Original Research Article

Bacterial profile of blood stream infections and antibiotic susceptibility pattern of isolates

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A B S T R A C T

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Blood culture provides essential information for the evaluation of a variety of diseases like endocarditis, pneumonia and pyrexia of unknown origin particularly, in patients with suspected sepsis. Septicaemia is one of the leading causes of neonatal mortality along with perinatal hypoxia. This work aimed to study the bacterial flora of blood stream infections in adults and paediatric patients including neonates, to study the risk factors and duration of incubation for obtaining positive cultures; to study their antibiotic susceptibility pattern and compare results with those of other investigators in this field. This study was conducted in the department of microbiology, NRI medical college and general hospital over a period of six months, from Jan 2009 to June 2009. During the six months period, 200 samples received from various departments were processed and relevant findings were noted. Out of 200 blood samples growth was obtained in 34 samples (17%). Among them Gram positive bacteria were 11 (5.5%). Gram negative bacilli were 23 (11.6%). Among the Gram positive bacteria maximum susceptibility was seen with Erythromycin and Vancomycin, maximum resistance was seen with Methicillin and Imipenum. The timely detection of bacteraemia can have profound influence on the final clinical outcome. The causative agents of septicaemia are acquired in hospital.

Introduction

Microorganisms present in the circulating blood whether continuously or intermittently are threat to every organ in the body (Usha and Pushpa Devi, 2007). Approximately 200,000 cases of bacteraemia and fungemia occur annually with mortality rates ranging from 20–50% (Dharm Raj *et al.,* 2013). The detection of microorganisms in a patient's

blood has great diagnostic and prognostic significance (Weinstein *et al.,* 1997). Blood culture provides essential information for the evaluation of a variety of diseases like endocarditis, pneumonia, and pyrexia of unknown origin particularly, in patients with suspected sepsis. Septicaemia is one of the leading causes of neonatal mortality along with perinatal hypoxia (Beebe and Koneman, 1995).

Many infections in neonatal and paediatric age group can only be established on the basis of etiological agent recovered from blood. Neonatal septicaemia refers to systemic infection in the new born confirmed by a positive blood culture (Klein *et al.,* 1983). It remains a major cause of morbidity and mortality amongst newborn especially in developing countries, where its incidence is higher than in the developed world (Lawn *et al.,* 2005) Apart from the relative immaturity of the immune system of the neonate risk factors predisposing to neonatal septicaemia in developing countries include prolonged rupture of membranes, prematurity, birth asphyxia, length of time spent in hospital, invasive procedure, delivery outside a hospital, material used in cutting and dressing the cord and maternal infections during pregnancy (Elbayoumi and Elmanama, 2011). A positive blood culture does not necessarily confirm infection, since contamination of blood can occur. The recovery of organisms traditionally considered as pathogens pose no problems in interpretation (Phillips *et al.,* 1990).

However, recovery of organisms such as coagulase negative *Staphylococci* (CONS), *Corynebacterium* or *Candida* spp is often difficult to interpret (Murty and Gyaneshwari, 2007). Additional information like the density of bacteraemia, number of positive cultures, duration of incubation of the broth to obtain a positive culture, presence of risk factors or an underlying

disease, is required in order to determine whether infection is truly present (Mane *et al.,* 2010). Since early 1950's there is striking increase in incidence of bacteraemia caused by members of *Enterobacteriaceae* and non fermenting gram negative rods (Leclerc *et al.,* 2001). Escherichia coli which was reported to be common in the past is being replaced by other multidrug resistant bacteria like *Klebsiella, Enterobacter, Salmonella, Citrobacter, Pseudomonas, Acinetobacter,* etc. (Maja *et al.,* 2006).

One key determinant in the ultimate outcome of patients with sepsis is institution of early and appropriate antimicrobial therapy (Lodise *et al.,* 2007). It is a common practice to institute early empirical therapy with broad spectrum antibiotics in patients presenting with clinical features suggestive of septicaemia or bacteraemia (Sipsas *et al.,* 2005) Given the severity of septicaemia, such empirical therapy may be justified, but the specific therapy based on the antibiogram of the isolate will definitely improve the therapeutic outcome (Owens, 2008). Sometimes even after receiving antibiotic susceptibility report, physicians may prefer to maintain the original regimen in setting of clinical improvement (Tamma and Cosgrove, 2011) There is conflicting information in how much attention the physician should pay to the cultures and antibiotic susceptibility reports (Keith *et al.,* 2014). The infection caused by MDR organism is more likely to prolong the hospital stay, increase the risk of death and require treatment with more expensive antibiotics (French, 2005). Early diagnosis and appropriate treatment of blood stream infections can make the difference between life and death. It would reduce mortality from septicaemia, reduce turnaround time and improve patient management (Bhattacharya, 2005).

