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Research Article

A study profile of UTI with reference to ESBL producers.

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Abstract

Beta lactamases enzymes are produced by certain bacteria that provide resistance to certain antibiotics. It is produced by both Gram positive and virtually all Gram negative bacteria, which is found on both chromosomes and plasmids. The beta lactamases provides primary mode of resistance to beta-lactam antibiotics. ESBL is the resistance produced through the actions of beta-lactamases. The extended spectrum cephalosporins, such extended as the third generation cephalosporin's were originally thought to be resistance to hydrolysis by beta-lactamases. The ESBLs summary have been reported for E.coli, Klebsiella, Enterobacter, Proteus, Pseudomonas, Salmonella, Serratia etc., This study was carried with an aim to isolate bacteria from urine sample collected from 75% of pregnant womens and 25% of old age person. The nature of bacterial isolates was identified to include in species level using standard morphological, biochemical and cultural characteristics. E*scherichia coli, Klebsiella pneumonia* and *Pseudomonas aeruginosa*. Resistant development was more for Ampicillin and then to Amoxycillin and the same was less for Ceftriaxone. The reason is not clear but totally, resistance to penicillin derivative was more compared to Cephalosporin derivative. Any way it could be confirmed after analyzing more Cephalosporin derivatives. Susceptibility to various antibacterial agents was studied to know the effective therapeutic agent as well as to know the resistant pattern, particularly to penicillin derivatives such as Amoxycillin, Ampicillin and Cephalosporin derivatives, Ceftriaxone. Sulbactam and Clavulanic acid was used to overcome to Penicillin and Cephalosporin derivative resistant problem. This study has clearly revealed that the Sulbactam and Clavulanic acid has anti beta lactamase producers, are becoming susceptible to the same antibiotics. It was confirmed for the penicillin and Cephalosporin derivatives.

Keywords: Beta lactamases, ESBL , Resistant development , antibiotics.

Introduction

Urinary tract infection (UTI) is bacterial infection affects any part of the urinary tract.Although urine contains a variety of fluids, salts and waster products,it is usually sterile.When bacteria get into the bladder or kidney and multiply in urine, they cause a UTI.The most common type of UTI is a bladder infection is also often called cystitis.Another kind of UTI is a kidney infection, known as pyelonephritis, and is much more serious.Although they cause by doing appropriate culture and initiate early antibacterial therapy.

Symptoms & Signs

For Bladder Infection

- Frequent urination along with the feeling of having to micturate even though little or no urine actually comes out.
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- Nocturia: Urinate during the night
- Urethritis: infection of the urethre.
- Cystitis: infection of bladder.
- Pyuria/Hematuria: pus or blood in urine.
- Pyrexia: Mild fever.
- Discharage from the urethra.
- Increased confusion and associated falls are common presenation for elderly pantients with UTI.
- Some urinary tract infections are asymptomatic.

For pyelonephritis kidney functions

- The above symptoms
- Emesis: Vomiting is common
- Back,side (flank) or groin pain
- Abdominal pain or pressure
- Shaking chills and high spiking fever
- Night sweats
- Extreme Fatigue

Diagnosis

A patient with dysuria (painful voiding) and urinary frequency generally has a spot mid-stream urine sample sent urine analysis, specifically the presence of nitrites, leukocytes or leukocyte esterase. If there is a high bacterial load without the presence of leukocytes,it is most likely due to contamination.The diagnosis of UTI is confirmed by a clean catch midstream urine.

If the urine culture is negative;

- Symptoms of urethritis may point at chlamydia trachomatis or Neisseria gonorrheae infection
- Symptoms of cystitis, may point atinterstitial cystitis
- In men,prostatitis may present with dysuria
- In severe infection, characterized by fever, rigors or flank pain, urea and creatinine measurements may be performed to assess whether renal function has been affected.

Causative Agents

Common organisms that cause UTIs include: *1. Escherichia coli 2. Klebsiella 3.Enterococcus faecalis 4. Proteus mirabilis 5.Enterobacter spp 6. Pseudomonas* and others.Rarely Staph. Aureus encountered in immunocompremised patients. A pneumonic that can be used to remember the bacteria that cause UTIs is SEEK PP (*Staph.aureus, E.coli, Enterococcus, Klebsiella, Proteus and Pseudomonas).*

Prevention

The following are measures that studies suggest may reduce the incidence of urinary tract infections. These may be appropriate for people, especially women, with recurrent infections:

