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Prevalence of gram positive and gram negative organisms in various clinical samples

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ABSTRACT

Background

The present work was aimed to depict the prevalence of Gram positive and Gram negative organisms isolated from different clinical samples.

Materials and Methods

Different pathogenic organisms were isolated from Males and females of different clinical samples such as Midstream Urine, Miscellaneous (Sputum, Pus, HVS, Body fluids, endotracheal aspirates, Eye swabs) and Blood samples, during the period of October 2015 to December 2015. Samples were processed and identification of isolates were done by gram staining, biochemical test. Antibiotic sensitivity testing of isolated pathogenic organisms was done by a disk diffusion technique using Mueller Hinton Agar plates according to CLSI guidelines.

Results

Out of total 770 samples analyzed, 414 samples showed the presence of bacteria, whereas 356 samples showed no growth of organisms on culture medium. Microbial growth revealed the presence of 11 different organisms which are reported here (*Escherichia coli*, *Klebsiella pneumoniae*, *Klebsiella oxytoca*, *Pseudomonas aeruginosa*, *Acinetobacter baumannii*, *Enterococcus faecalis*, *Citrobacter koseri*, *Proteus mirabilis*, *Staphylococcus epidermidis*, *Staphylococcus aureus*, *Streptococcus pyogenes)*.

Conclusion

The present study provided the most needed information on the prevalence of both Gram positive and Gram negative bacteria which were also responsible for infections in Urine, Miscellaneous and Blood samples.

Keywords: Prevalence, Disc Diffusion, Midstream Urine, Miscellaneous (Sputum, Pus, HVS, Body fluids, Endo Tracheal aspirates, Throat swabs, Eye swabs) and Blood samples.

INTRODUCTION

Microorganisms are living things, ordinarily too small to be seen without magnification, in terms of numbers and range of distribution. Microbes are the dominant organisms on earth. A large and diverse group of microscopic organisms that exists as single cells or cell clusters; it also includes viruses, which are microscopic but not cellular. [(1)] A nosocomial or hospital acquired infection develops during the patient's stay in the health care center and is generally not present or incubating at the time of admission. The microbes that grow in health care settings, along with patients weakened defense mechanisms help in setting the stage for nosocomial infections, which are mostly responsible for lengthening the patient's stay in the hospital and increasing the risk of death besides making the diagnosis and treatment of such patient's expensive. [(2)]

Besides this, usage of poor aseptic protocols by health care service providers also increases the risk of infections. [(3)] Nosocomial Infections most commonly invade the body through the urinary tract while other common portals of entry will be through the surgical wounds, the respiratory tract and the blood stream. [(4)] The number of infections caused by microorganisms is increasing significantly over the last few decades. One of the reasons for this increase is a development of microbial resistance to drugs used to treat these infections. About 50-60% of noscomial infections in the United States are now caused by antibiotic resistant strains of bacteria. [(5)]

Nowadays, difficulties in treating infectious diseases like Malaria, Tuberculosis (TB), diarrhoea, Urinary Tract Infections (UTI) etc. are because of development of drug resistance in infecting microbes. The threat to health care associated infection has been a matter of concern due to development of multidrug resistant (MDR) strains among common isolates in hospital. Applying antibiotics caused a breakthrough in treatment stepping out in ill's group infections treated in hospital, however, enlarging resistance, the formation of new mechanisms of resistance, and/or the spreading of the gene of resistance has become the shortcomings of antibiotic therapy. [(6)]

Urinary Tract Infection (UTI) remains the most commonly existing bacterial infection in human population, especially in nosocomial infected patients. [(7)] UTIs refer to the presence of microbial pathogens within the urinary tract and it is usually classified by the site of infection such as bladder (cystitis), kidney (pyelonephritis) or urine (bacteriuria). They are asymptomatic or symptomatic. UTIs that occur in a normal genitourinary tract with no prior instrumentation are considered as "uncomplicated", whereas "complicated" infections are diagnosed in genitourinary tracts that have structural or functional abnormalities including instrumentation such as indwelling urethral catheters, and are frequently asymptomatic. [(8)]

