fructose	+	+	-
5	Dextrose	+	+	+
6	Galactose	+	+	+
7	Raffinose	-	-	-
8	Trehalose	+	+	-
9	Melibiose	-	-	+
10	Sucrose	-	-	-
11	L- arabinose	-	-	+
12	Mannose	-	+	-
PART B				
13	Inulin	-	-	-
14	Sodium gluconate	+	-	-
15	Glycerol	+	+	+
16	Salicin	-	+	+
17	Dulcitol	-	-	-
18	Inositol	-	-	-

19	Sorbitol	-	-	-
20	Mannitol	+	-	-
20	Mannitol	+	-	-
21	Adonitol	-	-	-
22	Arabitol	-	-	-
23	Erythritol	-	-	-
24	α – methyl –D-glucoside	-	-	-
PART C				
25	Rhamnose	-	-	+
26	Cellobiose	-	-	-
27	Melezitose	-	-	-
28	α –methyl-D-mannoside	-	-	-
29	Xylitol	+	-	-
30	ONPG	+	-	+
31	Esculin hydrolysis	-	+	+
32	D-Arabinose	+	-	+
33	Citrate utilization	-	-	-
34	Malonate utilization	-	-	-
35	Sorbose	+	-	-

5.2. RESULTS FOR ANTIBIOTIC DISK DIFFUSION TECHNIQUE:

1. In this study of the isolates, the highest frequencies of antimicrobial-resistant were observed for *E. coli* isolates from humans. Maximum resistance was showed by E.coli towards all the antibiotic except chloramphenicol. Sensitive to chloramphenicol.
2. *Klebsiella* showed 50% resistance towards antibiotics such as vancomycin,ciprofloxacin,amoxicillin. The rest 50% was sensitive towards antibiotic tetracycline,chloramphenicol and kanamycin.

3. Bacillus shows resistance to most of the antibiotics used for antibiotic test such as tetracyclin,vancomycin,ciprofloxacin,kanamycin,chloramphenicol.

Table 3 Antibiotic disk diffusion result.

Antibiotics organisms	Tetracycline (30 mcg)	Vancomycin (30mcg)	Ciprofloxacin (30mcg)	Amoxicilin (30mcg)	Chloramphenicol (30mcg)	Kanamycin (30mcg)
E. coli	Resistance	Resistance	Resistance	Resistance	Sensitive	resistance
Klebsiella	Sensitive	Resistance	Resistance	Resistance	Sensitive	sensitive
Bacillus	Resistance	Resistance	Intermediate	Resistance	Resitance	Resistance

Table 4: Antibiogram of disk diffusion test

organism	antibiogram
E.coli	$T^RV^RCf^RAm^RC^SK^R$
klebsiella	$T^SV^RCf^RAm^RC^SK^S$
Bacillus	$T^RV^RCf^IAm^RC^RK^R$

Fig 15: *Bacillus* **(** Ciprofloxacin, chloramphenicol,tetracycline)

Fig 16: *Bacillus* (kanamycin,vancomycin,amoxicillin) Antibiotic disk diffusion identification

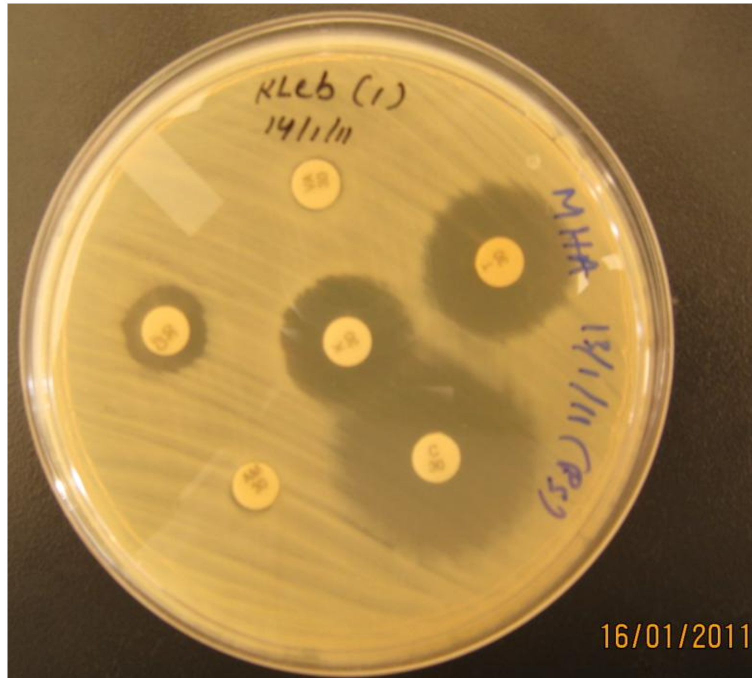

Fig 16: ***Klebsiella*** **Antibiotic disk diffusion identification**

Fig 17***: E coli .Antibiotic disk diffusion identification.***

5.4. Minimum inhibitory concentration result:

Conc- µg/ml

Table 5: minimum inhibition concentration test

	Vancomycin	Penicillin	Chloramphenicol	amoxicillin
E. coli	4	2	2	Resiatance
Klebsiella	4	2	16	Resistance
Bacillus	4	Resistance	2	Resistance

1. *E.coli* is resistance towards amoxicillin.
2. *Klebsiella* is also resistance to amoxicillin.
3. *Bacillus* was also found to be resistance towards amoxicillin and penicillin.