- Cleaning the urethral meatus (the opening of the urethra) after intercourse has been shown to be of have some benefit with large amount of intake of fluids.
- It has been advocated that cranberry juice can decrease the incidence of UTI (some of these opinions are referenced in External Links Section).A specific type of tannin found only in cranberries and blueberries prevent the adherence of certain pathogens (eg. E.coli) to the epithelium of the urinary bladder. A review by the Cochrane collaboration of cranberries (juice and capsules) can prevent recurrent infections in women.Many people in the trials stopped drinking the juice, suggesting it may not be a popular intervention.
- For post-menopausal women, a randomized controlled trial has shown that intravaginal application of topical estrogen cream can prevent recurrent cystitis.In this study,patients ion the experimental group applied 0.5 mg of estriol vaginal cream nightly for two weeks followed by twice – weekly applications for eight months.
- Acupuncture has been shown to be effective in preventing new infection in recurrent cases. One study showed that urinary tract infection occurrence was reduced by 50% for 6 months.However; this study has been criticized for several reasons.Acupuncture appears to reduce the total amount of residual urine in the bladder all of the studies are done by one research team without independent reproduction of results.

The following measures seem sensible, but have not been studied:

- Cleaning genital areas prior to and after sexual intercourse
- For sexually active women, and to a lesser extent men, urinating within 15 minutes of sexual intercourse to allow the flow of urine to expel the bacteria before speacilized extensions anchor the bacteria to the walls of the urethra.
- Having adequate fluid intake, espeacially water.
- Not resisting the urge to urinate
- Taking showers, not baths, or urinating soon after taking a bath.
- Practicing good hygiene, including wiping from the front to the back to avoid contamination of the urinary tract by fecal pathogens.

Epidemiology

UTIs are most common in sexually active women, and men increase in diabetics and people with sickle-cell disease or anatomical malformations of the urinary tract. Although can be a hidden factor in urinary tract infections. The use of urinary catheters in both men and women who are elderly people experiencing nervous system disorders and people who are convalescing or unconscious for long periods of time may result in an increased risk of urinary tract infection for a variety of reasons. Elderly individuals, both men and women, are more likely to harbor bacteria in their genitourinary system at any time. These bacteria may be associated with symptoms and thus require treatment with an antibiotic.Women are more prone to UTIs because in females, the urethra is much shorter and closer to the anus than in males. The article on vulvovaginal health has some health tips for preventing UTIs.

Treatment

Most uncomplicated UTIs can be treated with oral antibiotics such as trimethoprim, cephalosporins, nitrofurantoin, or a fluoroquinolone (Eg. Ciprofloxacin, levofloxacin). In older the last decade co-trimoxazole was used to treat UTI and now stains have developed drug resistance to most of the antibacterial.

Recurrent UTIs

Patients with recurrent UTIs may need further investigation. This may include ultrasound scans of the kidneys and bladder or intravenous urography (X-rays of the urological system following intravenous injection of iodinated contrast material). If there is no response to treatments, interstitial cystitis may be a possibility. During cystitis, uropathogenic *Escherichia coli* (UPEC) subvert innate defences by invading superficial umbrella cells and rapidly increasing in numbers to form intracellular bacterial communities (IBCs).

Urinary tract infections

Although it is more common in female due to the shorter urinary tract. Urinary tract infection is seen in both males and females. It is found in roughly equal proportions in elderly men and women. Since bacteria invariably enter the urinary tract through the urethra (an ascending infection),poor toilet habits can predispose to infection (doctors often advice women to wipe front to back, not back to front) but other factors are also important; (pregnancy in women, prostate enlargement in men) and in many cases the initiating event is unclear.While ascending infections are generally the rule for lower urinary tract infections and cystitis, the same may not necessarily hold for upper urinary tract infections like pyelonephritis which may be hematogenous in origin. Most cases of lower urinary tract infections in females are begin and do not need exhaustive laboratory work-ups. However, UTI in young infants must receive some imaging study, typically a retrograde arterigram, to ascertain the presence/ absence of congenital urinary tract anomalies. Males to must be investigated further, the way to investigate it are to use specific methods of X-ray, MRI and CAT scan technology.

How Antibiotic Resistance Happens

Antibiotic resistance results from gene action. Bacteria acquire genes conferring resistance in any way of three ways.In spontaneous DNA mutation, bacterial DNA (genetic material) may mutate (change) spontaneously (indicated by starburst). Drug-resistant tuberculosis arises this way. In a form of microbial sex called transformation, one bacterium may take up DNA from another bacterium. Penicillin-resistant Gonorrhea results from transformation.

Most frightening, however, is resistance acquired from a small circle of DNA called a plasmid that can flit from one type of bacterium to another. A single plasmid can provide a slew of different resistances. In 1968, 12.500 people in Guatemala died in an epidemic of Shigella diarrhea. The microbe harboured a plasmid carrying resistances to four antibiotics.

Towards Solving the Problem

Antibiotic resistance is inevitable, say scientists, but there are measures we can take to slow it. Efforts are under way on several fronts improving infection control, developing new antibiotics and using drugs more appropriately. Drug manufacturers are once again becoming interested in developing new antibiotics. These efforts have been both by the appearance of new bacterial illnesses, such as Lyme disease and Legionnaire's, and resurgences of old foes, such as tuberculosis, due to drug resistance.