The present study was undertaken to determine the bacterial flora of the blood stream infections in adults and pediatric patients including neonates and their antibiotic susceptibility pattern.

Materials and Methods

This prospective study was conducted in The Department of Microbiology, NRI Medical College and General Hospital over a period of six months, from Jan 2009 to June 2009. During the six months period, 200 samples received from various departments were processed and relevant findings were noted. Patients presented with prolonged fever or clinical impression of septicaemia / bacteraemia. Patients having prolonged fever in the post – operative period, despite atibiotic coverage were included in the study. Detailed history was taken to identify the possible risk factors. History of antibiotic usage empirically either before or after admission was also obtained. Data was collected on a pre-designed proforma. Blood samples for culture were collected following strict aseptic precautions. If empirical antibiotics were already started, the collection was timed before the next dose of antibiotic was due or about half-an-hour before the predicted peak of temperature. A second set was also collected in all patients about an hour later from a different venipuncture site. Three samples were collected in cases of suspected or sonographically diagnosed congenital heart disease. About 1 ml of blood in case of neonates and about 5ml in case of children was collected in each set. Immediately after collection, the blood was inoculated into BHI broth without switching needles. The bottle containing 10ml BHI broth was used in case of neonates and 5ml were used for children and adults to allow 1:10 dilution. The culture bottles were incubated at 37ºC aerobically. After overnight incubation, the samples were subcultured onto blood agar, Mac Conkey's agar and chocolate agar. If no growth observed on plates by the next day, subcultures were again repeated from the broth on day 3, day 4 and finally on day 7. Antibiotic susceptibility tests were performed according to the standard methods.

Procedure: Heat fixed smear is flooded with crystal violet and allowed to act for 30 seconds. The stain is poured off and is washed with iodine solution. Smear is covered with fresh iodine solution and allowed to act for 30 seconds. Iodine solution is drained off and smear is washed freely with water. The smear is flooded with iodine acetone mixture and is allowed to act for 30 seconds. Smear is washed with water and counter stained with carbol fuchsin and is allowed to act for 30 seconds. Smear is again washed with water, dried between folds of filter paper and observed under oil immersion objective.

Culture media: The media used in blood culture bottles are multipurpose and nutritionally enriched. Tryptic (or) trpticase soy, supplemented peptone, BHI, Columbia CNA Agar and Brucella broths are commonly used. Most commonly used blood culture media contain the anticoagulant sodium polyenethol sulfonate.

1) **Biphasic media: brain heart infusion agar and broth:** A biphasic (Castenada's) medium is one that combines on agar slope with a broth medium. The blood broth is allowed to run over the slope by tipping the bottle at regular intervals. Microbial activity can be seen by growth on the slope. This avoids the need to subculture from bottle and therefore reduces risk of contaminating the culture.

2) **Nutrient agar:** Sterilized by autoclaving at 15 lbs pressure (121ºC) for 15 minutes

and poured into sterile Petridishes.

3) **Blood agar:** Nutrient agar + 5% sheep blood at 50ºC, final pH - 7.4.
4) **MacConkey agar:** Sterilized by autoclaving at 15 lbs pressure (121ºC) for 15 minutes and poured into plates.

General procedure: The inoculated blood culture media should be incubated at 37ºC for 7 days. If any growth appears during incubation period, it should be proceed with gram stain. Then based on gram stain smear, it should be subcultured in corresponding media. The further tests such as biochemicals should be performed.

Biochemical reactions

Indole test: The test demonstrates the ability of certain bacteria to decompose the amino acid tryptophan to indole, which accumulates in the medium. Medium: The pH was adjusted to 7.4. The medium was dispensed and sterilized by autoclaving at 121ºC for 15 minutes.

Kovac's reagent: Aldehyde was dissolved in alcohol layer and slowly the acid was added. The reagent was prepared in small quantities and stored in refrigerator. The medium was inoculated and incubated for 24 hrs at 37ºC. 0.5 ml of Kovac's reagent was added and shaken gently before use.

