MICROBIOLOGICAL ANALYSIS OF BLOOD AND URINE SAMPLES FROM THE PATIENTS OF HOSPITAL OF JALANDHAR

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Abstract

Urinary tract infection (UTI) and blood infection are very common microbial infectious disease occurring in the clinical health care centers across the globe. The pathogens responsible for these infections are mostly accountable for infections in the hospitals. The blood infections are mostly known as septicemia, sepsis and bacteremia which are just the presence of some particular bacteria in the blood leading to some diseases. The uropathogens and microorganisms responsible for blood infection are varying from place to place and they also vary in their antibiotic sensitivity pattern. The purpose of the study was to assess the distribution of urinary tract and blood bacterial pathogens with respect to gender and to determine the antibiotic susceptibility pattern of isolates. This study is done in the hospital of Jalandhar and from 214 urine and blood samples out of 290 samples in total in which there were 96 positive samples and 118 negative samples for a period of 4 months..

Key words: Urinary tract infection (UTI), uropathogens, bacteremia, antibiotic sensitivity, infectious disease

Introduction:

Diseases related to blood and urine fluids are most common in the infections of human beings around the world. Currently those infections or diseases are more present in women than men due to some parameters like age, body physiology, lifestyle, and metabolism [1-5]. In the urine, there are many infections caused by E. coli bacteria known as the uropathogenic E. coli (UPEC) which are the primary cause of urine tract infections including both cystitis and pyelonephritis and The urine infections can be caused by the catheter contamination The presence of K antigens in the urine which leads to the urine tract infection [6]. Escherichia coli is the predominant uropathogen (80%) isolated in acute community-acquired uncomplicated infections, followed by Staphylococcus saprophyticus (10% to 15%), Klebsiella, Enterobacter, and Proteus species, and Enterococci (5% to 10%) infrequently cause uncomplicated cystitis and pyelonephritis [7]. The mode of action of the E. coli is described by many ways which involve the action of particular elements present in the bacteria. The presence of different strains of E. coli which is also a big factor of the diseases caused by urine infections [8].

The blood is a tissue which has many components such as white blood cells, red blood cells, platelets. The red blood cells are 5 millions in number and they are important in transportation of oxygen in the body and other elements, then there are white blood cells which are helpful for the antimicrobial defence of the body, to protect the body against foreign organisms through the actions of eosinophil, neutrophils, basophils, lymphocytes and there are platelets which are involved in coagulation process of the blood. The blood has
also the plasma which is 60% of the blood’s volume and it is mainly made of water but there are proteins and other chemical elements such as hormones, antibodies, salts, enzymes, glucose, fats particles [9].

The blood infections are mostly known as septicaemia, sepsis and bacteraemia which are just the presence of some particular bacteria in the blood leading to some diseases. The bacteria responsible of the blood infection are such as \textit{Pseudomonas spp}, \textit{Staphylococcus spp}, \textit{Klebsiella spp}, \textit{Streptococcus spp}. The diseases involve in this situation are peritonitis, pneumonia, cirrhosis in case of liver failure, meningitis, gastro intestinal bleeding which are very common in the life and these bacteria are the main cause [10, 11].

The mode of transmission will different according to type of infections involved; in case of urine infections, the transmission can be done from catheter which is the common way to get urinary tract infections the contamination of urine can be done by bacteria also and contamination by some contaminated tools can lead to urinary tract infections. In case of blood infections, the blood transfusion which is the most common way to get infection, the contamination through the contaminated food, water and other liquids. It can be done also through droplets from an infected person who is coughing, sneezing, and talking [12].

Many bacteria are resistant to different antibiotics due to some changes occur in their metabolic functions, due to some mutations and others parameters such incomplete course of the medicines leading to strong resistance of bacteria after sometimes. There is one particular test involved to check the sensitivity which is known as antibiotics sensitivity test in which we can determine the degree of resistance of a bacterium against a particular antibiotic. Most of the bacteria are gram negative bacteria which cause many diseases such as urinary tract infections, pneumonia, bacteraemia, diarrhoea and other abnormalities but the Gram positive bacteria are also involved in the infections (Hoffman et al., 2015. Many bacteria which are multiple drugs resistant such as \textit{Staphylococcus aureus} which are resistant to methicillin (MRSA – Methicillin Resistant \textit{Staphylococcus aureus}), \textit{Pseudomonas aeruginosa} which are resistant to polymyxin drug (Polymyxin Resistant \textit{pseudomonas aeruginosa}), the \textit{E. coli} which are also resistant to some drugs, the \textit{Acinobacter baumanii}, \textit{Klebsiella}, \textit{Pneumonia} strains were also identified as resistant to quinolones, cephalosporins, penicillins, monobactams [13].