Narrowing the spectrum

Appropriate prescribing also means that physicians use "narrow spectrum" antibiotics- those that only a few bacterial types- whenever possible, so that resistances can be restricted.

The Greatest Fear-Vancomycin Resistance

When microbes began resisting penicillin, medical researchers fought back with chemical cousins, such as methicillin and oxacilllin. By 1953, the antibiotic armamentarium included chloramphenicol, neomycin, terramycin (tetracyclin), and cephalosporins. But today, researchers fear that we may be bearing an end to the seemingly endless flow of antimicrobial drugs. At the center of current concern is the antibiotic vancomycin which for many infections is literally the drug of "last resort", says Michael Blum, medical officer in FDA's division of anti-infective drug products. Some hospital-acquired Staphylococcus aureus infections are resistant to all antibiotics except vancomycin. Now vancomycin resistance has turned up in another common hospital bug, *Enterococcus.*, S.aureus may pick up vancomycin resistance from Enterococci, says Madden.

Beta lactams (Penicillin and Cephalosporin) and Beta lactamase inhibitors (Clavulanic acid, Sulbactam, Tazobactam)

Beta-lactams have a long history in the treatment of infectious diseases, though their use has been and continues to be confounded by the development of resistance in target,organisms.,Beta-lactamases, particularly in Gram-negative pathogens, are a major determinant of this resistance, although alterations in the beta-lactam targets, the penicillin-binding proteins (PBPs) are also important, especially in Gram-positive pathogens. Mechanisms for the efflux and/or exclusion of these agents also contribute, though often in conjunction these other two. Approaches for overcoming these resistance mechanisms include the development of novel beta-lactamase-stable beta-lactams,beta-lactamase inhibitors to be employed with existing beta-lactams,beta-lactam compounds that strongly to low-affinity PBPs and agents that potentiate the activity of exixting beta-lactams against low-affinity PBP-producing organisms.

Beta-lactamase inhibitors are proteins designed to inhibit or destroy the effectiveness of beta-lactamase enzymes. Inhibitors generally have little antimicrobial properties themselves and so are combined with a beta-lactam antibiotic. These inhibitors (Clavulanic acid, Sulbactam, Tazobactam) function by binding to the beta-lactamase enzymes more "efficiently" than the actual beta-lactam antibiotic itself.This combination allows the antibiotic "do its job" without being degraded by the beta-lactamase enzymes.Several antibiotic/beta-lactamase inhibitor combinations exist on the market.

The bacterial infections, which contribute most to human disease, are also those in which emerging and microbial resistance is more evidenty diarrhoeal disease, respiratory tract infections, meningitis, sexually transmitted infections, and hospital acquired infections. Some important examples include penicillin-resistant Streptococcus pneumoniae, Vancomycin resistant Enterococci, methicillin-resistant Staphylococcus aureus. Multi resistant Salmonellae and multi-resistant *Mycobacterium tuberculosis*. The development of resistance to drugs commonly used to treat malariae is of particular concern, as is the emerging resistance to anti-HIV drugs.

When infections become resistant to first-line antimicrobials, treatment has to be switched to second or third line drugs, which are nearly always much more expensive and sometimes more toxic as well,

e.g, the drugs needed to treat multi drug resistant forms of tuberculosis are over 100 times more expensive than the first-line drugs used to treat non-resistant forms.In many countries, the high cost of such replacement drugs is prohibitive, with the result that some diseases can no longer be treated in areas where resistance to first line drugs are widespread.Most alarming of all are diseases where resistance is developing for virtually all currently available drugs, thus raising the spectrum of a post-antibiotic era. Even if the pharmaceutical industry were to step up efforts to develop new replacement drugs immediately, current trends suggest that some diseases will have no effective therapies within the next ten years.

The World Health Organization (WHO) estimates that 50 percent of all medicines are inappropriately prescribed, dispensed or sold, and that 50 percent of all patients fail to take their medicine properly.As a result, antimicrobial resistance (AMR) has become a major public health problem worldwide. Penicillin-resistant gonorrhea strains now appear in 60 percent of those infected with those bacteria, and in 98 percent of all strains found in Southeast Asia. Tuberculosis, Malaria,Meningitis, and other major diseases have also developed at least partial resistance to drugs that once effectively treated them.

Drug resistance is not limited to antibiotics. Chloroquine, once tthe drug of choice for malaria, is no longer effective in 81 of 92 countries where that disease is a problem. Multiple drug-resistance tuberculosis (MDR-TB) has appeared through out the world (from Eastern Europe to Sub-Saharan Africa to Asia) in those co-infected with HIV, among health care workers, and in the general population. Resistance has also begun to appear to some anti retrivirals.