Interpretation: A red colour in alcohol layer indicates a positive reaction. Yellow colour indicates negative reaction.

Methyl red test (Mackie and Mccartney, 1996)**:** This test is employed to detect the production of sufficient acid during fermentation of glucose and maintenance of pH below 4.5 The peptone and phosphate were dissolved; the pH was adjusted to 7.6, filtered, dispensed in 5 ml amounts and sterilized at 121ºC for 15 minutes. The glucose solution was sterilized by filtration

and 0.25 ml was added to each tube.

Methyl red indicator solution: The liquid medium is inoculated lightly and incubated at 37ºC for 48 hrs. About 5 drops of methyl red reagent (0.04) was added, mixed and read.

Voges-Proskauer (acetoin production) test (Mackie and Mccartney, 1996)**:** The test depends on the production of acetoin (acetyl methyl carbinol) from pyruvic acid in media. In presence of alkali and atmospheric oxygen acetoin is oxidized to diacetyl which reacts with alpha-napthol to give red colour. Glucose phosphate peptone water is inoculated and incubated at 37ºC for 48 hrs. 1 ml of 40% KOH and 3 ml of 5 solution of alpha naphthol were added in absolute alcohol. A positive test was indicated by the development of pink colour with in 2–5 min, becoming crimson in 30 min.

Citrate utilisation test (Mackie and Mccartney, 1996)**:** This is a test for ability of an organism to utilize citrate as the sole carbon and energy source for growth and an ammonium salt as the sole source of nitrogen. Simmon's citrate medium Organism is inoculated and incubated at 37ºC. Blue colour and streak of growth-indicates positive. Original green colour and no growth indicates negative.

Urease test (Mackie and Mccartney, 1996)**:** The glucose and urea solutions were sterilized by filtration. Basal media was prepared and pH is adjusted to 6.8-6.9 and sterilized by autoclaving at 121ºC for 15 min and cooled to about 50ºC glucose and urea were added and the medium was tubed as deep slopes. The entire slope was inoculated and incubated at 37ºC. Urease positive cultures change the colour of indicator to pink ($NH_2.CO-NH_2+H_2O$ ---> $2NH_3+CO_2$).

Triple sugar iron agar (Mackie and Mccartney, 1996)**:** Ingredients were heated to dissolve the solids the indicator solution mix was added and tubed. Sterilized at 10 lbs pressure (115ºC) for 15 minutes and cooled to form slopes with deep 3cm butts**.** Heavy inoculums was streaked over the surface of slope and stabbed into the butt and incubated aerobically at 37ºC for 24h.

Antibiotic sensitivity tests: Carried out by Kirby − Bauer disc diffusion method.

Muller − Hinton agar medium: The pH was adjusted to 7.4 and sterilized by autoclaving at 121ºC for 15 min and poured into plates.

Inoculum: Isolated colonies were inoculated in peptone water and incubated at 35-37ºC for 4–6 hrs the density was adjusted to approximately 10 CFU/ML by comparing its turbidity with that of 0.5 McFarland standard opacity tube.

Antibiotic discs: Antibiotic discs are erythromycin, piperacilin, penicillin, cefazolin, vancomycin, imipenum, ciprofloxacin, tobramycin, ceftazidime, amikacin and ceftizoxime.

Method: A cotton swab was dipped into inoculums. The Muller − Hinton agar plate was inoculated by streaking the swab three times over the entire agar surface and allowed for 3–5 minutes before applying antibiotic discs, using sterile forceps the antibiotic discs were placed and incubated. After incubation, the antibiotic sensitivity pattern was determined by measuring the diameter of zone of inhibition.

Results and Discussion

A total of 200 biological specimens were sent for culture to the microbiology lab during the period Jan 2009 to June 2009. There were 200 blood culture samples, of which 34 (17%) were identified as culture positive samples. Blood culture positive in hospitalised patients and out patients, out of 200 cases studied maximum blood samples 150 (75%) were received from ICU and 24 (12%) from paediatric ward with PUO, 22 (11%) from cardiology ward with the history of infective endocarditis and 4 samples from surgery ward with history of wound infection (2%) (Table 1). Out of 200 cases 47 (23.5%) were below the age of 20 years, among them 12 (6%) were neonates, 55 (28%) were between the age of 20–40 years, 48 (24%) were in the age group of 41−60 years. Number of cases above 60 years was 50 (25%) (Figure 1). Positive cases were predominant in males 21 (61.7%) than in females 13 (38.2%) (Figure 2).