The multi drugs resistant bacteria are those which have ability to escape the action of antibiotics and they use many ways to survive such as mutation, changes of metabolic functions, they are able to remove the drugs from inside to outside by using some pumps which help them to survive [14]. So to manage all these situations, many ways should be applied to kill them by the action of antibiotics. The combination of some antibiotics in the aim to increase the efficiency of the drugs. The challenges are many because today many bacteria become resistant due to many parameters that we have already talked about in the previous lines so we have to explain in the details those challenges like we have to take care of the patients in the hospitals to reduce the number of nosocomial infections, we have to take care of hygienic conditions in the hospitals to avoid the contamination of tools such as catheters, syringes and the improvement of the techniques used in the hospitals for the diagnostic purpose. The patients should be advised to complete the course of medicines during the required time for the treatment, they should not stop the treatment until it is allowed by the
medical specialist and the populations should be aware about the MDR bacteria and the dispositions required to avoid them in the life [15]

METHODS AND MATERIALS

The study was done on the patients from hospitals of Jalandhar. There were 290 samples collected such as pus, urine, sputum, wound, blood, C.S.F and further the reporting was done on most leading samples urine and blood samples for a period of 4 months (January 2019 to April 2019).

The study was done from 214 urine and blood samples out of 290 samples in total in which there were 96 positive samples and 118 negative samples for a period of 4 months (January 2019 to April 2019).

Collection of samples

In case of blood sample, the sterile blood (not contaminated blood) was used for the microbiological examination; the fresh blood sample was collected from the patients. The transport of the blood sample was done in sterile conditions to avoid the contamination of the blood.

The 24 hour urine was the best sample for the urine analysis of bacteria collected from the patients with urinary tract infections, catheters problems, renal failure and the urine was collected in a sterile container to avoid the contamination during the transport in the lab which could lead to false positive results in case of contamination [16].

Processing of urine samples

The urine sample was processed by following examinations:

Physical examination

There was a physical examination of urine and blood samples to check the colour, pH, odour and specific gravity of urine. The 5 major components of urine are: Urea 9.3 g/L; chloride 1.87g/L, Sodium 1.17 g /L, Potassium 0.750g/L and creatinine 0.670 g/L. Other physical characteristics that can apply to urine include turbidity (transparency), smell (odor), pH (acidity - alkalinity), and density. In case of the colour, it is typically yellow-amber but varies according to recent diet and the concentration of the urine (Travis J et al., 2013).

3.2.2. Culturing technique of urine

The streaking of the plates was used as the technique for the culture of the urine samples. The 24 hour urine sample was collected from patients with urinary tract infections, catheters problems, and renal failure. The media were prepared in the aseptic conditions especially nutrient agar as for the growth of the non-fastidious bacteria with the pH adjusted at 7.4, the temperature was 25°C; Mc conkey agar as differentiator medium for the growth of Gram’s negative bacteria only with pH adjusted at 7.1, the temperature was 25°C and blood
agar as enriched medium for the growth of *Streptococci* which could not grow on the ordinary medium with the pH adjusted at 7.2-7.6, temperature was at 25°C. The samples were streaked on the agar plates by the help of the sterile stick loop to avoid contamination. The plates were incubated in an incubator for 24 hours at 37°C. After 24 hours, the presence of the growth on the plates was observed (nutrient agar and MacConkey agar) and checked the haemolysis on the blood agar plates. Further some examinations were done to confirm the presence of bacteria [17].

**Culturing technique of blood**

The blood samples were collected in a sterile containers from patients with bacteraemia, fever, and cough condition though the venepuncture process with the help of sterile syringe. The nutrient broths were (2-3 bottles) prepared in aseptic conditions with the pH adjusted at 7.4 and the temperature was 25°C. The blood samples were inoculated in the bottles. The incubation was done at least 48 hours (2 days) at 37°C. The turbidity inside the bottles, the change of the colour in the bottles, the production of gases were checked to detect the presence or the absence of the growth of bacteria.

**Microscopic examination**

The microscopic examination was performed to differentiate the Gram’s negative and Gram’s positive bacteria from cultural growth [18].