Aim and objectives

- To isolate bacteria from urine samples from UTI suspected patients.
- To identify the nature of bacteria to include in species level using standard morphological, biochemical and cultural characteristics.
- To identify their susceptibility to various antibacterial agents to know the effective therapeutic agent as well as to know the resistant pattern, particularly to 1.1st, 2nd and 3rd generation Cephalosporin.2. Fluroquinones such as Norfloxacin, pefloxacin, levofloxacin and 3. Aminoglycoside groups such as Amikacin and Gentamycin.
- Sulbactum and Clavulanic acid was used to overcome the penicillin and cephalosporin derivate resistant problem.If a different susceptibility pattern is identified, and if Sulbactum and Clavulanic acid found to be better to cope up with beta lactamase problem, then it would be possible to pass it to the medical community to overcome this problem in a better manner compared to the present status.

Materials and Methods

This section involves the methods of sample collection, studies of commonly occurring microorganisms, staining techniques, different culture media, and various biochemical tests.The above will be dealt in brief in the following lines.

STERILIZATION

For the sterilization of media and other glasswares the standard microbiological sterilization techniques were followed.

SAMPLE COLLECTION

Clean catch midstream urine samples from 50 patients with urinary tract infection (UTI) problems were collected in sterile container from government hospital, Tamilnadu.Their urine samples were processed for any bacterial population as indicator of Urinary tract infection (UTI).

CLEANING OF GLASSWARE

The glassware of Borosil grade was used in all the experiments.The glassware was cleaned by soaking in chromic acid solution (100g potassium dichromate dissolved in one litre water with 500ml concentrated sulphuric acid) for two hours and washed in water.

Chemicals

All the chemicals were used of high purity analar specifications and whenever necessary sigma grade chemicals were used.

Sterilization Techniques

All the glasswares was sterilized in a hot air oven at 180° C for two hours.All the prepared media and water blanks were sterilized in an autoclave at 1 atm for 95 minutes.All the antibiotics were filter sterilized using sintered glass filter for the isolation,purification,inoculation and other microbiological works were carried out in a laminar air flow chamber (AirFlow, India).

EXAMINATION OF THE SPECIMEN
MICROSCOPIC EXAMINATION
STAINING

A thin smear was prepared from the urine as such on a grease free slide and a Gram's staining was done.It was flooded with crystal violet solution and allowed to stand for one minute.Then it was washed with water and then flooded with Gram's iodine solution.It was drained and decolourized with 95% ethanol, which then washed gently in running water.Then the same was stained with a counter stain called saffranin for 30 seconds. After drying the stained smear was observed under microscope to identify the organisms and pus cells to detect bacteriuria and Pyuria.0.01 ml of urine with calibrated loop 6mm diameter was used to inoculate on sheep blood agar and sterilized for colony count on Macconkey agar and thus two plate were incubated at 37° C for overnight to 48 hrs.

The colony was carried out after 24 hrs and 48 hrs and interpreted as

- <1000 colonies/ ml of urine – In significant bacteriuria.
- <1000 – 100,000 colonies / ml of urine – Probably significant of bacteriuria.
- Above 100,000 colonies / ml of urine – significant bacteriuria.

MOTILITY TEST

The hanging drop method was followed to observe the motility of the drop of suspension of culture was placed at the center of a coverslip and placed in an inverted position over a cavity slide so that the drop is hanging over the cavity.The edge of the drop was observed under low power lens of a microscope where the darting or corkscrew movement of the organism can be seen.The results were noted.

ISOLATION OF BACTERIA FROM SAMPLES

The urine sample were processed in such a way that they were streaked on the prepared nutrient agar,Blood agar and Mannitol salt agar plates aseptically.Then by using inoculation needle, it was streaked for the growth of isolated colonies and then the plates were incubated at 37° C for 24 hrs for bacteria. After 24 hrs the colonies grown on the plates were examined for their morphology, haemolysis and the same type of colonies was used for Gram's staining.The golden yellow colonies in Mannitol salt agar medium indicate the presence of S.aureus.

Morphology

The morphology of each type of colony was examined and the results were noted.

Staining

Each type of colony was aseptically taken from the plate and a thin smear was prepared on a glass slide.The preparation was heat fixed, Gram stained and observed under microscope. The results were noted.

Subculture

The same type of colony was simultaneously taken from the plate aseptically and streaked on the prepared nutrient agar plate. Then the plates were incubated at 37°C for 24 hrs.After one day, the results were noted for their colony morphology and pigment production and also the colonies grown on the plate were used for performing biochemical tests and antibiotic sensitivity tests.

BIOCHEMICAL TESTS
SUGAR FERMENTATION TEST

The term sugar in microbiology denotes any fermentable substance. They may be monosaccharides, disaccharides, trisaccharides, alcohols, glycosides and non-carbohydrate substances such as inositol.