Out of 200 blood samples, growth was obtained in 34 samples, among them 15 isolates obtained after 48 hours incubation, 6 isolates after 72 hours and 2 isolates after 7 days of incubation (Table 2). In the present study risk factors were identified in 25 cases out of which 11 (44%) had positive cultures and the following bacteria *Pseudomonas, Staphylococcus aureus, Acinetobacter* and *Klebsiella* were isolated. CONS were isolated in 2 cases but repeat isolation confirmed only one. This isolates were from a patient with post operative wound infection and peritonitis (Table 3). Out of 200 blood samples growth was obtained in 34 samples (17%). Among them Gram positive bacteria were 11 (5.5%). Gram negative bacilli were 23 (11.6%). The most commonly isolated gram-positive bacteria were *Staphylococcus aureus* in 9 (4.5%) and Coagulase negative *Staphyolococci* in 2 (1%) blood cultures. The most gram-negative bacteria found in positive cultures were *Pseudomonas aeruginosa* in 13 (6.6%), *Acinetobacter* in 6 (3%) and

Klebsiella pneumoniae in 4 (2%) (Figure 3). The antibiotic sensitivity and resistance patterns of gram-positive organisms were obtained from the laboratory reports. *Staphylococcus aureus* was found to be highly sensitive to erythromycin (100%) and vancomycin (100%) followed by pencillin (75%), piperacillin (50%), cefazolin (50%) and imipenum (25%).

Maximum resistant was seen with methicillin (100%) followed by imipenum (75%), cefazolin (50%), piperacillin (50%) and pencillin (25%) (Figure 4). Among gram-negative organisms, the sensitivity patterns were as follows: *Pseudomonas* was highly sensitive to ciprofloxacin (100%), followed by tobramycin (90%), piperacillin (80%), ceftazidime (80%), netillin (80%), imipenum (50%), amikacin (30%), amoxyclav (30%), ceftizoxime (20%) and cefepime (20%).

Klebsiella pneumoniae was highly sensitive to tobramycin (80%), amoxyclav (80%) and netillin (80%), followed by imipenum (60%), ciprofloxacin (60%), piperacillin (10%) and cefepime (10%). *Acinetobacter* was highly sensitive to ciprofloxacin (100%) and netillin (100%) followed by ceftazidime (60%), cefepime (38.3%), tobramycin (33.3%), imipenum (10%). Of the gram-negative bacteria isolated, *Pseudomonas* was highly resistant to cefepime (90%), ceftizoxime (80%), amikacin (70%), amoxyclav (70%), imipenum (50%), ceftazidime (20%), netillin (20%), ciprofloxacin (20%), piperacillin (10%) and tobramycin (10%).

Klebsiella pneumoniae was highly resistant to ceftazidime (100%) and ceftizoxime (100%), followed by piperacillin (90%), amikacin (90%) and cefepime (90%), ciprofloxacin (40%), tobramycin (20%), amoxyclav (20%) and netillin (20%).

Acinetobacter was hig hly resistant to tobramycin (100%), amikacin (100%), ceftizoxime (100%) and amoxyclav (100%) followed by imipenum (90%), cefepime (71.7%), tobramycin (66.7%) and ceftazidime (40%) (Table 4).

Septicaemia is a clinical syndrome associated with considerable morbidity and mortality. The timely detection of bacteraemia can have profound influence on the final clinical outcome. One blood culture set is rarely sufficient to establish or rule out bacteraemia, and multiple cultures could maximize sensitivity. In our study, the total number of positive cultures was 34 (17%). In India, the variation might be due to the fact that most of the patients are given the antibiotics before they come to the tertiary care hospital and other reason is that in most of the cases self medication is very common. On the present study, the highest rate of prevalence 27.5% was found of age group 21–40 years. In age group 61 years and above the prevalence rate was 25% and lowest rate of prevalence 6% was found in age group (0–1 year).