**Biochemical tests:**

Biochemical tests were performed for further confirmation and differentiation of microbes

**Oxidase Test:**

Method: A clean glass slide was taken. The pure culture was added on the slide. 1-2 drops of the oxidase reagent were added on the same slide and finally the change of the colour was observed after 30 seconds.

**Catalase**

Method: A clean glass slide was used. The pure culture was put on the slide by the help of a stick. A drop of catalase reagent was put on the same slide. The bubbling was observed after few seconds.

**Antibiotics sensitivity testing**

The method used was the Kirby- Bauer disc diffusion method [19]. The Mueller Hinton agar was prepared with the pH adjusted at 7.3 and the temperature was 25°C. The agar and other materials were autoclaved at 15lbs. The agar was put in the sterile plates. The inoculum was prepared by collecting the samples with the help of a sterile loop and it was suspended in 2ml saline solution already prepared. The turbidity of the suspension was adjusted to 0.5 Mc Farland standard by adding more organisms if the suspension is too light.
or diluting with sterile saline if the suspension is too heavy. Wickerham card was also used to adjust the turbidity of the suspension. The suspension was used within 15 minutes. A sterile cotton swab was used to get the bacterial suspension. The streaking was done on the different plates (the rotation was repeated for 3 times). The plates were allowed to dry for 5 minutes. The antibiotic disc dispenser was used to dispense discs containing specific antibiotics on the plate the sterile loop was used to press gently each disc on the agar plate to ensure the disc to attach on the agar plates. The plates were incubated overnight at 37ºC. The zone formation was measured in millimetres by using a ruler the next day to determine the inhibition of microorganisms; and finally some references according to different microorganisms were used to check the zone of inhibition for detecting the resistance or sensitivity of bacteria.

**Results and discussion**

The study was done on the patients from hospital of Jalandhar. There were 290 samples collected from hospital such as pus, urine, sputum, wound, blood, C.S.F and further the reporting was done on most leading samples urine and blood samples for a period of 4 months (January 2019 to April 2019).

The study was done from 214 urine and blood samples out of 290 samples in total in which there were 96 positive samples and 118 negative samples for a period of 4 months.

**Prevalence of bacteraemia (blood infection) and urinary tract infection (urine infection)**

The study included the 214 blood and urine samples from the patients with the age of 19 to 93 years. Among these 214 samples, 129 samples were from urine samples (60, 28%) and 85 samples were from blood samples (39.72%). Among the urine samples, 75 (58.14%) were females and 54 (41.86%) were males. Among the blood samples, 53 (62.35%) were females and 32 (37.65%) were males. Out of the 214 blood and urine samples studied, 96(43.46%) were shown significant positive growth bacteria and 121(56.54%) were shown negative.

**Prevalence of bacteraemia and urinary tract infection in over all patients according to gender**

The study was done from 214 blood and urine samples in which 86 males (40.19%) and 128 females (59.81%) were susceptible to get bacteraemia or urinary tract infection. Among the 96 positive samples, 60.42% of women and 39.58% of men developed the infections (either blood infection or urine infection). The prevalence of the infections were significantly higher in women than men (p<0.01)
Table 1: Shown the prevalence of infections according to Gender

<table>
<thead>
<tr>
<th></th>
<th>Prevalence of bacteremia and UTI in male patients (No)</th>
<th>Prevalence of bacteremia and UTI in male patients (%)</th>
<th>Prevalence of bacteremia and UTI in female patients (No)</th>
<th>Prevalence of bacteremia and UTI in female patients (%)</th>
<th>Total</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patients with infections</td>
<td>32</td>
<td>37.2</td>
<td>58</td>
<td>45.3</td>
<td>90</td>
<td>Chi square 5.565</td>
</tr>
<tr>
<td>Patients without infections</td>
<td>54</td>
<td>62.7</td>
<td>70</td>
<td>54.6</td>
<td>124</td>
<td>P=0.01</td>
</tr>
<tr>
<td>total</td>
<td>86</td>
<td>100</td>
<td>128</td>
<td>100</td>
<td>214</td>
<td></td>
</tr>
</tbody>
</table>

Isolated pathogens from blood and urine culture in all the patients

The isolated pathogens from 96 positive growth cultures were distributed with different percentage in both females and males in our general studied population. The most causative agent of the infections during the study was *E. coli* 45 cases (46.88%), and then there were *Staphylococcus albus* 24 cases (25%), *Pseudomonas aeruginosa* 13 cases (13.54%), *Klebsiella pneumonia* 11 cases (11.96%), *Mycobacterium tuberculosis* 3 cases (3.12%).