Discussion:

E. coli is defined as a Gram-negative, non-spore forming, rod-shaped micro-organism that is often motile by means of flagella or may be non-motile, and which can grow with or without oxygen. It is catalase positive, oxidase negative, fementative (glucose, lactose, D-mannitol, arabinose and maltose), reduces nitrate and is β-galactosidase positive. Approximately 95 % of *E. coli* strains are methyl red positive, but are Voges-Proskauer and citrate negative Indole positive bacteria such as *Escherichia coli*produce tryptophanase, an enzyme that cleaves tryptophan, producing indole and other products. *E. coli*does not produce acetyl methyl carbinol**,** When Kovac's reagent (p-dimethylaminobenzaldehyde) is added to a broth with indole in it, a dark pink color develops**.** Pathogenic isolates of *E. coli* has widely been implicated in various clinical infections as hospital *coli* have relatively high potentials for developing resistance. High resistance of *E. Coli* to antimicrobial agents tested was observed in this study and it was observed that E.coli has highest resistance to vancomycin, tetracyclines, kanamycin,ciprofloxacin,amoxcilin.It was also found that E.coli is sensitive towards chloramphenicol. Beta-lactam antibiotics are widely used in the treatment of bacterial infections. However, the production of extended spectrum beta-lactamases (ESBLs), one of the resistance mechanisms encountered in Enterobacteriaceae, mainly Escherichia coli and Klebsiella pneumoniae, has been associated with several treatment failures. Bacillus cereus responds positive towards citrates,lysine glucose ,ornithin,nitrate and oxidase.It is negative for indol methyl red and urease test. *Klebsiella* and *Enterobacter* produce more neutral products from glucose (e.g. ethyl alcohol, acetyl methyl carbinol). In this neutral pH the growth of the bacteria is not inhibited. The bacteria thus begin to attack the peptone in the broth, causing the pH to rise above 6.2. At this pH, methyl red indicator is a yellow color (a negative MR test). The citrate test utilizes Simmon's citrate media to determine if a bacterium can grow utilizing citrate as its sole carbon and energy source. *Klebsiella pneumoniae* are citrate positive. klebsiella do not produces H_2S gas. It utilizes glucose as the sole source of carbon when grown on glucose rich medium.Antibiotic resistant *E. coli* isolates indicate the unwise and excessive consumption of antimicrobial drugs which in turn has brought about failure in treatment, and consequently concerns about the related issues. *E. coli* has widely been implicated in various clinical infections as hospital acquired and community infections as reported by Shah et al. (2002). Pathogenic isolates of *E.coli* have relatively high potentials for developing resistance (Karlowsky et al., 2004). High resistance of *E. Coli* to antimicrobial

agents tested was observed in this study. Data shows that the prevalence of resistance to most drugs tested in *E. coli* isolates. The greatest resistance in the present study was to tetracycline. The growing antimicrobial resistance may be due to unrational use of antibiotics and the transfer of resistance genes by transport means including antibiotic resistant plasmids, bacterio-phages, transposons and integrons. Since a plasmid or transposon can carry several resistance indexes, simultaneous resistance to multiple antimicrobial agents maybe developed and the result would be MDR organisms. For example, resistance to is usually accompanied by resistance to Tetracycline.

CONCLUSION

Resistance to antimicrobial drugs increased based on the evolution of bacteria, including multidrug resistance, is unavoidable because it represents a particular aspect of the general evolution of bacteria that is unstoppable. Delay the emergence of resistant bacteria or resistance genes or dealing with this situation. Mutations in housekeeping structural or regulatory genes in bacteria increases resistance to antimicrobial drugs. Alternatively, the horizontal acquisition of foreign genetic information can give rise to the resistance. The 2 phenomena are associated in the emergence and more efficient spread of resistance. .The resistant strains produce “extended-spectrum beta-lactamases”, enzymes that destroy penicillin .These very common bacteria, are much harder to kill with antibiotics, when they produce these enzymes.

Future prospects

1 Antimicrobial susceptibility enhancement using agents .

2 Phage therapies, an approach that has been extensively researched and utilized as a therapeutic agent. Bacteriophages or "phages" are the viruses that invade bacterial cells and, in the special case of the lytic phages, they disrupt bacterial metabolism and cause the bacterial lysis . The therapeutic use of lytic bacteriophages is the phase therapy- to treat the pathogenic bacterial infections.

3 Emergence and increasing prevalence of bacterial strains that are very much resistant to available antibiotics that helps in the discovery of new therapeutic approaches.
4 An alternative approach for targeting bacterial virulence is antimicrobial therapy that offers promising opportunities to inhibit pathogenesis.
5 Certain virulence factors are very good potential targets for drug design and therapeutic intervention, whereas new insights are crucial for exploiting others. Targeting virulence is representing a new way to empower the clinician to prevent and treat infectious diseases.

APPENDIX I

Nutrient agar

Ingredients Gms / Litre

Peptic digest of animal tissue 5.000

Sodium chloride 5.000

Beef extract 1.500

Yeast extract 1.500

Agar 15.000

Final pH (at 25°C) 7.4±0.2

Muller-Hinton agar

For 1 liter of medium:

- Acid hydrolysate of casein ....17.5 g
- Beef infusion ....2.0 g
- Soluble starch ....1.5 g
- Bacteriological agar ....17.0 g

pH of the ready-to-use medium at 25°C : 7.3 ± 0.2

APPENDIX II

0.5 McFarland standard

Sulphuric Acid ,1%   995.00 ml

Barium Chloride, 1%   5.00kovac

Kovac's reagent

p-Dimethylaminobenzaldehyde 7 4.8%

Isoamyl Alcohol 4.8%

Hydrochloric Acid 23.8%

TDA reagent

Ferric chloride 10g/100ml (in distilled water)

Baritt reagent

Methyl reagent

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