Sepsis and septic shock occur at all ages but most often in elderly patients. At present, most sepsis episodes are observed in patients older than 60 years. Advanced age is a risk factor for acquiring nosocomial blood stream infection in the development of severe forms of sepsis. Neonates are vulnerable to infections because of their weak immunological barrier. In our study males were predominantly effected 21 (61.7%) and females 13 (38.2%), it is in accordance with the study of other workers. Most studies of septic shock report a male preponderance. The percentage of male patients varies from 52-60%. Although any localized infection can disseminate to the blood stream, the most common primary foci are intravascular devices of the

respiratory tract, urinary tract and various intra abdominal sites. In the present study, risk factors were identified in only cases of which 11 (44%) had positive cultures. The CONS, previously considered as a contaminant, has been recognized increasingly as a cause of bacteraemia. The ascendance of this group of *Staphylococci* has created increased interpretative difficulties for the clinician, since the great majority of CONS isolates continue to represent contamination rather than true bacteraemia.

In the present study, CONS was initially isolated in 2 cases but repeat isolation confirmed only one. This isolate was from a patient with postoperative wound infection and peritonitis. The positive predictive value for the isolation of CONS in the present study is 12.5%. In their study CONS was initially isolated in 4 cases but repeat isolation confirmed only one (25%). Isolation of *S. aureus* from blood usually signifies infection, but bacteraemia has been observed in only two cases in the present study, which were having underlying cardiac pathology. According to one study, up to 57% of cases where *S. aureus* was repeatedly isolated will have a cardiac pathology and all such patients with *S. aureus* bacteraemia should be thoroughly evaluated for the presence of any cardiac pathology as the cardiac vegetations serve as an important source of persistent *S. aureus* bacteraemia. Prior empirical antibiotic therapy before collection of the samples for culture may result in negative blood cultures.

In the present study empirical antibiotics were already started by the time of collection of samples for culture in 50 (25%) of the cases of these only 6 (12%) had positive cultures as compared to 26 (17.4%)

positive cultures from 150 patients who did't receive any antibiotics before collection of samples for blood culture. The duration of incubation of the broth to obtain a positive culture was observed to be more among the samples from the patients who were already on antibiotics by the time of collection. This was observed for two isolated of *Acinetobacter* spp and 4 isolates of Pseudomonas so the growth was obtained on third and seventh day respectively (Table 5). Though this was an interesting finding, the number was too small for a statistical evaluation for its significance. Among the positive cultures, we have observed that (32%) of the cultures were positive by first subculture itself (after 24 hours of incubation of BHI broth) 43.4% and 20% of the culture were positive by second subculture (After 48 hrs) and third subculture after 72 hrs respectively, while virtually no isolates were obtained later (subcultured on day 7).

In our study the incidence of gram negative bacilli was (11.5%) while 5.5% isolates were gram positive organisms. In our study *Staphylococcus aureus* was isolated in 4.5% of cases and CONS in (1%) of the cases. The CONS (16.5%) and *S. aureus* 14% in neonatal septicaemia. 9% of incidence of both CONS and *S. aureus* has been reported by Surinder *et al.* (2004). Amongst gram negative organisms, *Pseudomonas*, one of the important non fermented was isolated in 6.5% of cases where as it has been reported to be 7.62% and 5.9 in other studies. It might be because that most of the patients studied were indoor patients where these nonfermenters are important pathogens, because of the invasive procedures used both for diagnostic and treatment purposes. *Acinetobacter* was isolated in 3% of the cases.

Table.1 Studied maximum blood samples Number of Positive, Negative and Percentages

Sample Category	Hospitalised patients	Percentage	Positive culture	Percentage
Peadiatrics	24	12%	2	8.33%
Cardiology	22	11%	8	36.3%
Male ICU	84	42%	14	16.6%
Female ICU	66	34%	6	8.82%
Surgery Ward	4	2%	4	2%

Table.2 Various isolates in relation to the duration of incubation of BHI broth before plating

Time of inoculation	24 hours	48 hours	72 hours	7 days	Total
Klebsiella	3	1	-	-	4
Acinetobacter	3	1	2	-	6
Pseudomonas	1	8	2	2	13
S. aureus	2	5	2	-	9
CONS	2	-	-	-	2
Total	11 (32%)	15	6	2	34

Table.3 various risk factors identified and the spectrum of isolates obtained

Risk factors	No. of patients	No. of isolates	Microorganisms isolated
Endocarditis	6	4	*Pseudomonas*
Valvular heart disease	4	2	*S. aureus*
Gastroenteritis	6	2	*Acinetobacter*
UTI	2	1	*Klebsiella*
Bronchitis Pneumonia	4	0	-
Post-operative wound infection	2	1	CONS-1
Peritonitis	1	1	CONS -1
Total	25	11	

