A STUDY ON INCIDENCE OF BETA LACTAMASE AND METHICILLIN RESISTANT STAPHYLOCOCCUS AUREUS IN URINARY TRACT INFECTIONS

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INTRODUCTION

Urinary tract infections are serious health problems affecting millions of people every year. Women are especially more prone to UTI for reasons not very well understood but most likely it is because of close proximation of urethral vaginal and anal openings. One woman in five develops UTI during her lifetime. UTI in men are not as common as in women but can be very serious when they do occur. Normally urine is sterile. It is free from bacteria, viruses and fungi but does contain fluids, salts and waste Products. An infection occurs when living organisms usually bacteria from digestive tract adhere to the opening of urethra and begin to multiply. The urethra is the tube that carries urine from bladder to outside the body. Most infections occur from one type of bacteria i.e. *E. coli* which normally lives in colon.

In many cases bacteria first travel to urethra, multiply and cause urethritis. If they move to bladder and multiply it is called cystitis. If infection is not treated promptly they may travel up to involve kidney and is known as pylonephritis.

Microorganism called *Chlamydia* and *Mycoplasma* may also cause UTI in both men and women but these infections tend to remain limited to urethra unlikely *E-coli, mycoplasma*, may be transmitted sexually and infections require treatment of both partners.

Some people are more prone to getting infections than others. Any opportunity of the urinary tract that obstructs the flow of urine e.g. kidney stone increases the chances of infection. Similarly enlarged prostate gland can slow the flow of urine thus raising the risk of infection.

A common source of infection is catheters placed in urethra and bladder. A person who cannot void or unconscious or critically ill often needs a catheters

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for a long time. Bacteria on the catheters can cause infection of urinary tract.

People with diabetes have higher risk of UTI because of poor response of immune system. Any other disorder like cancers, patient on chemotherapeutic drugs and steroids which lower the immunity of the person raises the risk of UTI.

Pregnant women are more prone to UTI then other women. It is most likely due to hormonal changes and change in the position of urethra during pregnancy which make it easier for the bacteria to move up the ureters and then to the kidneys. Despite wide spread availability of antibiotics, urinary tract infection remain the most common bacterial infections in human population. Antibiotics are usually given empirically before the labortary results of urine culture are available. To ensure appropriate therapy current knowledge of the organisms that cause UTI and their susceptibility pattern is mandatory. Emergence of resistance to beta lactam antibiotic begin even before the first beta lactam antibiotic i.e penicillin was developed. Resistance to *Staphylococcus aureus* was due to plasmid encoded penicillinase. This beta lactamase generally spread to most isolates of *Staphylococcus aureus* as well as other species of *Staphylococcus*. The beta lactamases are the the major defence of Gram Negative Bacteria against beta lactam antibiotics. On the basis of molecular structure they can be classified into four molecular classes (A, B, C and D). class A and class C are the most common beta lactamases.beta lactamases belonging to class A and D are plasmid encoded.TEM-1 and SHV belong to class A while oxa 1 belong to class D.Class B and C enzymes have a broad spectrum of activity because they are   always   encoded   by chromosal genes and so confined to particular bacterial species.

ESBLs are defined as beta lactamases capable of hydrolyzing oximino- cephalosporins (cefotaxime, ceftazidime, ceftrioxone) that are inhibited by clavallinic acid (ESBL in 21ST) ESBL are not active against cephamycins and most strains are susceptible to cefoxitin and cefotexan. However ESBL producing strains can become resistant to cephamycins due to loss of outer membrane proteins (porin loss)

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There are some varients of beta lactamases which are resistant to inhibitors like clauvalinic acid and sulbactum there by showing resistance to bêta lactum-beta lactamases inhibitor combination of amoxy clavulanate and ampicillin –sulbactum, while they remain susceptible to inhibition by tazobactum and so the combination of pipracillin and   tazobactam. There are another group of enzymes known as carbapenaces which are active against cephamycins and carbapenums like imipenums and carbapenums. Despite world wide use of beta lactum antibiotics the distribution of ESBLs responsible for resistance to oximino cephalosporins like cefotaxime, ceftazidime, ceftrioxone etc. and carbapenums is not uniform. hospitals of USA no ESBL has been detected while in other hospitals as many as 40% of *Klebsiella* isolates have been reported to have producing ESBL. ESBL are most likely found in *Klebsiella* and *E. Coli* but also have been found in other gram negative bacteria like *Citrobacter*, *Proteus, Salmonella* and other enteric organisms and in non enteric organisms like *Acinetobacter* and *Pseudomonas* aeruginosa. Their prevalence is higher in isolates from intensive care units than in isolates from other hospital sites.

MATERIAL AND METHOD

Present study was conducted in Microbiology Department in Oswal Cancer Hospital. It is a 300 bedded referral hospital which receives patients from different parts of Punjab, Haryana and Himachal Pradesh. It provides specialized services to patients suffering from all kinds of cancer. This study was conducted during period from May 2009 to June 2010.

A total of 1500 urine samples were collected aseptically in sterile containers. All samples were collected before start of any antibiotic. 950 samples were of OPD patients while 550 samples were from IPD patients (Table 1).

Patients diagnosed as having urinary tract infection in the outpatient clinic or patients diagnosed within 48 hrs after hospitalization were classified as having outpatient urinary tract infection while patients diagnosed during the hospitalization were classified as IPD patients.