Pseudomonas aeruginosa frequently isolates as an opportunistic pathogen in recurrent infections of hospitalized patients and has been isolated from a number of sites in the hospital environment. [(9)] It is the most relevant, resistant and dangerous organism infecting the burn patients. [(10)] It is the fifth most common pathogen among hospital microorganisms and causes 10% of all hospital acquired infections. [(11)] Bacteremia and other bloodstream Infections (BSIs) are a major cause of morbidity and mortality worldwide. Usually, the bloodstream is sterile. Individuals with bacteremia may develop Septicemia, a life-threatening condition in which multiplying bacteria release toxins into the bloodstream. Microorganisms present in circulating blood, whether continuously or intermittently, a threat to every organ in the body. Approximately 200,000 cases of bacteraemia and fungemia occur annually with mortality rates ranging from 20-50%. Blood culture remains a highly specific indicator of bacteriemia. Antimicrobial susceptibility testing assist a great deal in a precise identification of the most appropriate choice of drug to be administered. [(12)]

The present work was intended to depict the prevalence of both Gram positive and Gram negative organisms isolated from various clinical samples.

MATERIALS AND METHODS

Sample Collection

Clinical samples which include Midstream Urine, Miscellaneous (Sputum, Pus, HVS, Fluids, Endo Tracheal aspirates, Throat swabs, Eye swabs) and Blood were collected from both male and female patients. The present study was carried out over a period of 3 months from Oct 2015 to Dec 2015 in Microbiology department, SSSMC& RI.

Culturing, Characteristics and Identification of bacteria

Samples were processed according to their nature type. All the samples were cultured on MacConkey Agar, Blood agar, chocolate agar plates and incubated according to the sample nature type. The confluent growth of the pathogens isolated was included in this study. Isolates were subcultured and colonies were screened for Gram positive and Gram negative isolates. Identification of isolates were done by gram staining and biochemical test. Result interpretation was done based on the identifying characters as described by Cheesbrough. [14,15]

Antibiotic Sensitivity Testing

Antimicrobial sensitivity was determined by Kirby Bauer disc diffusion method on Mueller Hinton agar (MHA). A suspension of each isolate was made so that the turbidity was equal to 0.5 McFarland standards and then plated as a lawn culture onto MHA. Antibiotic discs were placed and plates were incubated at 37°C for 18-24 hrs. Results were interpreted in accordance with central laboratory standards institute (CLSI) guidelines. [16]

RESULTS

Table:1 Prevalence of bacterial growth in urine sample:

Organism isolated	No of isolates	No of Male	No of female	Total percentage (%)
Escherichia coli	105	45	60	50
Klebsiella pneumoniae	38	21	17	18
Klebsiella oxytoca	25	11	14	12
Pseudomonas aeruginosa	15	9	6	7
Staphylococcus epidermidis	8	3	5	4
Acinetobacter baumannii	4	0	4	2
Citrobacter koseri	4	0	4	2
Enterococcus faecalis	5	2	3	2
Proteus mirabilis	4	3	1	2
Streptococcus pyogenes	2	0	2	1
Total	210	94	116	100 %
Percentage (%)		45%	55%	

Table: 2 Prevalence of bacterial growth in miscellaneous samples:

Organism isolated	No of isolates	No of Male	No of female	Total percentage (%)
Escherichia coli	50	29	21	28%
Klebsiella pneumoniae	43	27	16	23%
Klebsiella oxytoca	27	11	16	15%
Pseudomonas aeruginosa	25	16	9	14%
Acinetobacter baumannii	16	8	8	9%
Enterococcus faecalis	6	3	3	3%

Citrobacter koseri	5	2	3	3%
Proteus mirabilis	4	3	1	2%
Staphylococcus epidermidis	2	1	1	1%
Staphylococcus aureus	1	1	-	1%
Streptococcus pyogenes	2	1	1	1%
Total	181	102	79	100%
Percentage (%)		56%	44%	

Table: 3 Bacterial identification in each sample of Miscellaneous:

0rganism isolated	**Sputum**	**Pus**	**HVS**	**Fluids**	**Endo Trachael**	**Throat swabs**	**Eye swabs**	**Total**
Escherichia coli	9	17	20	1	2	1	-	50
Klebsiella pneumonia	23	10	9	1	-	-	-	43
Pseudomonas aeruginosa	11	10	3	-	-	-	1	25
Klebsiella oxytoca	9	9	8	-	1	-	-	27
Acinetobacter baumannii	-	5	5	-	-	-	-	16
Proteus mirabilis	-	4	-	-	-	-	-	4
Enterococcus faecalis	1	2	2	1	-	-	-	6
Staphylococcus epidermidis	-	1	1	-	-	-	-	2
Citrobacter koseri	2	1	1	-	-	1	-	5
Staphylococcus aureus	-	1	-	-	-	-	-	1
Streptococcus pyogenes	2	-	-	-	-	-	-	2
Total	63	60	49	3	3	2	1	181
Percentage (%)	34%	33%	27%	2%	2%	1%	1%	100%