Table.4 Antibiotic susceptibility pattern of gram negative bacteria

Antibiotics	*Pseudomonas* N=10	*Klebsiella* N=5	*Acinetobacter* N=3
Ciprofloxacin	S-100, R-20	S-60, R-40	S-100, R-0
Tobramycin	S-90, R-10	S-80, R-20	S-33.3, R-66.7
Piperacillin	S-80, R-10	S-10, R-90	S-0, R-100
Ceftazidime	S-80, R-20	S-0, R-100	S-60, R-40
Imipenum	S-50, R-50	S-60, R-40	S-10, R-90
Amikacin	S-30, R-70	S-10, R-90	S-0, R-100
Ceftizoxime	S-20, R-80	S-0, R-100	S-0, R-100
Cefepime	S-20, R-90	S-10, R-90	S-38.3, R-71.7
Amoxyclav	S-30, R-70	S-80, R-20	S-0, R-100
Netillin	S-80, R-20	S-80, R-20	S-100, R-0

Figure.1 Age group wise no. of patients in percentages
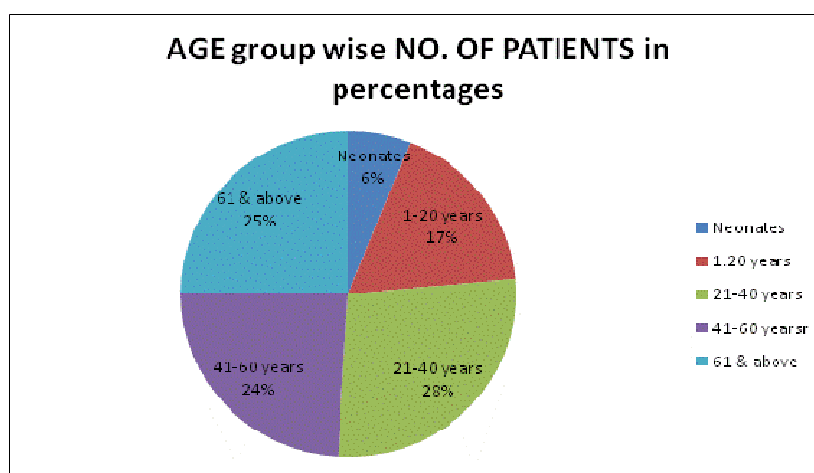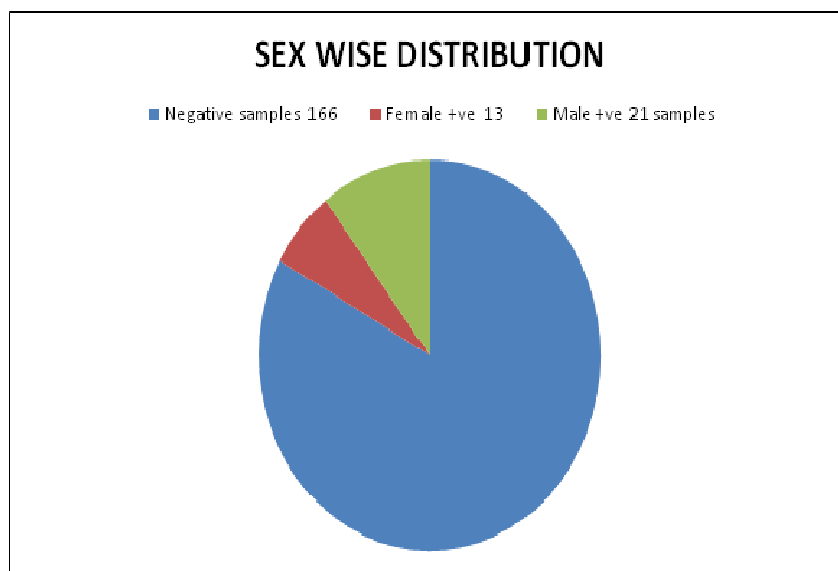