The sugars were fermented by various types of bacteria and in that process acid and gas was produced which were indicated by the colour indicated by the indicators for acid and gas collected into the inverted Durham's tube.The usual sugar media consist of 1% of

the sugar concerned in peptone water (or) Hi-media discs with an appropriate indicator. A Durham's tube was kept inverted in the sugar tube to detect gas production. The incubation period was 24 hrs and the temperature was generally 37° C.

The organisms grown in the plates were inoculated into

1. Mannitol,motility medium
2. Triple sugar iron agar
3. Peptone for indole production for presumption identification and further confirmation was carried out by including the following biochemical such as urease, citrate, lysine iron agar, IMViC test and others.

I-Indole, M-Methyl red, Vi-Voges proskauer's reaction, C-Citrate utilization.

INDOLE TEST

The colony from the plate was inoculated into the indole medium in a tube and then incubated at 37° C for 24 hrs. The formation of red ring upon the addition of Kovac's reagent indicates positive reaction.

METHYL RED TEST

The colony from the plate was inoculated into MR-VP broth tubes and incubated at 37°C for 24 hrs. The formation of red colour on the addition of methyl red indicator indicates the positive reaction.

VOGES-PROSKAUER TEST

The same type colony was inoculated into the MR-VP broth tubes and incubated at 37° C for 24 hrs. Development of pink to bright red colour on the addition of Barrit's reagent to the medium indicates positive reaction.

CITRATE UTILIZATION TEST

Slant of Simmon's citrate agar medium was inoculated with the organism wrong on the plate and incubated at 37° C for 24 hrs. Change of the colour from green to blue indicates the positive reaction.

OXIDASE TEST

The organisms taken from the plate was streaked on filter paper incorporated with oxidase reagent (1% tetramethyl paraphenylene diamine dihydrochloric acid in water). The appearance of purple colour indicates positive result.

UREASE TEST

This test is done in urease medium.Inoculate the slope heavily and incubate at 37° C. Urease positive cultures produce a purple pink colour.

CATALASE PRODUCTION

A loopful of 10% hydrogen peroxide on colonies of nutrient agar is added.

MICROORGANISMS TESTED

The clinical isolates were isolated from clinical samples and conducted antimicrobial susceptibility test by disc diffusion method (Kirby Bauer method).

PREPARATION OF INOCULUM

24 hours old cultures of selected bacteria and fungi were mixed with physiological saline until a Mc Farland turbidity standard of 0.5 (10 6 cfu/ml).

ANTIBIOTIC SENSITIVITY TEST
TEST FOR THE ISOLATES

After confirming the quality of medium and discs using standard strain antibiotic sensitivity test was performed in Muller Hinton Agar medium. A lawn culture was prepared on the media with the swab from the culture grown nutrient broth for 2 hrs and adjusted to Mc Farland's opacity no-1.Antibiotic disc like amoxicillin with clavulanic acid disc were placed on the media using sterile forceps.After 24 hrs of incubation the clear zone of inhibition around the disc was measured and the results were noted Modified Kirby-Bauer technique).

TEST FOR THE ISOLATES RESISTANT TO PENICILLIN AND CEPHALOSPORIN DERIVATIVES
SULBACTAM WITH AMPICILLIN/SULBACTAM WITH AMOXYCILLIN MEDIUM

Muller Hinton agar was prepared and sterilized at 115° C for 15 minutes,After sterilization,pinch of Ampicillin was added to the medium and poured into the sterile petri plates.After solidification different types of urine samples were streaked into the medium.Incubated at 37° C for 24 hours, on the next

day resistant bacterial strains were isolated and the resistant bacterial strains were streaked into sulbactam with ampicillin incorporate medium.Similar test was carried out for amoxicillin also separately.

CLAVULANIC ACID WITH AMPICILLIN/CLAVULANIC ACID WITH AMOXYCILLIN MEDIUM

Muller- Hinton agar was prepared and sterilized at 115° C for 15 minutes, After sterilization, pinch of ampicillin was added to the medium and poured in to the sterile petriplates.After solidification different types of urine samples were streaked into the medium.Incubated at 37° C for 24 hrs, on the next day, bacterial resistant strains were isolated and the bacteria resistant organisms were streaked into clavulanic acid with ampicillin incorporate medium.Similar test was carried out for amoxicillin also separately.

SULBACTAM WITH CEFTRIOXONE / CLAVULANIC ACID WITH CEFTRIOXONE MEDIUM

Muller Hinton agar was prepared and sterilized at 115° C for 15 mins, After sterilization, pinch of ceftriaxone was added in to medium and poured into the sterile petriplates. After solidification, different types of urine samples were streaked into the medium. Incubated at 37°C for 24 hrs, on the next day bacteria resistant organisms were isolated and the bacteria resistant organisms were streaked into sulbactam with ceftriaxone incorporate medium.Similar test was carried out for clavulanic acid also separately.