<table>
<thead>
<tr>
<th>Microorganisms</th>
<th>E. coli</th>
<th>Staphylococcus albus</th>
<th>Pseudomonas aeruginosa</th>
<th>Klebsiella pneumonia</th>
<th>Mycobacterium tuberculosis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Percentage %</td>
<td>45 cases</td>
<td>24 cases</td>
<td>13 cases</td>
<td>11 cases</td>
<td>3 cases</td>
</tr>
<tr>
<td></td>
<td>(46.88%)</td>
<td>(25%)</td>
<td>(13.54%)</td>
<td>(11.96%)</td>
<td>(3.12%)</td>
</tr>
</tbody>
</table>

Table 2: Shown the isolation of pathogens from cultures in all the patients
Figure 1: Shown graph of frequency of causative agents in all the positive blood and urine cultures

<table>
<thead>
<tr>
<th>Isolates</th>
<th>Gender</th>
<th>Total</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>male</td>
<td>female</td>
<td>Number</td>
</tr>
<tr>
<td>E. coli</td>
<td>17 (17.71%)</td>
<td>28 (29.17%)</td>
<td>45</td>
</tr>
<tr>
<td>Staphylococcus albus</td>
<td>10 (10.42%)</td>
<td>14 (14.58%)</td>
<td>24</td>
</tr>
<tr>
<td>Pseudomonas aeruginosa</td>
<td>5 (5.21%)</td>
<td>8 (8.33%)</td>
<td>13</td>
</tr>
<tr>
<td>Klebsiella pneumonia</td>
<td>4 (4.17%)</td>
<td>7 (7.29%)</td>
<td>11</td>
</tr>
<tr>
<td>Mycobacterium tuberculosis</td>
<td>1 (1.04%)</td>
<td>2 (2.08%)</td>
<td>3</td>
</tr>
<tr>
<td>Total</td>
<td>37 (38.55%)</td>
<td>59 (61.45%)</td>
<td>96</td>
</tr>
</tbody>
</table>

Table 3: Shown the isolation of the pathogens in the studied population according to gender, for this study of the pathogens according to the gender, the Chi square was 4.003 and p value=0.01

Susceptibility of organisms isolated from blood and urine cultures
Susceptibility of *E. coli* isolated from blood and urine cultures:

In the study, 45 isolated Gram’s negative *E. coli* were found to be sensitive to Meropenem (67%), Etrapenem (41%), Colinstin (100%), Amikacin (73%), Ceftazidine (7%), Fosfomycine (84%), Ceftriazone EDTA sulbectam (100%), Gentamycin (40%), Cefozolin (4%), Imipenem (7%), Cefixime (5%), Ampicillin (4%), Norfloxacin (5%).

![Graph of the level of sensibility of *E. coli* to antibiotics](image1.png)

**Figure 2:** Graph of the level of sensibility of *E. coli* to antibiotics

Susceptibility of *Pseudomonas aeruginosa* isolated from blood and urine

*Pseudomonas aeruginosa* were found sensitive to Meropenem (50%), Etrapenem (50%), Colinstin (100%), Amikacin (67%), Ceftazidine (0%), Cefoperazone – sulbactam (17%), Ceftraxone EDTA (100%), Ceftriaxone (6%), Imipenem (33%), Cefixime (0%), Aztreonam (17%), Co-trimoxozol (0%), Norfloxacin (50%).

![Graph of the level of sensibility of *Pseudomonas aeruginosa* to antibiotics](image2.png)

**Figure 3:** Graph of the level of sensibility of *Pseudomonas aeruginosa* to antibiotics
Susceptibility of *Staphylococcus albus* isolated from blood and urine cultures

*Staphylococcus albus* found in the study were sensitive to Linezolid (100%), Nitruforantoin (50%), Telcophanine (38%), Chloramphenicol (14%), Tetracycline (29%), Rifampicin (0%), Cotimoxazole (7%), and Ampicillin (15%).

![Graph of level of sensibility of *Staphylococcus albus* to antibiotics](image)

**Figure 4**: Graph of level of sensibility of *Staphylococcus albus* to antibiotics

Susceptibility of *Klebsiella pneumonia* isolated from blood and urine

*Klebsiella pneumonia* bacteria found were sensitive to Meropenem (37%), Etrapenem (21%), Colinstin (100%), Amikacin (16%), Ceftazidine (5%), Fosfomycine (68%), Ceftriaxone EDTA (100%), Imipenem (5%), Tigecycline (11%), Ofloxacine (5%), Ampicillin (0%), Norfloxacin (0%), Nitrofurantoin (21%), Gentamycin (5%), Cefazolin (0%).