Figure.2 Sex wise distribution

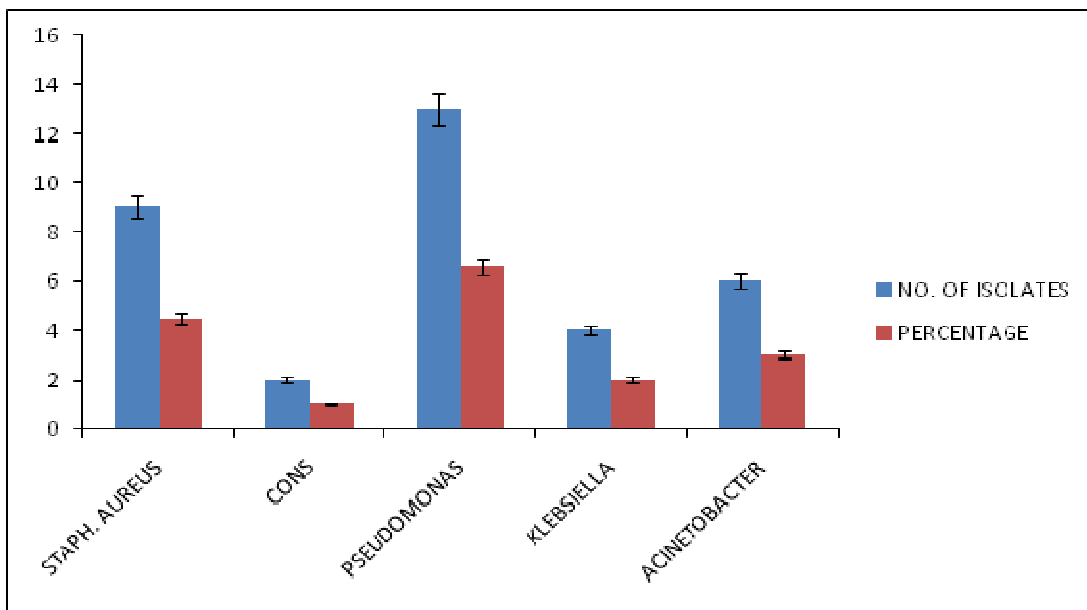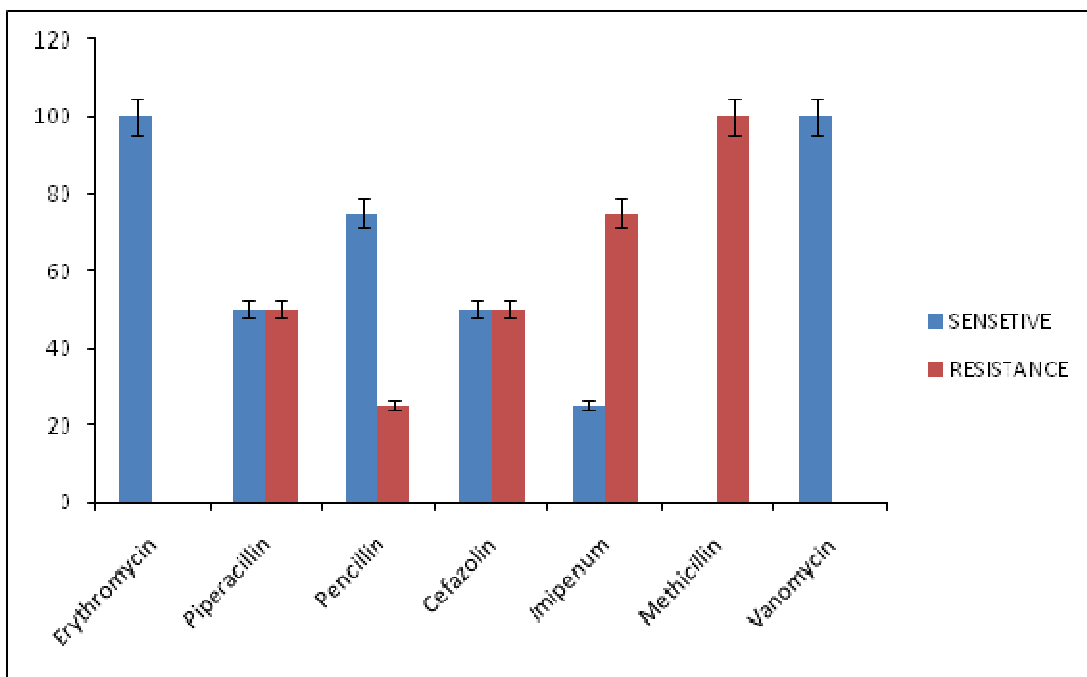**Figure.3** Distribution of bacterial pathogens

Figure.4 Antibiotic susceptibility pattern of gram positive bacteria

Acknowledgments

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