Table: 4 Prevalence of Bacterial Growth in Blood culture

Organism isolated	**No of isolates**	**No of Male**	**No of female**	**Total percentage (%)**
Staphylococcus epidermidis	8	5	3	37%
Klebsiella pneumoniae	4	3	1	17%
Acinetobacter baumannii	4	2	2	17%
Pseudomonas aeruginosa	2	1	1	9%
Staphylococcus aureus	1	1	-	4%
Klebsiella oxytoca	1	1	-	4%
Salmonella typhi	1	-	1	4%
Proteus mirabilis	1	1	-	4%
Escherichia coli	1	1	-	4%
Total	23	15	8	100%
Percentage (%)		65%	35%	

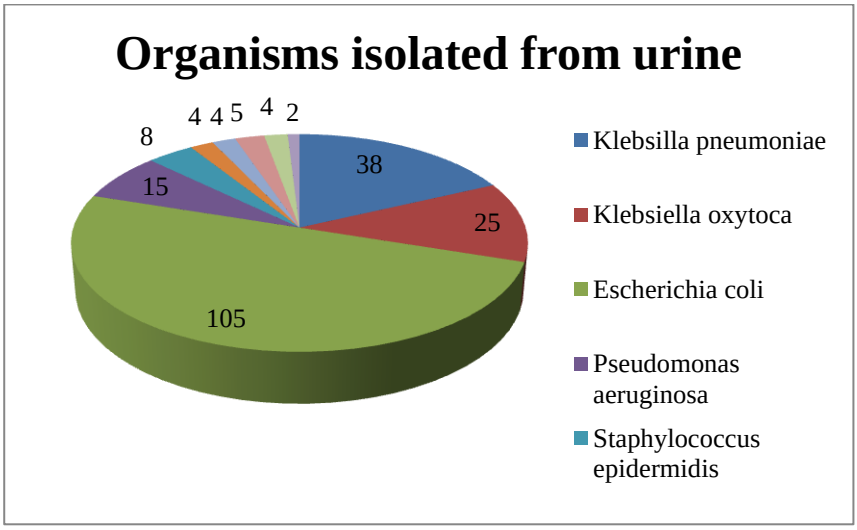

Fig: 1 Distribution of bacterial growth in Urine sample:

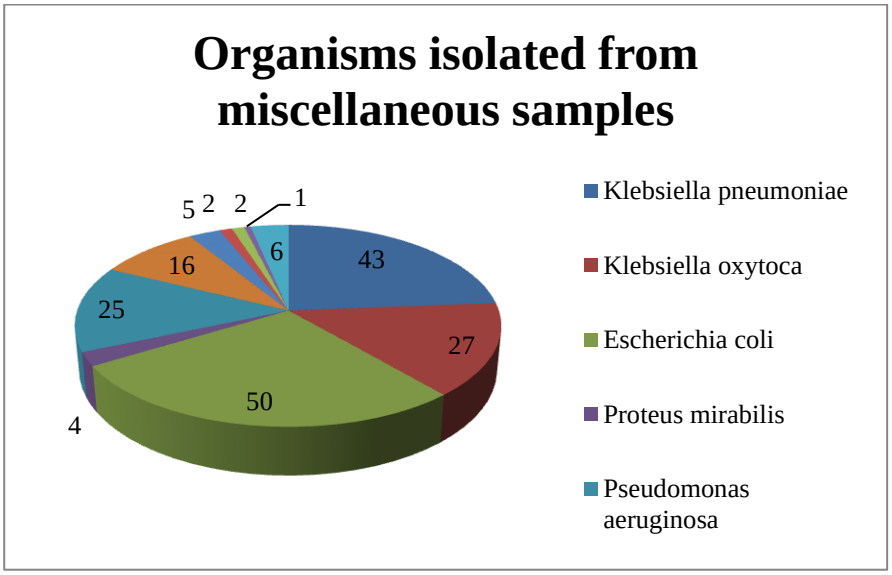

Fig: 2 Distribution of Bacterial Growth in Miscellaneous samples:

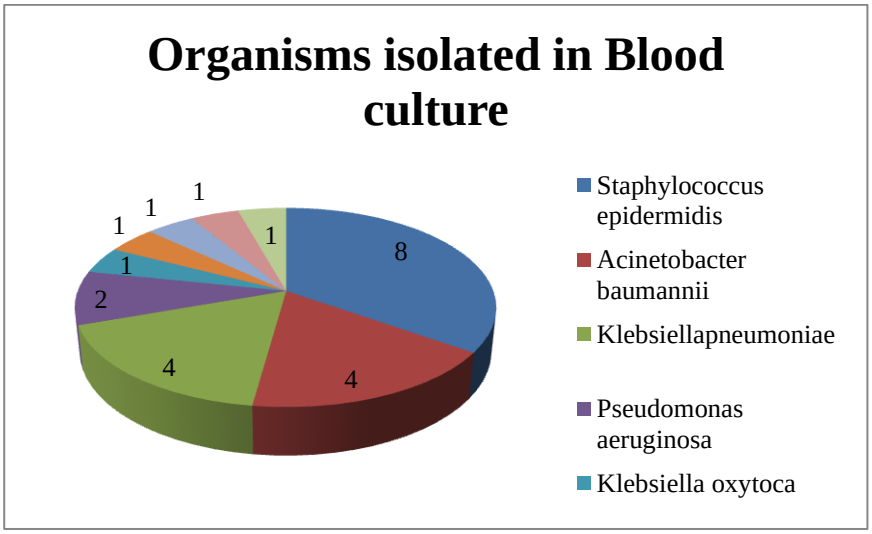

Fig: 3 Distribution of Bacterial Growth in Blood culture:

This study was conducted during a period of three months on clinical isolates from indoor patients in order to determine the prevalence of clinically significant Gram-positive cocci and Gram negative bacilli in various clinical samples at SSSMC & RI. Between October 2015 and Dec 2015. Midstream Urine, Miscellaneous and Blood cultures were examined for the presence of bacteria.

Out of total 770 samples analyzed, 414 samples showed the presence of infection while in 356 samples no growth of organisms was seen in culture medium. The 3 types of clinical samples such as Midstream Urine, Miscellaneous (Sputum (34%), Pus (33%), HVS (27%), Fluids (2%), Endo Tracheal aspirates (2%), Throat swabs (1%), Eye swabs (1%)) and Blood were observed which is shown in (Table 3).

The commonly isolated organisms from Mid-stream Urine samples include *Escherichia coli*(50%), *Klebsiella pneumoniae*(18%), *Klebsiella oxytoca*(12%), *Pseudomonas aeruginosa*(7%), *Staphylococcus epidermidis*(4%), *Acinetobacter baumannii*(2%), *Citrobacter koseri*(2%), *Enterococcus faecalis*(2%), *Proteus mirabilis*(2%), *Streptococcus pyogenes*(1%) whereas in Miscellaneous includes *Escherichia coli*(28%), *Klebsiella pneumoniae*(23%), *Klebsiella oxytoca*(15%), *Pseudomonas aeruginosa*(14%), *Acinetobacter baumannii*(9%), *Enterococcus faecalis*(3%),*Citrobacter koseri*(3%), *Proteus mirabilis*(2%), *Staphylococcus epidermidis*(1%), *Staphylococcus aureus*(1%),*Streptococcus pyogenes* (1%) and in Blood samples include *Staphylococcus epidermidis* (37%), *Klebsiella pneumoniae* (17%), *Acinetobacter baumannii*(17%), *Pseudomonas aeruginosa*(9%), *Staphylococcus aureus*(4%), *Klebsiella oxytoca*(4%), *Salmonella typhi*(4%),*Proteus mirabilis*(4%), *Escherichia coli*(4%) as shown in the (Table 1,2,4& Fig 1,2,3).

Gram negative organisms were dominant compared to Gram positive organisms in the nosocomial infections. *E. coli* was found to be the most dominant pathogen in the Mid-stream Urine (50%) and Miscellaneous samples (28%) but in Blood samples *Staphylococcus epidermidis* was the dominant pathogen. *Streptococcus pyogenes* (1%) were found to be the least responsible in the urine, *Staphylococcus aureus* (1%), *Staphylococcus epidermidis*(1%) were least in the Miscellaneous samples and *E.coli*(4%) was least in the Blood samples. *Salmonella typhi* (4%) was also observed to be present in collected Blood samples. Hence, in urine samples female (55%) were highly infected than Male (45%). Where as in Miscellaneous, male was 56% and female was 44% and in Blood samples, male was found to be affected more (65%) and female (35%).