THE DOUBLE DISC SYNERGY TEST

This phenotypic method may also use to detect ESBL, the four different cephalosporin discs such as ceftazidime,cefotaxime,ceftriaxone,cefepime should place around amoxicillin-clavulanic acid disc at the center to center distance of 15mm from center disc.An isolate would considered to be an ESBL producer if there is any enhancement between any of the four cephalosporins and clavulanate containing disc.

Results

CHARACERIZATION OF E.coli

Microscopy : G-ve Rods
Motility Test : Motile
Capsule Staining : Positive
Endospore Staining : Negative
Colony Morphology : Small colonies in Nutrient agar
On MacConkey agar : Pink colour non mucoid colonies
On Blood agar : Non haemolytic colonies
Triple sugar iron test : Acid butt – Acidslant,Gas production

TABLE-1

BIOCHEMICAL CHARACTERIZATION OF E.coli

S.No	Name of Test	Result
1	Catalase	Positive
2	Oxidase	Negative
3	Coagulase	Not done
4	Indole	Positive
5	Methyl red	Positive
6	Voges proskeur	Negative
7	Citrate	Negative
8	Urease	Positive
9	Gelatinase	Positive
10	Nitrate	Positive
11	O/F test glucose	Fermentative

CHARACERIZATION OF Klebsiella pneumonia

Microscopy : G-ve Rod (Diplobacilli)
Motility test : Motile
Capsule staining : Positive
Endospore staining : Negative
Colony morphology : Large mucoid colonies in nutrient agar
On MacConkey agar : Pink colour mucoid colonies
On Blood agar : Non haemolytic colonies
Triple sugar iron test : Acid butt – Acid slant,Gas production,H2S positive

TABLE-2
BIOCHEMICAL CHARACTERIZATION OF
Klebsiella pneumonia

S.No	Name of Test	Result
1	Catalase	Positive
2	Oxidase	Negative
3	Coagulase	Not done
4	Indole	Negative
5	Methyl red	Negative
6	Voges proskaeur	Positive
7	Citrate	Positive
8	Urease	Positive
9	Gelatinase	Positive
10	Nitrate	Positive
11	O/F Test Glucose	Fermentative

CHARACTERIZATION OF
Pseudomonas aeruginosa

Microscopy : G-ve Rods
Motility test : Motile
Capsule Staining : Negative
Endospore staining : Negative
Colony morphology : Bluish green large opaque irregular colonies
On MacConkey agar : Non lactose fermentor
On Blood agar : Non haemolytic colonies (or) Alpha Haemolytic colonies
Triple sugar iron test Acid butt – Alkaline slant, Gas +ve, H2S +ve

TABLE-3
BIOCHEMICAL CHARACTERIZATION OF
Pseudomonas aeruginosa

S.No	Name of the Test	Result
1	Catalase	Positive
2	Oxidase	Positive
3	Coagulase	Not done
4	Indole	Negative
5	Methyl red	Negative
6	Voges proskeur	Positive
7	Citrate	Negative
8	Urease	Negative
9	Gelatinase	Negative
10	Nitrate reduction	Positive
11	O/F Test glucose	Non-fermentative

TABLE-4
MAJOR ORGANISMS ISOLATED FROM URINARY TRACT INFECTION CASES

S.No	Total number of samples analyzed	Number of positive samples	Name of the organisms isolated
1	50	24 20 10	Eschericia coli Klebsiella pneumonia Pseudomonas aeruginosa

TABLE-5
ANTIBIOTIC SENSITIVITY FOR
Escherichia coli
(S)- Sensitive (I) – Intermediate (R) – Resistant

S.No	Name of the Antibiotic	Diameter of the Zone (Result)
1	Ampicillin	12mm (R)
2	Amoxycillin	08 mm (R)
3	Cefotaxime	17 mm (I)
4	Ceftriaxone	10 mm (R)
5	Cephalexin	19 mm (S)
6	Ciprofloxacin	26 mm (S)
7	Cloxacillin	18 mm (S)
8	Co-Trimaxazole	21 mm (S)
9	Erythromycin	08 mm (S)
10	Gentamycin	14 mm (I)
11	Penicillin	05 mm (R)
12	Norfloxacin	22 mm (S)
13	Ofloxacin	10 mm (R)
14	Methicillin	12 mm (R)
15	Tetracycline	22 mm (S)

TABLE-6
ANTIBIOTIC SENSITIVITY FOR
Klebsiella pneumoniae

(S)- Sensitive (I) – Intermediate (R) – Resistant

S.No	Name of the Antibiotic	Diameter of the Zone (Result)
1	Ampicillin	12mm (R)
2	Amoxycillin	07 mm (R)
3	Cefotaxime	17 mm (S)
4	Ceftriaxone	18 mm (R)
5	Cephalexin	19 mm (S)
6	Ciprofloxacin	26 mm (S)
7	Cloxacillin	18 mm (S)
8	Co-Trimaxazole	21 mm (S)
9	Erythromycin	08 mm (R)
10	Gentamycin	14 mm (I)
11	Penicillin	05 mm (R)
12	Norfloxacin	22 mm (S)
13	Ofloxacin	10 mm (R)
14	Methicillin	12 mm (R)
15	Tetracycline	22 mm (S)