![Graph of the level of sensibility of *Klebsiella pneumonia* to antibiotics](image)

**Figure 5**: Graph of the level of sensibility of *Klebsiella pneumonia* to antibiotics
Susceptibility of *Mycobacterium tuberculosis* in the blood and urine cultures

*Mycobacterium tuberculosis* bacteria were found sensitive to Cefoparazone (50%), Levofloxacin (68%), Gentamycin (85%), Polymycin-B (15%), Amikacin (0%), Ceftriazone, Fosfomycin (20%), Tobramycin (100%), Cefopara sulbactam (67%), Ciprofloxacin (33%), Azithromycin (2%).

![Graph of the level of sensibility of *Mycobacterium tuberculosis* to antibiotic](image)

**Figure 6:** Graph of the level of sensibility of *Mycobacterium tuberculosis* to antibiotic

Discussion

The study was conducted on the microbiological examination of urine and blood from patients suffering from UTI, bacteraemia, pneumonia, during a period of 4 months (January 2019 to April 2019) in microbiology laboratory of hospital. The study demonstrated that infections were very high in female (52, 62%) than male (47, 38%) due to some parameters like age, lifestyle, body physiology, physical exercises. The prevalence of the UTI was 42.17% and it was 36.07% in bacteraemia which have shown that the nosocomial infections were mostly the UTI. All age groups have shown the significance of the infections, although the higher number was observed for age group of 20-44 years old in which the routine tests have to be done. It was found that 43.46% of the patients were susceptible to get infections showing the impact and pertinence to control those infections in the population. The study has also demonstrated that UTI and bacteraemia are prominent responsible of nosocomial infections in that particular population *E. coli* was the most bacteria found in the study represented by 46.88% due to higher risk to get the infection through the catheters contamination but recently some studies have demonstrated that *Staphylococcus aureus* bacteria was a high cause of nosocomial infection. There has been an increase in the rate of antibiotic resistant bacteria associated with nosocomial infections in ICU. Bacteria develop resistance when they acquire new genetic material. Poor antibiotic prescribing selects for resistant bacteria. Some microorganisms were more prominent in the study like *E. coli* due their high ability to be resistant to many antibiotics by changing their metabolism, by mutating, by transferring some genetic materials from one strain to another one and they were directly linked to the nosocomial infections. Many challenges have to be adopted by the personal of
hospital to control and to decrease the number of nosocomial infections in the population. The Study of the Efficacy of Nosocomial Infection Control (SENIC) demonstrated that a third of nosocomial infections might be prevented with appropriate infection control measures. These comprise surveillance methods, prevention strategies and treatment programs. The population and health centres have to improve a lot of conditions especially hygienic conditions to decrease the high prevalence of the nosocomial diseases in the world because with time the number of those diseases is increasing.

**Conclusion**

Urine and blood samples are very helpful for the diagnostic of many infections and diseases especially the nosocomial infections where some bacteria as *E. Coli* (mostly), *Pseudomonas aeruginosa*, *Klebsiella pneumonia*, *Staphylococcus species* have developed the strategies to escape to the action of many antibiotics leading to increase of the prevalence of those diseases in the population. Women are more prone to get the infection than male because of some parameters like their lifestyle, the metabolism or body physiology and the number of the infections is increasing day by day in the hospitals. The mode of transmission is also mentioned in these infections because they are involved in how to handle the diseases by controlling the transmission through the contaminated instruments. The analysis have shown that the antibiotics play also a big role in the emergence of those infections in the population by the fact some bacteria are able to escape to their action and this will lead to nosocomial infections. The control of these infections is done through many processes which should be applied by the population to decrease the prevalence of the UTI and the bacteraemia which are the most nosocomial infections found in the hospital environment. The hygienic conditions should be applied in the whole population and at the hospital regarding the MDR bacteria to decrease the level of the diseases caused by the MDR bacteria so many centers of control should be created and also the education should be provided to the population about the risks, the causes, consequences, mode of action of the nosocomial infections and also the most important they have to be aware about the methods of prevention and protection against these particular diseases. People from hospital should take care of the different precautions to improve the environment and the conditions at the hospital for the main aim to decrease the level of nosocomial infections in the hospitals.

**References**