DISCUSSION

This study was targeted to depict the prevalence of Gram positive and Gram negative organisms isolated from different clinical samples such as Urine, Miscellaneous and Blood samples. The most common uropathogens in our study were *E. coli* (50%) and *Klebsiella pneumoniae* (18%). Although the percentage of *E. coli* is much higher in our study, it supports the previous findings indicating that the *E. coli* is the principal etiological agent of UTI, accounting for 46.98% of the screened cases. [17,18,19] In another study, it was reported that predominant uropathogens are *E. coli* followed by *Klebsiella* species which also support our study. [20] Infections of *Pseudomonas aeruginosa* (7%), *Staphylococcus epidermidis* (4%), *Acinetobacter baumannii* (2%), *Citrobacter koseri* (2%), *Enterococcus faecalis* (2%), *Proteus mirabilis* (2%), *Streptococcus pyogenes* (1%) were found to be very few in this study. The similarities and differences in the type and distribution of uropathogens may result from different environmental conditions and host factors, and also from some practices such as health care and education programmers, socioeconomic standards and hygiene practices in each country.

In Miscellaneous samples, *S. aureus* was found in the most prevalent organism and its prevalence was found to be 36.36%, 38.78%, 33.33% and 54.05% during the period 2000- 2003, respectively. It was followed *by E. coli* and *Proteus spp*. In Miscellaneous includes *Escherichia coli*(28%), *Klebsiella pneumoniae*(23%), *Klebsiella oxytoca*(15%), *Pseudomonas aeruginosa*(14%), *Acinetobacter baumannii* (9%), *Enterococcus faecalis*(3%),*Citrobacter koseri* (3%), *Proteus mirabilis*(2%), *Staphylococcus epidermidis* (1%), *Staphylococcus aureus*(1%), *Streptococcus pyogenes* (1%)

The most common Gram negative and Gram positive organism isolated from the blood was

Staphylococcus epidermidis (37%), *Klebsiella pneumoniae* (17%), *Acinetobacter baumannii* (17%), *Pseudomonas aeruginosa* (9%), *Staphylococcus aureus* (4%), *Klebsiella oxytoca* (4%), *Salmonella typhi* (4%), *Proteus mirabilis* (4%), *Escherichia coli* (4%). We considered the growth of *Staphylococcus epidermidis* to be indicative of its presence in the patient as opposed to being a contaminant. *Staphylococcus* seems to be emerging as the dominant organisms in bloodstream infections. Nosocomial infection due to *Staphylococcus aureus* constitutes a major part of the total annual nosocomial infection. [21]

On the other hand, the variation in blood culture positivity depends on several other factors such as the amount of blood taken for screening [22] formulation of the blood culture medium used for bacterial detection or administration of antibiotic therapy prior to taking blood for culture (23). Our study showed that the rate of isolation of Gram negative bacteria is higher than Gram positive bacteria. In contrast to our study, Kavitha P, et al and Swain B et al [24,25] reported Gram positive bacteria were dominant than Gram negative bacteria. Bloodstream infections with *Pseudomonas* spp. have been associated with increased morbidity in some studies. Another significant finding of the study is the isolation of *Salmonella* typhi in 4% of the cases. An increasing incidence of *Salmonella* species has also been reported in some recent studies. [26, 27]

In most of the studies, Gram-negative bacilli have taken over the Gram-positive organisms, especially in hospital settings. Mehta et al [28] has reported the incidence of 80.96% for gram-negative and 18% for Gram-positives which was similar to present findings. Our study revealed that Gram-negative bacteria were predominant than Gram positive bacteria. This has been an observation among similar studies done in the patients of developing countries. [29-32]

CONCLUSION

The present study provided much necessary information based on the prevalence of both Gram positive and Gram negative bacteria were responsible for infections in Urine, Miscellaneous and Blood samples. Infections in the patients are notifiable problems. Adherence to infection prevention protocols, and the proper monitoring and the judicious use of antibiotics are important in preventing such infections. The solution can be planned by continuous efforts of microbiologist, clinician, pharmacist and community to promote greater understanding of this problem. Frequent hand washing, prevent spread of the organism should be encouraged in a hospital environment. Better medical care should be provided to patients during hospital stays.

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