TABLE-7
ANTIBIOTIC SENSITIVITY FOR
Pseudomonas aeruginosa

(S)- Sensitive (I) – Intermediate (R) – Resistant

S.No	Name of the Antibiotic	Diameter of the Zone (Result)
1	Ampicillin	12mm (R)
2	Amoxycillin	06 mm (R)
3	Cefotaxime	16 mm (I)
4	Ceftriaxone	08 mm (R)
5	Cephalexin	18 mm (S)
6	Ciprofloxacin	27 mm (S)
7	Cloxacillin	18 mm (S)
8	Co-Trimaxazole	22 mm (S)
9	Erythromycin	09 mm (R)
10	Gentamycin	13 mm (I)
11	Penicillin	12 mm (R)
12	Norfloxacin	22 mm (S)
13	Ofloxacin	10 mm (R)
14	Methicillin	13 mm (I)
15	Tetracycline	20 mm (S)

Discussion

Diseases, particularly microbial diseases are increasing day by day.Diagnostic and treatment measures are also in increasing trend. Antibiotics are gaining more importance from time to time from the time of it's first discovery for the treatment of bacterial and fungal diseases. Indiscriminate use of antibiotics leads to development of resistance to various antibiotics, particularly by beta-lactamase production.

Urine collected from 75% of pregnant womens, those and 25% of old age persons.

The nature of bacterial isolates was identified to include in species level using standard morphological, biochemical and cultural characteristics. Escherichia coli, Klebsiella pneumonia and Pseudomonas aeruginosa.

The tremendous therapeutic advantage afforded by antibiotics is being threatened by the emergence of increasingly resistant strains of microbes. In pregnant women, penicillin-resistant Klebsiella pneumonia is of greatest concern; recent reports also indicate the appearance of outpatient methicillin-resistant S.aureus (MRSA) infections. MRSA is a significant problem in the hospital, as are vancomycin-resistant Enterococcus, oxacillin-resistant S.sureus, and multidrug-resistant Gram-negative bacilli. Responsible antibiotic use and stringent infection control policies are needed to discourage the development of resistant strains (File,1999).

Out of 24 isolates of E.coli 15 (62.5%) were resistant to Ampicillin and all (100%) were susceptible to Ampicillin+sulbactam complex and Ampicillin+clavulanic acid complex. Out of 20 isolates of Klebsiella pneumonia 11 (55%) were resistant to Ampicillin and all (100%) were susceptible to Ampicillin+Sulbactam complex and Ampicillin+Clavulanic acid complex. Out of 10 isolates of Pseudomonas areuginosa 05 (50%) were resistant to Ampicillin and all (100%) were susceptible to Ampicillin + Sulbactam complex and Ampicillin + Clavulanic acid complex.

Out of 24 isolates of E.coli 11 (45.8%) were resistant to Amoxycillin and all (100%) were susceptible to Amoxycillin+Sulbactam complex and

TABLE-8
AMPICILLIN WITH SULBACTAM MEDIUM

S.No	Name of the Organisms	Total No	Ampicillin Medium	No	Ampicillin + Sulbactam	No
1	Escherichia coli	24	Resistant	15	Intermediate	15
2	Klebsiella pneumonia	20	Resistant	11	Intermediate	11
3	Pseudomonas aeruginosa	10	Resistant	05	Intermediate	05

TABLE-9
AMPICILLIN WITH CLAVULANIC ACID

S.No	Name of the Organisms	Total No	Ampicillin Medium	No	Ampicillin + Clavulanic acid	No
1	Escherichia coli	24	Resistant	15	Intermediate	15
2	Klebsiella pneumonia	20	Resistant	11	Intermediate	11
3	Pseudomonas aeruginosa	10	Resistant	05	Intermediate	05

TABLE-10
AMOXYCILLIN WITH SULBACTAM MEDIUM

S.No	Name of the Organisms	Total No	Amoxycillin Medium	No	Amoxycillin + Sulbactam	No
1	Escherichia coli	24	Resistant	11	Intermediate	11
2	Klebsiella pneumonia	20	Resistant	07	Intermediate	07
3	Pseudomonas aeruginosa	10	Resistant	03	Intermediate	03