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The diagnosis of urinary tract infection was based on the standards of the centre for disease control and prevention (CDC) of detection of (10)5 bacterial colonies /ml by urine culture with more than one of the following signs and symptoms. Fever higher than 38 C, tenderness in suprapubic area, painful urination , urgency and frequency.

During study of patients suffering from urinary tract infection , various parameters were considered like age , sex and whether the patient is from OPD or IPD and isolates are producing beta lactamase or not. Out of 950 OPD patients growth was obtained from 515 samples and357 samples out of 550 IPD samples were found positive for one or other bacteria (Table-2).Thus 54% samples from OPD and 65% samples from IPD were found positive.

Table -3 is showing positivity considering age of the patients. Highest percentage (56%) were from patients between 22 to 45 years of age. Table   -4   shows tha rateof urinarytract infection is more among females i.e.59.4% as compared to males (44.2%). Similarly sex ratio was analysed in indoor patients. Not only overall positivity was more in indoor patients , higher percentage (69.3%) of females were found to be suffering from urinary infection than male patients whose positivity was 60.5%. Midstream urine samples were collected from all the suspected patients in wide mouthed sterile container and inoculated on McConkey agar and blood agar plates without delay to avoid unnecessary multiplication of the organism. These plates were incubated at 37 C for 24 hrs. After 24 hrs of incubation plates were screened for growth of bacteria. The bacteria were identified on the basis of their morphology, gram staining and biochemical reactions. On the basis of morphology they can be easily differentiated into gram positive and gram negative organism. In case of any difficulty Gram staining is done.

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IDENTIFICATION OF GRAM POSITIVE ORGANISM

They can be identified on the basis of their morphology and by biochemical tests.

MORPHOLOGY---Colonies of *Staphylococci* on blood agar are bigger in size (pinhead) and are either golden, white or yellow in colour. Golden colour colonies are *S. aureus*, white colour colonies are of *S. albus* while yellow coloured colonies are of *S. citreus*. Coagulase test is done to find out pathogenicity of the Staph.

COAGULASE TEST---Place a drop of human plasma on the slide and mix with growth to be tested. Appearance of clumping indicates positive coagulase test and the species is confirmed as *Staph aureus*. Colonies of *Streptococcus* are smaller in size (pin point) and are either beta haemolytic, alpha haemolytic or non haemolytic. *Streptococcus* does not grow on McConkey agar except *Enterococcus.* In cases *Staphylococcus* and *Streptococcus* were not identified just on the basis of their morphology catalase test was done.

CATALASE TEST---- Place a drop of Hydrogen peroxide on the slide and mix with growth to be tested. Appearance of effervasence indicates positive catalase test.

STAPHYLOCOCCUS   - CATALASE POSITIVE

STREPTOCOCCUS   -   CATALASE NEGATIVE

IDENTIFICATION OF GRAM NEGATIVE BACTERIA

The gram negative bacteria were divided into two groups on the basis of lactose fermentation i. e. lactose fermenters and non lactose fermenters. Lactose fermentors produce pink coloured colonies and non lactose fermenters produce pale coloured colonies. Further gram negative bacteria were identified on the basis of different biochemical tests.

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A study was conducted during May 2009 to July 2010   in oswal cancer hospital to find out incidence of MRSA and MSSA and other gram positive cocci. Similarly incidence of ESBL positive and negative gram negative organisms in patients suffering from urinary tract infection were also found. Since treatment of patients suffering from urinary tract infection depends on whether organism is being capable of producing beta lactamase or not. We have also studied the sensitivity pattern of such organisms.

A total of 1500 urine samples were collected from patients suspected to be suffering from urinary tract infection.950 urine samples were collected from patients attended out patient department while 550 urine samples were collected from indoor patients.Table 1 shows the distribution of such samples. Table 2 gives information of sterile and unsterile samples. Out of 950 urine samples from OPD patients growth of one or the other organism was found in 515 samples (54%) while remaining samples were sterile. Similarly 357 samples from 550 indoor patients showed infection (65%). Indoor patients tend to suffer more with urinary tract infection than OPD patients. Many factors contribute to high infection rate among indoor patients like insertion of catheter and other ailments like malignancies, chemotherapeutic drugs, steroids and infected environment of the hospital etc. According to statistics analysis also positivity is significantly higher in IPD (p-value = 0.006)

Table-3 shows that infection of urinary tract is more in both the sexes in the reproductive age group i.e.54% while in children it is 24% while 22% in patients above 45 years of age. The reason of this disparity is not clear. According to age there is no difference between OPD and IPD (p-value =

0.255, 0.580,

0.168)

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From Table 4 &5 it is concluded that infection rate is more in female patients than in male patients whether they are outdoor patients or indoor patients. Studies by other auothers have also the similar kind of results.one factor may be that woman's urethra is short allowing bacteria quick access to the bladder. Also a woman's urethral opening is near sources of bacteria from the anus and vagina. According to many studies woman who use diaphragm are more likely to develop UTI than woman who use other forms of birth control devices. Postivity is significantly higher among females (p-value = 0.005) From Table -6 overall positivity was calculated among gram negative and gram positive organisms. It was seen that 48.8% of gram negative organisms were ESBL positive while 31 % of *S. aureus* were ORSA and 48% of *staph albus* were ORSA.Positivity is significantly higher in gram negative as compared to gram positive. (p-value = 0.008)

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