TABLE-11
AMOXYCILLIN WITH CLAVULANIC ACID

S.No	Name of the Organisms	Total No	Amoxycillin Medium	No	Amoxycillin + Clavulanic acid	No
1	Escherichia coli	24	Resistant	11	Intermediate	11
2	Klebsiella pneumonia	20	Resistant	07	Intermediate	07
3	Pseudomonas aeruginosa	10	Resistant	03	Intermediate	03

TABLE-12
CEFTRIAXONE WITH SULBACTAM MEDIUM

S.No	Name of the Organisms	Total No	Ceftriaxone Medium	No	Ceftriaxone + Sulbactam	No
1	Escherichia coli	24	Resistant	08	Intermediate	08
2	Klebsiella pneumonia	20	Resistant	06	Intermediate	06
3	Pseudomonas aeruginosa	10	Resistant	04	Intermediate	04

TABLE-13
CEFTRIAXONE WITH CLAVULANIC ACID

S.No	Name of the Organisms	Total No	Ceftriaxone Medium	No	Ceftriaxone + Clavulanic acid	No
1	Escherichia coli	24	Resistant	08	Intermediate	08
2	Klebsiella pneumonia	20	Resistant	06	Intermediate	06
3	Pseudomonas aeruginosa	10	Resistant	03	Intermediate	03

Amoxycillin+Clavulanic acid complex. Out of 20 isolates of K.pneumoniae 7 (35%) were resistant to Amoxycillin and all (100%) were susceptible to Amoxycillin+Sulbactam complex and Amoxycillin + Clavulanic acid complex.Out of 10 isolates of P.aeruginosa 3 (30%) were resistant to Amoxycillin and all (100%) were susceptible to Amoxycillin+Sulbactam complex and Amoxycillin + Clavulanic acid complex.

Out of 24 isolates of E.coli 8 (33.33%) were resistant to Ceftriaxone and all (100%) were susceptible to Ceftriaxone + Sulbactam complex and Ceftriaxone + Clavulanic acid complex. Out of 20 isolates of K.pneumoniae 6 (30%) were resistant to Ceftriaxone and all (100%) were susceptible to Ceftriaxone + Sulbactam complex and Ceftriaxone + Clavulanic acid complex. Out of 10 isolates of P.aeruginosa 4 (40%) were resistant to Ceftriaxone and all (100%) were susceptible to various antibacterial to Ceftriaxone + Sulbactam complex and Ceftriaxone + Clavulanic acid complex.

Resistant development was more for Ampicillin and then to Amoxycillin and the same was less for Ceftriaxone. The reason is not clear but totally, resistance to penicillin derivative was more compared to Cephalosporin derivative. Any way it could be confirmed after analyzing more Cephalosporin derivatives.

Susceptibility to various antibacterial agents was studied to know the effective therapeutic agent as well as to know the resistant pattern, particularly to penicillin derivatives such as Amoxycillin, Ampicillin and Cephalosporin derivatives, Ceftriaxone.

Sulbactam and Clavulanic acid was used to overcome to Penicillin and Cephalosporin derivative resistant problem. This study has clearly revealed that the Sulbactam and Clavulanic acid has anti beta lactamase producers, are becoming susceptible to the same antibiotics. It was confirmed for the penicillin and Cephalosporin derivatives.

Infections were as follows: Surgical wound 19; Tracheobronchitis 12; Urinary tract 7; Catheter-related bacteremia 2; and Pneumoniae 2. Eighteen patients received intravenous sulbactam alone (1gram every 8 hours) and 24 patients received intravenous sulbactam/ampicillin (1:2 gram every 8 hours) with on major adverse effects. Of the 42 patients, 39 improved or were cured and showed Acinetobacter baumanni eradication and one patient had persistence of wound infection after 8 days of Sulbactam/Ampicillin requiring surgical debridement. Two patients died after 3 days of therapy (one of the deaths was attributable to A.baumanni infection). The in-vitro activity of the sulbactam/ampicillin combination was by virtue of the antibacterial activity exhibited by sulbactam. Killing curve showed that sulbactam was bacteriostatic; no synergy was observed between ampicillin and sulbactam. Our result indicate that sulbactam may prove effective for non-life-threatening A.baumanni infections. Its role in the treatment of severe infections is unknown. However, the current formulation of sulbactam alone may allow its use at higher doses and provide new potential synergic combinations, particularly for those infections by A.baumanni resistant to imipenem (Corbella, 1998).

Different susceptibility pattern was identified for the penicillin and cephalosporin derivative, Sulbactam and Clavulanic acid was found to be better to hope up with beta lactamase problem. Though it is being used in the animal diseases and rarely in human diseases, it has to be spread among the medical community to overcome this problem in a better manner compared to the patient status. The resistant pattern was studied in detail for E.coli, Klebsiella pneumonia and Pseudomonas aeruginosa. By further analysis and by finding a new alternative to those beta lactamases, in near future, it is possible to find out safer and effective drug against those or other drugs. Further study must be carried out to other bacteria able to produce beta-lactamase.

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